Biodiversity of the Vireya group of *Rhododendron* L. (Ericaceae) collections in New Zealand and their potential contribution to international conservation

A thesis presented in partial fulfilment of the requirements for the degree of

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Abstract

Rhododendron Subgenus *Vireya sensu* Argent (2006), commonly known as vireyas is an example of a group of plants with numerous taxonomic and conservation issues, making this group a challenge for development of conservation plans. In addition, recent molecular studies on the vireyas have revealed unexpected relationships that contrast with many of the previously known classification systems. Vireyas have been evaluated by the IUCN and have 63 taxa Red-Listed as threatened, and as New Zealand has 17 of these, they could have the potential to contribute to international conservation. Prior to developing a conservation plan, molecular techniques were used in an attempt to resolve some of the remaining taxonomic and conservation issues around the vireyas, utilizing a total of 352 accessions from approximately 160 taxa.

The initial phylogenetic analysis of 87 vireya accessions, using maximum parsimony analysis of the *rpb*2i intron 23 nucleotide sequences revealed that the sections *Pseudovireya* and *Discovireya* are monophyletic and sister to the rest of the vireyas. The remaining sections were paraphyletic or polyphyletic. Further phylogenetic analyses that included an additional 84 published sequences of the same nuclear region presented improved phylogenetic resolution, and the maximum parsimony analysis showed that the vireyas are monophyletic. This analysis also showed that the sections *Pseudovireya* and *Discovireya* are basal clades and not monophyletic, but sister to the rest of the vireyas. The phylogenetic analyses confirmed several known vireya relationships and resolved several taxonomic issues, and a new classification of vireyas is proposed. The genetic diversity analyses using microsatellite, RAPD and *rpb*2i sequence data were carried out on taxa with multiple accessions indicated that 14 out of 16 taxa showed significant genetic diversity indicating suitability for *ex situ* conservation.

This study confirmed that vireyas form a large and complex group with several remaining taxonomic issues, and it is clear that more taxa need to be studied to unravel its taxonomic complexity. This study has identified several vireya taxa in New Zealand collections that could be used as part of an *ex situ* conservation programme and a Conservation Plan for these is presented.

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Preface

The work described in this study was carried out as part of the requirements for the degree of Doctor of Philosophy (PhD) in Plant Science at the Institute of Natural Resources, Massey University, New Zealand. The study was commenced on 1 November 2007, and was based on the biodiversity of the Vireya group of *Rhodododendron* L. (Ericaceae) collections in New Zealand and their potential contribution to international conservation. The research was sparked by the recent renewed interest in the conservation of *Rhododendendron* by the BGCI under the auspices of Ms Sara Oldfield and in collaboration with various international biodiversity conservation organizations. As an initial step and a contribution towards the project, a preliminary list of vireya taxa was generated and presented to the stakeholders in early 2008.

The next step leading up to the Red-Listing of the rhododendrons was the meeting held in Singapore in 2008 to evaluate the conservation status of the rhododendrons worldwide. The outcome of this meeting was a preliminary list of rhododendron with provisional IUCN Red List categories assigned to them. This list enabled a wider selection of taxa for the present study than previously planned. The research into vireyas also showed renewed international interest with the publication of the molecular phylogenetic analyses by Brown et al. 2006a, 2006b, 2006c, which gave an insight into the complex taxonomy of vireyas and their placement within the genus *Rhododendron*. Some results were at odds with the previously accepted classification and those data exposed more taxonomic problems. However, these studies initiated a *de novo* classification of vireyas based on molecular phylogenetic analyses.

The present study was conducted in light of these recent molecular studies and plant specimens obtained from the Pukeiti Gardens (Taranaki, New Zealand) as the major source of plant material, with additional specimens obtained from smaller collections around the country. Coincidentally, the Pukeiti Gardens acquired several new vireya taxa and new accessions of existing taxa, which facilitated the present study further.

The main aim of this study was to introduce molecular techniques that would enable conservation biologists to easily identify, classify and make calculated decisions during the assessment of vireyas for conservation.

Acknowledgements

هُوَ الَّذِي خَلَقَ لَكُمْ مَا فِي الْأَرْضِ جَمِيعًا ثُمَّ اسْتَوَىٰ إِلَى
السَّمَاءِ فَسَوَّاهُنَّ سَبْعَ سَمَاوَاتٍ وَهُوَ بِكُلِّ شَيْءٍ عَلِيمٌ

It is He who hath created for you all things that are on Earth. Moreover, His design comprehended the heavens, for He gave order and perfection to the seven firmaments; and of all things He hath perfect knowledge (*Al-Baqara, Ayah* 29).

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List of Publications

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Fayaz, A (2009) **Classification and conservation of *Rhododendron* Section *Vireya* (vireyas) using DNA fingerprinting**. *Bulletin of the New Zealand Rhododendron Association* **97**: 35–44.

MacKay, M B, Fayaz, A, Gardiner, S E, Wiedow, C, Smith, G & Oldfield, S (2010) **Meeting Target Eight: *Rhododendron* Subgenus *Vireya* in New Zealand as an example of *ex situ* conservation**. Paper presented at the Proceedings of the 4th Botanic Gardens Congress, 14–18th June, Dublin. URL: http://www.bgci.org/files/Dublin2010/papers/MacKay-Marion.pdf

MacKay, M B, Fayaz, A, Wiedow, C, Reddy, S, Smith, G & Gardiner, S E (2012) **Vireya *Rhododendron* in plant collections in New Zealand: potential for international conservation – A project for the Peter Skellerup Plant Conservation Scholarship**. *New Zealand Garden Journal (Journal of the Royal New Zealand Institute of Horticulture)* **15**(1): 23–29.

List of Abbreviations

AFLP – Amplified Fragment Length Polymorphism

***atp*B** – ATP synthase subunit beta

BGCI – Botanical Gardens Conservation International

bp – base pair

Bot. Jahrb. – *Botanische Jahrbücher*

BS – Bootstrap

CI – Consistency Index

cpDNA – chloroplast DNA

DI – Decay Index

DNA – deoxyribonucleic acid

EST – Expressed Sequence Tag

GS – Genetic Similarity

ISSR – Inter-Simple Sequence Repeat

ITS – Internal Transcribed Spacer

IUCN – International Union for Conservation of Nature

kb – kilo bases (1,000 bases)

L. – Linnaeus

***mat*K** – maturase K

MCMC – Markov Chain Monte Carlo

MPI – Ministry for Primary Industries (New Zealand)

mtDNA – mitochondrial DNA

mya – million years ago

nr – nuclear

NCA – Nested Clade Analysis

NJ – Neighbour Joining

PCR – Polymerase Chain Reaction

PD – Phylogenetic Diversity

PFR – The New Zealand Institute for Plant & Food Research Ltd.

PNG – Papua New Guinea

RAPD – Random Amplified Polymorphic DNA

***rbc*L** – ribulose 1,5-bisphosphate carboxylase/oxygenase (RuBisCo) large subunit

rDNA – ribosomal DNA

RI – Retention Index

***rpb*2i** – one of two genes encoding second-largest RNA polymerase II subunit

RBGE – Royal Botanic Gardens Edinburgh, UK

RFLP – Restriction Fragment Length Polymorphism

RSBG – Rhododendron Species Botanical Garden, USA

Sect. – Section

Ser. – Series

sp. – species (singular)

spp. – species (plural)

ssp. – subspecies

Subgen. – Subgenus

Subsect. – Subsection

TBR – Tree Bisection Reconnections

***trn*F** – transfer RNA Phenylalanine

***trn*K** – transfer RNA Lysine

***trn*L** – transfer RNA Leucine

Conventions Followed

rhododendron – common name of the genus *Rhododendron*

azalea – common name for the flowering shrubs belonging to the subgenera *Pentanthera* (deciduous) and *Tsutsui* (evergreen)

vireya – common name referred to the Section *Vireya* Sleumer, Subgenus *Vireya*, Section *Schistanthe*, or the (majority of) tropical rhododendrons of Malesia

genus, ***subgenus***, ***section***, ***subsection***, ***species, subspecies, variety, form*** – these are all written in *italics*

ORDER, **SUB-ORDER**, **FAMILY**, **SUB-FAMILY**, **TRIBE**, **SUB-TRIBE** – (those above the genus in hierarchy) these are all written in SMALL CAPS

Series – these are written in normal text

Thesis Structure

Front Matter

Chapter 1 **General Introduction**

This chapter gives an introduction to this research project, outlining the aims and objectives of the study.

Chapter 2 **Literature Review I**

This chapter is a literature review of the rhododendrons and the vireyas, its classification, systematics, and conservation.

Chapter 3 **Literature Review II**

This chapter is a literature review of biodiversity in general, including causes of biodiversity loss, measurement of biodiversity and methods used in conservation of biodiversity. The genetic methods reviewed include molecular laboratory methods and data analysis methods associated with plant systematics and biodiversity conservation.

Chapter 4 **Materials and Methods**

This chapter describes the field work, molecular laboratory methods and data analysis methods.

Chapter 5 **Results and Analyses**

This chapter discusses the results of the methods described in Chapter 4, and its implications on the taxonomic and conservation issues.

Chapter 6 **Discussion**

This chapter discusses the results of the Chapter 5. The chapter is divided into two major sections: one section dealing with the phylogenetic analyses and the other dealing with the genetic diversity analyses.

Chapter 7 **Conservation Plan for Vireyas**

This chapter proposes a conservation plan in light of the results and analyses of this study.

Chapter 8 **Conclusions**

This chapter summarizes the findings of the Chapter 5, and draws conclusions on the taxonomic and conservation issues. Also discussed are the future research prospects driven from these conclusions.

End Matter

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Table of Contents

List of Tables

List of Figures

List of Photographs

All photos are by the author unless specified.

Chapter 1

Introduction

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1 Introduction

The genus *Rhododendron* L. belonging to the family ERICACEAE (commonly known as the heath family), consists of 850–1,000 species (Argent 2006; Brown et al. 2006a, 2006b; Brown et al. 2006c; Gibbs et al. 2011; Stevens 2001 onwards) classified in a complex hierarchical paradigm based on morphological characters and more recently, molecular data. The group *Vireya* (subgenus or section, depending on which classification is followed) commonly referred to as vireyas consists of approximately 320[1] species (Goetsch et al. 2011), found in an area ranging from mainland India through Malesia to northern Australia and westward to the Philippines and Taiwan (Argent 2006). Vireyas are an example of a group of plants with numerous taxonomic and conservation issues, making this group a challenge for development of conservation plans. The morphological and molecular classification systems for vireyas agree on the definition of some subgroups while the others are open for debate (Goetsch et al. 2011).

A recent IUCN Red-List assessment indicates about 63 taxa (about 17%) of vireyas are under threat in the wild (Argent 2006; Gibbs et al. 2011), mainly due to loss of habitat. A further 84 (22%) taxa were classified as Data Deficient (DD[2]), which is a consequence of the difficulty and high cost of sampling them in the wild. Conservationists are utilizing numerous techniques to save the remaining wild species, and understanding the population structures, species relationships and conservation issues are essential in designing and implementing any conservation project. This study uses conventional morphological techniques for description, as well as a selection of the more modern molecular techniques to examine the taxonomic and conservation issues of vireyas, to develop an *ex situ* conservation plan.

[1] Total number of taxa including subspecies, forms and varieties is approximately 380 (Argent 2006).
[2] A category which holds species for which there is inadequate information to assess extinction risk based on distribution, population status, or both (Pfab et al. 2011).

1.1 Work Contained in this Study

The purpose of this research is to examine and inform the taxonomic and conservation issues associated with selected vireyas held in New Zealand collections, using both morphological and molecular techniques. The elements of research contained in this study are:

1. A detailed literature review of taxonomic and conservation issues related to vireyas, and methods used in their conservation.

2. Identification and selection of vireya taxa with taxonomic and conservation issues, focussing on the material present in New Zealand collections.

3. Physical examination (macroscopic and microscopic) of plant material, to relate physical characters to species identity and molecular data.

4. Molecular studies (RAPD, microsatellite and DNA sequencing) of vireyas.

5. Identification of the vireya accessions in New Zealand that could be used for future *ex-situ* conservation exercises.

1.2 Objectives of the Study

The hypothesis of this study is that vireya accessions held in New Zealand collections may represent genetic diversity that may be useful in the *ex situ* conservation of vireyas in New Zealand and has the potential to contribute to international conservation programmes. The study aims to investigate the taxonomic and conservation issues of selected vireya taxa using molecular and morphological techniques, and use these findings to develop an appropriate conservation plan for *ex situ* conservation of vireyas. The main objectives and areas of study contained in thesis are:

1. To estimate the phylogeny of the Subgenus *Vireya sensu* Argent using molecular data. The phylogenetic analyses aim to reveal relationships within and between species, examine the monophyly[3] of vireyas and also examine the monophyly of traditional sections and subsections within vireyas. These analyses will also be used to examine the taxonomic boundaries of the vireya taxa.

2. To determine the genetic diversity of vireya accessions within New Zealand collections, and assess the genetic distinctiveness of these collections. The genetic distinctness of the New Zealand collections will be determined by comparing the accessions in New Zealand collections with that of the international collections (using published data). This exercise will also aim to provide a better understanding of the genetic variation between international collections.

3. To propose a Conservation Plan for vireyas of conservation interest in New Zealand collections using the phylogenetic analyses and the genetic diversity analyses.

[3] A group of organisms forming a clade that includes all of the descendants of a single common ancestor.

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Chapter 2

Literature Review I: Rhododendrons and Vireyas

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2 Literature Review I: Rhododendrons and Vireyas

This chapter describes the systematic and conservation of the genus *Rhododendron* L. and the *Rhododendron* L. Subgenus *Vireya* Argent. It discusses the various classifications systems used since the inception of this genus (Section 2.1) and subgenus (Section 2.2), to the present day classifications based on molecular systematics. These sections investigate the development of the classification systems and the basis of these systems.

2.1 The Genus *Rhododendron* L.

Except for the rose, also known as the Queen of Flowers, not many plants have generated a more lively interest throughout Europe than the many species of the genus *Rhododendron* (Hooker 1849). It is thus not surprising that many of the initially described species were commonly referred to as Alpine Roses. Rhododendrons, as they are called commonly, were mainly grown for their profusion of large inflorescences and for the striking foliage of the many evergreen species. The poisonous qualities of these plants were also well known, as the fate of many Greeks (with symptoms of mental disorders) during the celebrated retreat of the Ten Thousand[4], which was attributed to honey produced from the plant now known as *Rhododendron luteum* (Partington 1835).

A large number of studies have been carried out on *Rhododendron* since its discovery, including those related to its description, cultivation and systematics. The *Rhododendron* genus name is composed of the Greek words *rhodon* = rose and *dendron* = tree. The name was already known in ancient times, but was referred to oleander (*Nerium oleander*, family APOCYNACEAE), which also grows in the same Mediterranean region. The Italian doctor, botanist and philosopher, Ernst A Caesalpino[5] (circa 1519–1603), introduced alpenrose (Alpine Rose – *Rhododendron ferrugineum* L., Photo 1) as *Alpinum*

[4] Their march to the Battle of Cunaxa and back to Greece (401–399 BC) was recorded by Xenophon (one of its leaders) in his work, 'The Anabasis'.
[5] Professor of botany and medicine at Pisa in Rome, and since 1592, physician to Pope Clement. He classified plants according to their fruits and seeds.

rhododendron[6]. The *Rhododendron* as a genus was first described by Linnaeus in 1753 with just 5 species (Linnaeus 1753).

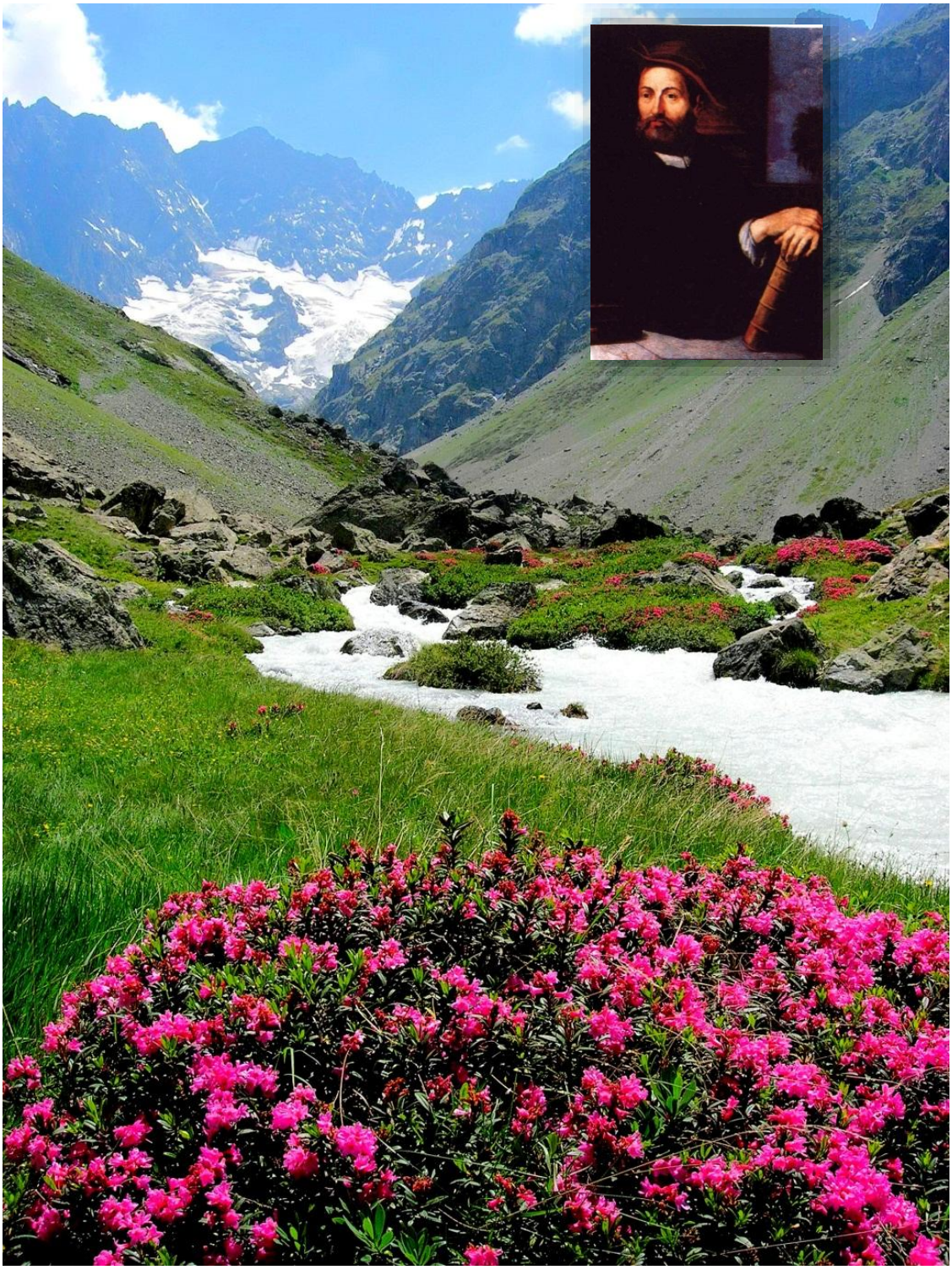

Photo 1 *Rhododendron ferrugineum* L. growing on the European Alps. [Photo: Bas Vrins]. **Inset:** Ernst A Caesalpino [Courtesy of Wikipedia].

[6] The name 'rhododendron' used as the specific epithet.

The genus *Rhododendron* L. currently includes about 850–1,000 species (Argent 2006; Brown et al. 2006a, 2006b; Brown et al. 2006c; Gibbs et al. 2011; Stevens 2001 onwards) distributed through Asia, Malesia, Australia, Europe and North America. Two main centres of diversity can be identified, one in SW China (mainly temperate species) and the other in tropical SE Asia (mainly tropical species). The genus is nearly cosmopolitan and found in very diverse habitats, which has given rise to very complex morphological characteristics. This morphological diversity has led many authors to recognize different internal divisions of the genus, as well as assignment of several taxa to other genera.

Rhododendron L. is a large genus and fairly distinct from other genera of the family ERICACEAE, however there is a great deal of internal variation. The plant form for instance, ranges from large 30 m high trees to alpine shrubs, tropical epiphytes and mat-forming subshrubs. The current delineation of *Rhododendron* L. consists of trees and shrubs, terrestrial or epiphytic, variously hairy, with hairs usually tangled and coming away as a layer. The leaves are evergreen, deciduous or semi-deciduous, arranged alternately, sometimes clustered at the stem apex. The leaf margins are usually entire, and very rarely crenulate. The inflorescences are racemes or corymbs, mostly terminal, sometimes lateral, usually few- to many-flowered, and sometimes reduced to a single flower. The flowers have persistent calyces, which are 5–8-lobed, sometimes reduced to a rim. The corolla are funnelform, campanulate, tubular, rotate or hypocrateriform, regular or slightly zygomorphic, 5(rarely to 8)-lobed, lobes imbricate in bud. The stamens are 5–10(rarely to 27) in number, inserted at the base of the corolla and usually declinate. The filaments are linear to filiform, anthers are without appendages. The anthers open by terminal or oblique pores. The disk is usually thick, 5–10(rarely to 14)-lobed. The ovary is 5(rarely to 18)-loculed, with a straight or declinate to deflexed, persistent style. The stigma is capitate-discoid, and crenate to lobed. The fruits are capsules, usually cylindrical, coniform, or ovoid, sometimes curved, dehiscent from top, septicidal, with the valves thick or thin, straight or twisted. The seeds are numerous, minute, fusiform, always winged, or both ends with appendages or thread-like tails (Linnaeus 1753; Sleumer 1966a).

(a) *(b)*

Photo 2 The first rhododendron collected and the collector. (a) *Rhododendron hirsutum* L. [Photo: Nicholas Turland, Saint Louis, USA]. (b) Portrait of Charles de l'Écluse (Carolus Clusius). Engraving by Martinus Rota, Italy, 16**th** century. [Courtesy of University Library, Prentenkabinet I152 Rot/1].

The first species of *Rhododendron* introduced into cultivation was the temperate species *R. hirsutum* L. (Photo 2a) from the European Alps in the 16th century by Charles de l'Écluse[7] (Photo 2b) (l'Écluse 1576). The introduction of this species into cultivation was followed by subsequent introductions of the many other species of *Rhododendron* that we know today (Postan 1996). These temperate rhododendrons and their hybrids have now become very popular garden plants, especially in the West. In contrast the first tropical rhododendron or vireya, *R. malayanum*, was formally described by William Jack[8] in 1822 (Argent 2006). Since then, the vireyas themselves have gained popularity amongst growers, along with the temperate rhododendrons.

2.1.1 Classical Systematics of *Rhododendron* L.

The family ERICACEAE presently consists of 8 subfamilies, 126 genera and about 3,995 species that are distributed worldwide from temperate and subarctic regions to high elevations of tropical regions. *Rhododendron* L. belongs to the subfamily ERICOIDEAE

[7] Also known as Carolus Clusius, a Flemish doctor and pioneering botanist. He is renowned for his extensive study of the Spanish flora

[8] A Scotsman from Aberdeen, who worked for the East India Company, and was renowned as a botanist and the author of the publication *Malayan Miscellanies*.

Endl. (Gillespie & Kron 2010; Kron et al. 2002) and was previously placed under the subfamily RHODODENDROIDEAE (Copeland 1943; Cox 1948; Stevens 1971) and tribe RHODOREAE G Don. RHODODENDROIDEAE was divided by de Candolle, which he recognized as a tribe, into two groups depending on whether or not the corolla lobes were free (de Candolle & de Candolle 1838). Klotzsch (1851) did not use this character, and recognized MENZIESIACEAE (with squamose buds and anthers dehiscing by more or less elongated slits) and RHODORACEAE (with strobiliform leaf buds and anthers dehiscing by pores, the pollen being mixed with viscin threads).

Copeland (1943) recognized 20 genera and four tribes within the subfamily RHODODENDROIDEAE (Kron et al. 2002). These 20 genera are currently recognized in the subfamily ERICOIDEAE (Kron 1997). Copeland (1943) based his circumscription of the subfamily on anatomical and embryological characters. He defined the ERICOIDEAE by the presence of deciduous corollas, anthers without awns, and septicidally-dehiscent capsular fruits. The subfamily ERICOIDEAE presently includes 19 genera in five recognized tribes, with the tribes separated by the pattern of anther dehiscence. Cox (1948) studied the wood anatomy of RHODODENDROIDEAE and described five tribes, four of which including RHODOREAE were classified based on anatomical characters similar to those of Copeland's study.

The formal classification of the rhododendrons began when Linnaeus first instituted the genus *Rhododendron*[9] in 1753 and he also created a separate genus *Azalea* which contained 6 species. The division of these species into *Rhododendron* and *Azalea* was based on the number of stamens, 10 and 5 respectively. Salisbury (1796) remonstrated that *Azalea* and *Rhododendron* could not be maintained as distinct genera. In early 19th century, George Don (1834) recognized 57 species of *Rhododendron* and divided the genus into 8 sections based on floral and foliar characteristics, as shown below:

[9] The name 'Rhododendron' referred to the rhododendrons however appeared in literature as early as the year 1535.

Order[10] **ERICACEAE** G. Don
Subfamily **RHODOREAE** G. Don
Genus ***Rhododendron*** L.
Section ***Ponticum*** G. Don
Section ***Boòram*** G. Don
Section ***Pogonanthum*** G. Don
Section ***Lepípherum*** G. Don
Section ***Chamaecístus*** G. Don
Section ***Tsutsutsi*** G. Don
Section ***Pentanthera*** G. Don
Section ***Rhodora*** G. Don
Genus ***Vireya*** Blume

Don differentiated the genus *Vireya* from the genus *Rhododendron* with the following characters: (i) having flowers with small calyces, (ii) the stamens not being attached to the corolla in any way, (iii) the majority of the species are epiphytic shrubs, (iv) leaves scattered and verticillate, (v) leaf lamina with margins quite entire, coriaceous, and covered with scales, and (vi) flowers borne in terminal fascicles.

Indiscriminate naming of rhododendrons continued, with authors placing species in both *Rhododendron* and *Azalea*. Planchon (1854) reviewed and placed all the 25 then known taxa from the Far East in *Rhododendron*, increasing the number of species considerably. Until 1860 all the known species of azaleas introduced from the Far East were from gardens, the majority from China. In 1870, botanist and plant collector Carl Johann Maximowicz[11] (1870) used living collections and herbarium material to develop a series of new diagnostic characters (firmness of the testa and the persistence or otherwise of the bracteoles), which enhanced Don's work. Their combined natural system of classification much improved and revised the delimitation of the genus. Maximowicz recognized two subtribes, EURHODODENDREAE corresponding to the RHODORACEAE of Klotzsch, and PHYLLODOCEAE corresponding to the MENZIESIACEAE.

In 1882 Charles Baron Clarke published a treatment (with keys) of Indian *Rhododendron* in J D Hooker's *Flora of British India* (Clarke 1882), in which the genus was divided into the four subgenera, as shown below:

[10] Classified as an 'Order' as opposed to a 'Family', containing 5 tribes.
[11] Curator/Chief Botanist of St Petersburg Botanic Gardens; Keeper of the Herbarium at the Imperial Botanic Garden, St Petersburg. He is also recognized as the first person to enumerate the flora of Japan.

Genus ***Rhododendron*** L.
- Subgenus ***Vireya*** Blume
- Subgenus ***Pseudovireya*** G. Don
- Subgenus ***Eurhododendron*** (DC.) Maxim.
  - Series A (non-lepidote species)
  - Series B (lepidote species)
- Subgenus ***Rhodorastum*** Maxim.

The subgenera were separated according to the characters: capsule and seed morphology, capsule dehiscence, phyllotaxy, and inflorescence arrangement. The species of Subgenus *Eurhododendron* were separated into the two series (A and B) mainly according to foliar characters (Photo 3):

Series A

- Elepidote (non-lepidote): leaves with abaxial surfaces glabrous, or tomentose without glandular scales.
- Plants are usually large shrubs or trees.

Series B

- Lepidote: leaves with abaxial surfaces covered with sessile, round, glandular scales (which in *R. anthopogon* coalesce, forming a tomentum).
- Large or small shrubs.

Photo 3 Examples of elepidote and lepidote rhododendrons. (a) Elepidote (Series A) species: *Rhododendron arboreum* ssp. *zeylanicum*, growing wild on the Horton Plains (Sri Lanka), at ~2,000 m altitude. (b) Lepidote species (Series B): *R. lindleyi*, cultivated at the Pukeiti Rhododendron Garden (New Zealand).

In 1889, Leopold Dippel[12] published an account of trees and shrubs cultivated in Germany, which included several species of *Rhododendron* and its allies (Dippel 1889). The classification he followed was mainly that of Maximowicz, but also utilized additional morphological characters such as inflorescence, floral and foliar to demarcate the various subgroups, and the classification used is as shown below:

Suborder **RHODODENDRINEAE** J. Presl.
Family **ERICACEAE** G. Don
Tribe **RHODODENDREAE** (Juss.) Colla
Subtribe **EURHODODENDREAE** Maxim.
Genus ***Rhododendron*** L.
Subgenus ***Osmothamnus*** Maxim.
Subgenus ***Eurhododendron*** Maxim.
Section ***Candalbra*** Dippel
Section ***Chrysantha*** Dippel
Section ***Lepidota*** Maxim.
Subgenus ***Therorhodion*** Maxim.
Subgenus ***Azalea*** L.
Section ***Euazalea*** Maxim.
Section ***Rhodora*** G. Don
Subgenus ***Tsusia*** (Planch.) Maxim.
Subgenus ***Rhodorastrum*** Maxim.
Subgenus ***Azaleastrum*** Planch.

Dippel described 39 species of *Rhododendron* using the above classification, and his work became an excellent account of the majority of species of *Rhododendron* in cultivation at that time.

The treatment of ERICACEAE by Drude (Drude 1897) in the monumental work on plant taxonomy and phytogeography, *Die Natürlichen Pflanzenfamilien* ('The Natural Plant Families' by Adolf Engler and Karl von Prantl) is notable. This treatment, even though minor compared to some of the previous works such as those by Maximowicz, forms an integral part of the proposed plant classification system (the 'Engler System'), which is still in use by many herbaria and authors.

Smaller number of species were included in various subsequent publications such as the works by Koehne (1893), Zabel (1902) and Schneider (1906) all adopting the

[12] Professor of botany and director of the Botanical Garden in Darmstadt.

classification by Maximowicz. The classification system of Dalla-Torre and Harms (von Dalla-Torre & Harms 1903) provided a comprehensive synonymy of *Rhododendron* L. and its subgenera. The hierarchy is arranged as below:

Family **ERICACEAE** DC.
Subfamily **RHODODENDROIDEAE** Drude
Tribe **RHODODENDREAE** Spreng.
Genus ***Rhododendron*** L.
Subgenus ***Eurhododendron*** Drude
Section ***Eurhododendron*** Drude
Section ***Vireya*** Hook. f.
Section ***Osmothamnus*** Maxim.
Subgenus ***Azalea*** Planch.
Section ***Rhodora*** G. Don
Section ***Azaleae*** Drude
Section ***Tsusia*** Planch.
Subgenus ***Rhodorastrum*** Maxim.
Subgenus ***Azaleastrum*** Planch.
Subgenus ***Keysia*** Drude
Subgenus ***Choniastrum*** Franch.
Subgenus ***Therorhodion*** Maxim.

In the above classification *Vireya* was re-introduced as a section, and *Pseudovireya* is reduced to a synonym of *Vireya*. There are a few notable hierarchical changes compared to Maximowicz's and Dippels's classification, such as *Osmothamnus* and *Tsusia*.

The years that followed saw the discovery of numerous new species of *Rhododendron*, many of them described by Ernest Henry Wilson. In 1913, E H Wilson collaborating with Alfred Rehder published an account of the genus *Rhododendron* (Wilson 1913). The classification he followed was principally that of Maximowicz, but differed widely in the conception and limits of certain sections, mainly the arrangement of the species with revised nomenclature. The main characters used to classify the subgenera were: (i) scaliness and hairiness of the leaves, (ii) leaves persistent or deciduous, (iii) ovary surface (iv) ovary locules 5 or more than 5, (v) number of corolla lobes, and (vi) number of stamens either 5–10 or 10–20.

Family **ERICACEAE** G. Don
Genus ***Rhododendron*** L.
Subgenus ***Lepidorhodium*** Koehne.
Section ***Pogonanthum*** G. Don.
Section ***Lepipherum*** G. Don (Syn: *Osmothamnus* Maxim.)
Section ***Rhodorastrum*** Maxim.
Section ***Lepidota*** Maxim.
Subgenus ***Eurhododendron*** Maxim.
Subgenus ***Azalea*** Planch.
Section ***Chionastrum*** Franch.
Section ***Azaleastrum*** Planch.
Section ***Tsutsutsi*** G. Don (Syn: *Tsusia* Planch.)
Section ***Pentanthera*** G. Don (Syn: *Euzalea* Maxim.)

Following on from their previous work, Wilson and Rehder (1921) published a monograph on azaleas for the Subgenus *Anthodendron*, which was divided into two major parts, one for azaleas of the Old World (the Asiatic sections) and the other for the azaleas of North America. In this monograph in addition to the description of novel species and a new Section *Sciadorhodion*, they rectified previous errors and misconceptions:

Azaleas of the Old World

Genus ***Rhododendron*** L.
Subgenus ***Anthodendron*** Endlicher
Section ***Tsutsutsi*** G. Don
Section ***Sciadorhodion*** Rehder & Wilson
Section ***Rhodora*** G. Don
Section ***Pentanthera*** G. Don

Azaleas of North America

Genus ***Rhododendron*** L.
Subgenus ***Anthodendron*** Endlicher
Section ***Rhodora*** G. Don
Section ***Pentanthera*** G. Don

The work carried out by Schlechter (1919) for the *Rhododendron* species of New Guinea and Copeland (1929) for the *Rhododendron* ofPhilippines, showed similar trends in their classifications. Notably, these two studies also suggested subdivisions for the vireyas (which are discussed further in Section 2.2.1).

In the early part of the 20th century a large number of plants were acquired by the Royal Botanic Garden Edinburgh (UK) from several collectors including Sir Isaac Bayley Balfour[13], Peter Hadland Davis[14], Reginald John Farrer[15], George Forrest[16], Henry Halcro Johnston[17], Frank Kingdon-Ward[18], and Frank Ludlow[19], leading to another dramatic rise in the number of *Rhododendron* species known. The genus grew immensely due to the influx of the new species, and some sections such as *Hymenanthes* grew very large. To break down these very large sections, Sir Isaac Bayley Balfour began to group many of the species into series (Balfour 1916, 1917, 1919, 1920).

Cataloguing the new living material of the many taxa that came to the garden led to the development of an artificial classification system, culminating in the publication of *The Species of Rhododendron* by the Rhododendron Society and edited by J B Stevenson (1930) under the auspices of the Regius Keeper of the garden, Sir Isaac Bayley Balfour. The morphological characters used for this classification system included: leaf-shape and size, leaf punctuation, presence or absence of hairs on the pedicels, ovary shape and surface features, etc. The new classification system gave rise to a very significant revision of the genus, in which all the temperate and subtropical species of *Rhododendron* known at that time were split into 39 series and several subseries (Hutchinson 1919, 1930; Rehder 1930; Stevenson 1930, 1947; Tagg 1930). This classification has been adopted by consequent botanists and researchers until recent developments in molecular systematics (Goetsch et al. 2005; Goetsch & Hall 2002; Kron 1997; Kron & Judd 1990), which have now begun to assist in the unravelling the many systematics issues associated with the

[13] Sir Isaac Bayley Balfour (1853–1922) collected in Rodriguez and Socotra Islands, describing over 300 new species of plants in his *Botany of Socotra*.
[14] Peter Hadland Davis (1918–1992) collected plants from almost all of the countries of the Mediterranean, and many other countries of the world. He was renowned as a major contributor to the *Flora of Turkey and the East Aegean Islands*.
[15] Reginald John Farrer (1880–1920) explored the Himalayas and the greater China, Burma and Ceylon. His collections together with illustrations, field notes, botanical specimens and seeds, provided valuable information to the Royal Botanic Garden Edinburgh, where the Regius Keeper Sir Isaac Bayley Balfour, took a special interest in Sino-Himalayan plants.
[16] George Forrest (1873–1932) ranks amongst the greatest of all collectors of rhododendrons, introducing hundreds of species from China and Tibet to the Royal Botanic Garden Edinburgh, notable species including *R. giganteum* and *R. sinogrande*.
[17] Henry Halcro Johnston (1856–1939) made significant contributions to botany and horticulture through his detailed collection of plant species.
[18] Frank (Francis) Kingdon Ward (1885–1958) went on around 25 expeditions over a period of nearly fifty years, exploring the Himalayas, Tibet, North Western China, Burma (Myanmar) and Assam (North Eastern India).
[19] Frank Ludlow (1895–1972) is well known for his discovery of rhododendrons on expeditions to Tibet, and also found better forms of many previously discovered rhododendron species.

genus *Rhododendron*. The work on the revision of the classification resumed with the revision of several series by J M Cowan and H H Davidian (Cowan & Davidian 1947, 1948, 1949, 1951; Davidian 1954, 1963, 1964).

2.1.2 Modern Systematics of *Rhododendron* L.

The modern debate over the delimitation of *Rhododendron* could be said to have started with the account of Copeland (1943) who split it into five separate genera (*Azalea, Therorhodion, Azaleastrum, Rhododendron* and *Hymenanthes*), adding more confusion than resolution to the then existing classification. Copeland (1943) based his classification on the morphological characters: anther opening (by long slits, short slits or by circular pores), flowers sympetalous or choripetalous, flowering axes with or without leaves, flowers terminal (leaves deciduous, or if evergreen with flattened bristles) or axillary (if leaves deciduous, then with lateral flowers; if evergreen, then without flattened bristles) and with or without glandular scales. The classification of Copeland (1943) is shown below:

Family **ERICACEAE** DC.
- Genus ***Azalea*** L.
  - Subgenus ***Rhodora*** H. F. Copeland
  - Subgenus ***Pentanthera*** (G. Don) K. Koch
  - Subgenus ***Sciadorhodion*** (Rehder & Wilson) H. F. Copeland
  - Subgenus ***Tsutsutsi*** (G.Don) K. Koch
- Genus ***Therorhodion*** (Maximowicz) Small
- Genus ***Azaleastrum*** (Planchon) Rydberg
  - Series 1 **Albiflorum** H. F. Copeland
  - Series 2 **Semibarbatum** H. F. Copeland
  - Series 3 **Stamineum** H. F. Copeland
  - Series 4 **Ovatum** H. F. Copeland
- Genus ***Rhododendron*** L.
  - Subgenus ***Rhodorastrum*** C. B. Clarke
  - Subgenus ***Vireya*** C. B. Clarke
  - Subgenus ***Eurhododendron*** C. B. Clarke
  - Subgenus ***Pogonanthum*** C. B. Clarke
  - Subgenus ***Keysia*** C. B. Clarke
  - Subgenus ***Rhodorastrum*** C. B. Clarke
- Genus ***Hymenanthes*** Blume

The next major revision to the classification of *Rhododendron* which was carried out by Sleumer initiated systematic studies that included all sections and subgenera of

Rhododendron including *Vireya* (Sleumer 1949). His classification was based on morphological characters such as inflorescence arrangement, plant scales, leaf abscission and seed appendages.

Genus ***Rhododendron*** L.

LEPIDOTE PLANTS

Subgenus ***Lepidorrhodium*** Koehne
- Section ***Lepipherum*** G. Don
  - Subsection ***Glauca*** Sleumer
  - Subsection ***Boothia*** Sleumer
  - Subsection ***Campylogyna*** Sleumer
  - Subsection ***Lepidota*** Sleumer
  - Subsection ***Baileya*** Sleumer
  - Subsection **Geinestieriana** Sleumer
  - Subsection ***Uniflora*** Sleumer
  - Subsection ***Edgeworthia*** Sleumer
  - Subsection ***Tephropepla*** Sleumer
  - Subsection ***Maddenia*** Sleumer
  - Subsection ***Camelliaeflora*** Sleumer
  - Subsection ***Micrantha*** Sleumer
  - Subsection ***Moupinensia*** Sleumer
  - Subsection ***Cinnabarina*** Sleumer
  - Subsection ***Ferruginea*** Sleumer
  - Subsection ***Lapponica*** Sleumer
  - Subsection ***Caroliniana*** Sleumer
  - Subsection ***Heliolepida*** Sleumer
  - Subsection ***Triflora*** Sleumer
- Section ***Pogonanthum*** G. Don
- Section ***Vireya*** [20] (Blume) H. F. Copeland

Subgenus ***Pseudazalea*** Sleumer

ELEPIDOTE PLANTS

Subgenus ***Eurhododendron*** Maxim.
- Subsection ***Auriculata*** Sleumer
- Subsection ***Barbata*** Sleumer
- Subsection ***Maculifera*** Sleumer
- Subsection ***Arborea*** Sleumer
- Subsection ***Thomsonia*** Sleumer
- Subsection ***Neriiflora*** Sleumer
- Subsection ***Pontica*** Sleumer
- Subsection ***Fortunea*** Sleumer
- Subsection ***Selensia*** Sleumer

[20] The subsections of this group are discussed in section 2.2.1.

Subsection ***Campylocarpa*** Sleumer
Subsection ***Irrorata*** Sleumer
Subsection ***Parishia*** Sleumer
Subsection ***Argyrophylla*** Sleumer
Subsection ***Lactea*** Sleumer
Subsection ***Falconera*** Sleumer
Subsection ***Grandia*** Sleumer
Subsection ***Fulva*** Sleumer
Subsection ***Campanulata*** Sleumer
Subsection ***Taliensia*** Sleumer
Subsection ***Floribunda*** Sleumer

Subgenus ***Pseudanthodendron*** Sleumer
Section ***Rhodora*** (L.) G. Don
Section ***Viscidula*** Matsumura & Nakai
Section ***Pentanthera*** G. Don.

Subgenus ***Anthodendron*** (Reichenbach) Rehder & Wilson
Section ***Brachycalyx*** Sweet
Section ***Tsutsusi*** Sweet
Section ***Tsusiopsis*** Sleumer

Subgenus ***Azaleastrum*** Planchon
Section ***Euazaleastrum*** Sleumer
Section ***Choniastrum*** (Franchet) Sleumer
Section ***Candidastrum*** Sleumer
Section ***Mumeazalea*** (Makino) Sleumer

Subgenus ***Pseudorhodorastrum*** Sleumer
Section ***Trachyrhodion*** Sleumer
Section ***Rhabdorhodion*** Sleumer
Section ***Rhodobotrys*** Sleumer

Subgenus ***Rhodorastrum*** (Maxim.) C. B. Clarke

The elaborate classification system above was provided with a detailed key to the subgenera, sections and subsections. Although comprehensive, this classification system was not widely adopted in its entirety. Instead, the period that followed saw the use of the systems of Stevenson (1930) and Copeland (1943) which contained several groups described by Sleumer.

A major revision to the classification of Stevenson (1930) and Copeland (1943) was made by Cullen & Chamberlain (1978, 1979), using the morphological characters: scale[21] shape, colour, size, spacing and stalk length. This was followed by the latest major revision using morphological characters by Chamberlain et al. (1996). Many minor

[21] The lepidote scales unique to the Subgenus *Rhododendron* are modified hairs on both leaf surfaces.

revisions of various subgenera and sections were carried out in the latter part of the twentieth century, these include the work by Cullen (1980), Chamberlain (1982), Philipson & Philipson (1982), Chamberlain & Rae (1990), Kron (1993), Judd et al. (1995) and Jin et al. (2007).

Historically, the most taxonomically problematic groups were the subgenera *Azaleastrum*, *Mumeazalea*, and *Candidastrum*. The classifications by Sleumer (1949) and Chamberlain et al. (1996) placed the sections *Azaleastrum* (with 5 stamens) and *Choniastrum* (with 10 stamens), which share the lateral inflorescence character, in Subgenus *Azaleastrum*, even though they differ consistently in the number of stamens and other characters (Philipson & Philipson 1986).

Spethmann (1980, 1987) split Copeland's delimitation of *Rhododendron* into three subgenera: *Rhododendron*, *Maddenodendron* and *Vireya*. Spethman utilized morphological, anatomical and some biochemical characters (flavonoids and carotenoids) in his classification. He further identified primitive (presence of anthocyan-monoglycosides and dihydroquercetin) and advanced characters (methylated flavonoids) for his phylogenetic study.

The current and most widely adopted classification is that by Chamberlain et al. (1996), also known as the Edinburgh Classification. It contains alphabetical and taxonomic lists of all the taxa in *Rhododendron* published up to the end of 1995 and is a summation of many of the works discussed earlier in this section (Chamberlain 1982; Chamberlain & Rae 1990; Cullen 1980; Judd & Kron 1995; Kron 1993; Philipson & Philipson 1982; Sleumer 1966a). It also contains adjustments resulting from recent international research, an alphabetical list of Biological Recording Unit codes along with a record of the accepted taxa that occur in each, and a list of the living collections of *Rhododendron* at the Royal Botanic Garden Edinburgh. The classification system is outlined below:

Family **ERICACEAE** DC.
- Genus ***Rhododendron*** L.
  - Subgenus ***Rhododendron*** Endlicher
    - Section ***Vireya***[22] (Blume) H. F. Copeland
    - Section ***Pogonanthum*** Aitch. & Hemsl.
    - Section ***Rhododendron*** D. F. Chamberlain
  - Subgenus ***Hymenanthes*** (Blume) K. Koch
    - Section ***Ponticum*** G. Don
  - Subgenus ***Tsutsui*** (Sweet) Pojarkova
    - Section ***Tsutsui*** Sweet
    - Section ***Brachycalyx*** Sweet
  - Subgenus ***Pentanthera*** (G. Don) Pojarkova
    - Section ***Pentanthera*** G. Don
    - Section ***Rhodora*** H.F.Copeland
    - Section ***Viscidula*** Matsum. & Nakai
    - Section ***Sciadorhodion*** Rehder & Wilson
  - Subgenus ***Azaleastrum*** Planch.
    - Section ***Azaleastrum*** (Planch.) Maxim.
    - Section ***Choniastrum*** Franch.
  - Subgenus ***Therorhodion*** (Maxim.) A. Gray
  - Subgenus ***Mumeazalea*** (Sleumer) W. R. Philipson & M. N. Philipson
  - Subgenus ***Candidastrum*** Franch.

Notable differences between this classification and Sleumer's (1949), include the placement of Subgenus *Therorhodion*, which Sleumer placed outside the genus *Rhododendron*, and placement of the four species of Section *Sciadorhodion*.

Chamberlain et al. (1996) classification recognized the major subgenera *Rhododendron*, *Hymenanthes*, *Tsutsusi*, *Pentanthera*, *Azaleastrum*, and the three minor ones *Therorhodion*, *Mumeazalea* and *Candidastrum*. The Subgenus *Rhododendron* consisted of the sections *Pogonanthum*, *Rhododendron*, *Vireya*, *Therorhodion* and *Tsutsusi*. The sections *Rhododendron* and *Vireya* consisting mainly of the subsections of Sleumer (1949). It is important to note that the Section *Rhododendron* now includes the genus *Ledum* as Subsection *Ledum* (Photo 4a) and sister to Subsection *Edgeworthii* (Photo 4b), as determined by K A Kron and W S Judd (1990). However, molecular studies by Kurashige et al. (Kurashige et al. 2001) does not support this placement, and show that the Subgenus *Rhododendron* is monophyletic, if Subsection *Ledum* is excluded. A molecular phylogenetic study of the subfamily RHODODENDROIDEAE Endlicher that

[22] The subsections of this group are discussed in section 2.2.1.

included a small number of *Rhododendron* taxa by Kron (1997) showed *R. tomentosum* of Subsection *Ledum* clustering with the rest of the *Rhododendron* taxa. Further molecular studies including more related taxa will perhaps ascertain an acceptable placement for *Ledum*.

Photo 4 *Rhododendron neoglandulosum* and *R. edgeworthii*. (a) *R. neoglandulosum*, from Subsection *Ledum* (L.) K. A. Kron & W. S. Judd, previously known as *Ledum glandulosum* var. *glandulosum*, growing in the Wenatchee National Forest, Wenatchee Mountains, USA. [Photo: Walter Siegmund]. (b) *R. edgeworthii* belonging to the Subsection *Edgeworthii*, a sister group to Subsection *Ledum*. [Photo: David Davies].

The Section *Vireya* consists of the subsections utilized in Sleumer's work on the *Flora Malesiana* project (Sleumer 1960, 1966a) and discussed in section 2.2. The Subgenus *Hymenanthes* in Chamberlain's (1996) classification is equivalent to Sleumer's (1949) Subgenus *Eurhododendron*, consisting of the single Section *Ponticum*, which in turn comprises 24 subsections. The majority of these subsections are those proposed by Sleumer (1949). The Subgenus *Pentanthera*, includes the major Section *Pentanthera* (comprising 15 species from the south-eastern United States), and three smaller sections *Sciadorhodion*, *Rhodora* and *Viscidula*. Sleumer (1949) however, had merged the sections *Rhodora*, *Viscidula* and *Pentanthera* into the Subgenus *Pseudanthodendron*. The only morphological characters linking these four sections are deciduous, tomentose leaves and terminal inflorescences (Cox & Cox 1997). The Subgenus *Tsutsusi* of Chamberlain et al. (1996) was based on the studies by Judd and Kron (1995), and is

equivalent to Subgenus *Anthodendron* of Sleumer (1949), less Section *Tsusiopsis*. Another major difference between the classifications of Sleumer (1949) and Chamberlain (1996) is that the Subgenus *Therorhodion* was placed outside the genus *Rhododendron* by Sleumer.

An interesting feature of Sleumer's classification system is the proximity of the deciduous Section *Pentanthera* G. Don to the evergreen Subgenus *Hymenanthes* (Blume) K. Koch (Subgenus *Pseudanthodendron* Sleumer), both of which lack lepidote scales. The leaves of rhododendrons in Subgenus *Hymenanthes* are generally thick and have, in many species, a thick coating of fuzzy hairs (indumentum) on the lower surface (Cox & Cox 1997). In the Subgenus *Pentanthera*, the Chamberlain et al. (1996) classification system included the major Section *Pentanthera*, comprising 15 species from the south-eastern United States plus three from other regions (belonging to the sections *Sciadorhodion*, *Rhodora* and *Viscidula*). Apart from having deciduous leaves covered with hairs, and terminal rather than axillary inflorescences, few morphological attributes link these four sections together (Cox & Cox 1997).

2.1.3 Molecular Systematics of *Rhododendron* L.

The systematics of *Rhododendron* has been studied in recent times using molecular phylogenies based on several DNA regions. Most of these studies were carried out to evaluate higher-level phylogenetic relationships. Studies on lower ranks, such as subgenera and sections, began much later. Molecular systematics of *Rhododendron* was initiated in 1997, by Kathleen A Kron. In this study, the phylogenetic analyses of the *mat*K sequences of 42 taxa from traditional subfamily RHODODENDROIDEAE and related clades with *Actinidia chinensis* (ACTINIDIACEAE) as the outgroup indicated that the RHODODENDROIDEAE are paraphyletic (Kron 1997). Phylogenetic trees obtained in the analyses indicated an expanded 'rhododendroid' clade (taxa belonging to the subfamily RHODODENDROIDEAE) that included four major subclades ('empetroid', 'rhodo', 'ericoid', and 'phyllodocoid') (Figure 1). The empetroid clade contains the taxa belonging to the genera *Empetrum* (previously placed under the family EMPETRACEAE) and *Ceratiola*. The 'rhodo' clade contains the taxa belonging to the genera *Rhododendron, Menziesia*, *Tsusiophyllum* and *Therorhodion*. The ericoid clade contains the taxa belonging to the genera *Bruckenthalia, Erica, Calluna* and *Daboecia*. The

'phyllodocoid' clade consists of the genera *Phyllodoce*, *Kalmiopsis*, *Epigaea*, *Rhodothamnus*, *Elliottia*, *Leiophyllum*, *Loiseleuria*, *Kalmia* and *Bejaria*. The 'ericoid' clade was found to be sister to the phyllodocoid clade and the empetroid clade sister to the 'rhodo' clade. Relationships within the clades were generally well resolved except within the 'rhodo' clade where *mat*K data indicated that *Rhododendron* is probably paraphyletic, indicating a re-classification of the genus *Rhododendron* was necessary.

The relationships indicated by the *mat*K data also suggested that 'ericoid' leaves and sympetalous corollas are the plesiomorphic condition in the 'rhododendroids', and further study will be needed to test this hypothesis (Kron 1997).

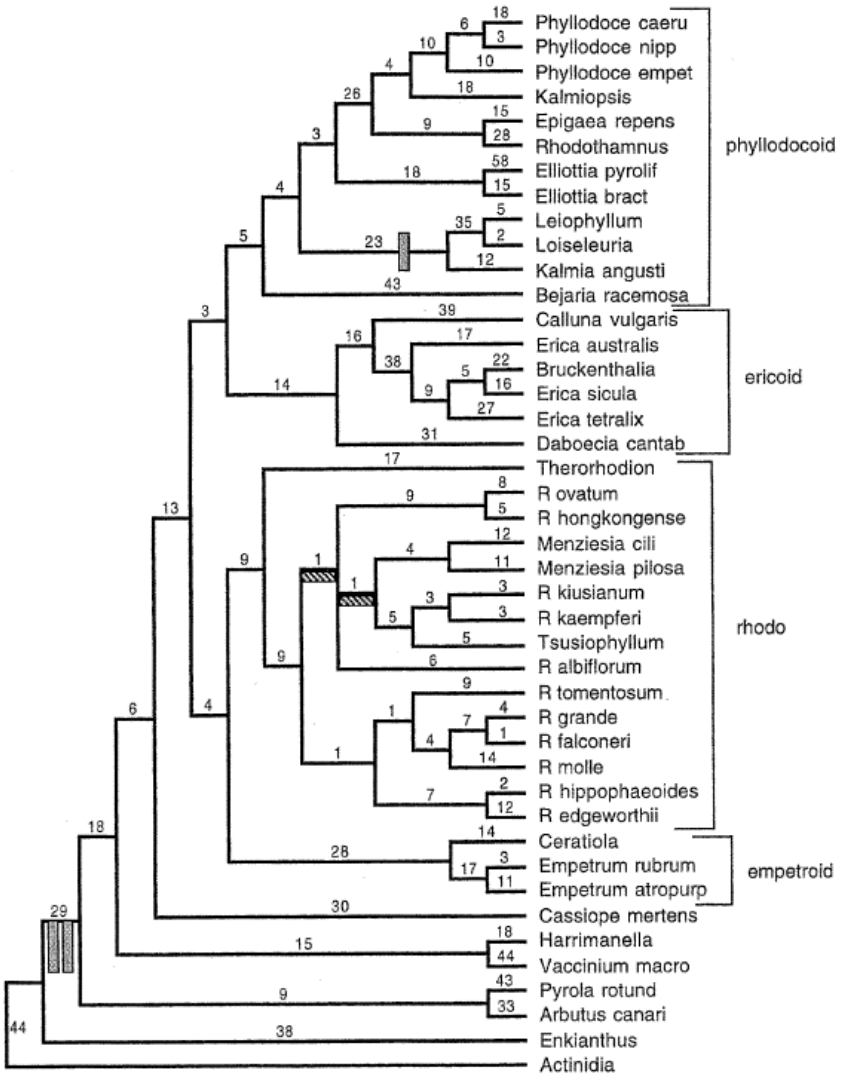

Figure 1 Single representative tree found in all analyses of *mat*K sequences (L=1197, CI=0.52, RI=0.63). Branch lengths are indicated above branches. Horizontal bars indicate clades that

collapse in the strict consensus of all most-parsimonious trees in all analyses. Vertical bars indicate phylogenetically informative insertions in the *mat*K sequence (Kron 1997).

Scheiber et al. (2000) carried out a genetic relationships study among specimens of the 15 recognized species in *Rhododendron* L. Section *Pentanthera* G. Don by comparing the sequences of the entire internal transcribed spacer (ITS) region (including ITS1, ITS2, and the 5.8S subunit). *R. vaseyi* A. Gray belonging to Section *Rhodora* (L.) G. Don was used as the outgroup. The bootstrap analysis showed divergence values among the taxa were extremely low ranging from 0.00–3.51%, providing support to traditional views of Section *Pentanthera* as a group of very closely related species (Figure 2).

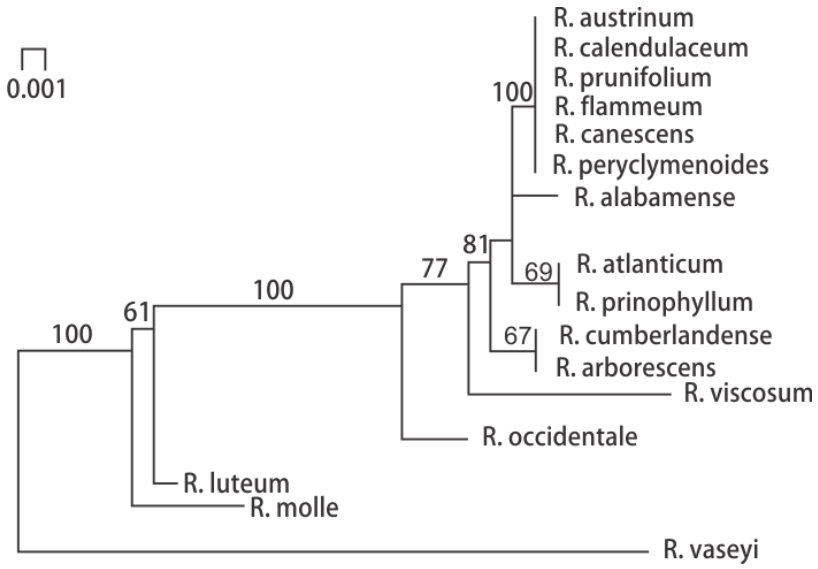

Figure 2 Dendrogram depicting genetic relationships in *Rhododendron* Section *Pentanthera*. Values above branches indicate bootstrap values supporting the respective cluster. Adapted from (Scheiber et al. 2000).

Hwang & Hsu (2001) carried out a study on the phylogenetic relationships among 8 species of *Rhododendron* by comparing the sequences of the chloroplast *trn*F–*trn*L intergenic spacer region. Neighbor-Joining (Figure 3) and parsimony analyses (Figure 4) showed identical topology with 3 major clades. Close phylogenetic relationships among *R. pseudochrysanthum*, *R. morii*, *R. rubropunctatum*, and *R. hyperythrum* based on the chloroplast DNA sequences agreed with those derived from morphological characters.

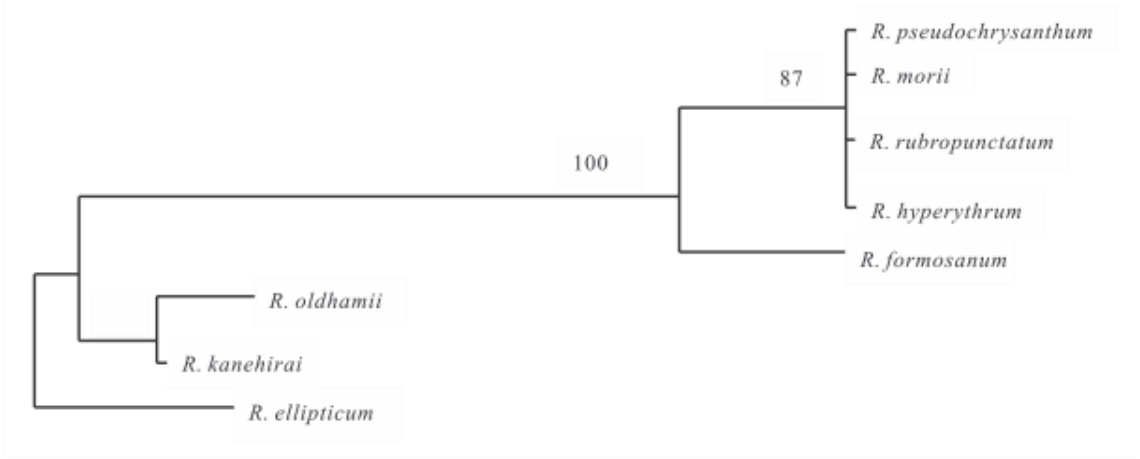

Figure 3 Neighbour-Joining tree of eight *Rhododendron* species based on *trn*F–*trn*L DNA sequences. Bootstrap values (500 replicates) are given above the nodes. Adapted from (Hwang & Hsu 2001).

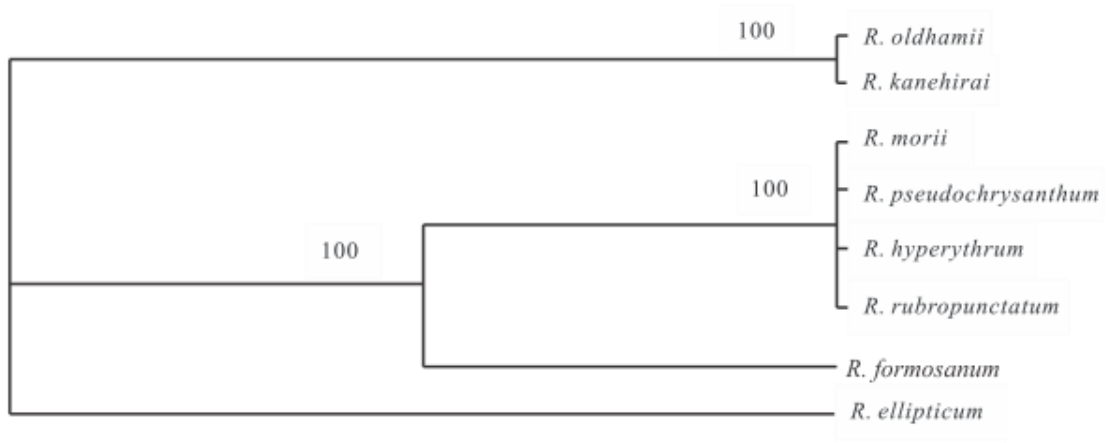

Figure 4 One of the 45 equally parsimonious trees generated by a branch-and-bound search of eight *Rhododendron* chloroplast *trn*F–*trn*L DNA sequences. Bootstrap values (500 replicates) are given above the nodes. Adapted from (Hwang & Hsu 2001).

A more recent phylogeographic study was carried out by Chung et al. (2007), which revealed the origin and evolutionary history of a *Rhododendron* species complex in Taiwan. This study inferred a single origin and a once-widespread distribution of the *R. pseudochrysanthum* species complex in Taiwan based on chloroplast DNA sequence variation (two chloroplast intergenic spacers: *trn*L–*trn*F and *atp*B–*rbc*L) of 124 individuals from five endemic *Rhododendron* species. The haplotype and nucleotide diversities were much lower for the *R. pseudochrysanthum* complex, comprised of the species *R. pseudochrysanthum*, *R. morii*, *R. rubropunctatum*, and *R. hyperythrum*, than for *R. formosanum*. Nested Clade Analysis (NCA) indicated a contiguous range

expansion for chloroplast DNA haplotypes of *R. formosanum*. The study suggested a once-widespread distribution of the *R. pseudochrysanthum* complex probably via north-to-south colonization of mid-elevations during low-temperature periods of the Pleistocene. Population fragmentation followed the warmer climate which began in the Holocene and resulted in the present-day range contraction into high elevations.

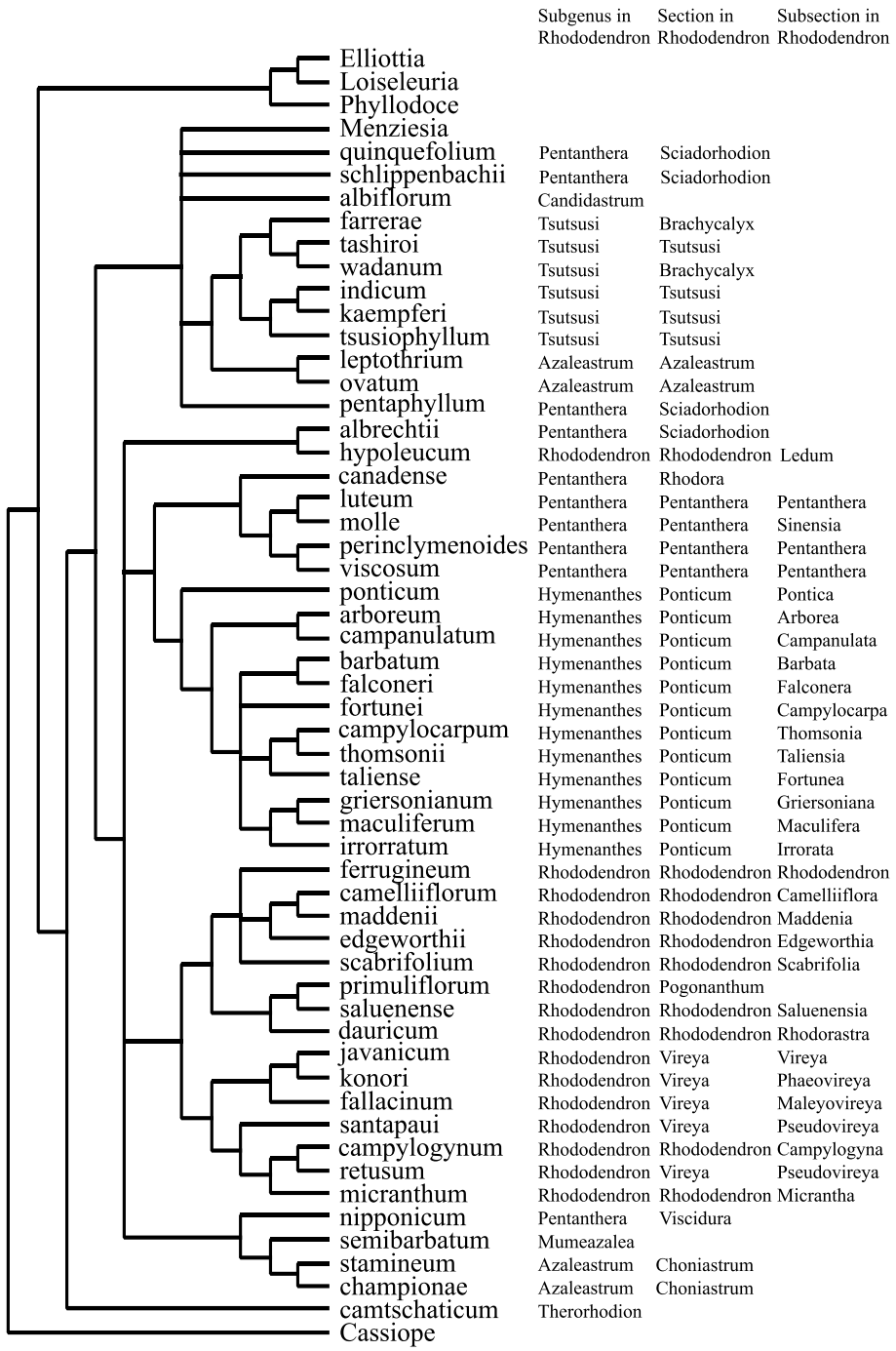

Figure 5 Strict consensus of 267 most parsimonious Fitch trees based on *mat*K and *trn*K intron sequences. Tree length = 744; consistency index (excluding uninformative characters) = 0.625; retention index = 0.805. Adapted from (Kurashige et al. 2001).

Kurashige et al. (2001) carried out a very comprehensive study of the phylogenetic relationships among all eight subgenera and 12 sections of *Rhododendron*, and related genera, inferred from *mat*K and *trn*K intron sequences (Figure 5). The results of this study showed that the genus *Rhododendron* is paraphyletic because the genus *Menziesia* is nested within the genus *Rhododendron*, Subgenus *Therorhodion* forms a basal lineage of tribe RHODOREAE, subgenera *Hymenanthes* and *Tsutsusi* are monophyletic, and subgenera *Azaleastrum* and *Pentanthera* are polyphyletic. However, Subgenus *Rhododendron* is monophyletic, if Subsection *Ledum* is excluded, which is contradictory to the findings of Kron & Judd (1990) with fewer *Rhododendron* and related taxa (Section 2.1.1). *Ledum* was initially described as a genus by Linnaeus in 1753, and a major difference between *Rhododendron* and *Ledum* is that the latter has the corolla divided to base, and the corolla lobes are imbricate in bud.

The study by Kurashige et al. (2001) thus supports the traditional classifications to a large extent, and similar studies with larger number of taxa representing more sections and subsections will perhaps improve the phylogeny.

Maximum Parsimony analysis utilizing the internal transcribed spacer (ITS) regions of nuclear ribosomal DNA (including 5.8S rRNA) of 15 *Rhododendron* species (representing most sections of the genus) by Gao et al. (2002) explored the infrageneric and sectional relationships of *Rhododendron* (Figure 6). The study also included one *Ledum* species, *Cassiope fastigiata*, and ITS sequences of 13 selected *Rhododendron* species and *Bejaria racemosa* from GenBank. *C. fastigiata* and *B. racemosa* were designated as the outgroup for the study. The tree inferred for the ITS data showed that the genus *Rhododendron* was a well-supported monophyletic group, while the Subgenus *Therorhodion* was basal to the rest of the genus. *Ledum* was shown to be a member of *Rhododendron*, and its close relationship with the lepidote rhododendrons was confirmed. The elepidote rhododendrons formed a weakly-supported clade within which the monophyly of subgenera *Hymenanthes* and *Tsutsusi* were strongly supported, while the subgenera *Pentanthera* and *Azaleastrum* were shown to be polyphyletic. The monophyly of Section *Choniastrum* (Subgenus *Azaleastrum*) was strongly-supported, while Subgenus *Tsutsusi* could be sister to a weakly-supported clade composed of two sampled species of Section *Azaleastrum* (Subgenus Azaleastrum) together with *R. semibarbatum*,

of Subgenus *Mumeazalea*. The arrangement of the subgenera of the genus *Rhododendron* are thus in strong agreement with the classification of Chamberlain et al. (1996).

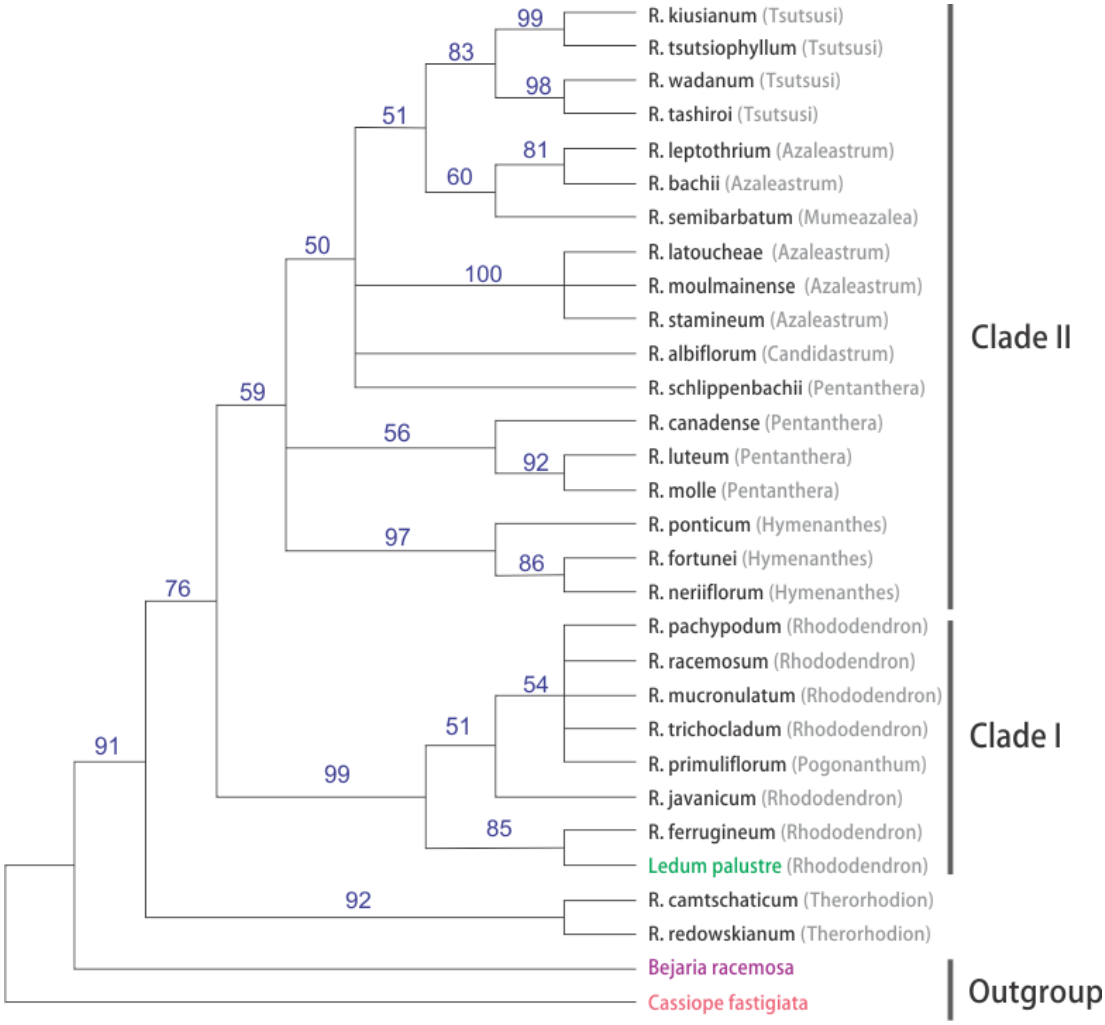

Figure 6 Strict consensus of 18 parsimonious trees based on ITS sequence data. All the characters were equally weighted and gaps treated as missing data (Tree Length = 270, CI = 0.7741, RI = 0.7707). Numbers above branches are bootstrap values with 1,000 replications. The subgenera of Chamberlain et al. (1996) are given in brackets after taxon name. Adapted from Gao et al. (2002).

Tsai et al. (2003) studied the genetic relationships of 20 *Rhododendron* species from Taiwan (Figure 7). Their study was based on the complete sequences of the ITS region of ribosomal DNA, including ITS1, 5.8S rDNA, and ITS2. According to their dendrogram, the 20 species formed four main clusters, corresponding to the subgenera *Tsutsusi*, *Azaleastrum*, *Rhododendron* and *Hymenanthes* (Figure 7). In this study, the findings based on the ITS sequences were in agreement with the traditional systematics of *Rhododendron*.

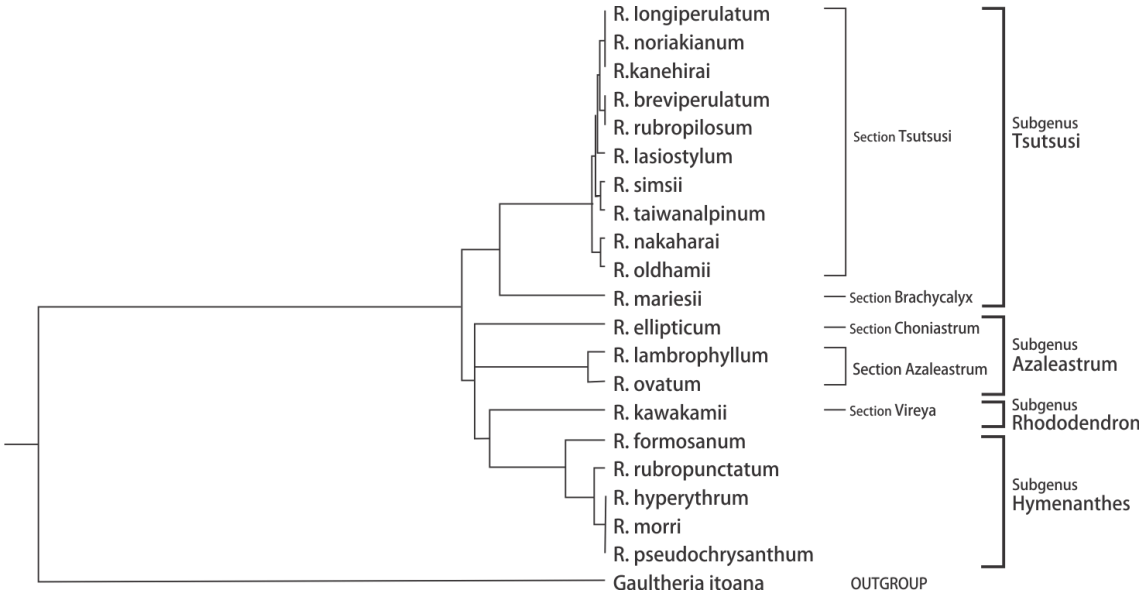

Figure 7 Dendrogram of the 20 species of *Rhododendron* in Taiwan obtained from sequence data of the nrDNA ITS region. Adapted from (Tsai et al. 2003).

A phylogeny based on cpDNA *mat*K and *trn*L–F carried out by Milne (2004) indicated that multiple *Pontica* lineages colonised each of SW Eurasia, SE North America, and NE Asia, with little or no speciation within regions thereafter (Figure 8), suggesting the survival of multiple (3–4) *Pontica* lineages in SW Eurasia. *Pontica* is, as suggested by its Tertiary relict distribution, probably the oldest group within Subgenus *Hymenanthes*, and comprises two major clades, one of which is wholly Eurasian, and paraphyletic with respect to at least some of the remaining 200 species of Subgenus *Hymenanthes*, which are all distributed in SE Asia. The other clade has species from W and SE North America, SW Eurasia, and NE Asia. Although the phylogeny indicates probable trans-Atlantic migration for one of two America-Eurasia disjunctions in *Pontica*, the timing supports migration via Beringia[23] for both.

[23] A vast area between the Kolyma River in the Russian Far East to the Mackenzie River in the Northwest Territories of Canada.

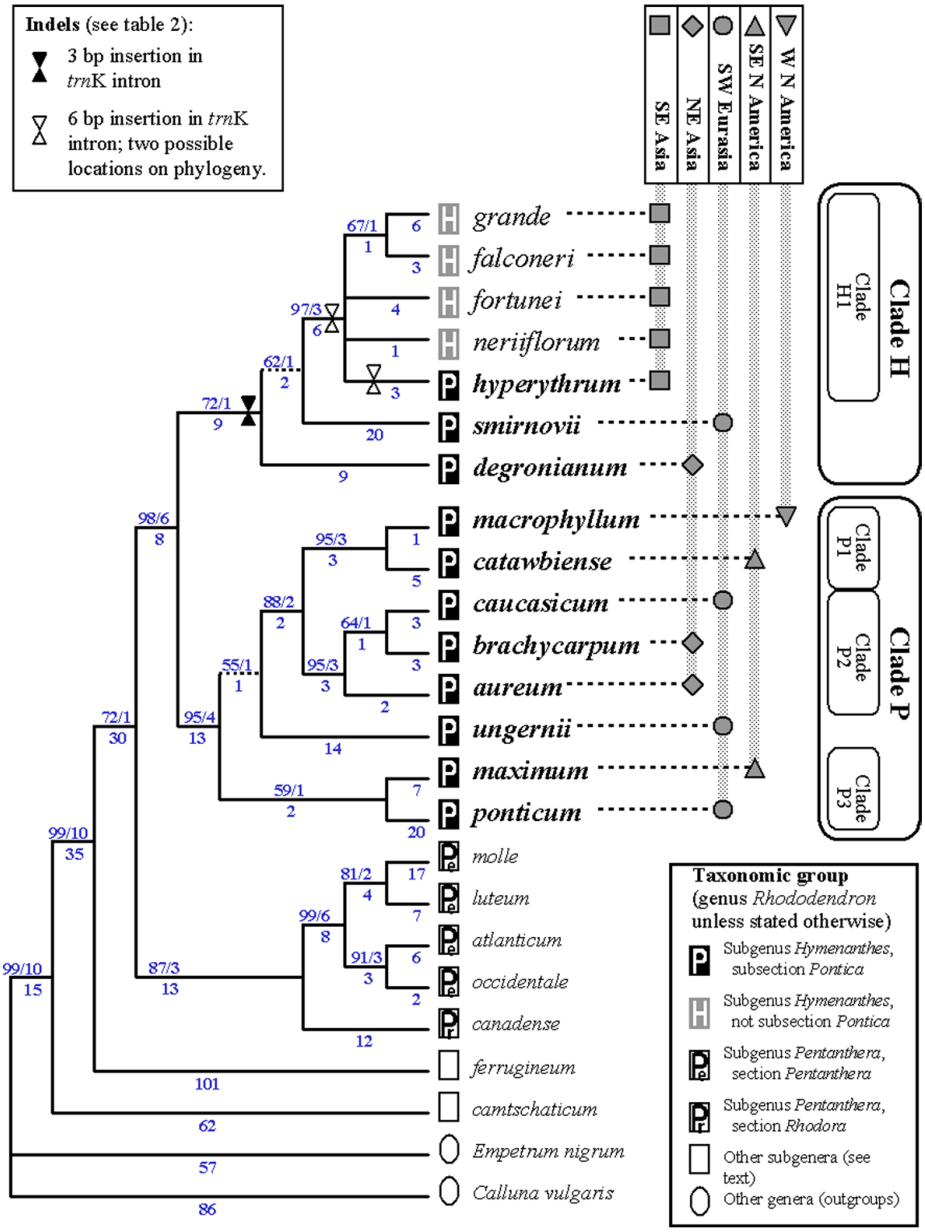

Figure 8 Phylogeny of *Rhododendron* based on cpDNA *mat*K and *trn*L–F sequences. The tree represents the Subsection *Pontica* (species names in bold), four representatives of other subsections of Subgenus *Hymenanthes*, five species of the Subgenus *Pentanthera*, two other *Rhododendron* species, and two outgroup taxa. The tree shown is one of 15 most parsimonious trees, which has identical topology to both the strict consensus and bootstrap consensus trees. Numbers above branches are bootstrap support (BS) and (after slash) decay index (DI); numbers below branches are branch lengths in the selected most parsimonious tree. Dotted branches are those not in the strict consensus tree based on *mat*K data alone. Areas of distribution indicated for species are as follows: 'SE Asia' comprises southern China, the adjacent islands south to Java, the Himalayas and outliers in India and Sri Lanka. 'NE Asia' comprises Japan and Korea with a narrow band stretching through Manchuria to eastern Siberia for *R. aureum* only. 'SW Eurasia' comprises the area around the southern Black Sea coast (mainly N Turkey and Caucasus) with outliers in Lebanon, Spain, and Portugal for *R. ponticum* only. 'SE N America' indicates parts of N America east of 86°, from N Georgia to Nova Scotia. 'W N America' indicates areas within 300 km of the west coast of the USA. Adapted from Milne (2004).

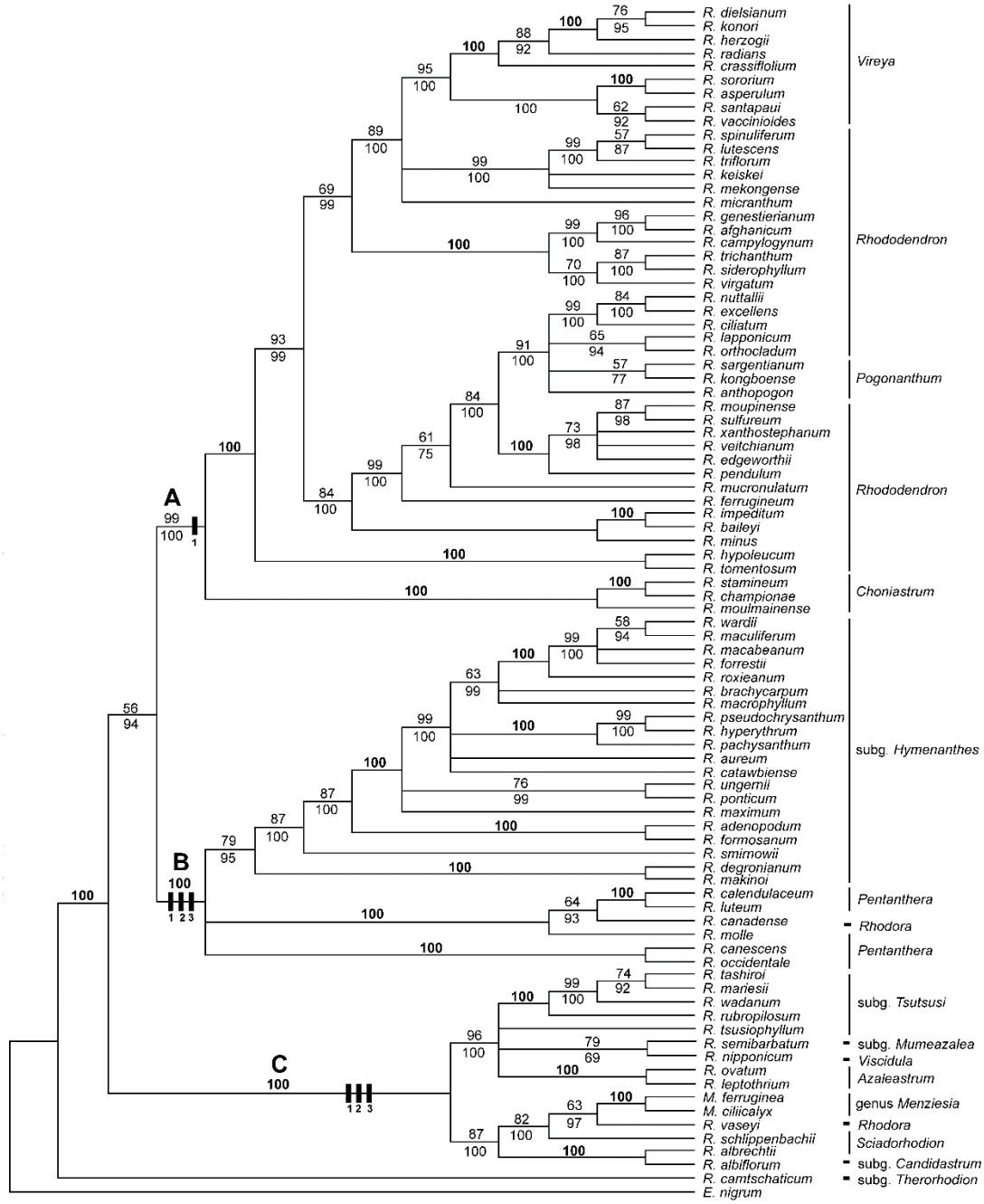

Figure 9 Maximum parsimony strict consensus tree based upon *rpb*2i gene sequences. Numbers above the branches give the bootstrap support for 1,000 replicates. Only those bootstrap values >50% are shown. Bayesian posterior probabilities (×100) are shown below the branches or, when equal to bootstrap values, as a single number (bolded) above the branch. Taxon names on the extreme right refer to sections of Chamberlain et al. (1996) unless otherwise indicated. The vertical bars represent unambiguous synapomorphic indels. Adapted from Goetsch et al. (2005).

The traditional classification of the genus *Rhododendron* based on morphological characters led to a consensus taxonomy recognizing the major subgenera *Azaleastrum*,

Hymenanthes, *Pentanthera*, *Rhododendron*, *Tsutsusi*, and three minor subgenera. To study whether these subgenera are monophyletic and to infer phylogenetic relationships between sections and species, Goetsch et al. (2005) carried out a cladistic analysis using *rpb*2i sequence data, including all groups within the genus. The results clarified the phylogeny of *Rhododendron*, and suggested that several changes to the infrageneric systematics of *Rhododendron* (Figure 9). Their results supported to a large extent, the classification of Sleumer (1949) over that of Chamberlain et al. (1996). For taxa outside of the Subgenus *Rhododendron*, this system rejects three subgenera and two sections that are present in the taxonomic system of Chamberlain et al. (1996). Inclusion of the Section *Pentanthera* within Subgenus *Hymenanthes* had 100% bootstrap and Bayesian inference support for a clade containing only these taxa (with *R. canadense* moved to the new Section *Pentanthera*). The sections *Sciadorhodion* and *Viscidula*, along with *R. vaseyi* (previously in Section *Rhodora*) from the previous Subgenus *Pentanthera* were combined with sections *Azaleastrum*, *Tsutsusi* and *Brachycalyx* to form a much broader Subgenus *Azaleastrum*. The sister groups in this subgenus are thus sections *Tsutsusi* and *Sciadorhodion*. The taxa belonging to the Subsection *Ledum* (*R. tomentosum* and *R. hypoleucum*) were shown to fall within the Subgenus *Rhododendron*, providing support to the studies by Kron and Judd (1990), and Kurashige et al. (2001). The *rpb*2i data showed that Section *Choniastrum* forms a well-supported cluster sister to Subgenus *Rhododendron*. Unlike Subgenus *Rhododendron*, leaves of Section *Choniastrum* lack lepidote scales, thus Goetsch et al. (2005) proposed that *Choniastrum* be promoted to a separate subgenus.

The studies carried out by Brown et al. (2006a, 2006b; 2006c) were focussed mostly on the *Vireya* group and will be discussed in Section 2.2.3. However, the study utilizing the ITS nrDNA region included representative taxa from the subgenera *Rhododendron* (sections *Rhododendron* and *Pogonanthum*), *Azaleastrum*, *Mumeazalea*, *Hymenanthes*, and *Pentanthera*. The results suggested that the Subgenus *Azaleastrum* Planch. (which contains the sections *Azaleastrum* (Planch.) Maxim. and *Choniastrum* Franch.) is monophyletic and sister to Subgenus *Mumeazalea*, when only the Section *Choniastrum* was sampled. Previous studies have found the two sections of the Subgenus *Azaleastrum* to be monophyletic, while the subgenus as a whole is polyphyletic (Gao et al. 2002; Kurashige et al. 2001). The Subgenus *Rhododendron*, the lepidote rhododendrons, was found to be monophyletic in all the ITS analyses, and this close relationship has long been

hypothesised on the basis of morphological similarities (Rouse et al. 1993; Williams & Rouse 1990; Williams et al. 1985) and molecular phylogenetic studies (Goetsch & Hall 2002; Kurashige et al. 2001). While the Subgenus *Rhododendron* is a natural group, the three currently recognised sections (*Pogonanthum*, *Rhododendron* and *Vireya*) are not, and this conclusion is supported by results from other molecular studies (Goetsch & Hall 2002; Kurashige et al. 2001).

The study carried out by Craven et al. (2008) for the Subgenus *Rhododendron* showed that this subgenus is not monophyletic. The study also showed that the traditionally recognized groups within the Subgenus *Rhododendron* are also not monophyletic, such as the *Vireya* group, and are further discussed in 2.2.3. Craven et al. (2008) proposed that the vireyas be classified within Subgenus *Rhododendron* in the sections *Discovireya*, *Pseudovireya*, and *Vireya* (now *Schistanthe*) with two subsections within Section *Schistanthe* (subsections *Euvireya* and *Malayovireya*). The remaining subsections of Sleumer's (1966a) classification were included by Craven et al. (2008) within the Subsection *Euvireya.*

The Parsimony and Bayesian analyses of Milne et al. (2010) utilizing cpDNA *mat*K and *trn*L–F sequence data (Figure 10 and Figure 8 respectively) divided the Subgenus *Hymenanthes* into two clades: clade H, in which two *Pontica* species and the SE Asian *R. adenopodum* were sister to a clade of 60 SE Asian species, and clade P comprising eight *Pontica* species plus *R. praevernum*, *R. calophytum*, and *R. insigne* from SE Asia. Their distribution within the Chinese/Himalayan range of *Rhododendron* indicates an ancestor that came from the north or east to meet the diversifying group of *Hymenanthes* in the Himalayas, making the SE Asian members of *Hymenanthes* a polyphyletic group. This is in contrast to the morphological studies by Wang et al. (Wang et al. 2007) and molecular studies by Kurashige et al. (2001) and Gao et al. (2002).

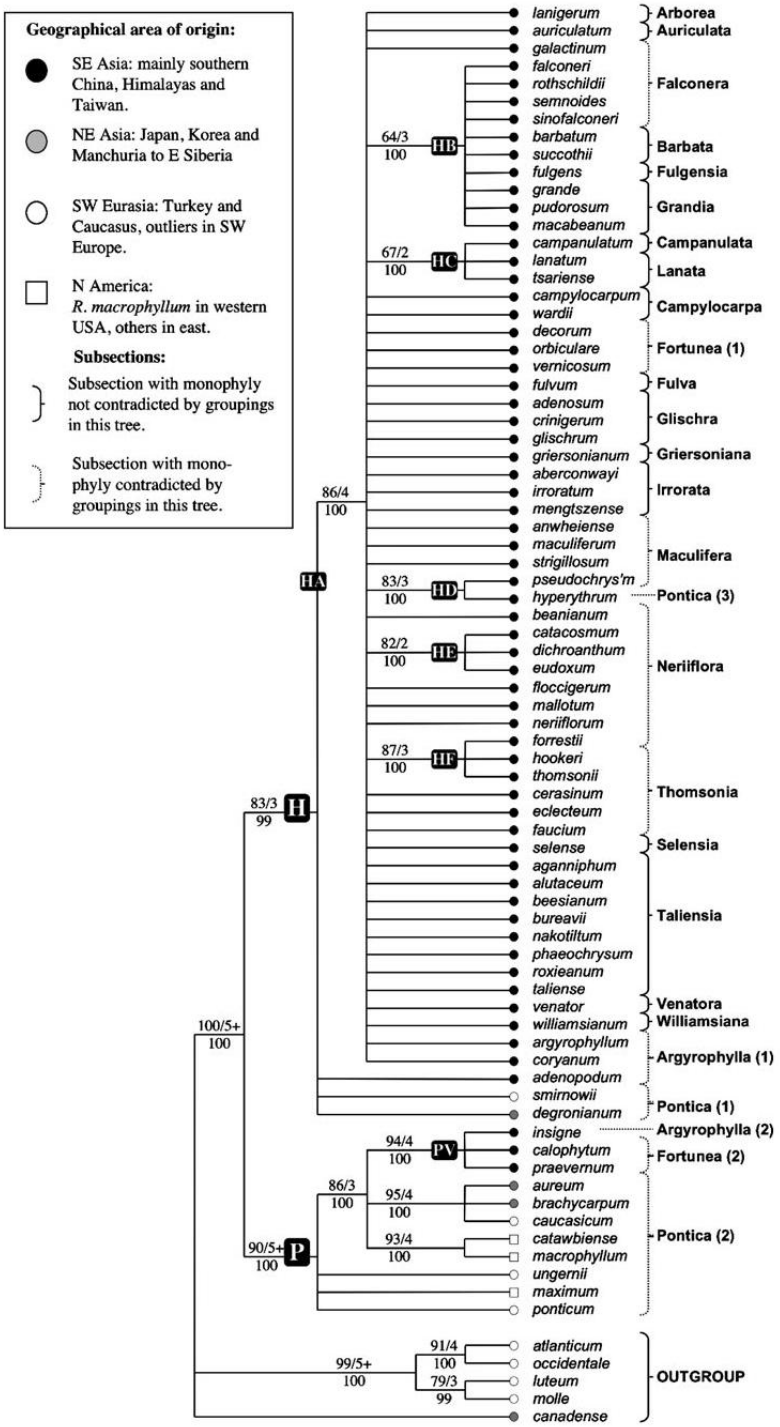

Figure 10 Consensus of parsimony strict consensus tree and Bayesian consensus tree of *Rhododendron* Subgenus *Hymenanthes*. Clades with ≥60% bootstrap, ≥2 Bremer and ≥98% Bayesian support are shown. Area of origin and with species arranged by subsection as far as possible are shown. Bootstrap and Bremer support values appear above branches, Bayesian posterior support below them. The two accessions of *R. praevernum* examined had identical sequences and are treated here as a single accession. Subsections with dotted line brackets are those whose monophyly is contradicted by the tree. A number after the subsection name [e.g. *Fortunea*] indicates the species of that subsection appear in two separate places in the phylogeny. Outgroup species are from Subgenus *Pentanthera*, sections *Rhodora* (*R. canadense*) and *Pentanthera*. Adapted from Milne et al. (2010).

Members of the *Rhododendron* subgenera *Pentanthera* (deciduous) and *Tsutsusi* and *Azaleastrum* (evergreen) are commonly referred to as azaleas. Both azalea types are important ornamentals with extensive breeding and hybrid groups are often named after the supposed principal ancestor species. The Subgenus *Pentanthera* is phylogenetically closer to evergreen rhododendrons (subgenera *Rhododendron* and *Hymenanthes*) than to the Subgenus *Tsutsusi* (Goetsch et al. 2005; Milne et al. 2010).

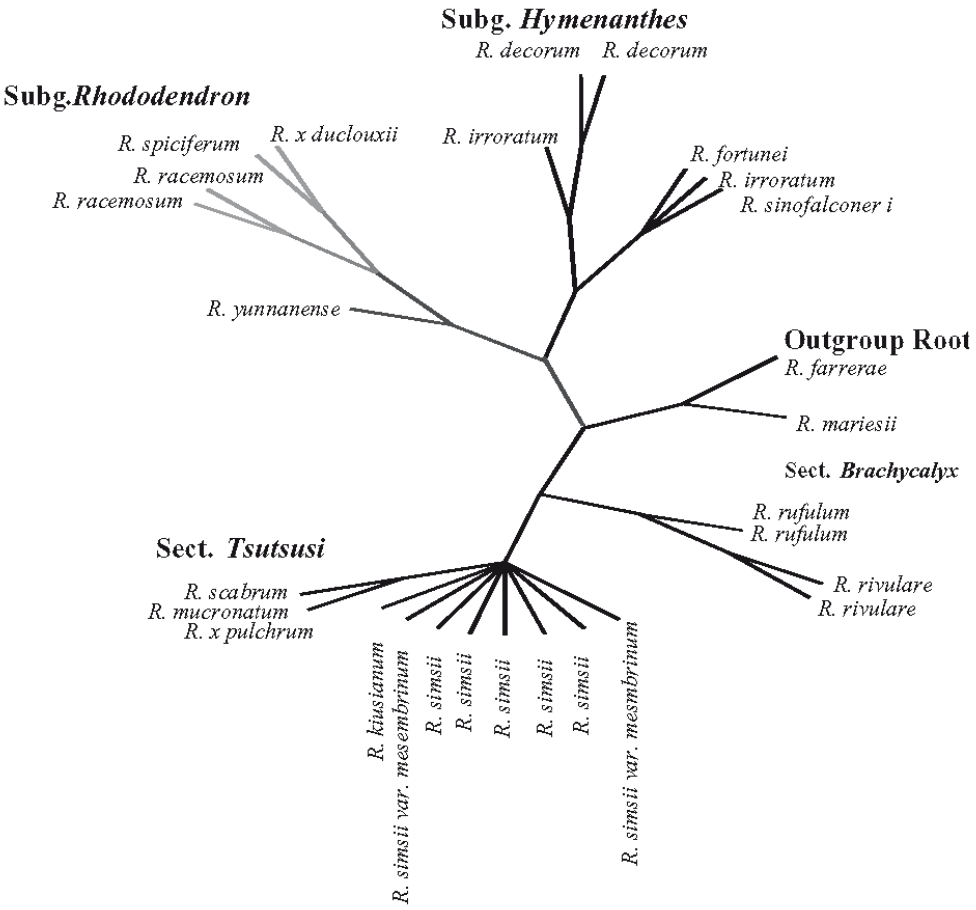

Figure 11 Consensus parsimony tree based on *mat*K data for a selection of Chinese *Rhododendron* species and cultivated *R. simsii* hybrids. Tree length = 98; consistency index (excluding uninformative characters) = 0.9403; retention index = 0.9987. Adapted from De Keyser et al. (2010).

Molecular techniques for phylogenetic and kinship research have been evaluated for azaleas in the study by De Keyser et al. (2010). Firstly, some studies using comparative gene sequencing were presented; this approach was then widened to the use of molecular markers to reveal more detailed genetic relationships. One of these studies included a phylogenetic analysis based on the *mat*K sequences used to confirm the taxonomic

position of the different species of the Subgenus *Tsutsusi*. Figure 11 shows a parsimony cladogram of the genetic similarity between the Chinese *Rhododendron* species and some *R. simsii* hybrids. Five major clusters can be distinguished. The first group contains *R mariesii* and *R. farrerae* (both species belonging to the Section *Brachycalyx* of the Subgenus *Tsutsusi*). The second cluster corresponds to the Subgenus *Rhododendron*. The third cluster corresponds to the Subgenus *Hymenanthes*. The fourth corresponds to the Subgenus *Tsutsusi*. The fifth and the largest cluster contain species belonging to the Section *Tsutsusi* and can be divided in two subclusters. The first sub-cluster contains four *R. simsii* populations, two *R. simsii* var. *mesembrinum* populations, *R. scabrum*, *R. kiusianum* and the Kurume hybrid 'Cupido.' A second sub-cluster contains five *R. simsii* hybrids, *R.* × *pulchrum*, *R.* × *mucronatum*, and *R. scabrum*. Finally, the use of candidate genes as functional markers for the assessment of genetic diversity was presented.

Another recent molecular study on the genus *Rhododendron* was carried out by Liu et al. (2011) using the *psb*A–*trn*H intergenic spacer region. This phylogenetic study utilized 40 accessions from 36 taxa, representing seven subgenera. The results showed that all the taxa are grouped into seven monophyletic clades with high bootstrap support, which belong to the seven subgenera according to the classification system of Mingyuan et al. (2005)[24] in Flora of China. *Pseudorhodorastrum* Sleumer was supported as an independent subgenus. The proposition of Chamberlain that the Subgenus *Pseudazalea* be placed in Subgenus *Rhododendron* as Subsection *Trichoclada* was not supported by this study.

[24] D F Chamberlain is an author in this publication.

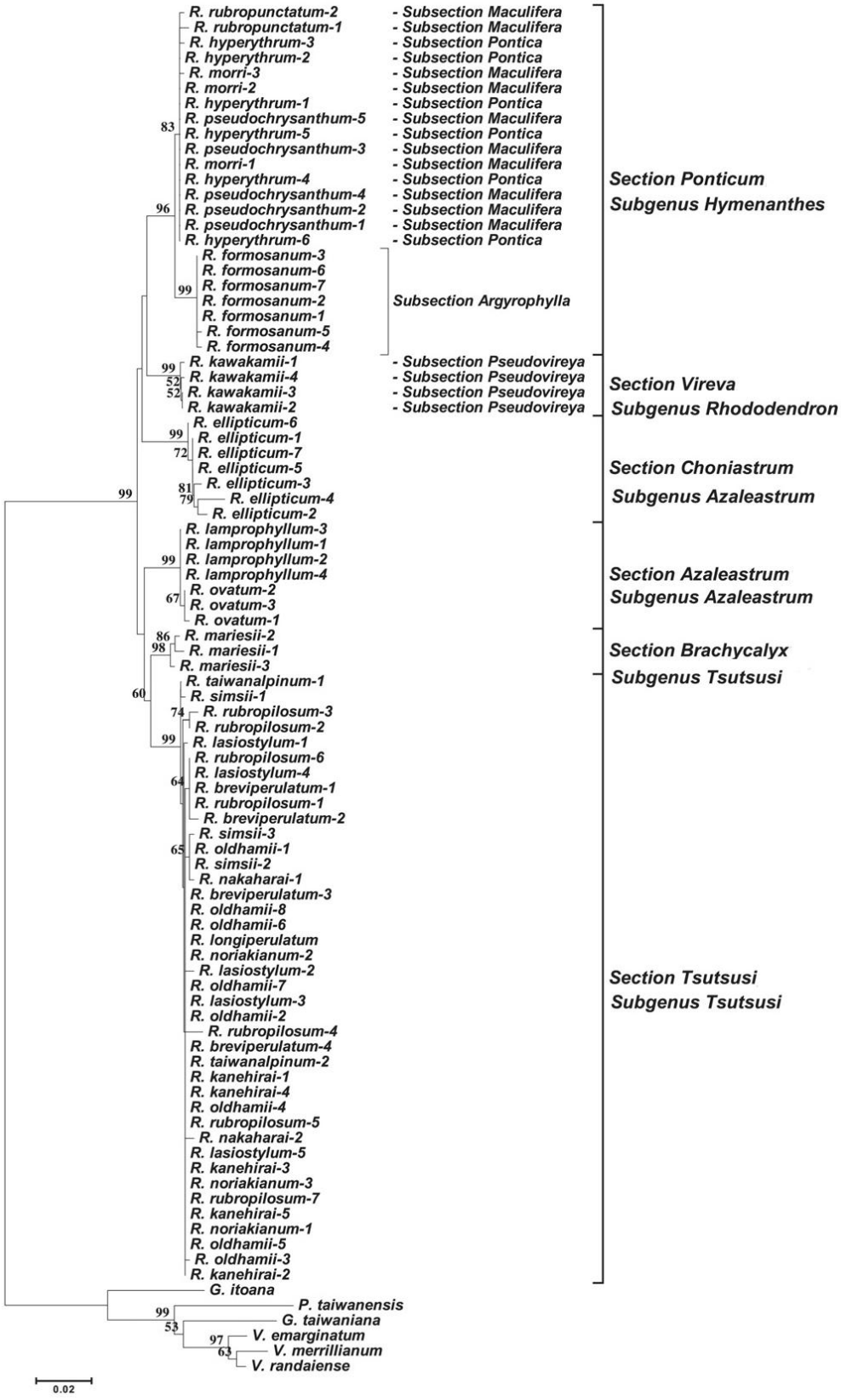

Figure 12 The Neighbour-Joining tree based on ITS sequences from 84 *Rhododendron* species and five outgroups. Numbers above internodes indicate bootstrap values from 500 replicates. Branch lengths are proportional to the number of base changes along each branch. Adapted from Tsai et al. (Tsai et al. 2012).

The most recent study on the systematics and genetic diversity (discussed further in Chapter 4) of *Rhododendron* was carried out by Tsai et al. (2012) on Taiwanese species sampled from museums using DNA data sequences for the internal transcribed spacer (ITS) region (Figure 12). The ten species sampled from the Section *Tsutsusi*, were not segregated from each other. However, within the Section *Ponticum* (Subgenus *Hymenanthes*), five *Rhododendron* species formed two clusters, one containing all the accessions of *R. formosanum* (Subsection *Argyrophylla*) and the other containing the accessions belonging to the subsections *Maculifera* and *Pontica*. Within the *Maculifera* / *Pontica* cluster, accessions of the four included species could not be genetically differentiated from each other. The accessions belonging to the subgenera *Rhododendron*, *Azaleastrum* (which contains the sections *Choniastrum* and *Azaleastrum*), were differentiated. The study thus shows that DNA data from ITS sequences can reveal high levels of morphological plasticity for *Rhododendron* species. Furthermore, the results also suggested that the classification of *Rhododendron* in Taiwan should be revised in the future, specifically those belonging to the Section *Tsutsusi* (Subgenus *Tsutsusi*), and the subsections *Maculifera* and *Pontica* (Subgenus *Hymenanthes*) may require species synonymies. The results also suggest that the ten Taiwanese species in the Section *Tsutsusi* could be treated as a single variable species, *R. simsii*, and the three species from the Subsection *Pontica*, and *R. hyperythrum*, could be treated as a single species, *R. pseudochrysanthum*. This study had huge implications on the delimitation of *Rhododendron* species worldwide, and similar studies for other hotspots of this genus such as China with very high species diversity, will greatly reduce the number of species currently recognized (approximately 1,000) to a more manageable number.

2.1.4 Summary

As with any group of plants, the similarities between rhododendrons were often unconsciously accepted through an association of observations and ideas. Many early botanists attempted to utilize artificial criteria of classification that had no scientific basis, often based on *a priori* grounds, including Linnaeus's sexual system which is also artificial. Using a few easily recognized features, each botanist then tried to improve on the last botanist's system.

The majority of the studies discussed in Sections 2.1.1 and 2.1.3 are concerned with the gradual improvement of the various infrageneric subdivisions of *Rhododendron*. Systematic studies that encompassed all sections and subgenera of *Rhododendron* was initiated by Sleumer (1949), who proposed a comprehensive system of *Rhododendron* classification in the form of a key to the subgenera and sections using morphological characters. Subsequently, a number of more narrower taxonomic studies using morphological characters (Chamberlain 1982; Cullen 1980; Judd & Kron 1995; Sleumer 1966a) were incorporated into a very comprehensive classification of *Rhododendron* by Chamberlain et al. (1996). This classification system is now generally accepted by researchers and growers, mainly because it represents the findings of the majority of all the systematic studies of *Rhododendron* using morphological characters. Significant differences between the classification systems of Sleumer (1949) and Chamberlain et al. (1996) include the Subgenus *Therorhodion*, which Sleumer placed outside the genus *Rhododendron*, and placement of the four species of Section *Sciadorhodion*. Based on morphological studies by Judd & Kron (1995), Chamberlain et al. (1996) assigned these species to the Subgenus *Pentanthera*, while Sleumer combined them with the Section *Brachycalyx* in Subgenus *Anthodendron*, equivalent to Subgenus *Tsutsusi* (Chamberlain & Rae 1990).

The molecular systematic studies of *Rhododendron* started very recently, about 20 years ago. The initial studies were carried out at family and subfamily levels, and it took nearly 10 years to initiate the studies at infrageneric and sectional levels. The molecular studies have in many instances reinforced the placement of certain groups or related groups based on morphological characters, while the others have been dissolved or incorporated into other groups. One example is the incorporation of the taxa within the genus *Menziesia* which Klotzsch (1851) placed in the family MENZIESIACEAE. This genus has been incorporated into the genus *Rhododendron* as a subgenus by Kurashige et al. (2001) based on molecular data.

The combination of studies such as those by Goetsch et al. (2005) and Liu et al. (2011) and other narrower studies (targeted at infrageneric levels) have led to a better understanding of the *Rhododendron* phylogeny. The genus *Rhododendron* is thus in need of a revised classification that takes into account the proposed phylogenies based on recent molecular studies.

2.2 The Subgenus *Vireya* Argent

The *Vireya* group of *Rhododendron* (tropical rhododendrons or vireyas) has in the past been demarcated by numerous authorities as a genus, subgenus or a section, with various subdivisions included or excluded (Argent 2006; Clarke 1882; Don 1834; Sleumer 1966a; von Dalla-Torre & Harms 1903) as discussed in Section 2.1. The circumscription of the *Vireya* group by Argent (2006) encompasses the largest number of taxa belonging to this group. The *Vireya* group is hereafter referred to as vireyas.

The *Rhododendron* Subgenus *Vireya sensu* Argent (2006) comprises 313[25] species, and several subspecies and varieties. The taxon rank *Vireya* was first used by Rafinesque (1814) in his publication *Specchio delle scienze o Giornale Enciclopedico di Sicilia*. However, the first vireya formally described was *R. malayanum*, based on material collected from Mt Bunko[26] by William Jack[27] in 1822. Carl Blume described 5 species under the new genus *Vireya* in his publication of 1826, *Bijdragen tot de flora van Nederlandsch Indië* (Blume 1825a; Blume 1825b, Blume 1825c). In 1845, Thomas Lobb introduced the first 5 live plants for cultivation in Britain. Subsequent description of new species in the 19th century included those by Charles Curtis, Odoardo Beccari and Sebastian Vidal (Argent 2006).

[25] Several new taxa have been described since the publication by Argent (2006).
[26] Now Mt Bengkoh (Sumatram Indonesia)
[27] A Scotsman from Aberdeen, who worked for the East India Company, and was renowned as a botanist and was the author of the publication *Malayan Miscellanies*.

Photo 5 *Rhododendron malayanum*, the first vireya described. The plant was found by Dr. William Jack in 1823, on the summit of the Mt Gunong Bunko, Bencoolen, Sumatra (Indonesia), at altitude ~1,000 m. [Drawing from Curtis's Botanical Magazine].

Tropical rhododendrons are at present among the most sought-after plants in SE Asia and Melanesia for their ornamental value. The horticultural popularity gained by the temperate rhododendrons however was not achieved by the vireyas until the latter part of the 20th century. Significant descriptions of new species and collections were made in the 20th century by Johannes Jacobus Smith (1932), Friedrich (Rudolf) Schlechter (1919), Herbert Copeland (Copeland 1929, 1932), Eric Holttum, Hermann Sleumer (Sleumer 1958, 1960, 1966a, 1973), John Womersley (Withers & Womersley 1986), Norman

Cruttwell (1984), George Argent (Argent 1982, 1988a, 1998, 2000, 2003, 2004, 2009; Argent & Barkman 2000; Argent & Chamberlain 1996; Argent & Dransfield 1989; Argent et al. 1984; Argent & Madulid 1995, 1998), etc. The majority of the vireyas that we know today were described by Sleumer (1960, 1966a). Only a handful of species and subspecies have been described in the 21st century (Brown & Craven 2003; Danet 2005, 2007, 2010; Takeuchi 2000).

2.2.1 Geographical Distribution of Vireyas

The vireyas comprises approximately 380 taxa (including several subspecies and varieties) that are predominantly distributed throughout Malesia, with a few outlying species found on mainland Asia and Australia (Brown et al. 2006c) (Figure 13). Brown et al. (2006c) suggested two possible origins for vireyas: (i) an old group, with ancestors present on Gondwana, rifting north in the Cretaceous, or (ii) a young group, which has dispersed eastwards from India to Australia and the Solomon Islands since the islands of Malesia were in, or close to, their present-day positions. At present, there is insufficient evidence to determine which of these is valid.

Almost all the *Rhododendron* species described in Malesia belong to Section *Vireya*, except for three species of Subgenus *Hymenanthes* (from West Malaysia and Sumatra), two species of Subgenus *Azaleastrum* (south to West Malaysia), one species of Subgenus *Tsutsusi* (from northern Philippines) and about six introduced species persistent in various forms in cultivation (Argent 1990).

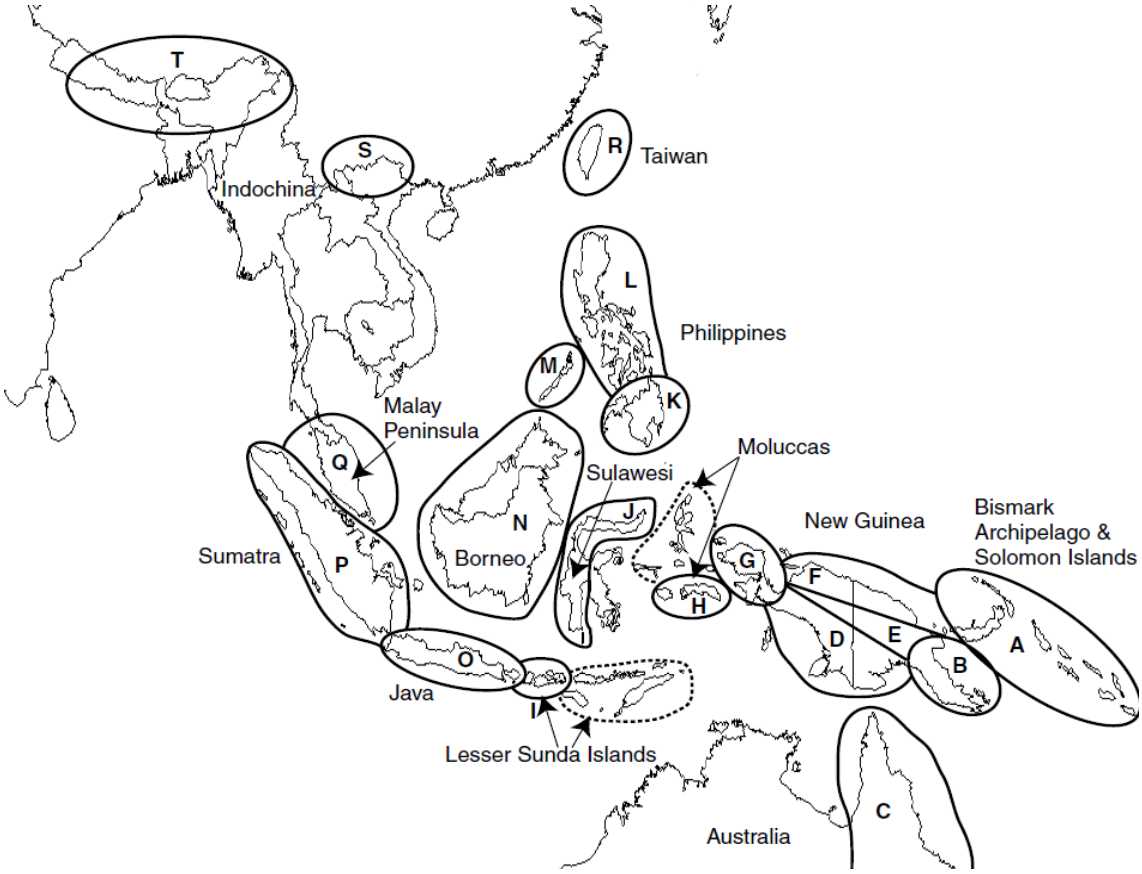

Figure 13 Distribution of Subgenus *Vireya* (sensu Argent). The areas marked are: Bismarck Archipelago and the Solomon Islands (A), Papuan Peninsular (B), northeastern Australia (C), New Guinea craton (D), central New Guinea (E), northern New Guinea (F), Vogelkop Peninsula (G), south Moluccas (H), Lesser Sunda Islands (I), north and west Sulawesi (J), southern Philippines (K), northern Philippines (L), Palawan (M), Borneo (N), Java and Bali (O), Sumatra (P), Malay Peninsula (Q), Taiwan (R), north Vietnam and south China (S) and the Himalayas (T) (Brown et al. 2006c).

The vireyas are found from sea level to alpine regions, with the majority in upper montane regions. Habitats vary from tropical very humid rainforests to subtropical alpine grasslands, and the vireyas have very effectively adapted to these habitats giving rise to various growth habits. Tropical lowland rainforests consists mainly of dipterocarp forests and heath forests, which are home to numerous vireyas, the majority growing as epiphytes. Tropical montane rainforests consists mostly of cloud forests with ample moisture giving rise to many epiphytes including vireyas and other epiphytes such as *Nepenthes* (the tropical pitcher plants). Tropical alpine forests are usually open or fragmented and the canopy is usually 6–10 m tall. This region consists mostly of dwarf forms of lower montane forest vireyas, and mat-forming vireya species (Argent et al. 2007; Brown et al. 2006c; Cortett 2009). Heads (2003) carried out a very comprehensive

study on the biogeography of the family ERICACEAE in general. The study also includes information on terrain tectonics and ecology giving an insight into the origins of the extant taxa of vireyas.

Vireyas are all woody, small trees to subshrubs, or trailing to mat-forming. Small trees and shrubs are usually restricted to lower altitudes while subshrubs and mat-forming species are found in high altitude alpine habitats. Vireyas are predominantly epiphytic and are found mostly growing on the trunk or large branches of rainforest trees. Several species are also lithophytic and found on rocky ground or on cliff sides (Argent 2006; Argent et al. 2007; Sleumer 1966a).

Sleumer (1966a) and Stevens (1982) have published very useful maps showing sections and subsections of Malesian ERICACEAE, but so far fewer than 20 species have been mapped (Croizat 1969; Takeuchi 2000; Van Welzen 1997).

2.2.2 Classical Systematics of Subgenus *Vireya* Argent

The numerous taxonomic issues associated with the vireyas are a consequence of the wide morphological variation (and diversity) seen within the group, leading to many authors to delimit different internal divisions based on characters such as leaf scales (Sleumer 1966a). The numerous synonyms of some of the taxa of vireyas can be attributed to homoplasy[28], where very similar characters have been observed in geographically isolated locations. The earliest classification of vireyas based on morphological characteristics was devised by George Don (1834):

[28] Describes characteristics of an organ that are shared by different species because of shared evolution.

Order[29] **ERICACEAE** G. Don
Subfamily **RHODOREAE** G. Don
Genus ***Rhododendron*** L.
Section ***Ponticum*** G. Don
Section ***Boòram*** G. Don
Section ***Pogonanthum*** G. Don
Section ***Lepípherum*** G. Don
Section ***Chamaecístus*** G. Don
Section ***Tsutsutsi*** G. Don
Section ***Pentanthera*** G. Don
Section ***Rhodora*** G. Don
Genus ***Vireya***[30] Blume

In this classification, the genus *Vireya* was characterized by the small, minutely-dentate calyx, sub-campanulate or funnelform corolla, ten stamens with oblong anthers dehiscing apically, style filiform, stigma capitate, capsule silique-formed, 5-angled and 5-celled and seeds expanded into bristles at each end. The genus *Vireya* differs from the genus *Rhododendron* in the calyx being small, the stamens both not being attached to the corolla in any way, leaves scattered and verticillate, quite entire, coriaceous, covered with scaly dots beneath, flowers disposed in terminal fascicles and the plants being epiphytic[31].

The earliest classification of vireyas based on morphological characteristics was devised by Schlechter in 1919 for New Guinean species. He used floral and foliar characteristics for this classification as shown below:

Genus ***Rhododendron*** L.
Group A
Section ***Schistanthe***[32] Schlechter
- ***R. hansemanni*** Warbg. (now *R. macgregoriae* F. Muell.)
- ***R. torricellense*** Schlechter (now *R. glabrifilum* J. J. Sm.)
- ***R. gorumense*** Schlechter (now *R. macgregoriae* F. Muell.)
- ***R. wentianum*** Koord.
- ***R. stolleanum*** Schlechter

Section ***Linnaeopsis*** Schlechter
- ***R. linnaeoides*** Schlechter (now *R. anagalliflorum* Wernham)

[29] Classified as an 'Order' as opposed to a 'Family', containing 5 tribes.
[30] Named by Blume after M Virey, a French physician.
[31] Cited as 'parasitic shrubs' in G. Don (1834).
[32] This is the earliest mention of the taxon *Schistanthe* in literature.

Group B

Section ***Zygomorphanthe*** Schlechter

- ***R. fuchsioides*** Schltr. (now *R. lindaueanum* var. *lindaueanum* Argent)
- ***R. yelliotii*** Warbg.
- ***R. saruwagedicun*** Förster (now *R. yelliotii* Warb.)
- ***R. podocarpoides*** Schltr. (not resolved, ? *R. purpureiflorum* J. J. Sm.)
- ***R. neriifolium*** Schlechter
- ***R. rarum*** Schlechter
- ***R. dielsianum*** Schlechter
- ***R. laureola*** Schlechter (now *R. dielsianum* Schlechter)
- ***R. warianum*** Schlechter (now *R. leptanthum* F. Muell.)
- ***R. melantherum*** Schlechter
- ***R. dasylepis*** Schlechter (now *R. beyerinckianum* Koord.)
- ***R. schultzei*** Schlechter (now *R. beyerinckianum* Koord.)
- ***R. commonae*** Förster
- ***R. keysseri*** Förster (now *R. culminicola* F. Muell.)
- ***R. christi*** Förster

Section ***Hapalanthe*** Schlechter

- ***R. zoelleri*** Warbg.
- ***R. baenitzianum*** Lauterb.
- ***R. laureola*** Schlechter (now *R. dielsianum* Schlechter)

Section ***Hadranthe*** Schlechter

- ***R. hellwigii*** Warbg.
- ***R. astrapiae*** Förster (now *R. konori* var. *konori* Schlechter)
- ***R. gardenia*** Schlechter
- ***R. schlechteri*** Lauterb.
- ***R. herzogii*** Warbg.

Photo 6 Taxa described in Group A of the classification by Schlechter (1919) In this group corollas are deeply-lobed to halfway. (a) *R. macgregoriae* F. Muell. (syn: *R. hansemanni*, *R. gorumense*) belonging to the Section *Schistanthe* Schlechter. (b) *R. anagalliflorum* Wernham (syn: *R. linnaeoides* Schlechter) belonging to the Section *Linnaeopsis* Schlechter [Photo: Chris Callard, www.vireya.net].

Photo 7 Taxa described in Group B of the classification by Schlechter (1919). In this group the corollas are shallowly-lobed to a third of the way or less. (a) *R. christi* Förster, belonging to the Section *Zygomorphanthe* Schlechter. (b) *R. gardenia* Schlechter, belonging to the Section *Hadranthe* Schlechter.

The sections *Schistanthe* and *Linnaeopsis* (Group A) were separated from the sections *Zygomorphanthe*, *Hapalanthe* and *Hadranthe* (Group B) based on how deeply-lobed the flower corollas are. The Group A has flowers with corollas 5-lobed to almost halfway (Photo 6), while those of the Group B are less deeply 5-lobed (only in the upper third or less) (Photo 7). The sections *Schistanthe* and *Linnaeopsis* were differentiated based on floral and foliar characters. Section *Schistanthe* is characterized by flowers borne in umbels, and with large thinly-leathery leaves, while the Section *Linnaeopsis* is characterized by flowers borne solitary, and with almost fleshy, thickly-leathery leaves, rarely 4 cm long. The Section *Zygomorphanthe* is differentiated from the sections *Hapalanthe* and *Hadranthe* by having strongly zygomorphic flowers, usually borne in few-flowered clusters. The sections *Hapalanthe* and *Hadranthe* flowers are not very clear zygomorphic. The Section *Hapalanthe* was differentiated by having thinly-leathery leaves and small delicate flowers, while the Section *Hadranthe* has stiff thickly-leathery leaves, and large robust, almost fleshy flowers.

The next significant classification of vireyas was carried out by Copeland (1929) for the rhododendrons of Philippines as shown below:

Genus ***Rhododendron*** L.
- Subgenus ***Eurhododendron*** Endlicher
  - Section ***Leiorhodion*** Rehder.
  - Section ***Lepipherum*** G. Don
    - ***R. quadrasianum***
    - ***R. apoanum***
    - ***R. nortoniae***
    - *R. catanduanense* (now a synonym of ***R. nortoniae***)
  - Section ***Vireya*** (Blume) H. F. Copeland
    - Subsection ***Malesia*** H. F. Copeland
      - ***R. bagobonum***
    - Subsection ***Linearanthera*** H. F. Copeland
      - ***R. vidalii***
      - ***R. whiteheadi***
      - ***R. taxifolium***
    - Subsection ***Solenovireya*** H. F. Copeland
      - *R. copelandi* (***R. jasminiflorum* ssp. *copelandii***)
    - Subsection ***Euvireya*** H. F. Copeland
      - ***R. mindanaense***
      - ***R. kochii***
      - ***R. williamsii***
      - ***R. brachygynum***
      - ***R. loheri***
      - ***R. leytense***
    - Subsection ***Leiovireya*** H. F. Copeland
      - *R. clementis* (***R. javanicum* ssp. *schadenbergii***)
      - ***R. xanthopetalum***
      - *R. spectabile* (***R. javanicum* ssp. *schadenbergii***)
      - *R. schadenbergii* (***R. javanicum* ssp. *schadenbergii***)
      - ***R. loboense***
- Subgenus ***Anthodendron*** Endlicher
  - Section ***Tsutsusi*** G. Don

The above classification became the basis for the subsequent classifications, and also saw the vireyas divided into subsections (some of which are still recognized today), a pattern that was followed by several subsequent authors. The first of such classifications was carried out by Sleumer (1949), as shown below:

Genus ***Rhododendron*** L.
- Subgenus ***Rhododendron*** Endlicher
  - Section ***Vireya*** (Blume) H. F. Copeland
    - Subsection ***Pseudovireya*** (C. B. Clarke) Sleumer
      - ***R. vaccinioides***
      - ***R. asperulum***

Subsection ***Solenovireya*** H. F. Copeland
- ***R. jasminiflorum***
- *R. copelandii* ***(R. jasminiflorum* ssp. *copelandii)***
- ***R. longiflorum***
- *R. chaemaepitys* ***(R. jasminiflorum* ssp. *chaemaepitys)***
- ***R. stapfianum***
- ***R. suaveolens***
- ***R. orbiculatum***
- *R. gracile* ***(R. javanicum* ssp. *gracile)***
- ***R. radians***
- ***R. rutenii***
- ***R. habbemae***[33]
- *R. filamentosum* ***(R. oreadum)***
- ***R. carstensense***
- *R. bodenii* ***(R. habbemae)***
- ***R. armitii***
- ***R. carringtoniae***
- *R. toverenae*[34]
- ***R. loranthiflorum***

Subsection ***Schizovireya*** Sleumer
- ***R. macgregoriae***
- ***R. wentianum***
- ***R. glabrifilum***

Subsection ***Discovireya*** Sleumer
- ***R. malayanum***
- ***R. retusum***
- ***R. pulleanum***
- ***R. saruwagedicum***
- *R. fuchsioides* ***(R. lindaueanum* var. *lindaueanum)***
- ***R. luraluense***

Subsection ***Astrovireya*** Sleumer
- ***R. commonae***

Subsection ***Phaeovireya*** Sleumer
- ***R. beyerinckianum***
- ***R. phaeochitum***

Subsection ***Linnaeopsis*** Sleumer
- ***R. anagalliflorum***
- ***R. bagobonum***

Subsection ***Linearanthera*** H. F. Copeland
- ***R. vidalii***

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[33] Original text quotes as '*R. Habbemai*'.

[34] *R. toverenae* was first described from the Horseshoe Mountains of New Guinea by Hunstein in 1884. Described as the species with the largest flowers of the genus *Rhododendron* (12 cm long and 17.5 cm across), it is now considered as a 'lost species'. The closest species fitting the description of *R. toverenae* is *R. schlechteri* from the same region (flowers 9–12 cm long and 15–17 cm across) or perhaps *R. konori* which boasts 12–16(–19) cm long and almost as wide flowers (Argent 2006; Coe 1960; Leach 1961; Sleumer 1966b).

R. whiteheadii
R. taxifolium
R. emarginatum
R. insculptum
R. kawakamii

Subsection ***Euvireya*** H. F. Copeland

R. javanicum
R. kochii
R. williamsii
R. mindanaense
R. brachygynum
R. leytense
R. loheri
R. teysmannii ***(R. javanicum* ssp. *teysmannii)***
R. multicolor
R. loerzingii
R. renschianum
R. lompohense
R. buruense
R. zoelleri
R. baenitzianum
R. moszkowskii ***(R. zoelleri)***
R. mollianum
R. lindaueanum
R. lochiae

Subsection ***Leiovireya*** H. F. Copeland

R. crassifolium
R. murudense ***(R. crassifolium var. pseudomurudense)***
R. javanicum* ssp. *schadenbergii
R. xanthopetalum
R. loboense
R. hellwigii
R. astrapiae ***(R. konori)***
R. englerianum
R. devresianum ***(R. superbum* ssp. *superbum)***
R. gardenia

In this comprehensive treatment, Sleumer outlined the complete hierarchy of the groupings of *Rhododendron*, and in particular of the vireyas, which formed the basis of the classifications that we are presently accustomed to. This classification was slightly revised in his subsequent publications (Sleumer 1958, 1961, 1963b, 1964), which were part of the precursors to his next major work on the flora of Malesia.

In 1966, Sleumer conducted a large survey of rhododendrons which formed part of the work carried out for the *Flora Malesiana* project and revised the classification of vireyas as shown below:

Genus ***Rhododendron*** L.
- Subgenus ***Rhododendron*** Endlicher
  - Section ***Vireya*** (Blume) H. F. Copeland
    - Subsection ***Pseudovireya*** (Clarke) Sleumer
    - Subsection ***Siphonovireya*** Sleumer
    - Subsection ***Phaeovireya*** Sleumer
    - Subsection ***Malayovireya*** Sleumer
    - Subsection ***Albovireya*** Sleumer
    - Subsection ***Solenovireya*** H. F. Copeland
    - Subsection ***Euvireya*** H. F. Copeland
      - Series **Linnaeoidea** Sleumer
      - Series **Saxifragoidea** Sleumer
      - Series **Taxifolia** Sleumer
      - Series **Stenophylla** Sleumer
      - Series **Citrina** Sleumer
      - Series **Buxifolia** Sleumer
      - Series **Javanica** Sleumer

The above classification was based on the type of scales on organs of vireyas (Figure 14). The Section *Vireya* (Blume) H. F. Copeland is differentiated from the other subgenera, and sections in Malesia were mainly based on: (i) the plants being lepidote, i.e. covered with scales (epidermal trichomes), at least on the abaxial surface of the young leaves, mostly also on the tips of the branchlets and in the floral region, and (ii) seeds distinctly appendaged by long tails or wings at both ends. The subsections *Pseudovireya* and *Siphonovireya* were differentiated from the other subsections on the basis of the shape of the scales, which are disc-shaped (Figure 14d), i.e. their marginal zone is ± entire (at most slightly-regularly or mostly irregularly-crenulate) and narrow in relation to the generally darker, often thick or swollen centre. The scales on the branchlets and/or the pedicels sometimes on top of thick persistent, epidermal, wart-like protuberances, never so on the leaf lamina. The Subsection *Pseudovireya* was differentiated from *Siphonovireya* on the basis of the corolla generally being shortly-tubular, rarely funnel-shaped or (tubular-)campanulate, and the lobes are usually erect or spreading. The subsection on the other hand has trumpet-like or salver-shaped corollas, with the tube elongate and ± narrow, straight or slightly curved, and the lobes equalling ¼ or less of the total length of the corolla, spreading ± horizontally.

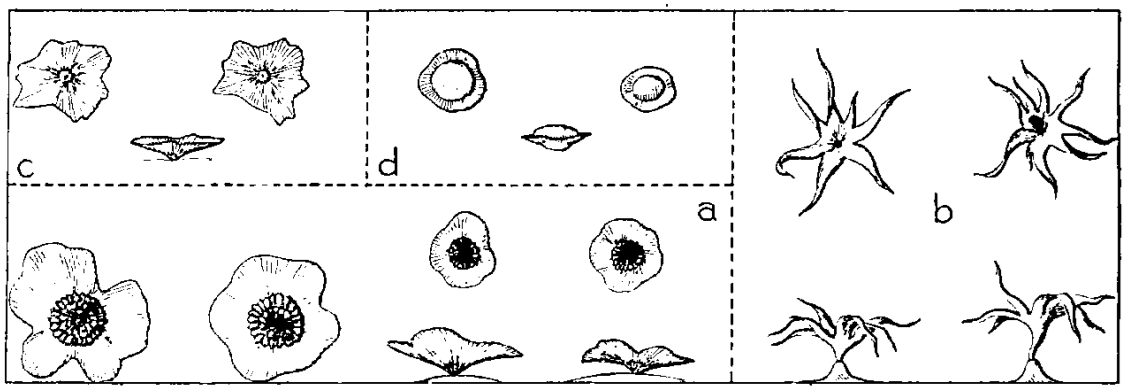

Figure 14 Main types of scales on the abaxial surface of leaves and used in the key to the subsections of *Rhododendron* Section *Vireya*. All figures at magnification ×70. **a.** Scale variously lobed, with dark chestnut coloured centre and of two different sizes, exclusively found in Subsection *Malayovireya* (*R. malayanum*). **b.** Scale deeply stellate-incised or -lacerate and stalked ('dendroid') to various degree (*R. konori*); on top of an epidermal tubercle exclusively found in Subsection *Phaeovireya*. **c.** Scale moderately substellately-angled or dented and sessile (*R. javanicum*). **d.** Scale entire or almost so and sessile, exclusively found in Subsection *Pseudovireya* (*R. quadrasianum* var. *cuneifolium*). Adapted from Sleumer (1966a).

The subsections *Phaeovireya*, *Malayovireya*, *Albovireya*, *Solenovireya* and *Euvireya* were differentiated from the subsections *Pseudovireya* and *Siphonovireya* on the basis of the scales being 'star-shaped' (Figure 14a–c). The marginal zone of these scales are distinctly-regularly or irregularly-lobed, dented, incised or lacerate to various degrees on the branchlets and the foliage, sometimes less deeply dented or even sub-entire and more disc-like on the corolla and/or the ovary, narrow to wide in relation to the mostly darker, generally but slightly or not thickened centre. The centre of the scales are either flat to slightly deepened (scale 'sessile'), or distinctly protracted downwards into a shorter or longer, slender foot, stem or column (scale 'dendroid', i.e. stalked).

The Subsection *Phaeovireya* differs from the subsections *Malayovireya*, *Albovireya*, *Solenovireya* and *Euvireya* by generally having markedly 'dendroid' scales, each borne on top of a distinct and permanent, blunt tubercle. The marginal zones of the scales are usually wide and deeply, often narrowly incised, and extreme forms are similar to stellate hairs (Figure 14b). The adaxial and/or abaxial surfaces of the leaves are rough to the touch after the scales are gone.

The subsections *Malayovireya*, *Albovireya*, *Solenovireya* and *Euvireya* possess usually 'sessile' scales, rarely 'subdendroid' (sometimes so in the Subsection *Solenovireya*, but

rarely in the Subsection *Euvireya*). Scales are usually borne on top of a low and rather inconspicuous, apparently not permanent epidermal tubercle, or mostly on no tubercles at all. The marginal zones of the scales are narrow to wide, and the centre often ± sunk into the epidermis of the leaves. Leaf surfaces are quite smooth at least at full maturity. The Subsection *Malayovireya* differs from the subsections *Albovireya*, *Solenovireya* and *Euvireya* by having the scales with rather large and dark centres. The marginal zones of the scales are relatively wide, ± deeply and broadly obtusely several-lobed, becoming silvery with age. The scales themselves are of two distinctly different sizes (numerous smaller and few much larger ones irregularly mixed, all very dense, touching or at least in part overlapping each other) (Figure 14a).

The subsections *Albovireya*, *Solenovireya* and *Euvireya* have scales with the centres mostly rather small and variously coloured (but never as dark as those of Subsection *Malayovireya*). The marginal zones of the scales are wider or narrower, mostly but moderately lobed or dented. The scales themselves are all equal in size, and very dense to well-spaced (Figure 14c). The scales of the Subsection *Albovireya* are very dense, touching or slightly overlapping each other, generally rather large, but forming a persistent and coherent layer on the abaxial surface of mature leaves. After the scales are eventually shed, prominent pits remain and are denser than those found in the Subsection *Euvireya*.

The subsections *Solenovireya* and *Euvireya* usually possesses lax to sub-dense scales, always distinctly spaced on the abaxial surface of mature leaves, generally small and often early caducous or the marginal zone early dissolute. The Subsection *Solenovireya* is differentiated from the Subsection *Euvireya* by having trumpet-like or salver-shaped corollas, and the corolla tube straight or somewhat curved, elongate and narrow. The corolla lobes are relatively short, equalling ½ of the total length of the corolla and spreading ± horizontally.

The Subsection *Euvireya* is the most species-rich and the most widespread, and can be distinguished from the Subsection *Solenovireya* by having flowers with either tubular, campanulate or funnel-shaped corollas. The corolla tube is generally wider and shorter than that in Subsection *Solenovireya*, erect or funnel-shaped, rarely horizontally expanded. The corolla lobes are ¼ or more of the total length of the corolla. The scales

are ‘star-shaped’ to various degrees, mostly small, placed distant from each other on the juvenile leaves, the thin marginal zone ± irregularly dented or lobed, often early dissolute. The remnant centres of the scales give the impression of a disc-shaped scale.

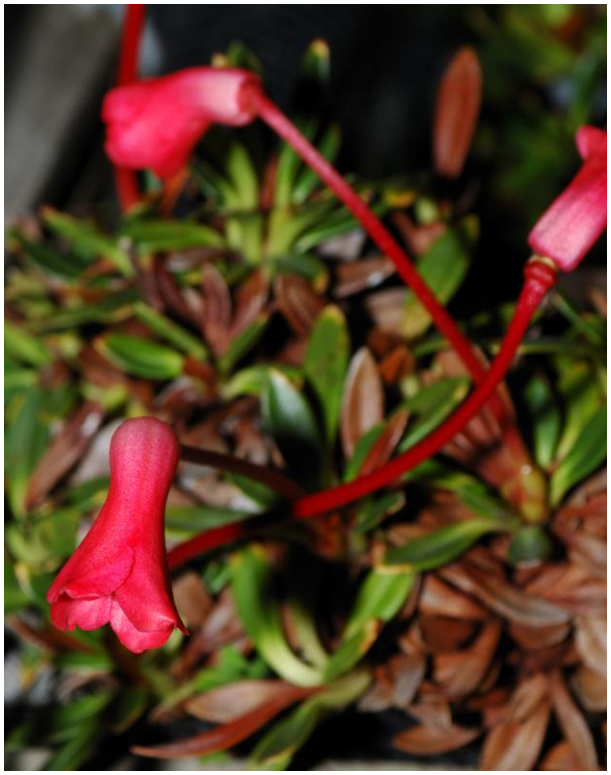

Photo 8 *Rhododendron saxifragoides* (EK541). The plant was growing at the Pukeiti Gardens (Taranaki, New Zealand).

The Subsection *Euvireya* is further divided into the seven series, Linnaeoidea, Saxifragoidea, Taxifolia, Stenophylla, Citrina, Buxifolia and Javanica. The series Linnaeoidea is differentiated from the other series by having very small leaves, usually 0.3–1(–2) × 0.2–0.6(–0.8) cm, while the other series have leaves usually >1.6 cm long. The monotypic series Saxifragoidea with the species *R. saxifragoides* (Photo 8) is distinguished by being a compact dwarf shrub, forming tussocks or mats, and the leaves are linear-lanceolate to oblanceolate. The nodding flowers are solitary (rarely paired) and the pedicels are stout, erect, distinctly emerging above the foliage. The series Taxifolia, Stenophylla, Citrina, Buxifolia and Javanica are erect shrubs or treelets.

The series Taxifolia and Stenophylla can be distinguished from the series Citrina, Buxifolia and Javanica by having linear to narrowly-lanceolate leaves. Leaves are usually

<7 mm wide. The series Taxifolia has leaves borne in pseudowhorls of 20 or more leaves, 1–1.5 mm wide, while the leaves of the series Stenophylla are borne opposite or on in pseudowhorls of 3–8(–15) leaves, 3–7(–11) mm wide. The series Citrina, Buxifolia and Javanica generally have much wider leaves, not linear or narrowly-lanceolate. The series Citrina has 5 stamens, while the series Buxifolia and Javanica has (7–)10(–14) stamens. The series Buxifolia can be distinguished from the series Javanica by having medium-sized leaves, i.e. 1–4(–6.5) cm long. The series Javanica contains the bulk of the taxa of the Subsection *Vireya*, and can be distinguished from the series Buxifolia by having usually large leaves, i.e. >4 cm long. This classification of Sleumer (1966a) was part of the account of *Rhododendron* in Malesia and does not include the Asian vireyas (predominantly belonging to the Subsection *Pseudovireya*) and Australian species (belonging to the Subsection *Euvireya*).

The most recent morphological classification of vireyas was carried out by Argent (2006), based mostly on the work by Sleumer (Sleumer 1949, 1966a) and Copeland (1929). An important feature of this classification is reintroduction of the rank of subgenus to *Vireya*, 'for practical purposes'. The subgenus rank for vireyas was first proposed by Clarke (1882) followed by Copeland (1943) and later by Spethmann (1980, 1987). The classification adopted by Argent (2006) is shown below:

Genus ***Rhododendron*** L.
- Subgenus ***Vireya*** (Blume) Clarke
  - Section ***Pseudovireya*** (Clarke) Sleumer
  - Section ***Discovireya*** (Sleumer) Argent
  - Section ***Siphonovireya*** (Sleumer) Argent
  - Section ***Phaeovireya*** (Sleumer) Argent
  - Section ***Malayovireya*** (Sleumer) Argent
  - Section ***Albovireya*** Sleumer
  - Section ***Euvireya*** (H. F. Copeland) Argent
    - Subsection ***Linnaeopsis*** (Schltr.) Sleumer
    - Subsection ***Saxifragoidea*** (Sleumer) Argent
    - Subsection ***Solenovireya*** H. F. Copeland
    - Subsection ***Malesia*** H. F. Copeland
    - Subsection ***Euvireya*** H. F. Copeland

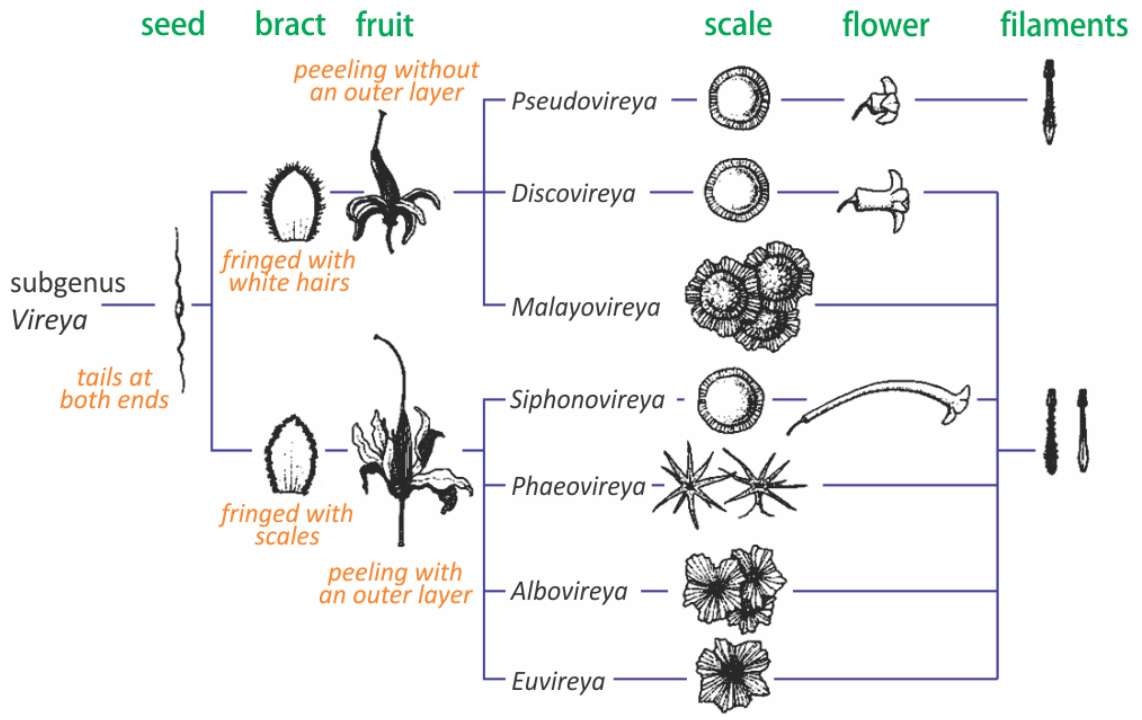

Figure 15 Diagrammatic representation of selected morphological characters used in the artificial key differentiating the sections of Subgenus *Vireya sensu* Argent (2006). Adapted from Argent (2006).

The above classification utilized similar morphological characters to those used by Sleumer (1966a), and shown in Figure 15. The Subgenus *Vireya* is differentiated from the other subgenera of *Rhododendron* by the presence of tails at both ends of the seeds. The only exception to this rule being *R. retusum* from Malesia, which has these tails reduced to minute tufts, somewhat similar to those found in some species of Subgenus *Hymenanthes* (e.g. *R. wrayi*). *R. kawakamii* from Taiwan also has reduced tails, but not to the extent seen in *R. retusum*. The sections *Pseudovireya*, *Discovireya* and *Malayovireya* are differentiated from the rest of the Subgenus *Vireya* by having bracts fringed with white hairs and fruits peeling without an outer layer. The sections *Siphonovireya*, *Phaeovireya*, *Albovireya* and *Euvireya* have bracts fringed with scales and fruits peeling with an outer layer. The sections *Pseudovireya* and *Discovireya* share the same type of scales, in which the scales have large dome-shaped centres. The Section *Pseudovireya* can be differentiated from the Section *Discovireya*, by having a significantly shorter corolla tube and stamen filaments hairy in the middle. Apart from Section *Pseudovireya*, all the other sections possess stamen filaments that are either completely hairy or completely glabrous. The Section *Malayovireya* has unique scales

variable in size, lobed, and with a swollen centre which in the largest scales are dark-coloured, the scales themselves usually at least touching, mostly forming a coherent layer over the epidermis of the leaf.

The Section *Siphonovireya* shares the same scale type characteristics as the sections *Pseudovireya* and *Discovireya*, in which the scales are mostly disc-shaped with a swollen centre, the scales themselves well-spaced such that the lower epidermis of the leaf is clearly visible between them. Additionally, flowers of Section *Siphonovireya* are trumpet-shaped, with the corolla lobes ¼ the length of the corolla tube. The Section *Phaeovireya* has unique dendroid scales, each borne from the apex of an epidermal tubercle on the lower epidermis of the leaf. The Section *Albovireya* can be identified by the deeply-lobed scales, which are mostly pale-coloured, with the scales usually at least touching and mostly forming a coherent layer over the lower epidermis of the leaf. The Section *Euvireya* has thin scales with small centres, moderately to deeply stellately-lobed, occasionally sub-dendroid or dendroid, the scales themselves well-spaced with the lower epidermis of the leaf clearly visible between them.

Argent's (2006) classification differs in having six of Sleumer's subsections being promoted to section rank, and Section *Vireya* promoted to subgenus rank. The taxon *Discovireya* was initially proposed by Sleumer (1949), which he then incorporated into a subsection in the *Flora Malesiana* treatment (Sleumer 1966a). Argent (2006) reinstated the taxon *Discovireya*, but as a section, containing some of the taxa contained in Sleumer's (1949) Subsection *Pseudovireya*. The remainder of taxa within Sleumer's (1949) Subsection *Pseudovireya* is amalgamated into Argent's (2006) Section *Pseudovireya*. This Section *Pseudovireya* now also includes the mainland Asian and Taiwanese species, that were excluded in Sleumer's (1966a) treatment.

The subsections *Siphonovireya*, *Phaeovireya*, *Malayovireya*, *Albovireya* and *Euvireya* of Sleumer (1966a) were transferred to the sections *Siphonovireya*, *Phaeovireya*, *Malayovireya*, *Albovireya* and *Euvireya* of Argent (2006) respectively. The Subsection *Solenovireya* of Sleumer (1966a) remains as a subsection, but under the Section *Euvireya* of Argent (2006). Argent's (2006) Subsection *Malesia* is formed from Sleumer's series Buxifolia (partial), Taxifolia, Stenophylla and Citrina. Argent's (2006) Subsection

Euvireya incorporates Sleumer's (1966a) series Javanica, Stenophylla and the majority of the taxa in Buxifolia.

Table 1 Comparison of the classifications of vireyas by Argent (2006) vs Sleumer (1966a).

Argent (2006)	**Sleumer (1966a)**
Subgenus ***Vireya***	Section ***Vireya***
Section ***Pseudovireya***	Subsection ***Pseudovireya*** *pro parte*
Section ***Discovireya***	Subsection ***Pseudovireya*** *pro parte*
Section ***Siphonovireya***	Subsection ***Siphonovireya***
Section ***Phaeovireya***	Subsection ***Phaeovireya***
Section ***Malayovireya***	Subsection ***Malayovireya***
Section ***Albovireya***	Subsection ***Albovireya***
Section ***Euvireya***	Subsection ***Euvireya***
Subsection ***Linnaeopsis***	Subsection ***Euvireya*** Series **Linnaeoidea**
Subsection ***Saxifragoidea***	Subsection ***Euvireya*** Series **Saxifragoidea**
Subsection ***Solenovireya***	Subsection ***Solenovireya***
Subsection ***Malesia***	Subsection ***Euvireya*** Series **Buxifolia** *pro parte* Subsection ***Euvireya*** Series **Taxifolia** Subsection ***Euvireya*** Series **Stenophylla** Subsection ***Euvireya*** Series **Citrina**
Subsection ***Euvireya***	Subsection ***Euvireya*** Series **Javanica** Subsection ***Euvireya*** Series **Stenophylla** Subsection ***Euvireya*** Series **Buxifolia** *pro parte*

Table 1 shows a comparison of the classifications systems followed by Argent (2006) versus that of Sleumer (1966a).

2.2.3 Other Studies on Subgenus *Vireya* Argent

Morphological characters have been widely used in the classification of the vireyas, especially prior to the development of molecular methods. Reviewed here are studies of vireyas based on morphological characters that are not discussed in the previous sections.

Karyological studies have been used for the classification of numerous flowering plants (Chiarini & Barboza 2008; Hynniewta et al. 2011; Tanaka et al. 2009), as they are an important tool in determining the ploidy level. The first known karyological studies of vireyas were carried out by Jones & Brighton (1972). Their study of 33 accessions of vireyas showed that they were all diploid ($2n = 26$), except for an unknown specimen of vireya from New Guinea which had a somatic number of $2n = 30$. The second study was carried out by Atkinson et al. (2000) and comprised 27 species (selected from a wide

geographical and altitudinal range) and one inter-subsectional hybrid. This study showed that all the specimens showed a uniform somatic number of $2n = 26$.

There is considerable morphological diversity within the Section *Vireya*, and seven subsections have been described based on floral and scale characters (Sleumer 1966a). Current taxonomic studies have indicated that floral characters are highly plastic and subject to considerable selection pressures because of their role in pollination biology (Argent 1988c; Stevens 1976). The study carried out by Stevens (1976) revealed that most species of *Rhododendron* Section *Vireya* from Papuasia can be assigned characteristic flower types. The study also revealed that the distribution of flower types within supraspecific taxa suggests that some rearrangement of the latter may be necessary, and some flower types were demonstrated to be polyphyletic. Other characters such as floral bracts and fruits have been examined, and these have indicated that the current infra-sectional classification is in need of revision (Argent 1988c).

Williams & Rouse (1990) carried out a study on the pollen grain size and pistil length in 93 species of *Rhododendron* belonging to a number of different subgeneric taxa. Their study showed that pollen volume is directly related to pistil length, where small pollen grains from a species with short pistils is placed on a very much longer foreign pistil, the pollen tubes may grow to 1.5–2 times the length of their own pistil but will not reach the foreign ovary if it lies beyond this distance. The results from 26 species belonging to the Section *Vireya* showed that where extreme disparity of pollen/pistil size causes failure of interspecific crosses, one or more bridging species with intermediate pollen/pistil size can generally be selected.

2.2.4 Molecular Systematics of Subgenus *Vireya* Argent

Although molecular studies focussing exclusively on vireyas started during the last decade, it is interesting to note that the first genetic study on vireyas was carried out by Henslow (1891) using non-molecular methods, and thus gave an insight into the complex relationships among vireyas. One interesting outcome of this study was the observation that the hybrid produced resembled either parent in either way and in various degrees, contrary to the popular belief at that time, that a hybrid resembles the male parent in the flowers, and the female parent in foliage. Moreover, Henslow also observed that

characters in the grandparents or higher ancestry were shown to reappear in the hybrid, having been more or less absent in the parents.

The majority of the molecular studies on *Rhododendron* in the past included a few representatives of vireyas: Kron & Judd (1990); Scheiber et al. (2000); Kurashige et al. (2001); Tsai et al. (2003); Milne (2004); Goetsch et al. (2005); Chung et al. (2007); Craven et al. (2008). These studies were aimed at resolving the phylogeny of the genus *Rhododendron* in general, and have been discussed in detail in the Section 2.1.3. The limited number of species used in these studies restricted the results to general comments on the relationship between vireya and other sections. There were insufficient taxa to draw any firm conclusions about relationships within vireya.

In contrast, the analyses carried out by Brown et al. (2006a, 2006b; 2006c) are the most comprehensive molecular studies on vireyas to date and marked a milestone in vireya research. These studies gave a new insight into the relationships within the vireyas, and relationships between the vireyas and the rest of the genus *Rhododendron*. The first of these studies by Brown et al. (2006a) was a phylogenetic analysis using the sequence data for the ITS nrDNA region, using 39 accessions of vireyas (representing 32 taxa). This study showed that Subgenus *Rhododendron* Endlicher is monophyletic (Figure 16).

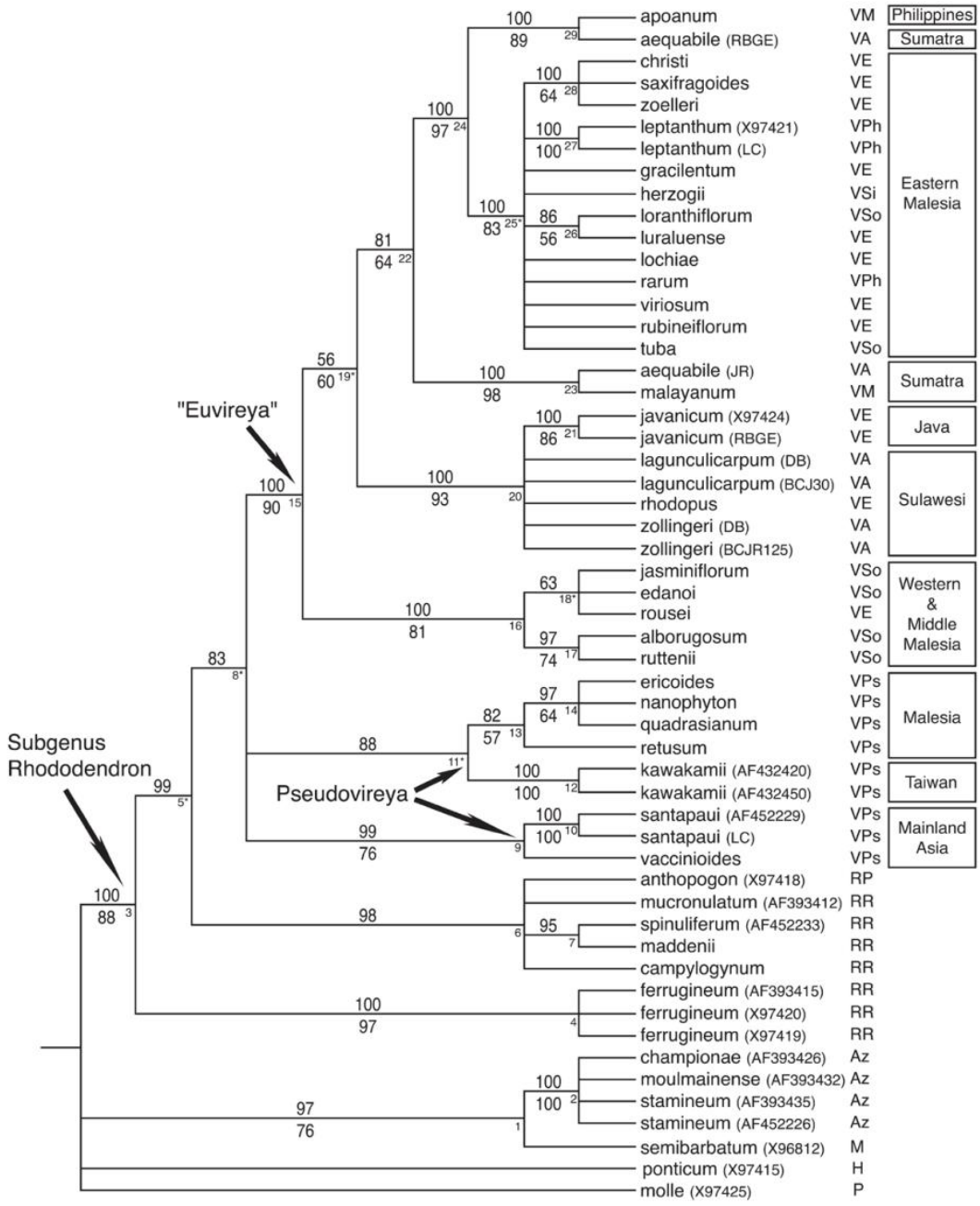

Figure 16 ITS nrDNA region 50% majority rule tree of vireyas from Bayesian analysis. Bayesian analysis iteration number 5; log-likelihood range −2470 to −2490. Nodes that are not resolved by the parsimony analysis (tree length 135, CI=0.72, RI=0.93) are marked with *. Bayesian posterior probability values are shown above the node and bootstrap values are shown below the nodes they support. All species of Section *Vireya* are indicated by a V and an abbreviation of the subsection: *Albovireya*, VA; *Euvireya*, VE; *Malayovireya*, VM; *Phaeovireya*, VPh; *Pseudovireya*, VPs; *Siphonovireya*, VSi; *Solenovireya*, VSo. The subgenus, or section of Subgenus *Rhododendron* taxa, and outgroup taxa are also indicated: Subgenus *Rhododendron*, Section *Rhododendron*, RR; Subgenus *Rhododendron*, Section *Pogonanthum*, RP; Subgenus *Azaleastrum*, Az; Subgenus *Mumeazalea*, M; Subgenus *Hymenanthes*, H; Subgenus *Pentanthera*, P. Parentheses are used to identify the different sequence accession (see Table 1) or the GenBank numbers of sequences sourced from the GenBank database. General area distributions are also shown. Adapted from Brown et al. (2006a).

Brown et al. (2006a) also showed that *Pseudovireya* was paraphyletic, and formed two clusters (nodes 9 and 11 in Figure 16), a small cluster corresponding to mainland Asian species (node 9), and a larger cluster (node 11) with two subclusters corresponding to Taiwanese (node 12) and Malesian (node 13) species respectively. *Pseudovireya* was also shown to be sister to the rest of the vireyas, labelled as '*Euvireya*' (node 15). This *Euvireya* clade consists of a mixture of all the subgroups within *Vireya* excluding *Pseudovireya*, and these subgroups were shown to be paraphyletic. Strikingly, the subclades formed within the *Euvireya* clade correspond to specific geographic regions (Figure 16). This research thus showed that the relationships among the species of Section *Vireya* do not correspond to the traditional classification based on morphology, instead correlate strongly with geographic areas, with a disjunction between an Australian–New Guinea clade and clades of west and middle Malesian taxa.

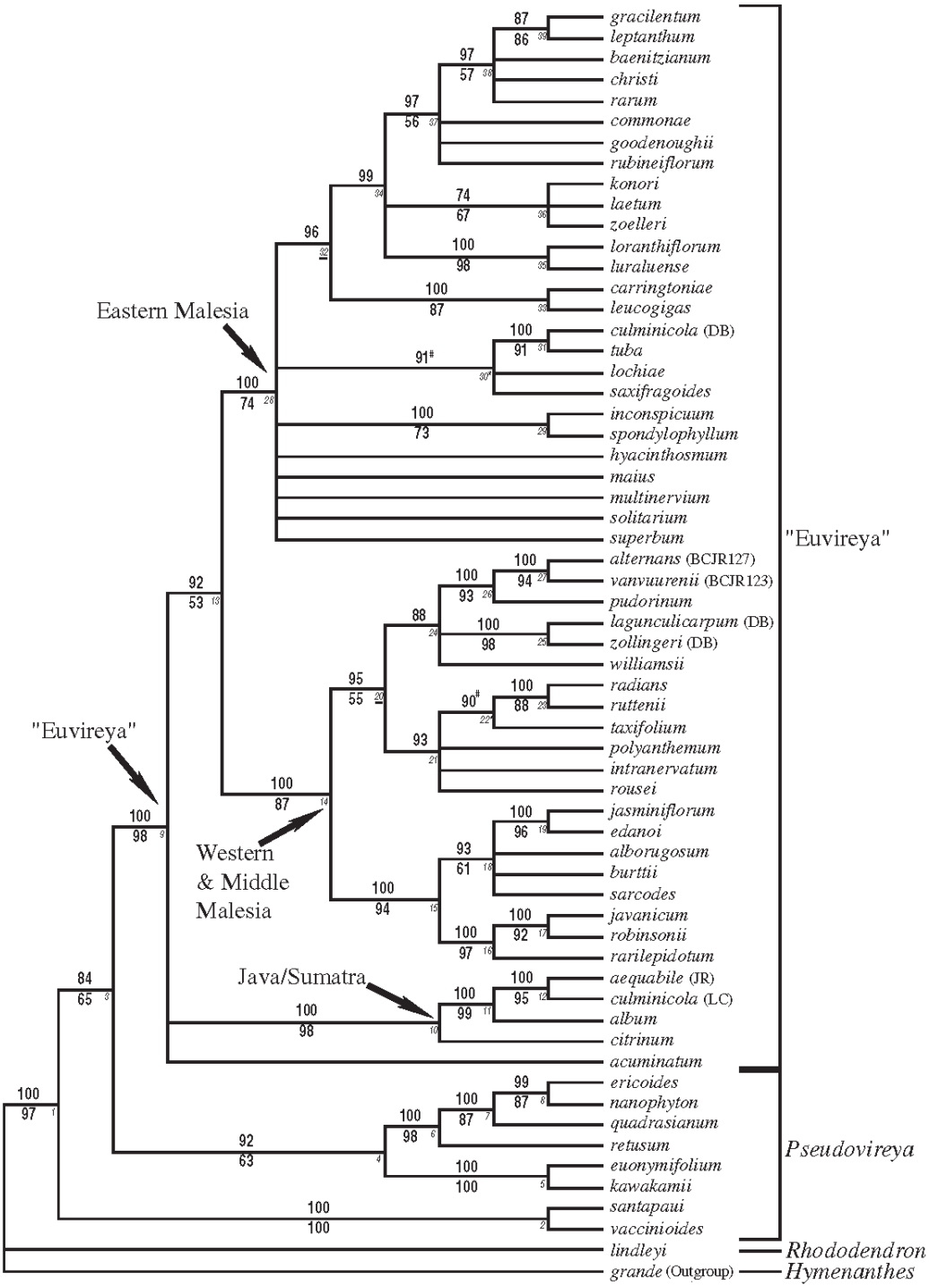

Figure 17 Combined cpDNA strict consensus tree for 59 vireya taxa. Strict consensus of 144 trees of length 191 from Parsimony analysis, using the *psb*A-*trn*H and *trn*T-*trn*L data sets combined, and including indel characters; CI = 0.62 and RI = 0.85. Nodes not resolved by the four long Bayesian analyses (log likelihood range –3970 to –4010) are indicated *. Nodes not resolved in the individual analyses, but resolved in all combined analyses, are underlined. Bayesian posterior probability values are shown above the node and bootstrap values are shown below the nodes that they support. Posterior probability values marked # were found in the short Bayesian analysis (log likelihood range -3690 to -3740) but not the long Bayesian analyses (log likelihood range –3970 to –4010). The three main clades of '*Euvireya'* are indicated by arrows. *Hymenanthes* = Subgenus *Hymenanthes*; *Rhododendron* = section *Rhododendron*. Adapted from Brown et al. (2006b).

The second study by Brown et al. (2006b) further examined the phylogeny of vireyas using two non-coding regions of cpDNA (*psb*A-*trn*H and *trn*T-*trn*L). The phylogenetic analyses of these two cpDNA regions, representing 75 vireya taxa, showed that the Section *Vireya* (Blume) H. F. Copeland was monophyletic (node 1 in Figure 17). Similar to the study of Brown et al. (2006a), *Pseudovireya* was shown to be paraphyletic, and formed two clades corresponding to mainland Asian species (node 2), and Malesian and Taiwanese species (node 4). The clade containing taxa belonging to the subsections *Euvireya*, *Siphonovireya* and *Malayovireya* were shown to be monophyletic, while the individual subsections were shown to be paraphyletic as in the ITS nrDNA study of Brown et al. (2006a). The groups supported by the cpDNA analyses strongly relate to geographic regions rather than taxonomic groupings, the most obvious of these being the general split between the regions eastern Malesia (node 28), and western and middle Malesia (node 14).

Brown et al. (2006c) is a cladistic biogeographic study of vireya rhododendrons, combining the results of Brown et al. (2006a) and Brown et al. (2006b). The molecular phylogenetic analysis of the vireya rhododendrons showed that a major clade divergence correlates with a distinct biogeographic pattern: one major clade restricted to the east of Wallace's Line[35] and another to the west. Based on geographic pattern, it was argued that the vireyas are an old Gondwanan group, the alternative hypothesis being that the group is young, assuming that low molecular distances between taxa within clades reflects a young age, which in turn requires long-distance dispersal to explain distribution patterns. It may be that deep divergences within the vireyas have an old history, but that diversification within clades is more recent (Brown et al. 2006c).

[35] An imaginary line drawn in 1859 separating the ecozones of Asia and Wallacea (a group of Indonesian islands separated by deep water straits from the Asian and Australian continent shelves), a transitional zone between Asia and Australia (Whitmore 1981, 1982).

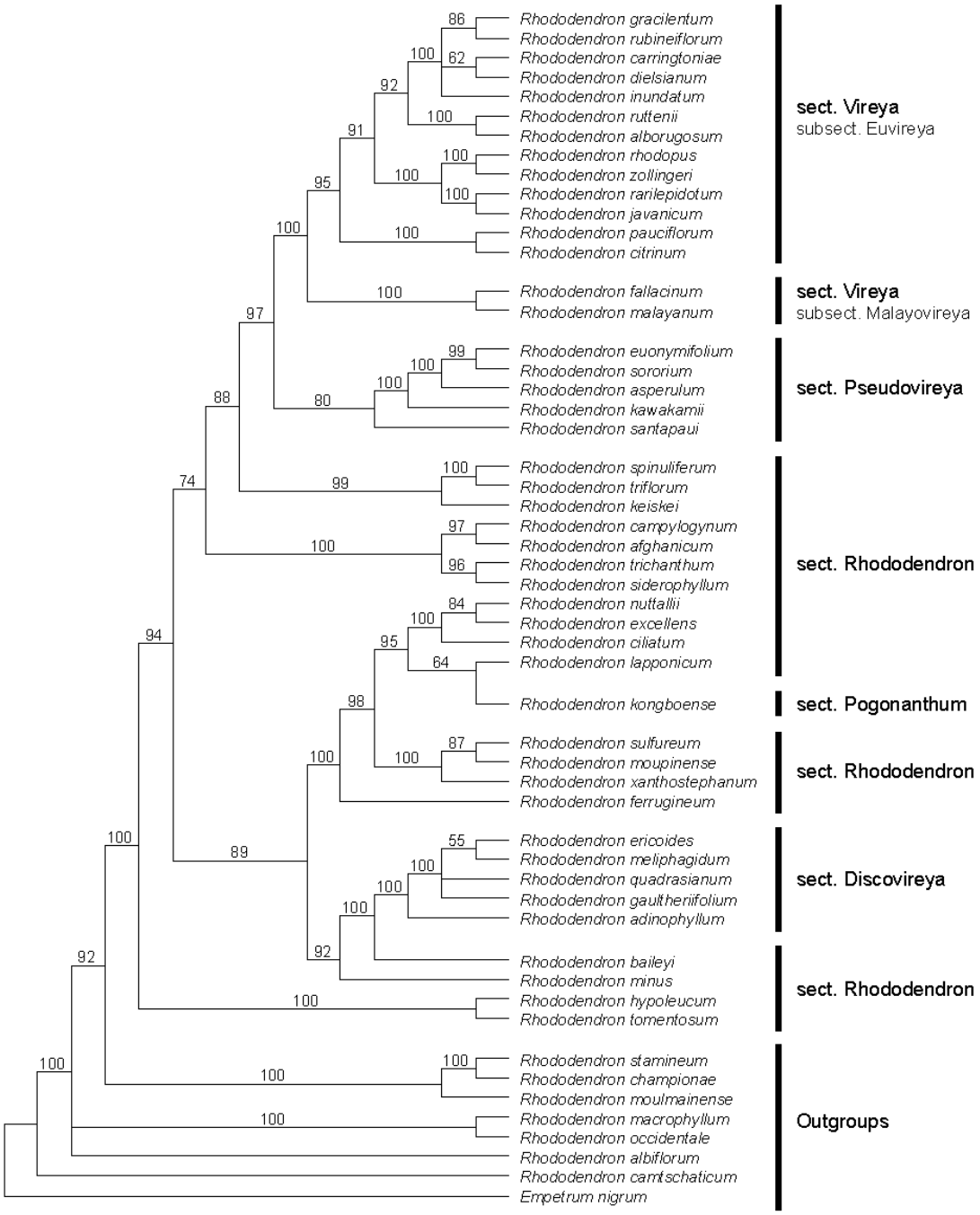

Figure 18 Maximum parsimony strict consensus tree for *Rhododendron* Subgenus *Rhododendron* based upon *rpb*2i sequences. All bootstrap values > 50% are shown. Adapted from Craven et al. (2008).

The study by Craven et al. (2008) included the results of the studies by (Brown et al. 2006a, 2006b; Goetsch et al. 2005; Hall et al. 2006), and marked another milestone in the classification of the genus *Rhododendron*. Based on molecular data, this study did not agree completely with the morphological classifications of Sleumer (1966a), Chamberlain et al. (1996) and Argent (2006). The relative positions of three vireya groups

were made clear in this study, agreeing with Argent's views that a practical method is required for dealing with the large number of vireya species (Argent 2006). This was achieved by treating the relevant subsections *sensu* Sleumer (1966a) as informal groups, thus facilitating identification but not compromising the principle that formal classification should be based on evolutionary relationships (Craven et al. 2008). Molecular data supported the taxonomic groups *Malayovireya* and *Euvireya sensu* Sleumer (1966a), however did not support the other subsections and series of Sleumer (Figure 18). The core vireya[36] complex was shown to be comprised of a large group of actively evolving (including radiating and interbreeding) species, of which the speciose and morphologically ultradiverse New Guinea clade was notable (Craven et al. 2008).

Craven et al. (2008) clearly showed that the Subgenus *Vireya* (*sensu* Argent) is polyphyletic and embedded within Subgenus *Rhododendron* (*sensu* Craven), and therefore cannot be a sister taxon to it. Figure 18 illustrates the maximum parsimony strict consensus phylogenetic tree for *Rhododendron* Subgenus *Rhododendron* based upon *rpb*2 sequences. A major difference seen is the paraphyly of *Vireya* (*sensu* Argent and Sleumer). Section *Pseudovireya* is sister to the Section *Vireya*. Section *Discovireya* is not sister to the clade formed by the sections *Vireya* and *Pseudovireya*, but more closely related to the temperate sections *Rhododendron* and *Pogonanthum*. The group *Euvireya* remains intact in all these classification systems (Brown et al. 2006a, 2006b; Craven et al. 2011). Distinguishing between the *Pseudovireya* and *Siphonovireya* can be difficult, but *Malayovireya*, *Albovireya* and *Phaeovireya* all seem coherent and largely monophyletic (Stevens 1985). The long, narrowly tubular corolla of *Solenovireya* and *Siphonovireya* is distinctive, but it is unclear whether these two sections are monophyletic (Heads 2003), and have not been shown to be so in any of the molecular studies discussed above.

[36] Taxa of Subgenus *Vireya sensu* Argent (2006) excluding those belonging to the sections *Pseudovireya* and *Discovireya*.

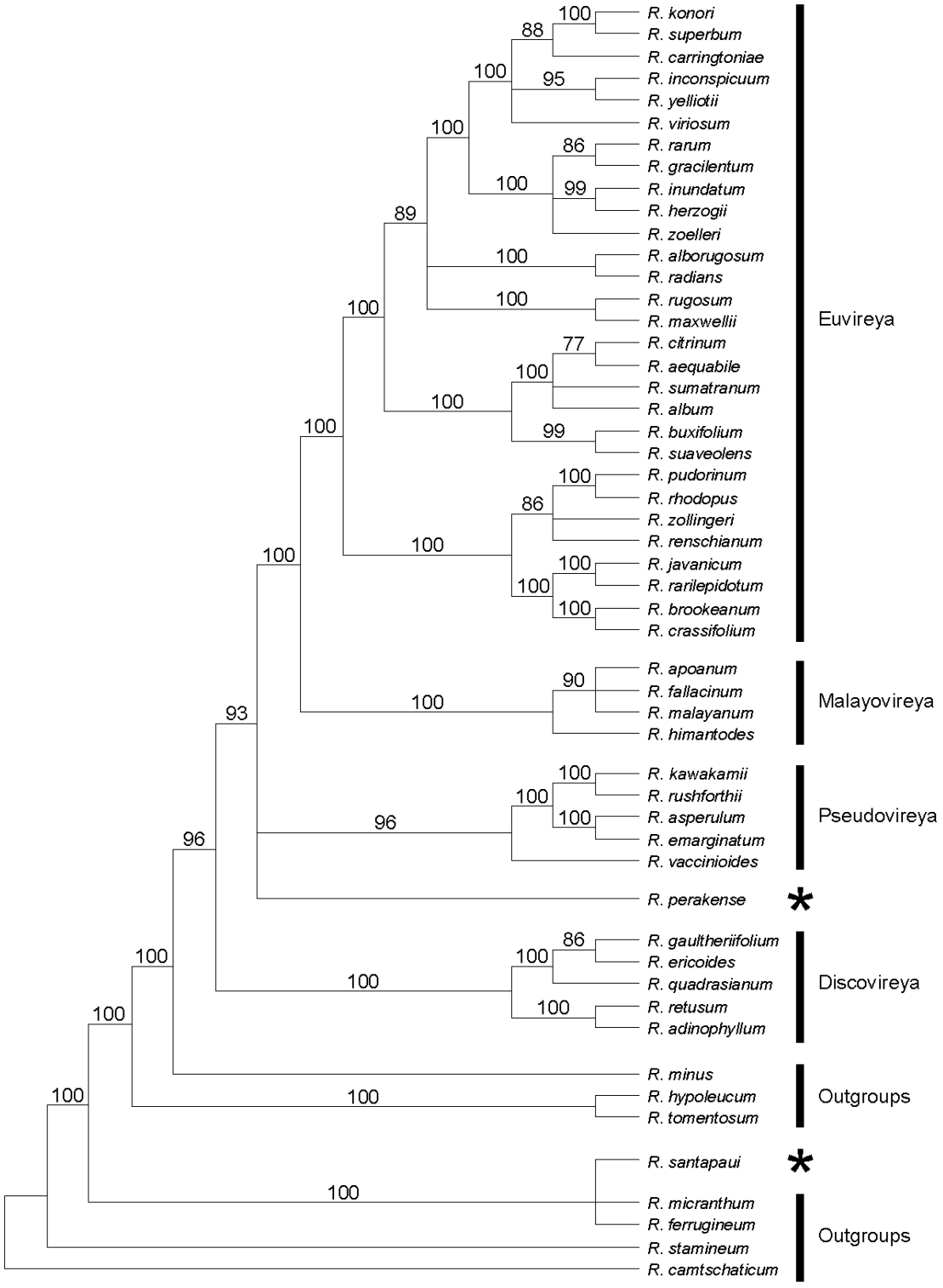

Figure 19 Inferred phylogeny of *Rhododendron* Section *Schistanthe* based upon *rpb*2i, *rpb*2d and *rpc*1 sequence data. The numbers indicate bootstrap support. Adapted from Craven et al. (2011).

Craven et al. (2011) further investigated the evolutionary relationships of the *Vireya* group of *Rhododendron*, utilising nuclear DNA sequence data, and demonstrated that this group of species is monophyletic, and a revised classification was presented (Figure 19). As the name *Vireya* was predated at sectional level by several other valid names, the correct name for the section is now *Schistanthe* (Craven et al. 2010). Within *Schistanthe*, four subsections are recognised: *Pseudovireya*, *Discovireya*, *Malayovireya* and *Euvireya*. The study proposed a revised classification of the vireyas (with identification keys):

Genus ***Rhododendron*** L.
- Subgenus ***Rhododendron***
  - Section ***Rhododendron***
  - Section ***Pogonanthum*** G. Don
  - Section ***Schistanthe*** Schlechter
    - Subsection ***Discovireya*** Sleumer
    - Subsection ***Pseudovireya*** (C. B. Clarke) Sleumer
    - Subsection ***Malayovireya*** Sleumer
    - Subsection ***Euvireya*** H. F. Copeland

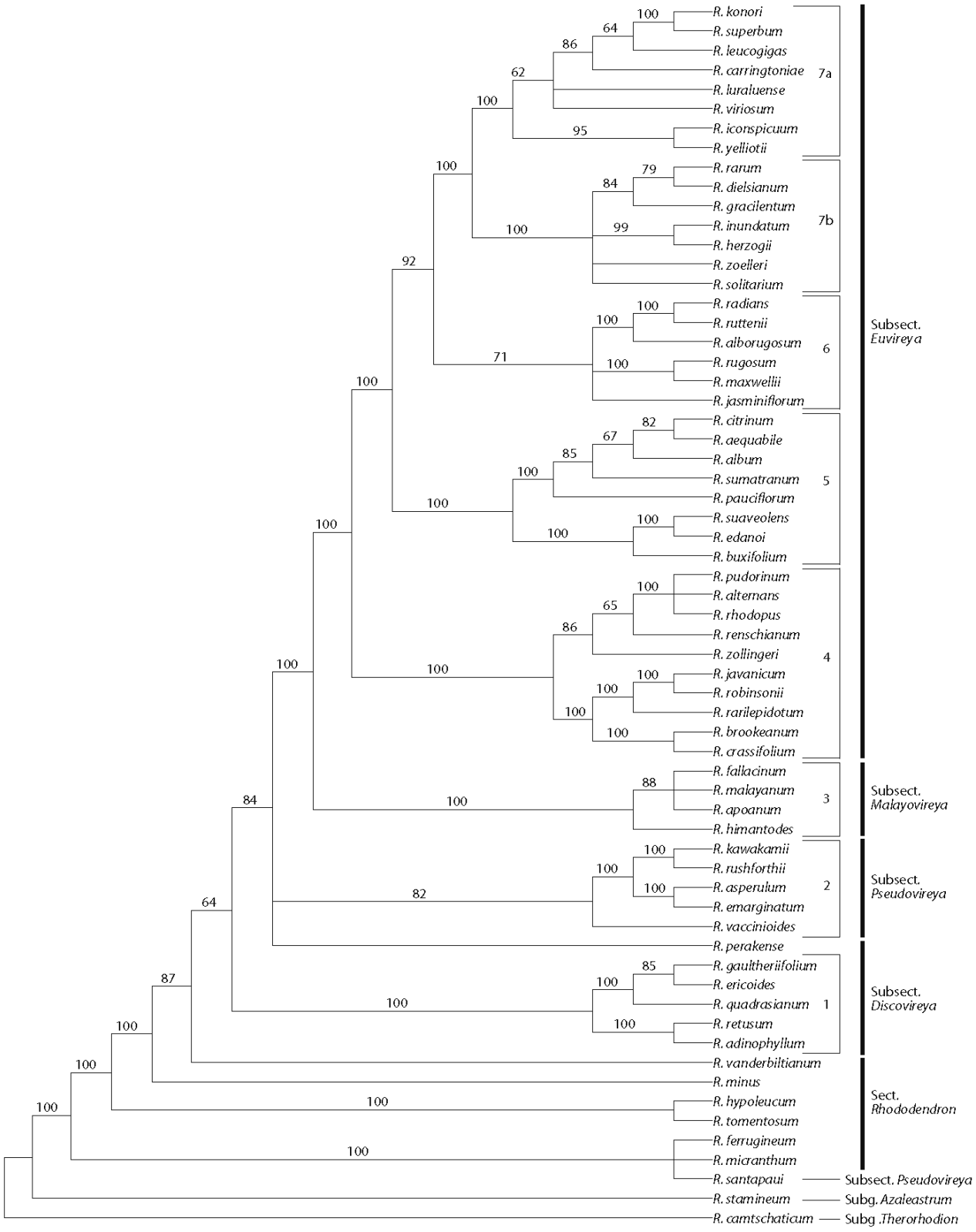

Figure 20 Maximum parsimony strict consensus tree based upon the combined data for *rpb*2i, *rpc*1, and *rpb*2d. Adapted from Goetsch et al. (2011).

Goetsch et al. (2011) is the most comprehensive molecular study of the *Vireya* group published to date, consisting of a phylogeny derived from analysis of sequences from multiple nuclear genes, *rpb*2i, *rpb*2d and *rpc*1. An analysis based on the combined sequences of these three nuclear genes supported a phylogeny in which the reinstated rank Section *Schistanthe* (excluding *R. santapaui*) is monophyletic, with well-defined clades corresponding to the subsections *Euvireya*, *Malayovireya*, *Pseudovireya*, and *Discovireya* (Figure 20). Within the Subsection *Euvireya*, the subclades follow geography more closely than traditional taxonomic groupings based on morphology. One of the two most derived clades contained exclusively species from New Guinea, Australia, and the Solomon Islands. The results are consistent with a stepwise phylogeographic history of Section *Schistanthe*, originating in Asia, spreading eastward to New Guinea within the last 15 mya, when movement of the Australian tectonic plate brought New Guinea into the Malesian domain.

A study conducted on the *Rhododendron* of Taiwan by Tsai et al. (2012) showed that *R. kawakamii* (belonging to the Subsection *Pseudovireya*) is separated from the other *Rhododendron* species in the study. The results were in agreement with the habitat preference of *Rhododendron* in Taiwan, in which *R. kawakamii* is epiphytic (a trait shared by the majority of the vireyas) and thus ecologically separated from the other *Rhododendron* species, which are either shrubs or trees. Molecular data showed that this species is distinct from the other *Rhododendron* species in Taiwan.

2.2.5 Vireyas in New Zealand Collections

New Zealand has acquired a large number of *Rhododendron* accessions over the last century, many of these from world renowned plant collectors. The vireya accessions were introduced during the latter half of the last century, and the majority of these accessions can still be seen at the Pukeiti Gardens (Taranaki). The majority of the accessions held at Pukeiti Gardens are of wild origin, many of which have been acquired within the last 15 years. Additionally, there are several significant private collections of vireyas around the country, which remain largely undocumented at present (Adams 1996; Allen 1971; Ballard 2006; Binney 2003; Black 1965, 1969; Postan 1996; Smith 2003). Pukeiti Gardens holds approximately 400 accessions of vireyas with several taxa containing multiple accessions (Smith 2009).

The Pukeiti Gardens and the Victoria Esplanade Gardens represent the largest collections of vireyas available to the public domain in New Zealand. However, there are several large gardens around the country that could in future play a leading role in the *ex situ* conservation of vireyas in New Zealand. These include the Pukekura Park (New Plymouth), Auckland Domain, Eden Garden, Auckland Botanic Gardens, Wellington Botanic Garden and Dunedin Botanic Garden. There are several smaller private collections around New Zealand such as the Mark Jury collection[37], Koromiko Nurseries[38], etc.

There have been numerous plant collectors who have contributed to the New Zealand collections of vireyas. The main collectors include Michael Black (Black 1965, 1969, 1970), David Binney (2003), Graham Smith (2003) and Oswald Blumhart (Ballard 2006). The majority of their collected accessions can still be found at the Pukeiti collection.

2.2.5.1 The Pukeiti Gardens (Taranaki)

The Pukeiti Gardens was established in 1951 by the Pukeiti Rhododendron Trust. Since 2010, the garden has changed ownership and is now managed by the Taranaki Regional Council. The garden is internationally recognised for its large collection of plants, but mainly for its huge collection of rhododendrons. Currently there are over 2,000 accessions of *Rhododendron* grown around the garden, with the temperate species growing in the open spaces, and the tropical rhododendrons (vireyas) sheltered from heavy rain and cold within display houses and covered walks laid out in a naturalistic style. The strength of this collection is held by the wild of the majority of the accessions. The vireya collection is represented by over 400 accessions representing approximately 160 taxa, with several taxa possessing multiple accessions (Smith 2009).

Many of the original acquisitions of vireya accessions still survive within the collection at Pukeiti Gardens. The garden staff regularly propagate the existing stock in a nursery and multiple accessions of several taxa are routinely raised (both for replacement of perished accessions and sale for the public). A complete inventory of the accessions held at the garden is yet to be drawn up. The recent acquisition of the garden by the Taranaki

[37] http://www.jury.co.nz
[38] http://www.thoughtware.co.nz/pages/Koromiko.htm

Regional Council, and specifically under the Taranaki Region Gardens, Pukeiti Gardens have acquired the BG Base Database for cataloguing the plants cultivated at the gardens (Smith 2009).

2.2.5.2 Victoria Esplanade Gardens (Palmerston North)

Victoria Esplanade Gardens is an inner city park located alongside the Manawatu River established to mark Queen Victoria's 60th Jubilee. The gardens are managed and operated by the Palmerston North City Council. The main features of the gardens include a Rhododendrons & Camellia Garden, Dugald McKenzie Rose Garden, bird aviaries, nature trails and a conservatory housing a large number of tropical and sub-tropical plants (consisting of numerous orchids and rhododendrons). In contrast to the Pukeiti Gardens, the Victoria Esplanade Gardens in Palmerston North is a significantly smaller garden and the conservatory which holds the vireyas is shared with numerous tropical and sub-tropical plant species.

There are over 1,000 accessions of rhododendrons (species and hybrids) within the gardens, with the temperate taxa found all around the garden while the tropical taxa restricted mainly to the conservatory. The conservatory houses 55 accessions of vireyas representing 22 taxa, several of these are thought to be of wild origin, many of which have been acquired from Pukeiti Gardens. The vireya accessions in the collection are well-established and are mature specimens (PNCC 2012).

2.2.5.3 Auckland Domain

Auckland Domain is Auckland's oldest park and its 75 hectares area has been developed around the cone of an extinct volcano. The garden consists of formal gardens, duck ponds, large green open spaces edged by mature trees and bush walks. The 'tuff rings' created by the volcanic activity thousands of years ago are still apparent in the land contours and forms a natural amphitheatre. Auckland having the largest population in New Zealand draws in a large number of visitors every year to the garden (Auckland Council 2012; Auckland Botanic Gardens 2012).

2.2.5.4 Eden Garden (Auckland)

Eden Garden is a privately owned botanic garden and the brainchild of Jack Clark who, with fifteen fellow enthusiasts, converted the wilderness and rubbish in an old quarry into a garden. The garden is 2.2 hectares (5.5 acres) and is located in central Auckland (Eden Garden 2012)

The garden boasts the largest and most varied collection of camellias in New Zealand, and a large number of vireyas. Other plants include a large collection of temperate rhododendrons, bromeliads and a large collection of New Zealand native plants. The large number of vireyas and temperate rhododendrons in this garden makes it an ideal site for *ex situ* conservation of these (Eden Garden 2012).

2.2.6 Vireyas in World-wide Collections

Following the discovery of the vireyas the number of *Rhododendron* species brought into cultivation and the creation of large collections of these species increased. The largest among these are the collections at the Royal Botanic Gardens Edinburgh (RBGE – Edinburgh, UK), the Royal Botanical Gardens at Kew (London, UK) and at Pukeiti Gardens (Taranaki, New Zealand). There are smaller, but significant collections in Europe, Australia and the USA. The largest collection of vireyas on the mainland USA is that at the Rhododendron Species Botanical Garden. There are also smaller collections in Hawai'i[39], where vireyas are widely cultivated for ornamental trade. Tropical Australia,

[39] http://www.whitecloudnursery.com

with weather more or less equalling that of the vireyas' native Malesia, also has significant collections[40].

Significant collections of rhododendrons and vireyas are held at the following collections:

1. Royal Botanic Garden Edinburgh, UK (RBGE 2011).
2. Rhododendron Species Botanical Garden, USA.
3. Royal Botanic Garden Kew, Richmond, Surrey, UK.
4. National Rhododendron Garden, Australia.
5. The Georgian Road, Olinda, Victoria, Australia.
6. Australian Rhododendron Society Garden, Mt Pleasant, Wollongong, NSW, Australia.
7. National Botanic Gardens, Glasnevin, Dublin, Ireland.
8. Botanika, Rhododendronpark GmbH, Deliusweg, Bremen, Germany
9. Hawai'i Tropical Botanical Garden, Papaiko, Hawai'i, USA
10. Lyon Arboretum, Honolulu, Hawai'i, USA
11. Eden Project, Cornwall, England
12. Royal Botanic Gardens Sydney, Sydney, NSW, Australia
13. San Francisco Botanical Garden, California, USA

2.2.7 Taxonomic & Conservation Issues of Vireyas

Similar to many plant groups, vireyas has several taxonomic issues as discussed in previous chapters (Argent 2006), making any conservation strategy difficult to plan. A large number of taxa are thought to be closely related to each other, and often confused with other similar taxa, while several taxa also known to hybridize with other taxa in the wild, often producing intermediaries. To investigate these issues, phylogenetic analyses using molecular data can be utilized to help elucidate the intricate relationships among these taxa. The Malesian region, home of the vireyas, is a biodiversity hotspot and a priority for biodiversity conservation worldwide. The major taxonomic issue faced in vireyas are those related to identity. Numerous taxa have been collected over the years in

[40] http://www.ncbg.org.pg, http://www.penangbotanicgardens.gov.my, http://www.sabahparks.org

the field under different names (Argent 2006). These errors gave rise to the numerous mistaken identities in the *ex situ* collections.

Taxonomy and conservation go hand-in-hand. We cannot expect to conserve organisms that we cannot identify, and our attempts to understand the consequences of environmental change and degradation are compromised fatally if we cannot recognize and describe the interacting components of natural ecosystems. Several recent reviews have emphasized the fundamental role that taxonomy plays in conservation, and significant high-level science policy reports have additionally drawn attention to the funding and credibility gap faced by taxonomic and systematic science (Mace 2004).

Taxonomic complexities which affect the delimitation of species cause problems for conservation. Making an assessment of the distribution and conservation status of any species first requires that the species can be recognised and delimited. If the unit to be conserved is in a taxonomically complex group, there can be major problems in assessing threats, devising conservation strategies and monitoring their success (Hollingsworth et al. 2006). Old and incomplete taxonomy constitutes a significant impediment for the conservation and sustainable use of plant taxa (Dransfield 2001).

The vireyas are taxonomically complex as detailed in the previous chapter, and setting conservation priorities is a challenging task. To determine the biodiversity and subsequent conservation activities, it is important to determine the taxonomic issues such as the correct identification (as numerous species are known to be confused with others) and examination of the inter-relationships among taxa. As the Malesian region is a diversity hotspot, species boundaries often overlap. This is evident especially in the numerous natural hybrids of vireyas (Argent 2006; Cruttwell 1984; Danet 2005; Sleumer 1966a). Further taxonomic errors can be attributed to the fact that, several taxa hybridizing with other taxa within its taxonomic section and also between other sections (Argent 2006).

The following subsections provide a summary of the taxonomic and conservation queries raised based on the issues detailed in Appendix 02. The classification system tentatively adopted here largely follows that proposed by Argent (2006) for easier management. The queries have been subdivided into their respective taxonomic groups according to the classification of Argent (2006). Figure 21 illustrates the map of Malesia showing the

region codes for the range of vireyas that are used in the queries tabulated in the following subsections.

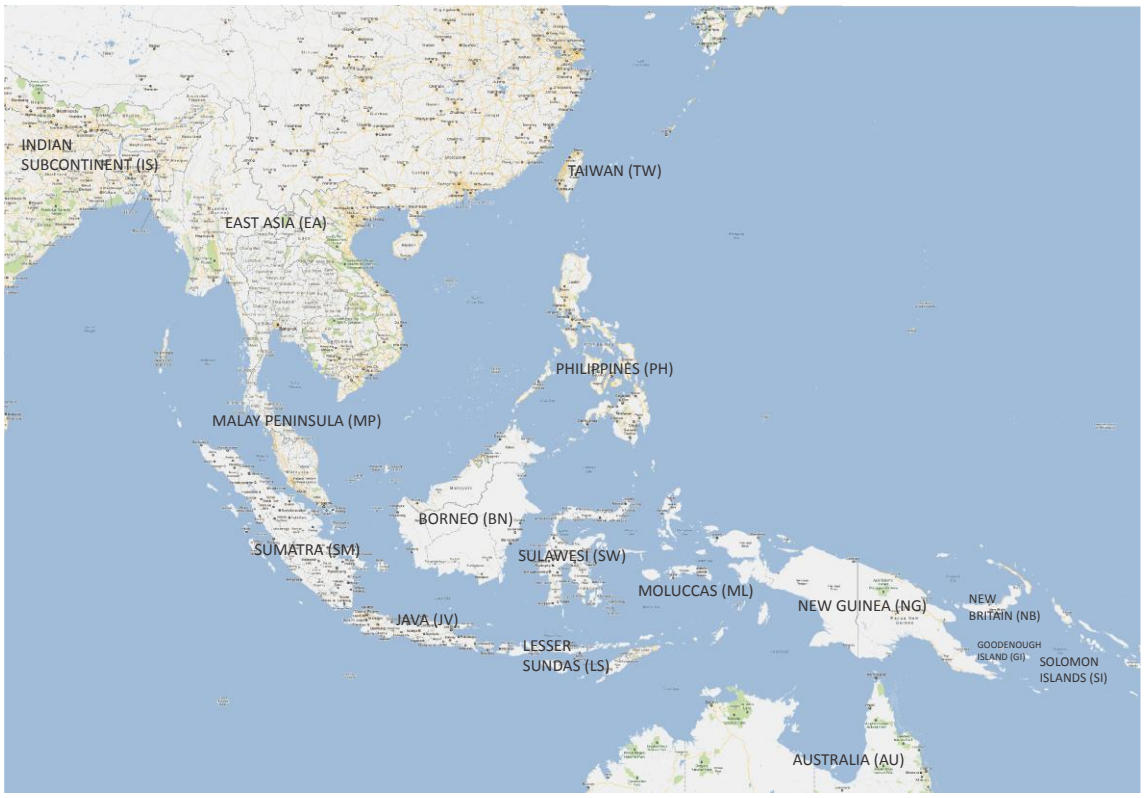

Figure 21 Map of Malesia showing region codes referred to in the subsequent sections. (Map: 2012 © Google Maps).

2.2.7.1 Section *Pseudovireya* (Clarke) Sleumer

Pseudovireya was first described by Clarke (1882) as a subgenus, which was later demoted to a section by Sleumer (1949). This section can be recognized by characters such as the fruits not peeling an outer layer before splitting, bracts fringed with simple white hairs, and scales mostly disc-shaped with a large swollen centre and a narrow flange (Argent 2006). *Pseudovireya* is distributed from India to Indo-China and Taiwan with 11 taxa (10 species and 1 subspecies), outside the Malesian region. *R. kawakamii* is found in Taiwan and the rest are all found on the continental Asia. Morphologically, the taxa of *Pseudovireya* show relatively minor variations. The corollas for example are generally campanulate, and white (mostly with varying shades of pink) or yellow (Photo 25a–b) (Argent 2006).

Recent molecular studies suggested that the clade of Asian mainland species of *Pseudovireya* are sister to a strongly-supported clade of the other six subsections of Section *Vireya sensu* Craven et al. (2008). The species *R. vaccinioides* and *R. santapaui* formed a clade with strong bootstrap support, and is sister to the rest of the taxa of *Pseudovireya* (*sensu* Brown et al. 2006c) (*R. meliphagidum, R. nanophyton, R. ericoides, R. quadrasianum, R. retusum, R. kawakamii* and *R. emarginatum* [Syn: *R. euonymifolium*]). According to the Argent's (2006) classification *R. meliphagidum, R. nanophyton, R. ericoides, R. retusum* and *R. quadrasianum* belong to the Section *Discovireya*, in contrast to the placement of these taxa in the Section *Pseudovireya sensu* Sleumer (1966a).

Hall et al. (2006) investigated the taxonomy of *Pseudovireya* and its relation to the rooting of Section *Vireya* within Subgenus *Rhododendron*. This study however utilized very few representative taxa of *Pseudovireya*, and the finer relationships between the taxa were not fully established. This section thus remains relatively poorly-studied, and has numerous taxonomic issues, which may affect the conservation of Red-Listed taxa of this group. The questions raised for the Section *Pseudovireya* are summarized in Appendix A2.1. The taxa are arranged according to the classification of Argent (2006), and the taxon numbers on the leftmost column correspond to the taxon number used in Argent's (2006) classification.

From Appendix A2.1, it is apparent that the majority of the taxa of Section *Pseudovireya* are related to each other. To understand and resolve the taxonomic issues within this group, accessions of each taxon must be analysed. However, in New Zealand, the only cultivated taxa are *R. vaccinioides*, *R. santapaui*, *R. emarginatum*, *R. kawakamii* and *R. rushforthii*, and these can therefore be used to partially resolve the taxonomic issues. *R. santapaui* and *R rushforthii* are of conservation interest as they are categorized as Data Deficient (DD) by IUCN, and are related to *R. kawakamii* and *R. emarginatum*. *R. santapaui* is also related to *R. vaccinioides*, as evident from molecular studies (Brown et al. 2006b). Accessions of these five taxa also need to be tested for genetic diversity within the taxa.

Additional to the questions raised in Appendix A2.1, there are a few general questions related to Section *Pseudovireya*. These include: (i) How closely related are the taxa within

this section? (ii) What are the interspecific relationships between the taxa of *Pseudovireya*? (iii) Do these taxa form separate groups or clusters within the phylogeny? (iv) If the taxa of this group cluster together, do they correspond to physical or geographic attributes? (v) What morphological traits separate these taxa from the rest of the vireyas?

2.2.7.2 Section *Discovireya* (Sleumer) Argent

This section was first described by Sleumer (Sleumer 1960) but has been given new status in Argent (2006). *Discovireya* is recognized by the fruits not peeling an outer layer before splitting, bracts fringed with simple white hairs and scales mostly disc-shaped with a large swollen centre and narrow flange, well-spaced with the lower epidermis of the leaf clearly visible between them (Argent 2006). The *Discovireya* species are widely distributed throughout the Malesian region and north to Peninsular Malaysia and eastward to the Philippines (represented by a single species). According to the classification by Craven et al. (2008) Section *Discovireya* is sister to sections *Rhododendron*, *Pogonanthum*, *Pseudovireya* and *Vireya*. There are 24 taxa in *Discovireya*, 13 of which have been Red-Listed. The taxon *R. ericoides* (VU D1) is of conservation interest, and cultivated in New Zealand. Photo 26a–f shows a selection of taxa from the Section *Discovireya*.

The vast geographic range of Section *Discovireya* across Malesia has given rise to several taxonomic issues. In the majority of the taxa, the geographic range of *Discovireya* often overlaps, and also with taxa of other sections, leading to taxonomic issues with non-*Discovireya* taxa. Appendix A2.2 summarizes the questions arisen from these taxonomic issues.

The majority of the questions raised in Section *Discovireya* are taxonomic issues related with confusion between taxa. Some species show huge morphological variation within the species, leading to the allocation of this variability to taxonomic varieties. The largest such variation is seen in the species *R. quadrasianum*, with six varieties described.

2.2.7.3 Section *Siphonovireya* (Sleumer) Argent

The Section *Siphonovireya* was first described by Sleumer (1960) and a new status is given by Argent (2006). This section can be recognized by characters such as fruits

irregularly peeling an outer layer before splitting, bracts fringed with scales and scales mostly disc-shaped with a large swollen centre and narrow flange, well-spaced with the lower epidermis of the leaf clearly visible between them (Argent 2006). According to a recent molecular study, *Siphonovireya* is absorbed into the large Subsection *Euvireya* (Craven et al. 2008). There are 11 taxa in this section, 5 of these are of conservation interest. The taxonomic and conservation issues related to the Section *Siphonovireya* are summarized in Appendix A2.3.

2.2.7.4 Section *Phaeovireya* (Sleumer) Argent

The Section *Phaeovireya* was first described by Sleumer (1949) and now given new status by Argent (2006). This section can be recognized by characters such as fruits irregularly peeling an outer layer before splitting, bracts fringed with scales and dendroid scales, each from the top of an epidermal tubercle on the lower epidermis of the leaf (Argent 2006). Molecular studies show that the Section *Phaeovireya* is polyphyletic, but are in the bulk of 'Euvireya' clade, specifically only in the clade 'Eastern Malesia' (Brown et al. 2006b). There are 50 taxa in *Phaeovireya*, and 25 of these are of conservation interest. The taxonomic and conservation issues of Section *Phaeovireya* are summarized in Appendix A2.4.

2.2.7.5 Section *Malayovireya* (Sleumer) Argent

The Section *Malayovireya* was first described by Sleumer (1958) with a new status given by Argent (2006). *Malayovireya* can be recognized by characters such as fruits not peeling an outer layer before splitting, bracts fringed with simple white hairs and scales very variable in size, lobed, with a swollen centre which in the largest scales is dark coloured. Scales usually at least touching, mostly forming a coherent layer over the epidermis of the leaf (Argent 2006). This section is now reduced to Subsection *Malayovireya* under the Section *Vireya* according to the molecular classification by Craven et al. (2008). This section contains 23 taxa in Section *Malayovireya*, and eight of these are of conservation interest. The taxonomic and conservation issues of Section *Malayovireya* are summarized in Appendix A2.5.

2.2.7.6 Section *Albovireya* Sleumer

The Section *Albovireya* was first recognized by Sleumer (1960) and recognized by characters such as fruits irregularly peeling an outer layer before splitting, bracts fringed with scales and scales deeply lobed, mostly pale in colour (Argent 2006). This section is now given the new status of Subsection *Euvireya* under Section *Vireya* according to the molecular classification of Craven et al. (2008). There are 15 taxa in this section, and eight of these are of conservation interest. The taxonomic and conservation issues of Section *Albovireya* are summarized in Appendix A2.6.

2.2.7.7 Section *Euvireya* (H F Copeland) Argent

The Section *Euvireya* was first described by Copeland (1929) as a subsection, and the new status of a section is given by Argent (2006). This section can be recognized by characters such as fruits peeling an irregular outer layer at maturity, bracts fringed with scales and scales thin with small centres, moderately to deeply stellately-lobed occasionally subdendroid or dendroid. Scales are well spaced with lower epidermis of the leaf clearly visible between them. This section is further divided into 5 subsections with very minor morphological differences. According to the molecular classification of Craven et al. (2008) this section is reduced to the Subsection *Euvireya* under Section *Vireya*, but retaining all of the species of the previous morphological classification. Furthermore, the 5 subsections *sensu* Argent (2006) are not recognized in the new molecular classification. There are a total of 238 taxa in Section *Euvireya*, and 102 of these are of conservation interest. The taxonomic and conservation issues of the Section *Euvireya* are summarized in Appendix A2.7.

2.2.7.7.1 Subsection *Linnaeopsis* (Schltr.) Sleumer

The Subsection *Linnaeopsis* was first described by Schlechter in 1917 as a section, but demoted to a subsection by Argent (2006). This subsection can be recognized by the small leaves, with the majority of the well-developed leaves less than 1 cm long, stomata only on the abaxial surface of the leaves. The plants are usually small creeping or erect shrubs (Argent 2006). There are 16 taxa in Subsection *Linnaeopsis*, and eight of these are of conservation interest. The taxonomic and conservation issues of the Subsection *Linnaeopsis* are summarized in Appendix A2.7.1.

2.2.7.7.2 Subsection *Saxifragoidea* (Sleumer) Argent

The Subsection *Saxifragoidea* was first described by Sleumer (1960) as a series, now given the status of a subsection by Argent (2006). This subsection consists of a single species and can be recognized by its cushion-forming habit and leaves with stomata on both sides. In cultivation, *R. saxifragoides* form extended horizontal branches when growing vigorously; these no doubt become buried by normal upward growth of bogs and thus form the rhizomes (Argent 2006). The single described taxon within the Subsection *Saxifragoidea* has no known taxonomic or conservation issues (Appendix A2.7.2).

2.2.7.7.3 Subsection *Solenovireya* H F Copeland

The Subsection *Solenovireya* was first described Copeland (1929), and can be recognized by its trumpet-shaped flowers, usually white or pale-pink, corolla lobes are less than ¼ the length of the tube, and the plants are usually medium to large shrubs with stomata on abaxial surface of the leaves only (Argent 2006). There are 45 taxa in Subsection *Solenovireya*, and 21 of these are of conservation interest. The taxonomic and conservation issues are summarized in Appendix A2.7.3.

2.2.7.7.4 Subsection *Malesia* H F Copeland

The Subsection *Malesia* was first described by H F Copeland (1929) and can be recognized by the medium-sized leaves, well-developed leaves being mostly 1–4 cm long and stomata on abaxial surface of the leaves only. Subsection *Malesia* consists of 63 taxa, and 30 of these are conservation interest. The taxonomic and conservation issues of Subsection *Malesia* are summarized in Appendix A2.7.4.

2.2.7.7.5 Subsection *Euvireya* H F Copeland

The Subsection *Euvireya* was first described by Copeland (1929) and can be recognized by its large leaves, with the majority of the well-developed leaves more than 4 cm long, and stomata found only on the abaxial surface of the leaf. The plants are usually medium to large shrubs or small trees (Argent 2006).

2.3 Summary

Since the conception of the genus *Rhododendron* in 1753, its classification has undergone a multitude of iterations with the improvement in the techniques used to study the taxa. *Rhododendron* has been widely studied as these plants are commonly cultivated, especially in the subtropical and temperate regions of the world. The classification of vireyas started more than a century ago and vireyas have been grouped into several subgroups based on morphological characters. The most useful of these morphological characters included: (i) the tails at the end of the seeds which differentiated the vireyas from the rest of the rhododendrons, and (ii) the scales, which to a certain extent have been used in demarcating the subgroups within the vireyas. Comprehensive studies on the classification of vireyas was initiated with the study of Copeland (1943), followed by the study by Sleumer (1949).

Following these studies several authors have carried out more detailed morphological studies, especially those of lower taxonomic groups. The most significant of these is the study of Malesian vireyas carried out by Sleumer (1966a) for the *Flora Malesiana*, and this work remained the foremost authority on this group for the next four decades. Numerous other revisions, and studies on subgroups continued, leading to the classification of Chamberlain et al. (1996) known as the Edinburgh Classification. This classification was widely adopted and persisted until the beginning of the 21st century, when molecular classifications have taken over. The monograph of vireyas by Argent (2006) is the next update to the work done by Sleumer and the subsequent authors, and have now become the most comprehensive study to date, which included all the species of vireyas described to that date. The major drawback of the classical classifications is that they were not based on any evolutionary process, and thus artificial (Argent 2006).

The move from the classical classifications to the recent molecular classifications saw much needed clarification, and thus a robust structure. To date, several regions of the nuclear and plastid DNA have been used to study the phylogeny of the vireyas, and the placement of the vireyas within the genus *Rhododendron*. These molecular studies enabled the inference of evolutionary relationships such as the one carried out by Craven et al. (2008). Craven et al. (2008) brought much anticipated resolution to many unsettled classification issues. The various molecular studies discussed in this chapter show only

minor differences between each other, i.e. the placement of the subgroups of the vireyas. However, the major trends are: (i) *Euvireya* forms a group (clade) with the bulk of the species, with the majority originating from eastern Malesia (mainly New Guinea), (ii) *Pseudovireya* forms a sister group to the rest of the vireyas (core vireyas), and (iii) the closest taxa to *Pseudovireya* being the *Discovireya*.

The classification and circumscription of the taxa of vireyas have been equally debated as for the genus *Rhododendron*. Molecular studies based on nuclear and plastid DNA sequences have now largely resolved the major classification issues with the vireyas. The most recent studies by Goetsch et al. (2011) and Craven et al. (2011) revealed the much anticipated relationships within the vireyas and its placement within the genus *Rhododendron*. These two studies have also enabled the restoration of the correct name for the vireyas, Section *Schistanthe*.

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Chapter 3

Literature Review II: Conservation of Biodiversity

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3 Literature Review II: Conservation of Biodiversity

This chapter investigates the general concepts of biodiversity conservation and the major causes of biodiversity loss. Areas investigated include the different methods used in the measurement and quantification of biodiversity, the methods and approaches used in the conservation of biodiversity and setting of conservation priorities and concepts of *in situ* and *ex situ* conservation.

Biodiversity[41] (or biological diversity) is defined as the total variety of living organisms that exist on Earth as well as the ecological complexes they are part of. There are three fundamental levels of biodiversity that need to be considered: (i) genetic diversity (the sum of the genetic variation within a species), (ii) species diversity (the number of species present and their relative abundance), and (iii) community diversity (the collection of organisms co-existing in their physical environment) (Magurran & McGill 2010; Primack 2010; Stuart et al. 1990). These three levels of biodiversity are related to each other. For example, loss of genetic diversity threatens species diversity. Diversity within a species is necessary to maintain diversity among species, and at the same time, diversity among species is necessary to maintain diversity within a species. And if any one type is removed from the system, the cycle can break down, and the community becomes dominated by a single species (Booy et al. 2000; Ehrlich 1988). Biodiversity is thus redefined to include the conservation of ecosystems, species and genes (Dyke 2008).

Conservation is about maintaining and improving the status of species and their habitats and can be initiated by a multitude of values. These values can range from relatively non-quantifiable ones such as aesthetic and cultural values, to more measureable ones such as economic value (Maclaurin & Sterelny 2008). To devise and carry out any biodiversity conservation plan, the following need to be determined: (i) causes of biodiversity loss, (ii) rationales for conservation of biodiversity, (iii) methods used in biodiversity conservation, and (iv) accurate measurement of biodiversity. The following sections

[41] 'Biodiversity' was coined by E O Wilson in 1988 (Wilson & Peter 1988).

discusses these four areas in detail and their relation to the biodiversity conservation of vireyas (Primack 2010).

3.1 Causes of Biodiversity Loss

Presently, the loss of biodiversity is on the increase, and largely due to human destruction of ecosystems, overexploitation of natural resources, overpopulation and the spread of agriculture pollution. Although, extinctions are part of the normal process of natural selection and evolution, we now face a massive human-induced extinction crisis, with extinction rates estimated at 1,000 to 10,000 times the expected rate (Pimm & Askins 1995). Many biological systems respond slowly to changes in their environment (Primack 2008), and the present rapid loss of biodiversity is much faster than they can recover naturally (Pimm & Askins 1995).

Factors resulting from human activities which threaten biodiversity include: (i) habitat destruction, (ii) habitat fragmentation, (iii) habitat degradation (including pollution), (iv) global climate change, (v) overexploitation of species for human use, (vi) the invasion of exotic species, (vii) the increased spread of disease, and synergisms among these factors. Most threatened species and communities face at least two or more of these problems that favours extinction and hinder efforts to protect them (Primack 2004).

3.1.1 Habitat Destruction

Alterations in ecosystem composition are detrimental to species and their interactions with their ecosystems, which are critical for the species survival and continuity of evolution (Wood et al. 2000). Habitat destruction is one of the major causes of depletion of plant populations worldwide and the main activities leading to habitat destruction include: (i) deforestation, and (ii) logging (Wood et al. 2000).

(i) **Deforestation** involves the conversion of forest habitats to farming (or non-forest) habitats. Only the most adaptable of wild species can survive the complete destruction of the habitat to which they have become adapted over evolutionary time (Corlett 2009).

(ii) **Logging** is the other main activity contributing to habitat destruction. Illegal logging is even more devastating to biodiversity, as it involves indiscriminate cutting down of trees, often within protected areas (Sodhi et al. 2007).

3.1.2 Habitat Fragmentation

In addition to outright destruction, habitats that formerly occupied wide, continuous areas are now often divided into pieces by roads, fields, towns, and a broad range of other human constructs (Primack 2008). Fragmentation usually occurs when forests are cut down leaving relatively small, isolated patches of forest known as 'forest fragments' or 'forest remnants'. The threat to biodiversity due to fragmentation is much greater in the tropics than other regions since the biodiversity is shown to be consistently higher in the tropics (Harris 1984; Primack 2004).

3.1.3 Habitat Degradation

Even when a habitat is unaffected by observable destruction or fragmentation, the communities and species in that habitat can be greatly affected by human activities. The most subtle and universal form of environmental degradation is pollution, commonly caused by pesticides, sewage, fertilizers from agricultural run-offs, industrial chemicals and wastes, emissions from factories and automobiles, and sediment deposits from eroded hillsides. Pollution and contamination cause irreversible damage to species and the entire ecosystem (Ladle 2009; Primack 2004). Although levels of air pollution in some parts of North America and Europe are in decline, in Asia home to the majority of the rhododendrons, it is on the rise (Primack 2004).

3.1.4 Global Climate Change

Catastrophic changes in the Earth's climate that precipitate mass extinctions may be relatively infrequent, however more modest changes occur quite regularly. These changes may lead to evolutionary bottlenecks, and cause biodiversity loss, especially when the changes are rapid. Species and populations may be lost permanently, if they do not have sufficient time to adapt themselves to the changing climate conditions (Hunter & Gibbs 2007; Ladle 2009; Langhoff 2007). Global climate change, in particular towards warmer

weather, is already occurring because of the large amounts of carbon dioxide and other greenhouse gases produced by the burning of fossil fuels and tropical deforestation entering the atmosphere. Future temperature changes could be so rapid that many species will be unable to adjust their ranges and will probably become extinct (Primack 2008).

3.1.5 Overexploitation of Species

Overexploitation refers to the harvesting of a renewable resource to the point of diminishing returns. Human economic activities that involve the taking of biological resources, or organisms, in larger numbers than their populations can withstand can lead to unsustainable levels of genetic diversity and often result in extinction. Overexploitation is not an activity limited to humans, introduced predators and herbivores, for example, can and do overexploit native flora and fauna (Rosser & Mainka 2002). The horticultural value of plants often leads to their overexploitation and is detrimental to their survival, as they are extracted from the wild in far larger numbers than they can regenerate. Several vireya species are now known to be under threat or even extinct due to overexploitation (Argent 2006; Gibbs et al. 2011; Wood et al. 2000).

3.1.6 The Invasion of Exotic Species

The great majority of exotics do not become established in the places in which they are introduced into, because the new environment is not suitable to their needs. However, a certain percentage of species do establish themselves in the new environment, and many of these become invasive, i.e. they increase in abundance at the expense of native species and often leading to the extinction of native species (Mooney 2005; Primack 2004).

3.1.7 The Increased Spread of Disease

A major threat to species and their environments is the increased transmission of diseases, which is often a result of human activities, such as habitat destruction, which may increase disease-carrying vectors (Primack 2004). For example, *Cornus florida* (flowering dogwood) which is one of the most common understory tree species in eastern North America, is currently threatened across much of its range by the introduced destructive fungus *Discula destructiva* that causes dogwood anthracnose. In infected trees, purple-

rimmed lesions first appear on leaf margins and expand up the veins into the petiole. As the infection progresses, the trees undergo twig and branch dieback and develop stem cankers which eventually leads to tree-death (Jenkins & White 2002). In case of vireyas, one of the recorded diseases is powdery mildew, which is sometimes found in cultivated specimens (Withers 1983).

3.2 Global Strategy for Plant Conservation (GSPC)

The Global Strategy for Plant Conservation (GSPC) has set several outcome-oriented global targets (CBD 1992, 2002). Botanic Gardens Conservation International (BGCI) in collaboration with international botanic gardens have actively promoted the Global Strategy for Plant Conservation (CBD 2002) by publishing and implementation of specific agenda for biodiversity conservation (Wyse Jackson & Sutherland 2000). The updated Global Strategy for Plant Conservation consists of five objectives that are further divided into a total of 16 targets (http://www.cbd.int/gspc/targets.shtml):

Objective I: Plant diversity is well understood, documented and recognized

- **Target 1:** An online flora of all known plants.
- **Target 2:** An assessment of the conservation status of all known plant species, as far as possible, to guide conservation action.
- **Target 3:** Information, research and associated outputs, and methods necessary to implement the Strategy developed and shared.

Objective II: Plant diversity is urgently and effectively conserved

- **Target 4:** At least 15 per cent of each ecological region or vegetation type secured through effective management and/or restoration.
- **Target 5:** At least 75 per cent of the most important areas for plant diversity of each ecological region protected with effective management in place for conserving plants and their genetic diversity.
- **Target 6:** At least 75 per cent of production lands in each sector managed sustainably, consistent with the conservation of plant diversity.
- **Target 7:** At least 75 per cent of known threatened plant species conserved in situ.
- **Target 8:** At least 75 per cent of threatened plant species in ex situ collections, preferably in the country of origin, and at least 20 per cent available for recovery and restoration programmes.
- **Target 9:** 70 per cent of the genetic diversity of crops including their wild relatives and other socio-economically valuable plant species conserved, while respecting, preserving and maintaining associated indigenous and local knowledge.

- **Target 10:** Effective management plans in place to prevent new biological invasions and to manage important areas for plant diversity that are invaded.

Objective III: Plant diversity is used in a sustainable and equitable manner

- **Target 11:** No species of wild flora endangered by international trade.
- **Target 12:** All wild harvested plant-based products sourced sustainably.
- **Target 13:** Indigenous and local knowledge innovations and practices associated with plant resources maintained or increased, as appropriate, to support customary use, sustainable livelihoods, local food security and health care.

Objective IV: Education and awareness about plant diversity, its role in sustainable livelihoods and importance to all life on earth is promoted

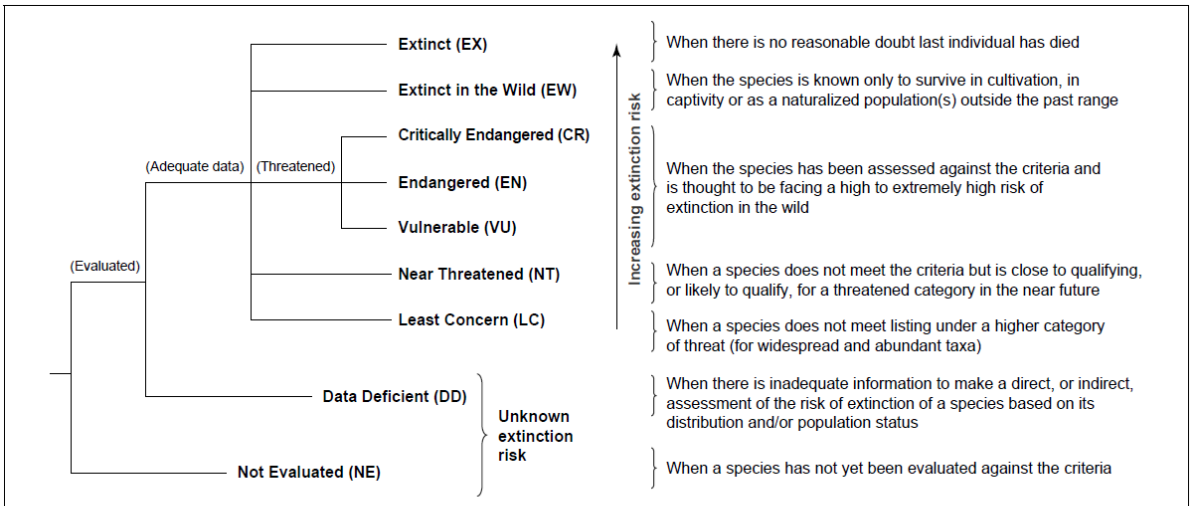
- Target 14: The importance of plant diversity and the need for its conservation incorporated into communication, education and public awareness programmes.

Objective V: The capacities and public engagement necessary to implement the Strategy have been developed

- Target 15: The number of trained people working with appropriate facilities sufficient according to national needs, to achieve the targets of this Strategy.
- Target 16: Institutions, networks and partnerships for plant conservation established or strengthened at national, regional and international levels to achieve the targets of this Strategy.

Among these targets, this study will be focused largely on the underlying principles and the measures needed to achieve and implement Target 8. Measures to implement GSPC are presently underway at international, regional and national levels globally. For example, BGCI has made available several publications imparting knowledge in the practical implementation of GSPC in botanic gardens (Hawkins et al. 2008; Oldfield & McGough 2007). Additionally, BGCI has also collaborated with international organizations such as IUCN and the Global Trees Campaign (GTC) in the production of the Red Lists for globally threatened species (Cicuzza et al. 2007; Gibbs et al. 2011).

3.3 The IUCN Red List and Conservation



Criterion	Critically Endangered	Endangered	Vulnerable	Qualifiers and notes
A1: reduction in population size	≥90%	≥70%	≥50%	Over ten years/three generations[c] in the past, where causes of the reduction are clearly reversible AND understood AND ceased
A2–4: reduction in population size	≥80%	≥50%	≥30%	Over ten years/three generations[c] in past, future or combination
B1: small range (extent of occurrence)	<100km^2	<5000 km^2	<20 000 km^2	Plus two of (a) severe fragmentation and/or few locations (1, ≤5, ≤10); (b) continuing decline; (c) extreme fluctuation
B2: small range (area of occupancy)	<10km^2	<500 km^2	<2000 km^2	Plus two of (a) severe fragmentation and/or few locations (1, ≤5, ≤10); (b) continuing decline; (c) extreme fluctuation
C: small and declining population	<250	<2500	<10 000	Mature individuals. Continuing decline either: (1) over specified rates and time periods; or (2) with (a) specified population structure or (b) extreme fluctuation
D1: very small population	<50	<250	<1000	Mature individuals
D2: very restricted population	N/A	N/A	<20 km^2 area of occupancy or ≤five locations	Capable of becoming Critically Endangered or even Extinct within a very short time frame
E: quantitative analysis	≥50% in ten years/ three generations	≥20% in 20 years/ five generations	≥10% in 100 years	Estimated extinction risk using quantitative models (e.g. population viability analyses)

Figure 22 A simplified overview of the IUCN Red List categories and criteria (Rodrigues et al. 2006).

The IUCN Red List of Threatened Species is the most comprehensive global inventory of the conservation status of plant and animal species. This list is based on the risk assessment of extinction to species within geographical (or political) demarcations. The IUCN Red List of Threatened Species is the most comprehensive resource available to date that catalogues the global conservation status of flora and fauna (Rodrigues et al. 2006).

The Red List assessment process has developed substantially over the past two decades, extending the value of the Red List far beyond the categorization of threat status of organisms. It has now a far wider reach and has become a powerful tool in conservation

planning, management, monitoring and decision making (Rodrigues et al. 2006). Figure 22 shows a simplified overview of the IUCN Red List categories and criteria used for assigning these categories. Assessments are based on published or unpublished information and usually include expert input. Those species evaluated for which insufficient data are available to make an assessment are classified as Data Deficient (DD) and those not assessed for conservation status as Not Evaluated (NE). Using the criteria a species is listed as Least Concern (LC) when a species does not meet listing under a higher category of threat (for widespread and abundant taxa). When a species does not meet the criteria but is close to qualifying, or likely to qualify, for a threatened category in the near future, it is listed as Near Threatened (NT). When the species has been assessed against the criteria and is thought to be facing a high to extremely high risk of extinction in the wild they are listed as Vulnerable (VU), Endangered (EN) or Critically Endangered (CR) depending on the level of threat and the population numbers. When the species is known only to survive in cultivation, in captivity or as a naturalized population(s) outside its original provenance it is categorized as Extinct in the Wild (EW). When there is no reasonable doubt that the last individual of a species has perished (i.e. not existing in the wild nor in cultivation) it is listed as Extinct (EX) (IUCN 2001).

3.4 Rationales for Conservation of Biodiversity

The global assessment of the conservation status of rhododendrons was one of a series of assessments initiated by the Global Trees Campaign (Oldfield 2009). Subsequently, several studies and workshops have been conducted that contributed towards the development of a Red List of Rhododendrons (Gibbs et al. 2011; MacKay et al. 2010). The publishing of the Red List of Rhododendrons marked a turning point in the development of conservation programmes for rhododendrons in general. The Red List identified globally threatened rhododendrons that included 63 vireya taxa. Notably, in the Red List, *R. retrorsipilum* Sleumer from Papua New Guinea has been cited as Extinct (EX). Fieldwork to find this species in the type location was futile, as the original forest cover at that locality was completely lost to agriculture and firewood collection (Argent 2006; Gibbs et al. 2011). A large number of taxa still remain as Data Deficient (DD) or Not Evaluated (NE), and further fieldwork is required to ascertain their distribution. The DD and NE category taxa does not mean that are of less conservation interest, instead it highlights the need to do more research and field studies to determine their present

distribution and population numbers. It is highly likely that many of these DD and NE taxa would contain threatened taxa that would require conservation. The Red List therefore will remain the foundation for the initial selection of taxa for conservation (Gibbs et al. 2011).

Rhododendrons in general are now a permanent feature of well-known gardens around the world. Rhododendrons are more commonly used as ornamental plants and are commercially produced around the world. Rhododendrons are also being studied for their use in medicine, as they have been shown effective as antibiotics, anti-inflammatories and for the treatment of diarrhoea. Some species of rhododendron have also been used for firewood, timber and honey (usually toxic), development of insecticides and as a potential narcotic, among other domestic uses (Chettri & Sharma 2007; Gibbs et al. 2011; Kerkvliet 1981; Singh et al. 2003; Wang et al. 2010). Rhododendrons usually grow in areas of high humidity and on acidic soils, which are unsuitable for other plants, and they play an important role in ecosystems by stabilizing slopes and protecting watersheds. Many vireyas in particular have adapted to epiphytic lifestyles and serve as an indicator species for the health of the ecosystem (Gibbs et al. 2011; Heads 2003; Stevens 1976).

The rationales for the conservation of biodiversity can be divided into three categories: (i) moral or ethical reasons (other species have a right to exist), (ii) aesthetic reasons (species have aesthetic value, like works of art, and thus need to be protected), and (iii) utilitarian or economic importance (humans obtain material benefit from extant species) (Crozier 1997). When developing conservation programmes for any species, the initial challenge is to determine which subpopulations should contribute to the population to be conserved. As there are several limiting factors for any conservation exercise, including finance, time and opportunity, establishing conservation priorities is very important. Additional factors that need to be considered for setting conservation priorities include: (iv) limited distribution and species rarity, (v) ecological importance, (vi) phylogenetic or evolutionary distinctiveness, and (vii) feasibility (Maxted et al. 1997b).

3.4.1 Moral or Ethical Reasons

The biodiversity on Earth was evolved over billions of years, and the humans that share this biodiversity do not have a right to destroy the extant biodiversity. Biodiversity is

intrinsically valuable, and we (humans) have moral reasons to conserve all aspects of this biodiversity, regardless of their economic values. It is however, difficult to convince people of the intrinsic value of biodiversity, although in practice any conservation work is heavily dependent on these values. More often, the economic value of biodiversity to humans surpasses other values such as aesthetic and ecological (Koricheva & Siipi 2004; Oksanen 1997).

3.4.2 Aesthetic Value

Biodiversity has aesthetic and cultural values, but these are harder to quantify than ecological value. Plants and animals are revered in many parts of the world for these values and have become symbols of cultural identity and heritage. The survival of natural areas and species are important to different cultures around the world, and these cultural groups have distinct traditions and knowledge for relating to the natural world (Guruswamy & McNeely 1998).

3.4.3 Economic Importance

Placing economic value on biodiversity is seen by many as the best and perhaps only successful way of preserving it while also protecting livelihoods of people living and benefitting around these areas (Ninan 2007). Economic forces drive much of the extinction of the world's biodiversity, yet biodiversity has economic value. Economists typically classify ecosystem resources according to how they are utilized. The main framework used is the Total Economic Value (TEV) approach that includes: (i) direct use value; (ii) indirect use value; (iii) option value; and (iv) non-use value. The first three are generally referred to together as 'use value' (IUCN 2005).

Direct use values refer to ecosystem goods and services that are used directly by humans: (a) value of consumptive uses (such as harvesting of food products, timber for fuel or construction, etc.) and (b) value of non-consumptive uses (such as the enjoyment of recreational and cultural activities that do not require harvesting of products). Direct use values are most often enjoyed by people visiting or residing in the ecosystem itself, while indirect use values are derived from ecosystem services that provide benefits outside the ecosystem itself. Option values are derived from preserving the option to use in the future

ecosystem resources that may not be used at present. Non-use values refer to the enjoyment people may experience simply by knowing that a resource exists even if they never expect to use that resource directly themselves, and is usually referred to as 'existence value' (IUCN 2005; Pearce & Moran 1994).

3.4.4 Limited Distribution and Species Rarity

Limited distribution and the rarity of a species is the most important factor in establishing conservation priorities (Lindenmayer & Burgman 2005). Decrease in numbers is a prime indicator of a vulnerable species (Baillie et al. 2004). The International Union for the Conservation of Nature and Natural Resources (IUCN) is the global authority on the conservation status of species.

Table 2 Summary of IUCN Categories designated for vireyas (Gibbs et al. 2011).

Not Evaluated (NE)	**118 taxa**
Least Concern (LC)	**134 taxa**
Data Deficient (DD)	**89 taxa**
Near Threatened (NT)	**4 taxa**
Vulnerable (VU)	**36 taxa**
Endangered (EN)	**4 taxa**
Critically Endangered (CR)	**7 taxa**
Extinct in the Wild (EW)	**0 taxa**
Extinct (EX)	**1 taxon**

Rhododendron was first assessed for risk of extinction, at a meeting held in Singapore in 2008 as an effort to initiate the Red Listing of threatened species of this genus. A total of 275 vireya taxa (species, subspecies, varieties and forms) were assessed from a total of 393 taxa, a summary of which is given in Table 2.

The categories DD, NT, VU, EN, CR and EW are suitable candidates for conservation and will be used as the basis of taxa selection for this study. The assignment of these categories was based mostly on field studies conducted by several experts on this genus, mainly those reported by George Argent. Categories have been applied mostly to

individual taxa rather than species, and hence understanding the delimitation of the species concept is very important. For example in the case of *Rhododendron jasminiflorum*, the subspecies *R. jasminiflorum* ssp. *copelandii* has been categorized as VU, while the other four subspecies have been categorized as LC (and thus of no conservation interest) (Gibbs et al. 2011). Molecular techniques for example can be used to determine the status of these five subspecies. If all the subspecies are very closely related genetically there is no need to conserve the ssp. *copelandii*. On the other hand if the ssp. *copelandii* were to exhibit significant genetic distinction from the other subspecies, conservation would be warranted, and perhaps need evaluation of its taxonomic status as a subspecies. Close relationships among the infrageneric taxa also demand conservation of all these taxa in order to conserve the genetic diversity of the species (Donaldson et al. 2004).

3.4.5 Ecological Importance

Plant species do not exist in isolation, and each forms part of a community with other species, with which it interacts to varying degrees and in various ways. Species thus have ecological importance due to their role in ecosystems and its impact on the other species (Hunter & Gibbs 2007; Maxted et al. 1997b). Trees, for example, carry higher ecological value as they are habitats for many other species (both plants and animals) and as primary oxygen producers and carbon sinks (Molles 2009).

3.4.6 Phylogenetic or Evolutionary Distinctiveness

Phylogeny depicts ancestor-descendent relationships and provides information about the overall pattern of biological diversification and extinction through time. Rather than setting conservation priorities based on limited distribution, aesthetic value, ecological importance and economic importance, phylogenetic information provides an easy and a quantifiable ‘unit’. A phylogeny not only depicts relationships among species but also provides estimates of amounts of divergence along lineages. The branch length and branching patterns provide a measure of the amount of evolution or genetic divergence that has occurred between species over time (Purvis et al. 2005). Conservation of biodiversity requires knowledge of its history. Each time a species becomes extinct, the independent evolutionary feature of that lineage is lost forever. Unfortunately, most

conservation efforts do not take history into account. Cladograms are a powerful tool with which to summarize the evolutionary history of life. Using a combination of cladograms and geographical information would represent a step toward including information in the conservation of biodiversity (Posadas et al. 2001).

3.4.7 Feasibility

Given the need to prioritize because of existing constraints on the availability of resources, it is important to assess the feasibility of a conservation programme, in terms of how easy or difficult it is to conserve and the long-term costs. Essentially this involves the assessment of the availability infrastructure and finance for the establishment and long-term management of the conservation programme (Scherr & McNeely 2008).

3.5 Methods Used in Biodiversity Conservation

When a species reaches very low numbers, or its habitat becomes critically endangered, the decision may be taken to remove some or all individuals from the wild and attempt to conserve them in captivity, either to breed or maintain a genetic stock. For plants, this is usually carried out using herbaria and botanical gardens (Pullin 2008). These botanical gardens now play an active role in conservation of many endangered species across the globe. The gardens can either be established either in the species' native country or offshore. Vireyas are for example managed *in situ* (in nationally protected parks and reserves) and well-established *ex situ* collections (in large botanical gardens worldwide).

3.5.1 *In Situ* Conservation

In situ conservation is defined as the conservation of plants in their original habitats and is the general consensus among conservation biologists as the best way of conserving a species and its genetic diversity (CBD 1992). This method ensures that the future generations of the natural populations of these species can evolve and adapt to the changing natural environment and the ecosystem. Biodiversity at all its levels, genetic species and as intact ecosystems can be best preserved *in situ* by setting aside an adequate representation of wilderness as 'protected areas'. The World Conservation Union defines a protected area as 'an area of land and/or sea especially dedicated to the protection and

maintenance of biological diversity, and of natural and associated cultural resources, and managed through legal or other effective means' (IUCN 2001).

Maintaining viable populations in natural ecosystems through the creation of protected areas is widely regarded as one of the most efficient ways to protect endangered biodiversity (Bruner et al. 2001; Chape et al. 2005; Groves 2003). Conservation targets that are a subset of the biodiversity of an ecoregion need to be assessed in order to capture the broad range of biodiversity available, since it would be impossible to assess each component of the biodiversity individually (Groves 2003; Redford et al. 2003). Most biodiversity conservation approaches aim to conserve as many taxa as possible (Gaston 1996), but the reasons used to motivate conservation are often utilitarian in nature (Pullin 2008) and should therefore take taxon distinctiveness into account. The objective of these areas should be expanded to the preservation of relatively intact natural ecosystems, where biological diversity – from microscopic unicellular plants and animals, to the giant trees and major mammals – can all be preserved. However species cannot be protected individually as they are all inter dependent on each other. Thus the whole ecosystem must be protected. The biologist's viewpoint deals with areas that are relatively species rich, or those where rare, threatened or endangered species are found, or those with 'endemic' species (which are not found elsewhere). As rare endemic species are found only in a small area these easily become extinct due to human activity. Such areas must be given an added importance as their biodiversity is a special feature of the region (Mace 2004; Maxted et al. 1997a). Conserving the areas where populations of these species exist naturally is thus an underlying condition for the conservation of biodiversity, and protected areas form a central element in any biodiversity conservation plan (Mace 2004; Maxted et al. 1997a).

The main advantages of *in situ* conservation include: (i) the dynamic conservation of the taxa relative to the changes in the ecosystem, and (ii) the ability to conserve a diverse range of related wild taxa. The main disadvantages of *in situ* conservation include: (i) vulnerability to natural and human-induced disasters, and (ii) relatively high cost of maintenance and monitoring, due to the usually large geographic extent of the conservation areas (Maxted et al. 1997a).

3.5.2 *Ex Situ* Conservation

Ex situ conservation is the preservation of components of biological diversity outside their natural habitats (Wilson & Peter 1988). This strategy is based on the long-term storage of taxa away from their native habitat (Maxted et al. 1997a). Although there is widespread agreement about conservation priorities with regard to biodiversity hotspots globally, conducting conservation programmes *in situ* is not always practical. *Ex situ* conservation is far easier to implement than *in situ* conservation in some cases, but comes with many disadvantages. The main disadvantage is that the complete biodiversity of the species cannot be conserved, but only a representative selection chosen for conservation (Guerrant et al. 2004).

For highly endangered species, *ex situ* conservation offers a feasible approach, and in some instances the only feasible approach. However, *ex situ* conservation should be used as a complementary method to *in situ* conservation where possible (Koskela & Amaral 2002). The main purpose of *ex situ* conservation is to secure and maintain representative samples of the existing genetic diversity of a taxon. The main advantages of *ex situ* conservation include: (i) the ability to conserve a greater diversity of taxa within a relatively smaller area, thus reducing the cost of maintenance and monitoring, and (ii) easier access for the evaluation and studying of the conserved taxa.

Unlike *in situ* conservation, *ex situ* conservation of plants requires significant human intervention, in the form of collection, seed storage, planting and maintenance of these. The other disadvantages of collecting germplasm samples for *ex situ* conservation are: (i) limited coverage of genetic variation – a limited number of accessions per each taxon is conserved, and closely related taxa are often not conserved, (ii) evolutionary development is limited or vastly reduced, due to the lack of genetic diversity within the small number of conserved accessions, and the disconnect from their natural ecosystem, (iii) bias during collection of plant material, and (iv) samples that are too large to deal with and hence are lost. *Ex situ* conservation therefore should target sampling and maintaining as much genetic variation as possible that is present within and among populations of selected taxa consistent with resources for storage (Brown & Hardner 2000; Maxted et al. 1997a).

Facilities for the *ex situ* conservation of biodiversity include: (i) gene banks (e.g. seed banks, sperm and ova banks, field banks), (ii) *in vitro* plant tissue and microbial culture collections, (iii) artificial propagation of plants, with possible reintroduction into the wild, and (iv) collecting for botanic gardens for research and public awareness. *Ex situ* conservation measures can complement to *in situ* methods as they provide an 'insurance policy' against extinction. These measures also have a valuable role to play in recovery programmes for endangered species. The main aim of *ex situ* conservation is to ensure the full range of genetic diversity is retained in botanical collections to safeguard them against extinction (Ashton 1988).

Botanic gardens play the leading role in many *ex situ* conservation programme for plants. However it is important to emphasize that *ex situ* conservation in botanic gardens is justifiable only as part of an overall conservation strategy that includes aspects of *in situ* conservation to ensure that threatened taxa ultimately survive in the wild (Guerrant et al. 2004; Maunder et al. 2001b). The role of botanic gardens should therefore complement *in situ* conservation programmes, and must:

- Serve as a source of material for reintroduction into degraded habitats and to enhance populations as part of ecosystem management.
- Support research and educational activities.
- Enable material selection for introduction into the nursery trade (FAO et al. 2004; Koskela & Amaral 2002; Li et al. 2002; Wyse Jackson & Sutherland 2000).

Storing genetic diversity as seed is the moist preferred, most widely used and the most convenient method of *ex situ* conservation of crops. Extensive research has been carried out to determine the optimum treatment and storage of seed of most of the major food crops. The biggest advantage of storing seeds is the relatively small amount of space required and thus the large number of accessions that can be stored for any given space. The seeds of vireyas in particular are highly suitable for storage as the number of seeds produced is relatively large and the small sizes of seeds require minimal storage space. The main disadvantage is that the high cost of setup and maintenance of these seed storage sites (Argent 2006; Harrington et al. 1970; Rouse 1985). Studies on collection, storage viability and propagation of *Rhododendron* and in particular, vireyas seeds have been

carried out. These studies showed that long-term storage and maintenance of viability of vireya seeds is possible (Arocha et al. 1999; Rouse 1985).

The majority of the gene banks in existence today are dedicated to food crops. However some of these gene banks do house several taxa of ornamental plants. At NIAS in Japan, several ornamental plant accessions including 120 cultivars of *Rhododendron obtusum* cultivars. Ornamental Plant Germplasm Center (OPGC)[42] of USA on the other hand is a dedicated storage facility for ornamental plant germplasm. OPGC holds approximately 3,200 accessions representing about 200 genera of ornamental plants including several hundred *Rhododendron* taxa.

A noticeable feature of threatened accessions within botanic gardens is limited diversity and limited number of accessions. Guerrant et al. (2004) described in detail the characteristics of threatened plant populations in such collections:

- Populations are small and often derived from a small number of closely related founder individuals.
- The cultivated stocks are subject to fluctuating population size as a result of changing horticultural practices and mortality events.
- Often very little or no associated ecological or biological information is available to guide *ex situ* conservation managers in cultivating and managing the accessions.
- There is limited information on the history of the taxa in cultivation and often satisfactory horticultural protocols are unavailable.
- Individuals are scattered through a number of collections with varying horticultural and curatorial capacity and hence differing patterns of regeneration and mortality.
- Individuals are susceptible to artificial selection, genetic drift, inbreeding, and hybridization.

[42] www.opgc.osu.edu (managed by the Ohio State University, USA)

- Persistence in collections is highest for horticulturally amenable taxa and particularly for taxa with little or no commercial value.

3.5.3 Conservation of Germplasm

Genetic resources are part of the global heritage and are essential for sustainable development of human life, and therefore need to be conserved. Germplasm collections are assemblage of accessions that characterize the genetic variability targeted for conservation and utilization of genetic resources. The number of accessions in germplasm collections can range from a single sample to thousands of samples, and are maintained in appropriate facilities under suitable environmental conditions (Jaramillo & Baena 2007). Plant germplasm conservation at present broadly encompasses the conservation of gene pools in natural forests, seedlings and clonal plantations, seeds, tissue cultures, pollen, genomic libraries, and cloned DNA sequences. Ideally, a combination of these components should be implemented in conservation programmes where possible. However, this may not be possible in most cases due to limited availability of resources (Engels & Visser 2003; FAO 2010a; Jaramillo & Baena 2007; Kaimowitz & Sheil 2007; Millar 1993).

In any conservation programme, it would be impractical to conserve every individual or the whole genetic diversity of a wild population. Due to limited resources, taxa and accessions representing these taxa need to be selected based on stringent criteria, and this is usually achieved by prioritizing one taxon over another. The IUCN Red List plays a pivotal role during the prioritization of taxa being selected for conservation by highlighting threatened taxa. Once the threatened taxon has been selected, further study is required to determine which accessions of a population need to be selected for conservation. At present this selection of accessions for germplasm conservation is aided by molecular techniques (Ford-Lloyd et al. 2008; Kaimowitz & Sheil 2007; Margules & Pressey 2000; Sarkar & Margules 2002).

Any available germplasm is valuable for use in conservation, in the case of vireyas this is represented by mainly mature plants in cultivation (Kaimowitz & Sheil 2007; Keppel

2002). Engels & Visser (2003) outlined the various levels of germplasm conservation that can be targeted (arranged in increasing size):

- **genes** – Important functional genes (for disease resistance, etc.) could be mapped using molecular markers (Harkins et al. 1998). With emerging new molecular technologies, these functional genes could be sequenced to examine the possible molecular basis of the resistance. Conservation of genes however involves the actual conservation of the individual genotypes in which the genes occurred.

- **genotypes** – Individuals that represents a particular population, or some trait of interest. To maintain the genotype, germplasm must be obtained through vegetative propagation, since seeds collected from an individual do not usually preserve the genotype of that individual.

- **populations** – The original provenances of a taxon. Even though the ultimate goal is to conserve genetic diversity, it is desirable to sample sufficient accessions that represent the gene frequencies in that population.

- **ecosystems** – A group of living and non-living things interacting with each other. This by far is the highest level of conservation, and is impractical to measure its genetic variation due to the complex relationships between the constituents of the ecosystem. Many threatened rare plants including vireyas have specialized pollination mechanisms that require specific pollinators. It would be difficult to preserve these pollinators in a repository, as one can with seeds (Guerrant et al. 2004).

The germplasm levels described above rely on utilization of appropriate molecular tools to determine the genetic variation. This is absolutely critical when dealing with small populations of threatened taxa, whether *in situ* or *ex situ*, where a few individuals exist and is necessary to capture what little genetic diversity is left (Guerrant et al. 2004).

In addition to storage of whole plants, seeds and tissue samples, DNA isolated from the plants could be maintained at low temperatures (-80°C) or electronically as sequence data

(*in silico*). The latter is becoming increasingly feasible as the cost of establishing and maintaining data storage and retrieval systems have decreased significantly at present. While current technology does not permit the regeneration of the original plant from isolated DNA or *in silico* data, they can however be used in areas such as genetic diversity and phylogenetic analyses (FAO 2010a).

3.6 Measurement of Biodiversity

The loss of biodiversity is now recognized as a global problem of significant magnitude. Conservation efforts focus on measuring species diversity and distribution, assessing biodiversity threats, and managing habitats to maintain that diversity. The accuracy of measuring biodiversity depends on the quality and scale of the data, and is essential in the development of any conservation plan, forming the basis for making conservation decisions (Crozier 1997; Ninan 2007; Primack 2008). In the following section, genetic diversity is discussed further, as the current study is based on the *ex situ* conservation and the accessions of interest are not examined in the wild.

3.6.1 Exploration of Genetic Diversity Using Molecular Marker Systems

Genetic diversity gave rise to the extant biodiversity (the basic units of all life on Earth), thus they are mutually dependent. Genetic diversity refers to the variety of genes (biochemical units of hereditary information), and can be used to measure the variability within and among species (Stuart et al. 1990). Genetic diversity includes the genetic variation within species (a measure of the diversity of information encoded in the genes of a species), both among geographically separate populations and among individuals within single populations, and responsible for both the similarities and the differences between organisms. Each species is made up of individuals that have their own unique genetic composition, thus a species may have different populations, each having different genetic compositions. To conserve genetic diversity, it is therefore important to conserve all the different populations of a species (Crozier 1997).

The core concept of conserving biodiversity is to ensure that there is sufficient genetic diversity to maintain the evolutionary processes within a species (Crozier 1997; Primack

2008). Conservation of genetic diversity is essential to the long-term survival of any species, and diminution of genetic diversity has a negative impact on the adaptive potential of any species. Loss of genetic diversity also leads to increased risk of inbreeding depression, by the intensification of harmful recessive alleles. Management of genetic diversity thus becomes an integral part of biodiversity conservation (Frankham et al. 2009). The extent to which biodiversity is lost depends on the extent to which genetic diversity is lost which, in turn, depends to the extent to which gene information is lost (Nunes et al. 2003).

Species with high levels of genetic diversity (i) are better equipped to evolve in response to, and adapt to changing environments, (ii) are less likely to suffer a loss of fitness because of the expression of deleterious recessive alleles in homozygous individuals, among other problems, and (iii) offer breeders greater scope for developing varieties with specific desirable traits, such as resistance to certain diseases (Hunter & Gibbs 2007; Iriondo et al. 2008). If allelic diversity becomes low at many genes of a species, that species becomes increasingly at risk. If new pressures (such as environmental disasters) occur, a population with high genetic diversity has a greater chance of having at least some individuals with a genetic makeup that allows them to survive (Primack 2008).

Accurate quantification of genetic diversity is crucial for biodiversity conservation, and several methods are currently in use. Only in the recent years has it become possible to evaluate intraspecific diversity, i.e. the genetic component of the diversity (Loeschcke et al. 1994). One of these methods utilizes the measurement of genetic variation based on continuous or quantitative characters (height, seed set, etc.) that are controlled by multiple genes as well as the environment. Another method is based on the distribution of different alleles of a gene among individuals, and can be expressed as polymorphism (the proportion of genes that have more than one common allele) and heterozygosity (the proportion of genes for which an average individual is heterozygous). This method utilizes molecular techniques to reveal the relationships within taxa, especially those within or among populations and is preferred by conservation biologists. Hence, this is a better measure of differences than evolutionary distances (Crozier 1997; Hunter & Gibbs 2007).

Molecular techniques are now routinely used to prioritize populations for listing and protection, mainly for *in situ* conservation (Bruni et al. 2012; Lemes et al. 2003; Maxted et al. 1997a; Song et al. 2003; Stefenon et al. 2007). To apply these molecular techniques to *ex situ* conservation we need to determine the biodiversity with respect to their natural populations in the wild. It is also important to understand that the distribution of genetic diversity within a species is uneven, indicating that disproportionate fractions of the diversity are concentrated in small sub-populations, even when the population is well-mixed (Rauch & Bar-Yam 2004). For example, if the taxa selected for this study have originated from such populations, low genetic diversity might be expected.

3.6.2 Molecular Marker Systems

The use of molecular markers has now become commonplace in biodiversity studies, especially in the analysis of differences among individuals within a population. Marker systems or fingerprinting methods are useful in phylogenetic analyses and the search for useful genes. They are also used extensively for marker-assisted selection in plant breeding programmes, paternity testing and food traceability (De la Rosa et al. 2004; FAO et al. 2004; Mohan et al. 1997; Pafundo et al. 2005; Xu & Crouch 2008). The marker analyses can be carried out by using several methods, and many of these methods are based on PCR. Some of these methods include: (i) Random Fragment Length Polymorphisms (RFLP), (ii) Random Amplification of Polymorphic DNA (RAPD), (iii) Amplified Fragment Length Polymorphisms (AFLP), (iv) microsatellites, (v) Sequence Tagged Microsatellite Site (STMS), (vi) Expressed Sequence Tag (EST), (vii) Inter-Simple Sequence Repeat (ISSR), and (viii) nucleotide sequence analysis.

3.6.2.1 Random Amplified Polymorphic DNA Analysis (RAPD)

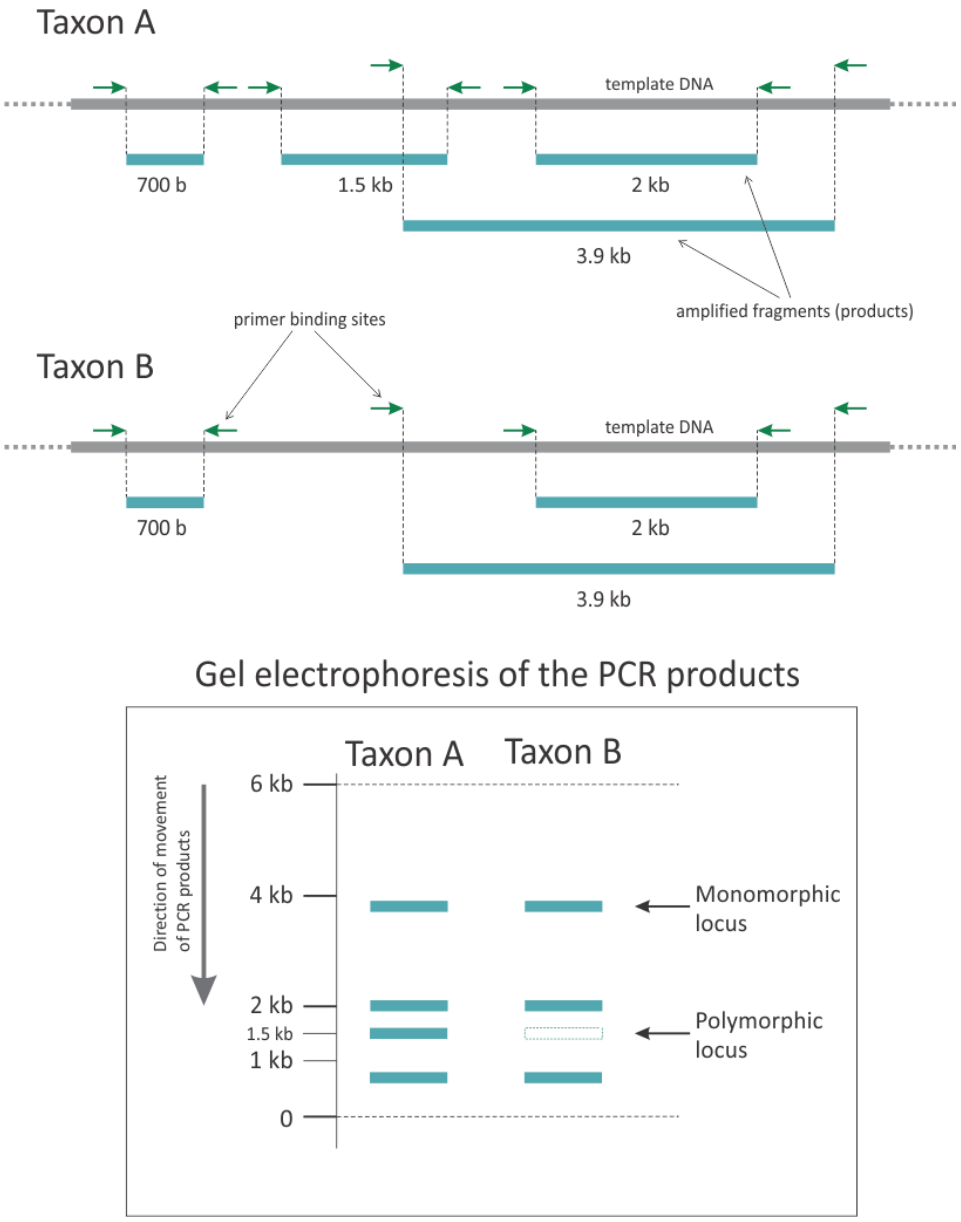


Figure 23 Random Amplified Polymorphic DNA (RAPD). During the PCR reaction the primers bind to the template DNA (grey line) at the binding sites (indicated by the arrows). The region of the template DNA between a pair of binding sites is amplified as a fragment (blue line). The resulting PCR products are then electrophoresed on an agarose gel, followed by staining with ethidium bromide and visualized under UV light. The scale shown is arbitrary.

Random Amplified Polymorphic DNA (RAPD) markers are arbitrary, short, random, synthetic oligonucleotides, 8–12 nucleotides long. A PCR reaction is carried out in which fragments of various lengths are amplified, depending on where the RAPD primers bind on the genomic template DNA sequence. The amplified fragments (or products) are separated on agarose gels and stained with ethidium bromide Figure 23. The banding

pattern formed for each individual (taxon) acts as a fingerprint, and can be used to compare with other individuals (Weising et al. 2005; Williams et al. 1990).

The main advantage of RAPDs is that only a single random primer is needed. Other advantages of RAPDs include: (i) primers are short synthetic oligonucleotides (~10 nucleotides long), (ii) primers are random sequences, and their development does not require cloning, sequencing or any other molecular characterization of the taxon studied, (iii) inexpensive, yet a powerful method to characterize and trace the phylogeny of diverse plant and animal species (Caetano-Anolles et al. 1991).

The main disadvantages of RAPDs include: (i) Nearly all RAPDs are dominant, i.e. it is not possible to distinguish whether a DNA segment is amplified from a locus that is heterozygous (single copy) or homozygous (double copies). Co-dominant RAPDs are observed as different-sized DNA segments amplified from the same locus, and are rarely detected. (ii) RAPDs are sensitive to reaction conditions, and since PCR is an enzymatic reaction, the quality and concentration of template DNA, concentrations of PCR components, and the PCR cycling conditions may greatly influence the outcome. Hence, the RAPD technique is notoriously laboratory-dependent and needs carefully developed laboratory protocols to be reproducible. (iii) Mutations in the binding regions cause mismatches between the primer and the template, thus resulting in the total absence of PCR product as well as in a merely decreased amount of the product, making the RAPD results difficult to interpret (Skov 1998; Weising et al. 2005).

The popularity of RAPD analyses as a tool for studying biodiversity has grown since its inception, mainly due to its ease of use and cost-effectiveness. RAPD analyses have been successfully used in studying numerous plant and animal species, including genetic diversity and fingerprinting studies of *Rhododendron* (Du et al. 2011; Iqbal et al. 1995; Manel et al. 2007; Milne et al. 2004; Zhou et al. 2009) (Bassam et al. 1992; Lanying et al. 2008). Some of these studies are discussed below.

Iqbal et al. (1995) carried out the first genetic studies of *Rhododendron* using RAPD markers. In this study three species of *Rhododendron*, *R. arborescens*, *R. atlanticum* and *R. yedoense* var. *poukhanense* were used to produce species-specific amplification profiles using ten random primers. The stability of amplification profiles among

individually cloned plants of each species was studied, and showed that the ten accessions of *R. atlanticum*, nine of *R. arborescens*, and ten of *R. yedoense* var. *poukhanense* showed no polymorphism among individual accessions. In order to ascertain that RAPD primers can indeed reveal real genetic differences among plants, F_2 plants of two hybrids were also analysed. In contrast to the clonally propagated plants, extensive polymorphisms were observed among the individual F_2 plants. This stability of RAPD profiles in clonally propagated rhododendron indicated the potential usefulness of RAPDs in identification of individual accessions.

Other studies on *Rhododendron* using RAPDs include: (i) Zhou et al. (2009) amplified DNA from 49 accessions of 43 *Rhododendron* taxa, to analyse their genetic diversity and phylogeny. (ii) Du et al. (2011) revealed the genetic diversity of four populations of *R. chrysanthum* (an endangered species endemic to NE China) at different altitudes.

3.6.2.2 Restriction Fragment Length Polymorphism (RFLP)

RFLP is a DNA fingerprinting technique that exploits variations in homologous DNA sequences, and was the first DNA profiling/fingerprinting technique devised. The differences between samples of homologous DNA sequences arise mainly from point mutations and indels (insertions and deletions). In the RFLP technique, the DNA sample digested using restriction enzymes that cut at sites characterized by short nucleotide sequences. The resulting ‘restriction fragments’ are separated according to their lengths by gel electrophoresis (Botstein et al. 1980).

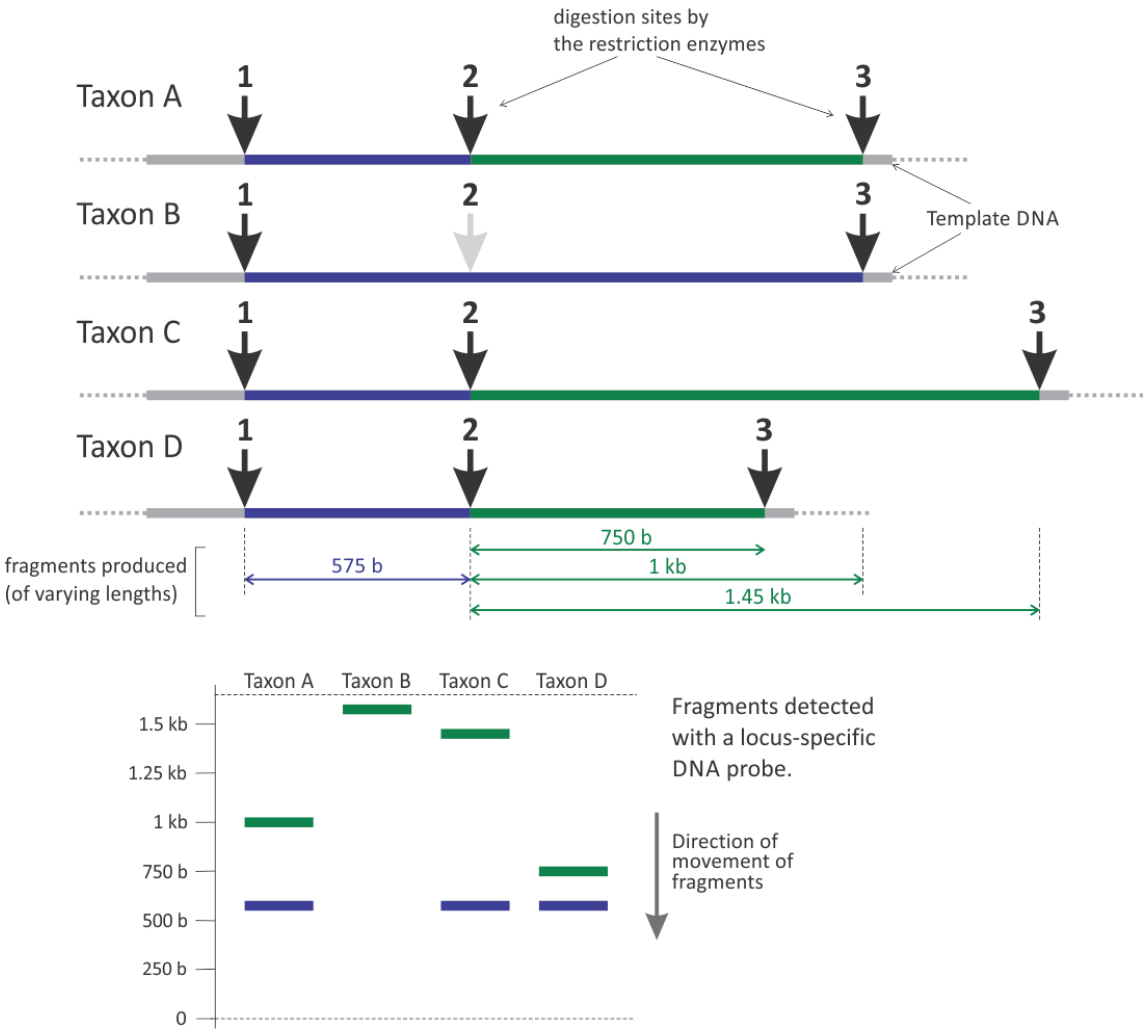


Figure 24 Restriction fragment length polymorphism (RFLP). The template DNA is digested using restriction enzymes that cut at sites characterized by short nucleotide sequences. The resulting 'restriction fragments' are separated according to their lengths by gel electrophoresis. A DNA probe (usually radioactive) is then used to detect the fragments, by hybridizing with the fragment. The scale shown is arbitrary.

Figure 24 illustrates a diagrammatic representation of the RFLP technique. When total genomic DNA is digested using restriction enzymes and all fragments stained, the fragments produced will be of such large number that they will appear as a smear on gel electrophoresis. To visualize specific restriction fragments (or loci), a DNA probe (usually radioactive) is used to detect the fragments, by hybridizing with the fragment.

RFLP markers have been used in the fingerprinting of *Rhododendron*, and the earliest of these studies include Milne & Abbott (2000) and Dunemann et al. (1999). In Dunemann et al. (1999) a genetic linkage map of *Rhododendron* was constructed using a segregating population from an interspecific cross. Parent-specific maps based on 239 RAPD, 38

RFLP, and two microsatellite markers were aligned using markers heterozygous in both parents. Two genomic regions bearing QTLs (Quantitative Trait Locus) with significant effects on the trait leaf chlorosis[43], were identified on two linkage groups of the chlorosis-tolerant parent. The study also found highly significant QTL effects for flower colour on two chromosomes indicating major genes located in these genome areas.

Milne & Abbott (2000) carried out a genetic diversity study on the invasive species *Rhododendron ponticum* in the British Isles. As previous studies suggested that the naturalized material of *R. ponticum* in the British Isles did not originate from Turkey, Spain or Portugal; this study assessed the extent of introgression that has affected the naturalized material. This study used RFLPs from chloroplast DNA (cpDNA) and nuclear ribosomal DNA (nrDNA) to distinguish between naturalized material in the British Isles of *R. ponticum*, and 15 other *Rhododendron* species including the closest relatives of *R. ponticum*.

3.6.2.3 Amplified Fragment Length Polymorphism (AFLP)

AFLP is a PCR-based DNA fingerprinting technique that combines RFLP analysis and PCR. The AFLP reaction comprises of two principal steps: (i) Genomic DNA is digested with two different restriction enzymes producing sticky ends, and double-stranded synthetic adapters of a defined sequence are ligated to both ends of all restriction fragments, (ii) selective amplification of some of these fragments with two PCR primers that have corresponding adaptor and restriction site specific sequences, (iii) electrophoresis of the resulting amplicons (amplified DNA fragments) on a gel matrix (Vos et al. 1995; Weising et al. 2005; Zabeau & Vos 1993).

[43] A condition in which leaves produce insufficient chlorophyll (a pigment responsible for the green colour of leaves), causing the leaves to become pale yellow to off-white.

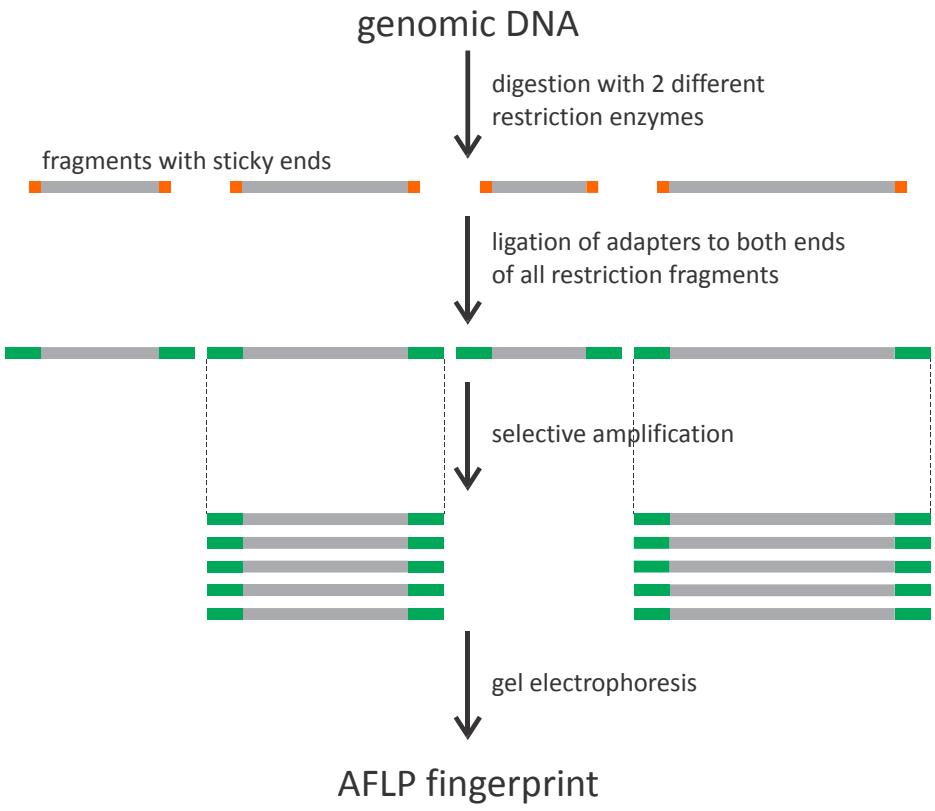


Figure 25 Simplified representation of the AFLP fingerprinting technique. Genomic DNA is digested with two different restriction enzymes producing sticky ends, and double-stranded synthetic adapters of a defined sequence are ligated to both ends of all restriction fragments. Selective amplification of some of these fragments with two PCR primers that have corresponding adaptor and restriction site specific sequences produces amplified DNA fragments. The resulting amplicons (amplified DNA fragments) are then electrophoresed on a gel matrix.

Figure 25 illustrates a diagrammatic representation of the AFLP technique. The AFLP technique has numerous advantages, such as producing a large number of amplified fragments (50–100), scoring is for presence or absence of fragments, and no prior knowledge of the sequence is required. AFLP analysis has been successfully applied in studies involving genetic diversity, varietal identification and genetic map construction, among others. However, only a few AFLP studies have been carried out for *Rhododendron*, with only a handful of these on studies of genetic diversity (Chappell et al. 2008; Dendauw et al. 2001b; Erfmeier & Bruelheide 2011; Ma et al. 2010).

Chappell et al. (2008) investigated the genetic diversity within and among seven deciduous azalea species (*Rhododendron* Section *Pentanthera*) native to the eastern United States. Ma et al. (2010) investigated the unusual patterns of hybridization

involving a narrow endemic species *Rhododendron cyanocarpum* (Ericaceae) that occurs sympatrically with potentially interfertile congeners (members of the same taxonomic genus) throughout its range within Yunnan, China. Erfmeier & Bruelheide (2011) investigated the maintenance of high genetic diversity within the invasive *R. ponticum* in British Isles.

3.6.2.4 Microsatellites (SSRs)

Microsatellite loci, also known as Simple Sequence Repeats (SSRs), short tandem repeats (STRs) or variable number tandem repeats (VNTRs), are repeating sequences of 2–6 base pairs of DNA dispersed throughout the eukaryotic genomes (Beyermann et al. 1992; Skinner et al. 1974). The high polymorphism seen in microsatellite arises from the variability in the number of repeats within a microsatellite locus. Microsatellites are thus ideal for application in plant breeding and genetic diversity of natural populations. The two main advantages of microsatellite markers are that they are highly variable, co-dominant and that they are PCR-based. The main disadvantage is the considerable amount of time needed for development of microsatellite loci (Byrne et al. 1996).

Microsatellites are extensively used as molecular markers in studies of population genetics, genetic diversity and in determination of kinship (Queller et al. 1993). Unlike RAPDs, the development of microsatellites is difficult in species for which Expressed Sequence Tag (EST, described below) or whole genome sequence is not available (as in *Rhododendron*), as the sequence for the target region is usually required, but once they are developed, they can be easily transferred between laboratories (Barbara et al. 2007; Squirrell et al. 2003; Weising et al. 2005). The conservation of microsatellites across species and genera has been revealed in several genetic diversity studies (Di Gaspero et al. 2000; Maroof et al. 1994; Ochieng et al. 2007; Thomas & Scott 1993) and a few phylogenetic studies (Hokanson et al. 2001; Stàgel et al. 2008), thus making microsatellites suitable for analysis of fine-scale genetic variation (Byrne et al. 1996).

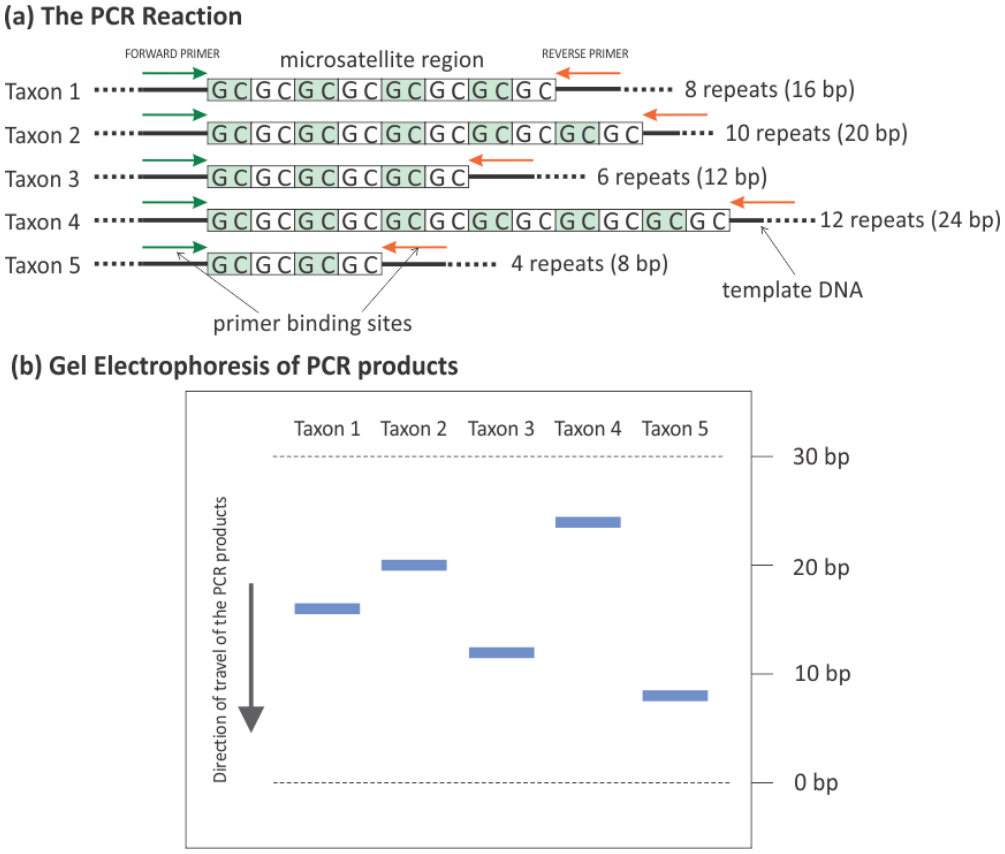


Figure 26 Microsatellite polymorphism and the analysis method. (a) Diagrammatic representation of microsatellite polymorphism. The example shows a dinucleotide (2 bp) microsatellite. The length of the microsatellite region is determined by the length of the microsatellite (2 bp in this example) and the number of repeats of the microsatellite (4–12 in this example). (b) Microsatellite analysis using gel electrophoresis. The scale shown is arbitrary.

Figure 26*a* illustrates an example of a dinucleotide (a repeat motif of 2 base pairs, in this case 'GC') in a particular locus for five taxa. To detect microsatellite polymorphism the sequence information for the microsatellite flanking regions need to be obtained. This information is then used to design locus-specific PCR primers (forward and reverse). In the next step, PCR is used to amplify the microsatellite regions and the resulting PCR products are denatured and separated on polyacrylamide gels and visualized by using fluorescent dyes (Weising et al. 2005).

Figure 26*b* illustrates a simplified representation of the microsatellite analysis using gel electrophoresis. The gel electrophoresis of the PCR products from the locus produces fragments of 16, 20, 24 and 8 bp in length for taxa 1–5 respectively. Fewer repeats correspond to shorter fragments and move faster through the gel, while the longer fragments lag behind. For a given taxon and locus, the number of repeats have been shown

to vary within and among populations, and therefore is a reliable character for use in population genetics and diversity studies (Cipriani et al. 1994; Terauchi & Konuma 1994; Yang et al. 1994a).

An alternative method of obtaining microsatellite markers that does not require genome sequencing was developed by Hayden & Sharp (Hayden & Sharp 2001). Their technique, known as the sequence-tagged microsatellite profiling (STMP), was used to rapidly generate large numbers of microsatellite markers from genomic or cDNA (complementary DNA, which is DNA synthesised from a messenger RNA (mRNA)). In this method, STMP generates short nucleotide sequence tags for fragments in a pool of SSR amplicons. These tags are then ligated to form concatemers for cloning and sequencing. Each tag contains sufficient nucleotide sequence for design of PCR primers, and allows the amplification of corresponding full length fragments from the pool of SSR amplicons. These fragments enable the characterization of an SSR locus by sequencing and thus the flanking sequence for the development of primers of the microsatellite marker.

Microsatellite markers have been developed for *Rhododendron*, mainly in temperate species (Dunemann et al. 1999; Naito et al. 1998; Tan et al. 2009). Dunemann et al. (1999) constructed a genetic linkage map for a *Rhododendron* population using microsatellites (among other markers). These microsatellites were the first of their kind for *Rhododendron*, since then, other microsatellite markers for *Rhododendron* have been developed. The most notable of these studies are those by Naito et al. (Naito et al. 1999), Kameyama et al. (2000; 2001, 2002), Wang et al. (2009), Kondo et al. (Kondo et al. 2009) and Caser et al. (2010). The study by Caser et al. (2010) was particularly interesting, as it was aimed at finding out whether *Rhododendron* hybrids were distinguishable on the basis of morphology and microsatellite polymorphism. The study showed that accessions can be uniquely identified using microsatellite markers. The genetic variation was shown to be consistent among the accessions; therefore these can be used for germplasm conservation and restoration of historical genetic resources.

The study by Naito et al. (1999) examined population structures in the morphologically variable *Rhododendron metternichii* var. *hondoense* using microsatellites and the contribution of these populations to conservation. *R. metternichii* var. *hondoense* is

known to propagate vegetatively (asexual), in addition to sexual propagation via seeds. Six microsatellite loci were analysed in two populations with differing habitats. One of these populations consisted of creeping individuals and few seedlings, while the other population consisted of mainly single-stemmed individuals and many seedlings. The results showed that sufficient polymorphisms were found within the population with creeping stems suggesting that the population had been maintained by both sexual and asexual reproduction in the past. One of the factors for the lack of seedlings at this population was due to dense litter cover, thus resulting in lower sexual propagation. The study concluded that the two populations had sufficient genetic variation to support conservation.

Kameyama et al. (2000) used microsatellites to characterize patterns of pollen-mediated gene flow in *R. metternichii* var. *hondoense*. The study utilized six microsatellite markers for 18 flowering accessions of *R. metternichii* var. *hondoense* within a 150 × 70 m plot. The results of the study showed that a directional flow of pollen from late-blooming trees to early-blooming ones was occurring.

Kondo et al. (2009) studied the effects of dispersal of seeds by water (hydrochory) on the formation of the present range of the Japanese endemic species *Rhododendron ripense* and the spatial distribution of its genetic variation. The study showed that the evolutionary history of dispersal of seeds by water in *R. ripense* appears to have been strongly shaped by both ancient and modern rivers. Microsatellites are hence very useful in determining the retention of genetic diversity within regions and determining evolutionary history of certain traits.

3.6.2.5 Sequence Tagged Microsatellite Site (STMS)

STMS is a PCR-based DNA fingerprinting technique in which a library of concatenated, large number of short (16 bp) sequence tags representing microsatellite-flanking regions is generated with the help of the restriction endonuclease *Bsg*I. In STMS, each sequence tagged microsatellite is amplified using a single primer which is specific to the conserved flanking region of the microsatellite, combined with a universal primer that anchors to the 5′-end of the microsatellite. Using additional PCR steps STMS markers can be

converted into conventional microsatellite markers by using two primer pairs for the region flanking the microsatellite repeats (Hayden & Sharp 2001; Weising et al. 2005).

STMS markers for *Rhododendron* have recently been developed and have shown their potential in genetic diversity studies (Caser et al. 2010; Caser & Scariot 2008; Dendauw et al. 2001a). Caser et al. (2010) carried out a genetic diversity study using STMS markers and morphological trait markers to evaluate 33 *Rhododendron* accessions and to determine the discrimination power of STMS markers.

3.6.2.6 Expressed Sequence Tag (EST)

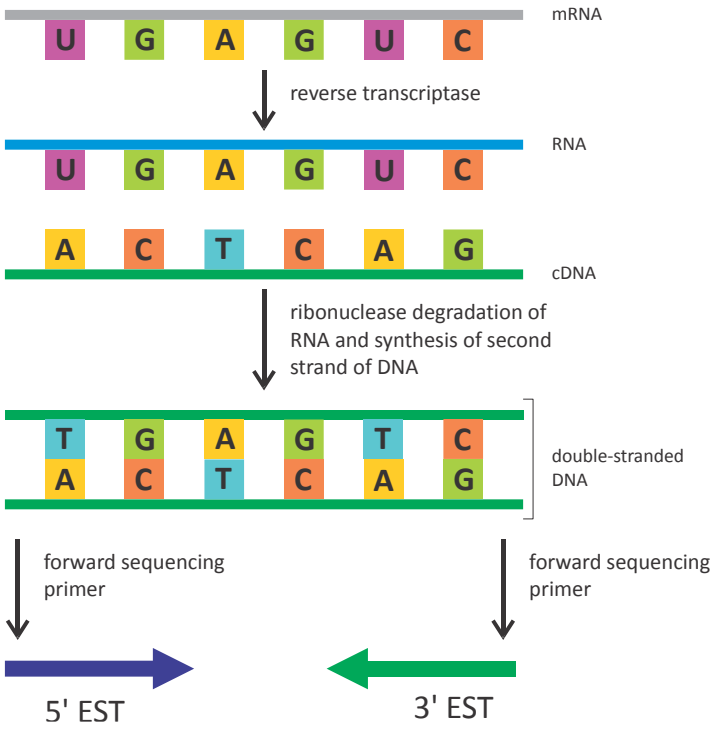


Figure 27 Diagrammatic representation of how ESTs are generated.

ESTs are short sequences of a random cDNA sequence (usually 200 to 500 bp) that are generated by sequencing either one or both ends of an expressed gene. ESTs result from single-pass sequencing of cloned mRNA (i.e. 200–800 bp of sequence starting from an end of a cDNA) (Figure 27). The mRNAs in a cell are copies of the genes that are being expressed, and do not contain sequences from intergenic regions, nor from non-coding introns. These cDNAs are typically individual clones from a cDNA library, and the resulting sequence is approximately 500 to 800 bases. The ESTs represent portions of

expressed genes as these clones consist of DNA that is complementary to mRNA (Adams et al. 1991; Parkinson & Blaxter 2009).

EST based markers have effectively been developed and used for *Rhododendron*, but very few studies have been carried out for determining genetic diversity (De Keyser et al. 2006; De Keyser et al. 2007; De Keyser et al. 2009; Li et al. 2011; Wei et al. 2005a; Wei et al. 2005b). A notable study by Scariot et al. (2007) investigated the discriminating capacity and effectiveness of AFLP, STMS and EST based markers in assessing genetic relationships among evergreen azaleas. The study revealed that STMS and EST based markers revealed a higher genetic distance detection capacity than AFLPs, which, nevertheless, were the most efficient marker system, due to their high polymorphism detection capacity.

3.6.2.7 Inter-Simple Sequence Repeat (ISSR)

Inter-Simple Sequence Repeat (ISSR) is a PCR-based genotyping technique based on the variation in the regions between microsatellites. This technique involves PCR amplification of genomic DNA using a single fluorescently labelled primer that targets the region between identical microsatellites, with 1–3 bases that anchor the primer at the 3' and 5' end. ISSRs are dominant markers, and hence amplified regions are scored as diallelic. Polymorphism in the amplified products between individuals within a population arises from structural changes to the regions via indels or mutations at the primer binding sites. In addition to freedom from the necessity of obtaining flanking genomic sequence information of the microsatellites, ISSR analysis is technically simpler than many other marker systems and provides highly reproducible results and generates abundant polymorphisms in many organisms. ISSRs have numerous applications such as in genetic diversity studies (population genetics, genotyping and conservation biology), phylogenetic analysis and assessment of hybridization (Davidson et al. 2010; Rout & Mohapatra 2006; Wolfe et al. 1998).

To date, very few studies utilizing ISSRs have been carried out on *Rhododendron* (Jin et al. 2006; Liu et al. 2010; Milne et al. 2004; Zheng et al. 2011). One notable study is the genetic analysis by Jin et al. (2006) on five natural populations of *Rhododendron fortunei* in Zhejiang province of China using 12 ISSR markers. This study revealed a total of 170

loci for the ISSR markers, of which 150 (88.24%) were polymorphic. The Shannon's Diversity Index was 0.4317 and Nei's Gene Diversity was 0.2848. This suggests that the genetic diversity of *R. fortunei* was relatively high; however the genetic diversity at population level (gene flow) of *R. fortunei* was relatively low. These results also suggest that population isolation and inbreeding regression may have played a major role in the genetic differentiation among *R. fortunei* populations.

3.6.2.8 Nucleotide Sequence Analysis

Although polymorphisms at nucleotide level can be determined using the various methods described above, the most direct method of determining nucleotide polymorphism is via sequencing. The basic unit of variation within the genome is the linear order of nucleotide bases that constitute the DNA. Ascertaining the order of these nucleotide bases is the most accurate way of sampling the genome for molecular characters (Maxam & Gilbert 1977; Weising et al. 2005). It therefore appears to be to be one of the most desirable molecular techniques. A recent study, however argued that markers targeted to specific gene sequences may still behave as anonymous markers, and that the type of marker system used is irrelevant at low taxonomic levels where a clear genetic structure is absent due to intensive breeding activities (van Treuren & van Hintum 2009).

DNA barcoding for species-level identification depends on distinguishing intraspecific from interspecific sequence variation; however, the appropriate amount of variation varies between different groups of organisms. To date, there is no universal DNA barcode gene for species discrimination among all organisms (Stoeckle 2003). In case of *Rhododendron*, a number of genes (or related regions, such as introns) have been sequenced, both from the plastid genome and the nuclear genome. The chloroplast genes sequenced for *Rhododendron* include: *rbc*L (rubisco large subunit 1) (Chung et al. 2007; Kron & Chase 1993), *mat*K (maturase K) (Dendauw et al. 2001b), *mat*K–*trn*K intron region (Kurashige et al. 2001), *trn*L–*trn*F intron region, *atp*B–*rbc*L (Huang et al. 2011). The nuclear genes sequenced for *Rhododendron* include: *rpb*2i (Goetsch et al. 2005; Goetsch & Hall 2002), ITS (Internal transcribed spacer) region (Brown et al. 2006a; Gao et al. 2002; Huang et al. 2011; Scheiber et al. 2000).

Nucleotide sequence data are commonly and extensively used in phylogenetic studies of *Rhododendron*, as discussed in Sections 2.1.3 and 2.2.3, however have rarely been used in genetic diversity or population studies of *Rhododendron* (Chung et al. 2007; Huang et al. 2011). Chung et al. (2007) is a phylogeographic study using chloroplast DNA (cpDNA), to reveal the origin and evolutionary history of a *Rhododendron* species complex in Taiwan. A total of 124 individuals selected from five endemic *Rhododendron* species were used for amplification of two chloroplast intergenic spacers: *trn*L–*trn*F and *atp*B–*rbc*L. This study inferred a single origin and a once-widespread distribution of the *R. pseudochrysanthum* species complex in Taiwan. The study also showed that restricted gene flow with isolation-by-distance characterized the re-colonization pattern of the *R. pseudochrysanthum* complex, while a contiguous range expansion was indicated for *R. formosanum*.

Huang et al. (2011) is a study carried out to investigate the genetic population structure of the alpine species *Rhododendron pseudochrysanthum sensu lato*, using chloroplast (cpDNA) and nuclear DNA (nrDNA) sequences. *R. pseudochrysanthum* forms a complex of incipient species with different degrees of morphological or ecological differentiation providing an ideal model for studying species divergence. The study examined the phytogeography and the evolutionary history of *R. pseudochrysanthum*, and found that systematic inconsistency existed between gene genealogies of the cpDNA and nrDNA. The resulting phylogenetic trees were rooted at *R. hyperythrum* and *R. formosana,* and both trees lacked reciprocal monophyly for all members of the complex *R. pseudochrysanthum*. The spatial distribution of the cpDNA for *R. pseudochrysanthum* had a noteworthy pattern showing high genetic differentiation (F_{ST} = 0.56–0.72) between populations in the Yushan Mountain Range and populations of the other mountain ranges. This study therefore showed that, both incomplete lineage sorting and interspecific hybridization/introgression may have contributed to the lack of monophyly among *R. hyperythrum, R. formosana* and *R. pseudochrysanthum*. Independent colonisations, plus low capability for seed dispersal in current environments, may have resulted in the genetic differentiation between populations from different mountain ranges.

The potential utility of the non-coding regions (introns) of the nuclear gene *rpb*2i in phylogenetic applications and especially in *Rhododendron* have been studied (Craven et al. 2011; Goetsch et al. 2011; Goetsch et al. 2005; Hall et al. 2006), and these have been

discussed in detail in Sections 2.1.3 and 2.2.3. The proteins encoded by the exon sequences of *rpb*2i are approximately 90% conserved, while their intron sequences show very high divergence. Diploid *Rhododendron* genomes contain only a single copy of *rpb*2i, which makes the sequencing of this region very easy (Goetsch et al. 2005).

The *rpb2* region itself is a low-copy region in the nuclear genome, and encodes the second-largest subunit of RNA polymerase II (Goetsch et al. 2005; Goetsch & Hall 2002). RNA Polymerase II is the multi-subunit enzyme that transcribes pre-mRNA from nuclear genes (Weinmann et al. 1974). The *rpb*2i gene of *Rhododendron* and of all ERICALES encodes one of two genes for the 140kd second-largest RNA Polymerase II subunit. *rpb*2i contains 25 exons, separated by 24 introns, of varying lengths, the intron 1 being the longest – 2.8kb (Figure 32) (Goetsch et al. 2005; Goetsch & Hall 2002; Woychik & Young 1990).

3.6.3 Measurement of Genetic Diversity

Genetic variation among individuals of a taxon within a population makes them different from one another and allows them to adapt to environmental changes over time. Maintaining genetic diversity within and between taxa is therefore important for the long-term survival of a taxon. The view taken is that conservation should seek to maximise the preserved information within the planet's biota, best expressed in terms of genetic information held in genes rather than in portions of the genome of uncertain or no function (Crozier 1997). Genetic diversity can be measured directly by sampling tissue from individual accessions and testing analysing those using genetic markers to detect the degree of difference among those accessions.

A variety of measures based on molecular marker systems have been proposed to quantify distinctiveness, which is often held to mark a taxon of high conservation worth. Crozier (1997) suggested several such measures including: (i) Gene Number, (ii) Higher-taxon Richness, (iii) Phylogenetic Measures, and (iv) Distance Measures.

3.6.3.1 Gene Number

Gene number is the total number of genes contained in the complete genome of an organism, and is not the total DNA content. This measure is based on the notion that genetic complexity increases during evolution. This is a reasonable measure of potential information content of organisms, their 'complexity', and sequences of the genes constitute the information content. Gene number suggests itself as a natural measure of complexity, indicating as it does the expected diversity of gene products (Bird 1995; Crozier 1997; Flowers & Purugganan 2008; Rokas & Carroll 2005; Sterck et al. 2007). Not all genes arise from pre-existing ones, but they can occasionally arise *de novo*, as in the incidental functionality of alternate reading frames, and new genes can be produced as the reshuffled subunits of existing genes (Crozier 1997). The gene number however is rarely used in genetic diversity studies, as this requires sequencing of the whole genome of that species (Wu et al. 2008).

3.6.3.2 Higher-taxon Richness

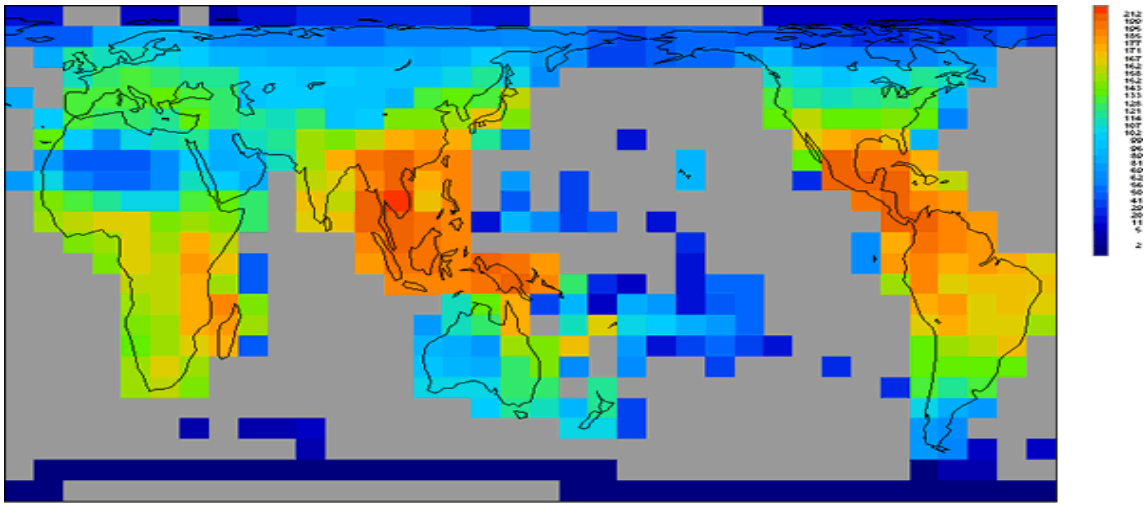


Figure 28 Map of family richness of seed plants worldwide. Grid-cell area ~611,000 km^2, for intervals of 10° longitude. Values are represented by logarithmic scale, with red for high richness and blue for low richness. Adapted from Williams et al. (Williams et al. 1994).

Higher-taxon Richness is also sometimes associated with the measurement of species diversity. This is a top down taxonomic approach in which higher taxa (families, genera, etc.) are surveyed rather than performing exhaustive species surveys. This is a moderately good indicator of Species Richness and is very cost-effective (Williams & Gaston 1994).

Several studies now support the idea of a relationship between the numbers of higher taxa and the numbers of species among areas (Williams et al. 1997). Higher-taxon Richness has also been used to investigate hotspots of endemism (Figure 21) (Humphries et al. 1995; Williams et al. 1994).

3.6.3.3 Phylogenetic Measures

Phylogenetics is the study of evolutionary history that reveals the intricate relationships among taxa, especially between species and higher hierarchies. This technique has been stimulated over the past three decades by the emergence of new molecular methods and statistical techniques for modelling the tree of life (Purvis et al. 2005). Phylogenetic analyses has now become a prerequisite for biodiversity conservation and its importance to conservation planning of diversity at species or up to ecosystem level has long been recognized (Magurran 2004; Magurran & McGill 2010).

3.6.3.4 Distance Measures

It is important to understand the difference between genetic fingerprinting and genetic diversity analysis. Genetic fingerprinting is the unambiguous identification of an individual (cultivar, inbred line or individual from a population) using molecular techniques, while genetic diversity analysis is a study undertaken to classify an individual or population or species compared to other individuals or populations or species using either molecular or other techniques, and is a relative measure (Escudero et al. 2003; Karp et al. 1996).

Several distance measures have been formulated in the study of genetics and specifically genetic diversity. Genetic Distance (*D*) is one of the most commonly used distance measures, and is a measure of divergence among populations (or species) that can be used to infer whether populations with all individuals are potential partners, or reproductively isolated (Goldstein & Pollock 1997; Hedrick 2005; Nei 1972).

The units of *D* depend on the type of data used (allozymes, nucleotide sequences, protein sequences, etc.). There are many different quantitative approaches to measure *D* (Goldstein et al. 1995; Takezaki & Nei 1996). They include: (i) Nei's Standard Genetic

Distance (Nei 1972), (ii) Nei's Minimum Genetic Distance (Nei 1973), (iii) Cavalli-Sforza and Edwards' Chord Distance (Cavalli-Sforza & Edwards 1967), (iv) Delta Mu-Squared or $(\delta\mu)^2$ (Genetic Distance based on the Stepwise Mutation Model – SMM) (Goldstein et al. 1995), and (v) Tamura-Nei Distance (Tamura & Nei 1993).

(i) Nei's Standard Genetic Distance (Nei 1972) (D_S) can be defined as:

$$D_S = -\ln \sum x_i y_i / \sqrt{\left(\sum x_i^2 y_i^2\right)}$$

where x_i and y_i are the frequencies of the *i*th allele, in the populations *x* and *y* respectively. This method assumes that differences arise due to both mutation and genetic drift. Under the Infinite Allele Model (IAM), D_S, is expected to increase linearly with time, if the mutation-drift balance is maintained throughout the evolutionary process.

(ii) Nei's Minimum Genetic Distance (Nei 1973), D_M, can be defined as:

$$D_M = \frac{1}{2}\left(\sum_{i=1}^{A} p_{ix}^2/L + \sum_{i=1}^{A} p_{iy}^2/L\right) - \sum_{i=1}^{A} p_{ix}p_{iy}/L$$

where p_{ix} and p_{iy} are the frequencies of the *i*th allele among the alleles, regardless of their locus affiliation and A is the total number of alleles at *L* loci considered ($A = \sum_{l=1}^{L} n_l$).

(iii) **Cavalli-Sforza and Edwards' Chord Distance** (Cavalli-Sforza & Edwards 1967), D_C can be defined as:

$$D_C = \sqrt{2\left(1 - \frac{\sum_{i=1}^{p} x_{1i}x_{2i}}{\sqrt{\sum_{i=1}^{p} x_{1i}^2 \sum_{i=1}^{p} x_{2i}^2}}\right)}$$

where x_i and y_i are the frequencies of the *i*th allele in the populations× and *y* respectively. The populations are conceptualised as existing as points in an *m*-dimensional Euclidean space which are specified by *m* allele frequencies (i.e. *m* equals the total number of alleles

in both populations). The distance D_C is the angle between these two points, which is a geometric distance points in multidimensional space. This method assumes genetic drift only, i.e. excludes mutation.

(iv) **Delta Mu Squared Distance** (Goldstein et al. 1995), $(\delta\mu)^2$ is defined as:

$$(\delta\mu)^2 = \sum_j^r \left(\mu_{x_j} - \mu_{y_j}\right)^2 /r$$

where

$$\mu_{x_j} = \sum_i i x_{ij}$$

$$\mu_{y_j} = \sum_i i y_{ij}$$

where x_{ij} and y_{ij} are the frequencies of the allele in state i at the jth locus in populations× and y, respectively, and r is the number of loci examined. Therefore, $(\delta\mu)^2$ therefore can be written as:

$$(\delta\mu)^2 = \sum_j^r \left(\sum_i i x_{ij} - \sum_i i y_{ij}\right)^2 /r$$

This method is specifically developed for microsatellite loci, and thus the most widely used measure of microsatellite genetic distance. The method assumes the Stepwise Mutation Model (SMM), in which an allele in state i (an allele with i repeats) is assumed to mutate to an allele either in state (i +1) or (i – 1) with an equal probability (Goldstein et al. 1995). An essential feature of a genetic-distance measure used to estimate relative times of divergence is that its expected value should increase linearly with time. This requirement is fulfilled by the $(\delta\mu)^2$ distance under the unconstrained SMM, and linearity is maintained even when the assumptions of single-step mutations and symmetrical mutation rate are violated (Kimmel et al. 1996).

(iv) **Tamura-Nei Distance** (Tamura & Nei 1993), *d* is defined as:

$$d = -k_1 \ln(w_1) - k_2 \ln(w_2) - k_3 \ln(w_3)$$

where

$$k_1 = \frac{2g_A g_G}{g_R}$$

$$k_2 = \frac{2g_T g_C}{g_Y}$$

$$k_3 = 2\left(g_R g_Y - \frac{g_A g_G g_Y}{g_R} - \frac{g_T g_C g_R}{g_Y}\right)$$

$$w_1 = 1 - \frac{P_1}{k_1} - \frac{Q}{2G_R}$$

$$w_2 = 1 - \frac{P_2}{k_2} - \frac{Q}{2G_Y}$$

$$w_3 = 1 - \frac{Q}{2g_R g_Y}$$

P_1 and P_2 are the proportions of transitional differences between nucleotides A and G, and between T and C, respectively. Q is the proportion of transversional differences.

g_A, g_C, gG, gT, are the respective frequencies of the nucleotides A, C, G and T, where

$$g_R = g_A + g_G$$
$$g_Y = g_T + g_C$$

This distance method corrects for multiple hits, taking into account the differences in substitution rate between nucleotides and the inequality of nucleotide frequencies. It also distinguishes between transitional substitution rates between purines and transversional substitution rates between pyrimidines. It also assumes equality of substitution rates among sites (Tamura & Nei 1993).

3.7 Data Analysis Methods

The data that is produced using the genetic marker systems need to be analysed, visualized and interpreted. Once the data has been captured and fine-tuned (such as base alignment of sequence data or base calling of microsatellite data), the next step is to carry out a phylogenetic or genetic diversity study. It is important to determine an evolutionary model and a suitable phylogenetic construction method to analyse the data produced. Also of importance is the evaluation of the reliability of these analyses with statistical support. This section describes in detail, the commonly used evolutionary models, phylogenetic tree construction methods and statistical support methods for the analyses.

3.7.1 Evolutionary Models

Evolutionary models are sets of assumptions about the process of nucleotide or amino-acid substitution (which aims to correct unseen changes along the phylogeny), and are often used in a phylogenetic analysis. These models can have a significant effect on the resulting tree and therefore on conclusions drawn in a phylogenetic study. In molecular phylogenetics, an evolutionary model can be used to define the probability of substitution from one nucleotide to another (Felsenstein 1981). The most popular evolutionary models used in phylogenetic inference are: (i) Markov Models, (ii) Nucleotide Substitution Models, (iii) Amino Acid Replacement Models (Liò & Goldman 1998).

3.7.1.1 Markov Models

The Markov Model is a stochastic model that assumes the Markov Property (in which the conditional probability distribution of future states of the process depends only upon the present state, not on the sequence of events that preceded it). The simplest Markov Model is the Markov Chain, which models the state of a system with a random variable that changes through time. Markov Chain Monte Carlo (MCMC) methods are a class of algorithms for sampling from probability distributions based on constructing a Markov chain that has the desired distribution as its equilibrium distribution (Larget & Simon 1999; Liò & Goldman 1998; Yang & Rannala 1997).

3.7.1.2 Nucleotide Substitution Models (NSMs)

In Nucleotide Substitution Models, the substitution of nucleotides in a sequence is assumed to be a random event. There are a large number of NSMs used in phylogenetic analysis, such as (i) Jukes-Cantor (the simplest model of DNA evolution, where all sites are assumed to change independently) and (ii) Tamura-Nei (assumes variable base frequencies and variable transition rates). Mathematical methods are then used to determine the genetic distances from molecular data, which in turn can be used to infer the phylogeny (Lemey et al. 2009; Liò & Goldman 1998).

3.7.1.3 Amino Acid Replacement Models (AARMs)

Amino Acid Replacement Models are empirical models and have advantages over NSMs. However, these models are suited more for protein coding sequences (Liò & Goldman 1998).

3.7.2 Phylogenetic Tree Construction

The most common method of visualizing and interpreting the resulting analysed data, is to represent this data graphically using a phylogenetic tree or an evolutionary tree, depending on the data type and interpretation required. The construction of a phylogenetic tree consists of three major steps: (i) utilization of a suitable evolutionary model, (ii) construction of the phylogenetic tree, and the (iii) evaluation of the reliability of the phylogenetic tree.

Phylogenetic analysis has become commonplace in plant systematics, especially with the advent of molecular techniques. With the availability of DNA sequence data through publicly accessible databases such as GenBank, there is an increased number of published phylogenetic studies. At present, molecular data sets have become the most important resources in plant phylogenetic reconstruction, genetic diversity studies and reconstruction of evolutionary processes (Purvis et al. 2005).

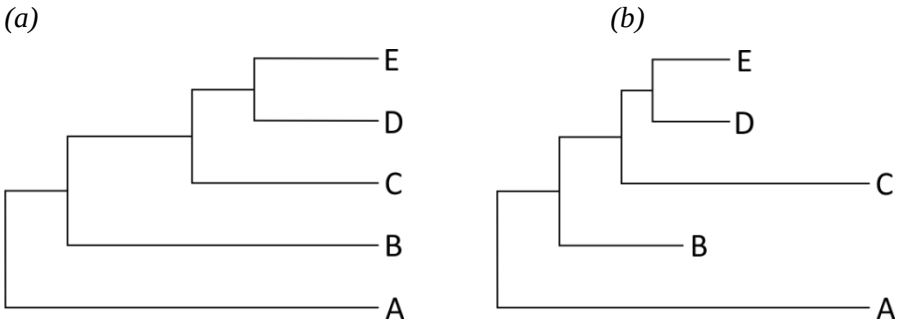


Figure 29 Rooted phylogenetic trees illustrating hypothesized evolutionary relationships of five imaginary taxa. The trees are rooted at the node containing the taxon A: (a) Unscaled rooted tree (branch lengths arbitrary); (b) Scaled rooted tree (branch lengths relative to evolutionary distance).

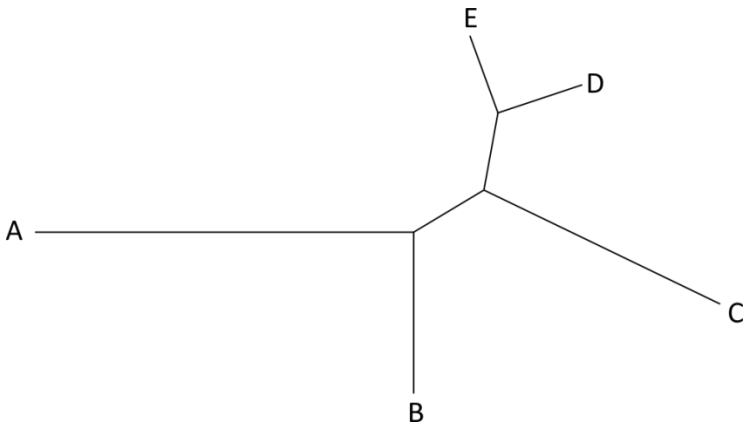


Figure 30 Unrooted scaled phylogenetic tree illustrating hypothesized evolutionary relationships of five imaginary taxa. The branch lengths are relative to the evolutionary distance.

Phylogenetic analysis is the inference of phylogeny, branching orders, and ultimately the evolutionary relationships, between taxa. The inference of phylogeny is often referred to as 'tree building'. A phylogenetic tree (or evolutionary tree) is a branching diagram (also called a dendrogram or a cladogram) that shows the inferred evolutionary relationships among taxa, based upon similarities and differences in their physical and/or genetic characteristics. The taxa joined together in the tree are implied to have descended from a common ancestor. In a rooted phylogenetic tree (Figure 29), each node with descendants represents the inferred most recent common ancestor of the descendants and the edge lengths may be used to interpret time estimates. Each node is called a taxonomic unit (TU), and internal nodes are generally called hypothetical taxonomic units (HTUs) as they cannot be directly observed. Phylogenetic trees form the basis of systematics and

diversity studies (Judd et al. 2008). Figure 30 illustrates an unrooted tree, in which the relatedness of the nodes is visualized without making assumptions about their ancestry.

Phylogenetic tree building methods are based on mathematical and/or statistical methods. There are two major types of data used in phylogenetic inference: (i) character-based methods (using characters such as nucleotide or protein sequences), and (ii) distance-based methods, in which the character data (or molecular sequence data) is transformed into pairwise distances (or dissimilarities). The resulting distance matrix can then be used to generate phylogenetic trees. A clustering method is then used to generate the phylogenetic or evolutionary tree. The most widely used clustering methods are: (i) Maximum Likelihood (ML), (ii) Maximum Parsimony (MP), (iii) Minimum-Evolution (ME), (iv) Neighbour Joining (NJ), and (v) Unweighted Pair Group Method with Arithmetic Mean (UPGMA) (Nei 1996).

The initial trees for the Maximum Likelihood trees can be determined using BIONJ method with Maximum Composite Likelihood (MCL) distance matrix. The MCL method in estimating evolutionary distances between DNA sequences can be employed for inferring phylogenetic trees, divergence times, and average sequence divergences between and within groups of sequences (Tamura et al. 2011). The BIONJ is an improved version of NJ, and is well adapted to studies where evolutionary distances are obtained from aligned sequences. BIONJ uses a simple model of the sampling noise of evolutionary distances, thus, taking into account that high evolutionary distances present a higher variance than short distances (Gascuel 1997). The initial trees for Neighbour Joining trees can be determined using the Maximum Composite Likelihood (MCL) distance matrix.

3.7.2.1 Maximum Likelihood

Maximum Likelihood (ML) method uses standard statistical techniques for inferring probability distributions to assign probabilities to particular possible phylogenetic trees, i.e. the analysis searches for the tree topology that maximizes the likelihood that the observed data have occurred under a given model of sequence evolution (Felsenstein 1981).

The ML framework provides a convenient approach to optimising models to data through a series of hierarchical likelihood ratio tests that test assumptions about how nucleotides evolve for a given dataset (Huelsenbeck & Crandall 1997). The advantages of phylogenies based on ML include: (i) suitability for distantly-related sequences, (ii) flexibility (i.e. works well under different molecular clock theories[44]), (iii) produces appropriate results under suitable evolutionary models, (iv) produces likelihood for all suboptimal trees – confidence/uncertainty. The disadvantages of phylogenies based on ML include: (i) production of bad approximations under bad evolutionary models, (ii) algorithms are computationally intensive. The ML method can therefore be applied to most phylogenetic studies (Guindon & Gascuel 2003; Yang et al. 1994b).

3.7.2.2 Maximum Parsimony

Maximum Parsimony (MP) analyses are based on the minimalistic principle of Ockham's razor, i.e. that the simplest, most parsimonious explanation of a problem should be preferred to more complex explanations. In phylogenetics, trees which correspond to the fewest character changes provide the most parsimonious results. This simple method is also computationally more efficient and thus can be used for generating preliminary trees. The disadvantage of this method is that under certain conditions (such as those with high degrees of sequence variability and homoplasy[45]) it can produce misleading results. MP analyses are also particularly prone to long-branch attraction, in which clades are reconstructed containing taxa which show long branches in comparison to other taxa in the phylogenetic tree (Bergsten 2005; Felsenstein 1978).

Initial trees are usually inferred using the Close-Neighbour-Interchange (CNI) method. The examination of all possible topologies for a given dataset (especially large datasets) is very time consuming. The CNI algorithm reduces the time spent searching by first producing a temporary tree (e.g. by using the NJ), and then examining all of the topologies that are different from this temporary tree by a topological distance. If this procedure is repeated several times, and all the topologies previously examined are avoided, an

[44] Molecular clock theories differ from other evolutionary theories in that they track molecular evolution rather than taxon evolution.
[45] A character shared by a set of species, but not originated from common ancestry. For example wings of insects and wings of birds are homoplasious structures, as they have been evolved from different structures, but used for the same purpose of flying.

improved tree can be generated. For the MP method, the CNI search can start with a tree generated by the random addition of sequences. This process can be repeated multiple times to find the final MP tree (Nei & Kumar 2000).

3.7.2.3 Minimum Evolution

The Minimum Evolution (ME) is a distance method used in the construction of phylogenetic trees by additive trees, and the topology shows the smallest value of the sum of all branches chosen as an estimate of the correct tree (Kidd & Sgaramella-Zonta 1971). However, the construction of a ME tree is time-consuming, and the number of possible topologies (unrooted trees) rapidly increases with the number of taxa, and it becomes very difficult to examine all the topologies. An alternative to the ME method is the NJ method. The ME method is more suitable for long nucleotide sequences, while sequences with relatively small number of nucleotides or amino acids, the NJ method generates the correct topology more often than does the ME method (Nei et al. 1998; Takahashi & Nei 2000). Initial trees are usually inferred using the Close-Neighbour-Interchange (CNI) method (Nei & Kumar 2000).

3.7.2.4 Neighbour Joining

The Neighbour Joining (NJ) algorithm of Saitou and Nei (1987) is one of the most popular methods for reconstructing phylogenetic trees from a matrix of pairwise evolutionary distances. The Neighbour Joining (NJ) is a bottom-up clustering method used for generating phylogenetic trees based on morphological, nucleotide or protein sequence data. The algorithm requires knowledge of the genetic distance between each pair of taxa. The NJ method is based on the minimum evolution criterion, i.e. the topology that gives the least total branch length is preferred at each step of the algorithm, and the tree is constructed in a step-wise fashion. This heuristic method has been extensively used in phylogenetics and usually finds a tree that is very close to the optimal tree. The main advantage of the NJ method is its relative computational efficiency (Saitou & Nei 1987; Tamura et al. 2004).

3.7.2.5 Unweighted Pair Group Method with Arithmetic Mean

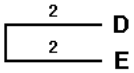
The Unweighted Pair Group Method with Arithmetic Mean (UPGMA) also known as Cluster Analysis is a simple agglomerative or hierarchical clustering method used for generating phylogenetic trees. UPGMA is often used to generate preliminary trees for more sophisticated phylogenetic reconstruction algorithms. The algorithm examines the structure present in a pairwise distance matrix to construct a rooted tree (or dendrogram).

	A	B	C	D	E	F
A						
B	2					
C	4	4				
D	6	6	6			
E	6	6	6	4		
F	8	8	8	8	8	

STEP 1
Pairwise evolutionary distances matrix. The shortest distance is 2 between A and B. Join A and B. Recalculate the distances to form a new matrix.

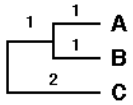
	AB	C	D	E	F
AB					
C	4				
D	6	6			
E	6	6	4		
F	8	8	8	8	

STEP 2
The shortest distance is 4, between D and E. Join D and E. Recalculate the distances to form a new matrix.



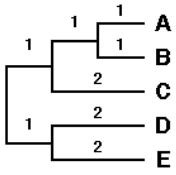
	AB	C	DE	F
AB				
C	4			
DE	6	6		
F	8	8	8	

STEP 3
The shortest distance is 4, between AB and C. Join AB and C. Recalculate the distances to form a new matrix.



	ABC	DE	F
ABC			
DE	6		
F	8	8	

STEP 4
The shortest distance is 6 between ABC and DE. Join ABC and DE. Recalculate the distances to form a new matrix.



	ABCDE	F
ABCDE		
F	8	

STEP 1
The shortest distance is 8 between ABCDE and F. Join ABCDE and F. Complete the tree.

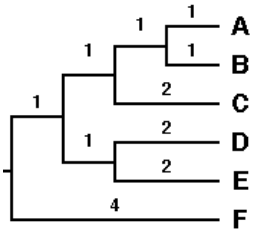


Figure 31 The UPGMA clustering method.

In the UPGMA method each taxon analysed is referred to as an operational taxonomic unit (OTU). At each step of this method, the nearest two OTUs (those OTUs with the shortest distance shown on the distance matrix) are combined into a higher-level cluster. The matrix is then recalculated with the remaining OTUs, with the combined OTUs represented as a single OTU. This process is repeated until all the OTUs have been clustered (Figure 31). The main disadvantage of the UPGMA method is that it assumes a constant rate of evolution, and thus is not a well-regarded method for inferring relationships (Sokal & Michener 1958). UPGMA was performed using MEGA version 5 (Tamura et al. 2011) using the nucleotide substitution model (Goldman & Yang 1994). The phylogeny was tested using the Bootstrap method with 1,000 replications (Efron 1979; Hedges 1992).

3.7.3 Evaluating the Reliability of Inferred Trees

When evolutionary history is inferred from the examination of the current state of things, this is inherently unreliable. However, a measure of how reliable the tree depicts current relationships can be obtained (Felsenstein 1988). Methods presently used in phylogenetic analyses include bootstrapping, Bayesian Inference (or Monte Carlo Testing) and Jacknifing.

3.7.3.1 Bootstrap Analysis

Bootstrap Analysis (or Bootstrapping) is a statistical method for estimating the sampling distribution, by assigning measures of accuracy to sample estimates. In molecular phylogenetics, this technique is used for assessing the robustness of the phylogenetic tree. In this technique, random samples (or subsets) of the original data are selected and the construction of the tree is repeated. Bootstrap support is slightly sensitive to the number of replicates used, and values around 1,000 replicates usually provide satisfactory results. In practice, although it is possible to randomize the taxa, the bootstrap method almost always randomizes the characters. If the tree is reliable, the same tree will be generated each time. The extent to which this is true gives us a measure of the robustness of the tree, and are usually given as percentages, where smaller numbers indicate poor bootstrap support and larger numbers indicate good bootstrap support (Davidson & MacKinnon

2000; Felsenstein 1985; Hedges 1992; Hillis & Bull 1993; Pattengale et al. 2009; Soltis & Soltis 2003).

In phylogenetic analyses, bootstrapping calculates a support value for each node based on the fraction of samples that support that node. The highest support value is 100%, while values below 70% are usually considered weak. As a rule of thumb, values below 50% are not shown, and numbers above 50% are shown near the nodes of phylogenetic trees. Sometimes branches below 50% are collapsed and are shown as a polytomies[46], suggesting that the evolutionary relationships of the taxonomic units within the branch cannot be fully resolved to dichotomies (Davidson & MacKinnon 2000; Purvis & Garland 1993; Soltis & Soltis 2003).

3.7.3.2 Bayesian Inference (Monte Carlo Testing)

Bayesian Inference (BI) is a method of statistical inference in which evidence is used to estimate parameters and predictions in a probability model. BI uses a likelihood algorithm similar to ML and an explicit model of sequence evolution, thus encompassing the strengths of the ML method. Bayesian inference in phylogenetics produces a posterior distribution for a parameter, composed of a phylogenetic tree and a model of sequence evolution, based on the prior for that parameter and the likelihood of the data, generated by multiple alignment (Box & Tiao 1973; Huelsenbeck et al. 2001).

Bayesian Inference is particularly useful when integrated with the MCMC model. With the easy availability of software such as BEAST and MrBayes, phylogenies based on BI and MCMC are efficiently inferred (Lemey et al. 2009; Song 2007).

3.7.3.3 Jackknifing

Jackknifing is a data resampling method similar to bootstrapping and is used to estimate the bias and variance (standard error). In this method one or more samples of the original dataset are systematically excluded (jackknifed) at a time and the estimate recalculated. Based on the remaining set of data, an estimate for the bias and an estimate for the

[46] In a phylogenetic tree, a polytomy represents a node that has more than two immediate descending branches.

variance are calculated (Shao & Tu 1995). The jackknifing method has advantages over bootstrapping, mainly due to its simplicity and relative ease of computation. However, jackknifing performs better with linear data, and irregular data often lead to erroneous results (Shao & Tu 1995).

3.8 Sampling Genetic Material

In molecular phylogenetics studies, appropriate and extensive taxon sampling is very important to ensure accurate phylogenetic estimation and inference of evolutionary history. Insufficient taxon sampling is often cited as a significant source of error in phylogenetic studies, and consequently, acquisition of large data sets is often advocated (Zwickl & Hillis 2002). However, the study by (Rosenberg & Kumar 2001) suggested that longer sequences, rather than extensive sampling, will better improve the accuracy of phylogenetic inference. Both (Zwickl & Hillis 2002) and (Hillis et al. 2003) found that increased taxon sampling resulted in greatly reduced phylogenetic estimation error, and (Pollock et al. 2002) showed that the benefits of increased taxon sampling were similar to adding an equivalent amount of sequence length for the same taxa (in the ranges simulated by (Rosenberg & Kumar 2001). A balance of thorough taxon sampling and sequence length is thus needed to significantly improve the accuracy of evolutionary inferences obtained from phylogenetic analyses. Genetic diversity studies on the other hand, require as many accessions as possible to reveal the genetic differences among the individuals of a selected taxon (Jansen et al. 2005).

To investigate any group of organisms using genetic marker systems, samples of individual accessions need to be collected. In plants, this is usually by collecting samples of tissue, often fresh leaves. The next step is the isolation and purification of genetic material (DNA, RNA or protein) from tissue samples. The purified genetic material is then quantified before analysis using the chosen genetic marker system.

3.8.1 Sample Collection

Sample collection is the first and the most important step in any genetic diversity study. When collecting samples from wild populations, generally the sampling of 30–50 individuals is considered sufficient to provide an insight into the distinctiveness among

the population. However, in *ex situ* collections, the number of individual accessions available for study is limited and is determined by the number of accessions available in such collections. Frequently only a single accession representing a taxon, or a small number of accessions are available for sampling (Jansen et al. 2005).

3.8.2 DNA Isolation

There are a variety of protocols for isolating DNA of both high quality and yield, however the fundamentals of DNA isolation remained the same. In all protocols, DNA is purified from cellular material with minimal degradation, and crude extraction protocols can be adapted to prepare sufficient quantities of DNA to allow for multiple end uses. The most commonly used methods for the isolation of DNA include (i) the CTAB (cetyltrimethylammonium bromide) method (Doyle & Doyle 1987), (ii) the Kobayashi method (Kobayashi et al. 1998), and (iii) the Miniprep Kit method (Qiagen 2006).

3.8.2.1 CTAB Method

The leaves of *Rhododendron* are generally thick and leathery, and those of vireyas in particular, are often covered with dense layers of scales. DNA extraction is known to be difficult, due to the large quantities of these extraneous tissues. Highly viscous polysaccharides are also isolated along with DNA in *Rhododendron* species, which cause problems in the end use (Brown et al. 2006a; Csaikl et al. 1998; Padmalatha & Prasad 2006). Pectin-like polysaccharides are often water soluble and extracted along with DNA, causing the DNA preparations to be highly viscous and inhibiting the activity of restriction enzymes (Do & Adams 1991; Porebski et al. 1997). Several techniques have been developed to overcome this problem. They include: (i) increasing the concentration of CTAB in the DNA isolation buffer when using the CTAB method, (ii) limiting the incubation time to a maximum of 15 minutes during CTAB extraction, and (iii) precipitating DNA at room temperature (Doyle & Doyle 1987).

Plant (and fungal) cells are protected with cell walls, and to isolate DNA from them, the cell walls must be first broken. By homogenizing, the tissue is separated into small groups of cells, and the individual cells can then be lysed (the cell wall and membranes broken down to allow access to nuclear material). A common method employed for lysis is

grinding the plant tissue with lysis chemicals and liquid nitrogen (that enable harmful cellular enzymes and chemicals to remain inactivated). Cell lysis can be achieved by a combination of a chelating agent (EDTA – ethylene diamine tetra-acetate, which also inactivates nucleases) and a detergent such as SDS (sodium dodecyl sulphate, also known as sodium lauryl sulphate). In the CTAB method, a CTAB mixture (commonly referred to as the 'extraction buffer' or 'CTAB buffer') is used for the lysis process. This mixture usually consists of CTAB (cetyl trimethylammonium bromide that acts as a detergent by inhibiting nucleases and helping separate proteins from the nucleic acids), Tris[47]-HCl @ pH 8.0, EDTA @ pH 8.0, NaCl and PVP). The NaCl in this mixture helps to remove proteins that are bound to the DNA, and PVP (polyvinyl-pyrrolidone or vinyl pyrrolidine homopolymer) removes polyphenols during DNA isolation. At pH 8.0 both DNA and RNA are retained in the aqueous phase, and at pH 5–6, DNA is retained in the organic phase while RNA is retained in the aqueous phase. The resulting 'crude' extract from this lysis process contains a complex mixture of DNA, RNA, proteins, lipids and carbohydrates (Dale et al. 2012; Doyle & Doyle 1987).

The next step is the separation of the DNA from the other components. RNA can be removed from DNA by treating with ribonuclease (RNase), which is usually heated to ensure that it is free from traces of deoxyribonuclease (DNase), which degrades DNA. Insoluble particulates are removed through centrifugation while soluble proteins and other substances are separated chemically. The most effective method of removing proteins is by extraction with a mixture of liquefied phenol (a strong denaturing agent for proteins) and chloroform (removes lipids and traces of remnant phenol). When this mixture is vigorously agitated, the proteins are denatured and precipitated at the interphase (and the organic phase), and the nucleic acids can then be recovered from the aqueous phase. An alternative method for the removal of proteins is by using a proteolytic enzyme such as proteinase K, which can digest protein and inactivates DNAse (Dale et al. 2012; Doyle & Doyle 1987).

DNA is then precipitated from the aqueous phase, by adding cold isopropanol or ethanol which precipitates DNA. The precipitated DNA can then be collected at the bottom of the test tube by centrifugation. Contaminating salts are also precipitated with the DNA and

[47] Tris(hydroxymethyl)aminomethane, $(HOCH_2)_3CNH_2$.

can be easily removed by subsequent washing of the precipitated pellet with 70% ethanol. The purified DNA pellet is then dried (to remove the alcohol) and re-suspended in TE (**T**ris-**E**DTA, which solubilizes and prevents degradation of DNA) buffer or sterile distilled water. This method has been shown to yield relatively intact genomic DNA from plant tissue of numerous woody plants (Dale et al. 2012; Doyle & Doyle 1987).

3.8.2.2 Kobayashi Method

An alternative method used for *Rhododendron* and ERICACEAE in general, is the method devised by Kobayashi et al. (1998) (hereinafter referred to as the Kobayashi method), which is a modified version of the CTAB method. The main difference between the CTAB and the Kobayashi methods is that the latter uses two buffers for the cell lysis process. Buffer 1 consists of Tris-HCl, EDTA, sorbitol and PEG 6000 and PVP. Buffer 2 consists of Tris-HCl, EDTA, sorbitol, sodium sarkosyl, NaCl and CTAB. DNA isolated from this method has been shown to be of high quality, free of polysaccharides and polyphenolics, and have been successful in *Rhododendron* (Kobayashi et al. 1998). PEG 6000 in Buffer 1 (Polyethylene glycol – MW 6,000) binds to hydrophobic sites of proteins, and also being an adsorbent removes coloured pigments during DNA isolation (Syamkumar et al. 2005).

3.8.2.3 Miniprep Kit Method

Another commonly used method is the pre-mixed kit method, which has gained popularity recently, mostly due to demand for DNA by high throughput analysis systems. The methods adopted by different manufacturers differ slightly, but all come as kits ready-to-use employing several steps that include spin columns, and enable relatively quick isolation of DNA. One of the most popular is the Qiagen DNeasy™ Plant Mini Kit (Qiagen, Mississauga, Ontario, Canada), which allows the isolation of DNA in less than an hour. The method uses approximately 100 mg of sample tissue, and can yield between 3–30 μg of DNA. The kit method employs a column with a silica-gel-membrane that adsorbs the DNA. This method uses buffers that do not include toxic substances such as CTAB, phenol, or chloroform. The resulting DNA does not require alcohol purification and is ready to use, as it is free from impurities and enzyme inhibitors. In this method, plant tissue is first mechanically disrupted and then chemically lysed, during which RNA

is also removed by digestion with RNase. The cell debris is then discarded and the resulting mixture is filtered in a spin column. Proteins and polysaccharides are precipitated using buffers, and the lysate is then loaded onto the column. During a brief centrifugation, the DNA is selectively bound to the silica-gel membrane while the contaminants are allowed to pass through the filter. Remaining contaminants and enzymes on the silica-gel membrane are removed by one or two wash steps. The resulting purified DNA is then eluted in water or low-salt buffer, ready to be used (Qiagen 2006).

3.8.3 DNA Quantitation

The extracted DNA needs to be quantified and checked for its purity, before any molecular studies can be carried out. There are several methods available to accomplish these goals, and the most common are: (i) direct measurement using spectrophotometry, and (ii) comparison with samples of known concentration using gel electrophoresis.

The spectrophotometric method is known to be inaccurate when samples are contaminated with impurities, such as the remnant chemicals from DNA isolation. However, this quick method requiring <1 μl of DNA provides an accurate estimation of quantity and purity, and is favoured for analysing large numbers of samples (Haque et al. 2003). The spectrophotometric method is based on the UV light absorption by nucleic acids. DNA absorbs light most strongly at the wavelength of 260 nm and the absorption value is denoted as A_{260}. Proteins absorb light most strongly at the wavelength of 280 nm and the absorption value is denoted as A_{280}. Salts and phenols absorb light most strongly at the wavelength of 230 nm and the absorption value is denoted as A_{230}. Other non-specific contaminants absorb at the wavelength of 320 nm and the absorption value is denoted as A_{320}. In older systems a diluted sample is placed in a quartz cuvette and the absorptions for various wavelengths measured. The latest instruments such as the NanoDrop® 2000 use a droplet (<1 μl) of undiluted DNA for measurement of absorption at the various wavelengths. Using undiluted DNA provides a more accurate reading than with the greatly diluted samples required by the older systems.

The DNA concentration is estimated using Beer's Law:

$$\text{Concentration } (\mu g/ml) = (A_{260} - A_{320}) \times 50$$

The purity of the DNA is estimated using the ratio:

$$\text{Purity} = A_{260}/A_{280}$$

Using this ratio, values ranging from 1.7–2.0 suggest good quality DNA samples. The disadvantage with this method is the overestimation of the concentration of DNA when excessive amounts of contaminants are present in the samples analysed. This method is therefore suitable for assessing reasonably pure DNA. The other disadvantage of this method is that there is no assessment of the integrity of the DNA sample, i.e. the sample may be completely degraded and still give a reading (Glasel 1995; Haque et al. 2003; Tataurov et al. 2008).

The gel electrophoresis method is slower, taking several hours, and involves the comparison of DNA samples with a sample of known concentration (also known as a standard or a molecular weight marker). The samples are electrophoresed with the standard in an agarose gel, and the gel is then stained with ethidium bromide, and the stained bands are visualised under UV light. This method can be used for estimating the amount of DNA (or RNA) in each band on the gel, by comparing the intensity of the fluorescence (brightness of the bands) with that of the standard. An additional advantage of this method is that the isolated DNA can be easily visualised and the quality assessed, as the presence of RNA is obvious as is DNA degradation (Dale et al. 2012; Johnson & Grossman 1977).

3.8.4 Preliminary Assessment of DNA Quality

Preliminary assessment of the quality of the DNA for PCR based studies can be carried out using a genetic marker system. RAPD analysis is among the quickest and economical, and can take up to half a day to perform. The assessment of the extracted DNA samples using the RAPD technique is useful as it is sensitive to the quality of the DNA template (Williams et al. 1990).

3.9 Summary

Biodiversity conservation relies on the knowledge of the causes of biodiversity loss and the accurate measurement of biodiversity. The main causes of biodiversity loss are human-related, and are a growing challenge. Various methods have been devised to measure the extant biodiversity, including numeric and genetic methods. Genetic methods are now the tools of choice in determining biodiversity accurately. The genetic method has also the added advantage of conserving the most important component of biodiversity – genetic diversity. It is also important to note that the genetic data can be further complemented with numeric methods such as species diversity indices. The conservation plan and method used for biodiversity conservation is highly dependent on the accurate measurement of biodiversity. Whether the conservation is *in situ* or *ex situ*, the accurate reporting of biodiversity measurements is of utmost importance.

Many molecular methods are available for genetic diversity studies, and the choice again depends on the economics, efficiency and applicability of the methods to the study in question. Scariot et al. (2007) has carried out a comparative study on the discriminating capacity and effectiveness of AFLP, STMS and EST markers in assessing genetic relationships among evergreen azaleas. This study concluded that the joint AFLP, STMS and EST data were remarkably effective for group discrimination and phylogenetic studies. De Keyser et al. (2006) compared EST markers with STMS markers, AFLP markers, microsatellite markers and morphological data. This study showed that although ESTs and STMSs appeared to be the most appropriate markers for paternity analysis and assessment of narrow genetic relationships, AFLP remained the most suitable technique for phylogenetic studies. Amongst all these methods, the microsatellite analysis has an additional advantage, i.e. once they are designed and characterized, they can be readily transferred between laboratories and even between related plant groups. The turnaround times, cost-effectiveness and ease of use of microsatellites are added advantages of these markers. For this study both RAPD markers and microsatellites were evaluated, mainly because the markers are easily obtainable and the facilities for the analysis were available locally.

The choice of the molecular region used to study the phylogeny of the vireyas (or any other group of plants for that matter) is important. Currently, only a few regions are being

employed in these studies, and results and phylogenetic inferences differ slightly. For example the studies using *rpb*2i shows that the vireyas are paraphyletic (Craven et al. 2008) while studies using other regions show them to be monophyletic (Brown et al. 2006a, 2006b). The combination of these DNA regions have given new insights into the relationships within vireyas (Goetsch et al. 2011), and have resolved the higher level classification issues.

There are numerous genetic methods and genetic data analysis methods to choose from in a biodiversity study. The methods chosen are often dependent on the availability of the resources, the ease of use or its efficiency. With regard to the molecular methods, the quickest and most cost-effective ones are preferable. The efficiency of a method is often evident in its popular use (in published literature) and promising results, for example the Kobayashi method for DNA isolation (Kobayashi et al. 1998) appears to be effective in many plant families, and in particular ERICACEAE, to which rhododendrons belong. This method, thus presents itself to be suitable for trial use in the isolation of DNA in rhododendrons. Several recent phylogenetic studies of *Rhododendron* utilized the kit method for the DNA isolation, particularly those studies based on DNA sequencing. The most popular DNA isolation method however still is the CTAB method, and has a proven track record for numerous plant families. Hence, the three DNA isolation methods needed to be evaluated for the vireyas at the beginning of this study, given that there is no proven method for this plant group.

The data analysis methods have their advantages and disadvantages too, but produce reasonably good phylogenetic trees when a sufficiently large number of nucleotides or amino acids are used. However, when the rate of evolution varies extensively from branch to branch, many methods may fail to recover the true topology (Nei 1996). A common practice seen in many studies is that each dataset is analysed using several data analysis methods. For example, a phylogeny can be inferred using any number of methods (such as MP, ME, NJ, etc.), and also can be combined with various combinations of evolutionary models. In this study all the tree inferring methods were evaluated, however the final conclusions were drawn from the Maximum Parsimony trees. Parsimony methods are intuitive, as they choose a minimum number of substitutions, and are deemed to be accurate for very closely taxa, such as the vireyas.

The three DNA isolation methods described in this section will be examined, as they are all available for this study. The most suitable method or combination of methods will be used for the DNA isolation of the selected taxa for this study. The isolated DNA will be quantified and quality-checked using the methods described in this section, and the most suitable method will be determined prior to the molecular analysis.

Chapter 4

Materials and Methods

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4 Materials and Methods

This chapter describes the general materials and methods used leading up to the molecular data analysis. The components described are data collection on all taxa available in literature, collection of plant material in the field, physical examination of the collected samples, molecular laboratory methods and data analysis methods.

4.1 Selection of Taxa for Study

The criteria for the selection of taxa for this study includes the following: (i) availability of the taxa in New Zealand, (ii) IUCN rating indicating taxa of conservation interest (taxa categorized as threatened or data deficient), (iii) taxa related to those selected in (ii). Additional taxa were selected to assist in the phylogenetic studies, which aims to answer the taxonomic and conservation issues. The taxa selected for the study are listed in Appendix 01.

The molecular data available in published literature were obtained from GenBank (www.ncbi.nlm.nih.gov/genbank/) and are listed in Appendix A4.

4.2 Plant Material Collection

The plant material was collected from several collections in New Zealand. The majority of the plant material was collected from Pukeiti Gardens (New Plymouth, New Zealand), with over 160 taxa, and over 400 accessions of vireyas. The second largest collection of vireyas in New Zealand is at the Victoria Esplanade Gardens (Palmerston North, New Zealand). This collection has over 50 accessions, and nearly 25 taxa. Smaller numbers of accessions were also collected from two collections: (i) Keith Adam's private collection (New Plymouth, New Zealand), and (ii) Pukekura Park (New Plymouth, New Zealand) collection. A total of 288 accessions representing approximately 130 taxa were collected from all the combined collections, and are listed in Appendices.

Samples of young, expanding leaves of the plants were harvested in plastic bags and immediately frozen on dry ice, in the field. Where very young leaves were not available,

older leaves, leaf buds or flowers were harvested, in the first instance, and in subsequent collections, young leaves were collected for those accessions. After the leaf harvest, all the samples were transported to the laboratory and transferred to -80ºC freezers until DNA extraction. Voucher specimens of the accessions for the herbarium and for morphological examination were also collected during the leaf harvest. Additional DNA vireya accessions were obtained from the US for selected taxa as extracted DNA, and are listed in Appendix A1 (last table) and A5 (accessions 1–17 in the list). These US samples were obtained for comparison with New Zealand accessions for the genetic diversity analyses, and as key taxa for the phylogenetic analyses in unravelling taxonomic issues.

4.3 Molecular Methods

This section describes the molecular methods used in the phylogenetic and genetic diversity studies. The areas covered include the methods for DNA isolation, DNA quantitation, DNA sequencing, microsatellite analyses and RAPD analyses.

4.3.1 DNA Isolation

There are several methods for DNA isolation, but due to the limited research on vireyas at the time of plant collection for this study, there was no standard reliable method for DNA extraction for this group of plants. To determine the best extraction method for vireyas three commonly used methods were investigated: (i) CTAB method, (ii) Qiagen DNeasy™ Plant Mini Kit (Qiagen, Mississauga, ON, Canada) method, and (iii) the Kobayashi method (Kobayashi et al. 1998).

4.3.1.1 CTAB Method

The accessions were randomly selected for the CTAB DNA isolation method, and are listed in Table 3. Total genomic DNA was extracted using the CTAB method by Doyle & Doyle (1990) as described in this section.

The CTAB buffer was prepared by adding 2.0 g CTAB (hexadecyl trimethyl-ammonium bromide) to 10.0 ml 1 M Tris (pH 8.0). 1 M Tris (pH 8.0) used in the buffer was prepared by dissolving 121.1 g of Tris base in 800 ml of double distilled water, and pH adjusted to

8.0 by adding 42 ml of concentrated HCl. 4.0 ml 0.5 M EDTA (ethylenediaminetetra-acetic acid di-sodium salt) (pH 8.0) was then added followed by 28.0 ml 5 M NaCl. 1 g PVP 40 (polyvinyl-pyrrolidone or vinyl pyrrolidine homopolymer, MW 40,000) was dissolved in 40.0 ml double distilled water. The PVP solution was then and added to the CTAB mixture, and the final mixture was adjusted to pH 5.0 with HCl and made up to 100 ml with double distilled water. The solution was allowed to cool, to room temperature before making the final adjustments to the pH. The volume was adjusted to 1 L with double distilled water, and sterilized using an autoclave.

Table 3 Accessions selected for the DNA isolation by the CTAB method.

#	**Accession No.**	**Taxon**
1	EI135	*R. jasminiflorum*
2	EI136	*R. jasminiflorum*
3	EI137	*R. jasminiflorum*
4	EI138	*R. maxwellii*
5	EI139	*R. bryophilum* (plant does not match the name, appears to be a hybrid involving *R. zoelleri*)
6	EI140	*R. loranthiflorum*
7	EI141	*R. luraluense*
8	EI142	*R. beyerinckianum* × *culminicola*

The plant tissue (~1 g) was ground in plastic bags with liquid nitrogen. The ground material was further ground in ~500 µl of CTAB buffer. The resulting mixture was transferred to a 2 ml Eppendorf™ tube and placed in ice until the remainder of the accessions were ground. The tubes containing the CTAB/plant material mixture were then incubated for ~15 min at 55°C in a recirculating water bath. At the end of the incubation, the tubes were centrifuged at 12,000 g for 5 min to spin down the cell debris. The supernatant was then transferred to clean 2 ml Eppendorf™ tubes, and the remaining plant debris carefully discarded. To each tube containing the supernatant, 250 µl of chloroform:isoamyl-alcohol (24:1) was added, and mixed by inversion, and the tubes were centrifuged at 12,000 g for 1 min. The upper aqueous phase (containing the DNA) was then carefully transferred to clean 2 ml Eppendorf tubes. 50 µl of 7.5 M ammonium acetate was added to each tube, followed by 500 µl of cold absolute ethanol. The tubes were then slowly inverted several times to precipitate the DNA, and centrifuged at 8,000 rpm for 5 minutes. The ethanol was then carefully discarded, leaving the DNA 'pellet' behind. The resulting DNA was re-suspended in cold 70% ethanol, and left to stand

overnight (in the fridge) or for a few hours (on the bench). The tubes were then centrifuged at 8,000 rpm and the ethanol discarded. If the DNA appeared coloured, they were re-suspended in cold 70% ethanol and left to stand for several hours and the ethanol discarded. The cleaned DNA pellet was then dried in a vacuum centrifuge (concentrator), for ~15 minutes (to remove any remaining ethanol). The dried DNA pellet was then suspended in double distilled water, depending on the amount of DNA isolated, and left on the bench until afternoon, or left in the fridge overnight, until fully dissolved.

4.3.1.2 Qiagen DNeasy™ Plant Mini Kit Method

The randomly selected accessions for the DNA isolation by the Qiagen DNeasy™ Plant Mini Kit are listed in Table 4. Total genomic DNA was extracted from ~1g of lyophilized leaf tissue using the DNeasy™ Plant Mini Kit (Qiagen, Mississauga, ON, Canada) following the manufacturer's instructions.

Table 4 Accessions selected for the DNA isolation by the Qiagen DNeasy™ Plant Mini Kit.

#	Accession No.	Taxon
1	EI135	*R. jasminiflorum*
2	EI136	*R. jasminiflorum*
3	EI137	*R. jasminiflorum*
4	EI138	*R. maxwellii*
5	EI139	*R. bryophilum* (plant does not match the name, appears to be a hybrid involving *R. zoelleri*)
6	EI140	*R. loranthiflorum*
7	EI141	*R. luraluense*
8	EI142	*R. beyerinckianum* × *culminicola*
9	EI143	*R. quadrasianum*
10	EI144	*R. quadrasianum*
11	EI145	*R. quadrasianum*
12	EI146	*R. goodenoughii*
13	EI147	*R. christi*
14	EI148	*R. gardenia* 'Odyssey'
15	EI149	*R. retivenium* var. *gracilentum*
16	EI150	*R. majus*
17	EI152	*R. christianae*

The plant material was ground to a fine powder in liquid nitrogen using a roller and a wooden board. The tissue was quickly transferred to 2 ml Eppendorf™ tubes, and 400 µl Buffer AP1 and 4 µl RNase A stock solution (100 mg/ml) was added to the disrupted

plant tissue and vortexed vigorously until no tissue clumps were visible. The material was then further vortexed or pipetted several times to remove any clumps. The mixture was incubated for 10 min at 65°C, and mixed 2 or 3 times during incubation by inverting the tubes. 130 µl of Buffer AP2 was then added to the lysate, mixed, and incubated for 5 min on ice. The lysate was then centrifuged for 5 min at 20,000 g, and was then pipetted into the QIAshredder Mini spin column which is placed in a 2 ml collection tube, and centrifuged for 2 min at 20,000 g. The flow-through fraction from was transferred into a new 2 ml Eppendorf™ tube without disturbing the cell-debris pellet. 1.5 volumes of Buffer AP3/E was added to the cleared lysate, and mixed by pipetting. 650 µl of this mixture was transferred, including any precipitate that have formed, into the DNeasy Mini spin column placed in a 2 ml collection tube. The column was centrifuged for 1 min at ~6,000 g, and the flow-through discarded. The collection tube was re-used and the centrifugation was repeated with the remaining sample. The flow-through and collection tubes were then discarded. The DNeasy Mini spin column was then placed into a new 2 ml collection tube, and 500 µl Buffer AW was added, and centrifuged for 1 min at ~6000 g. The flow-through was then discarded, and the collection tube kept for the next step. 500 µl Buffer AW was added to the DNeasy Mini spin column, and centrifuged for 2 min at 20,000 g to dry the membrane. The DNeasy Mini spin column was transferred to a clean 1.5 ml or 2 ml Eppendorf™ tube, and 100 µl Buffer AE was pipetted directly onto the DNeasy membrane. The tube was then incubated for 5 min at room temperature (15–25°C), and then centrifuged for 1 min at ~6000 g to elute the extract. The extract was purified using standard phenol/chloroform method, ethanol precipitated, and re-suspended in double-distilled water to obtain a concentration of ~200 ng/µl.

4.3.1.3 Kobayashi Method

To evaluate the Kobayashi DNA extraction method, 17 accessions of *Rhododendron* were randomly selected and are listed in Table 5. The Kobayashi method (Kobayashi et al. 1998) uses two separate buffers, and was used to isolate DNA. The Buffer 1 was prepared by mixing 50 mM Tris HCl (pH 8.0), 5 mM EDTA (pH 8.0), 350 mM sorbitol, and 10% PEG 6000. 1% of polyvinylpyrrolidone (PVP) was added to Buffer 1 on the day of extraction. Buffer 2 was prepared by mixing 50 mM Tris HCl (pH 8.0), 5 mM EDTA (pH 8.0), 350 mM sorbitol, 1% sodium sarkosyl, 710 mM NaCl, and 0.1% CTAB (prepared as described in Section 4.3.1.1).

Table 5 Accessions selected for the DNA isolation by the Kobayashi Method.

#	Accession No.	Taxon
1	EI135	*R. jasminiflorum*
2	EI136	*R. jasminiflorum*
3	EI137	*R. jasminiflorum*
4	EI138	*R. maxwellii*
5	EI139	*R. bryophilum* (plant does not match the name, appears to be a hybrid involving *R. zoelleri*)
6	EI140	*R. loranthiflorum*
7	EI141	*R. luraluense*
8	EI142	*R. beyerinckianum* × *culminicola*
9	EI143	*R. quadrasianum*
10	EI144	*R. quadrasianum*
11	EI145	*R. quadrasianum*
12	EI146	*R. goodenoughii*
13	EI147	*R. christi*
14	EI148	*R. gardenia* 'Odyssey'
15	EI149	*R. retivenium* var. *gracilentum*
16	EI150	*R. majus*
17	EI152	*R. christianae*

Frozen leaves (~1g) were ground to a homogeneous pulp with liquid nitrogen by applying a Teflon roller to the sealed bag placed on a wooden board. Two ml of Buffer 1 was added to the ground sample and mixed further. Using sterile scissors, a corner of the bag was cut and the homogenate squeezed into a 2 ml Eppendorf™ tube and placed in ice until the remaining samples have been homogenized. The samples were centrifuged at 2,600 g for 10 minutes, and the supernatant discarded. Samples with large amount of homogenate were re-suspended in Buffer 1 and re-centrifuged, and the supernatant discarded. The resulting pellets were re-suspended in 0.8 ml of Buffer 2, and incubated for 10 minutes in a water bath at 65ºC. The incubated samples were transferred to ice, and 0.8 ml of cold chloroform:octanol (24:1) was added and mixed gently every 2 minutes for 15 minutes. The mixed samples were centrifuged at 18,000 g for 15 minutes, until two separate layers were formed. The top phase was carefully transferred to a 2 ml Eppendorf™ tube using a pipette and 0.6 ml of cold isopropanol (100%) was added and mixed very gently. If no DNA precipitate was observed, the tubes were left on ice for a further 10 minutes. The tubes were further centrifuged at 18,000 g for 5 minutes and the supernatant discarded, leaving the DNA pellet. The precipitated nucleic acids were suspended in 70% ethanol and left overnight. The ethanol is then discarded, and if the pellet appears coloured it was again suspended in 70% ethanol and left overnight or till afternoon. The washed pellet

was dried in a vacuum centrifuge and re-suspended in 50–200 µl (depending on the size of the pellet) of PCR grade water, overnight, or until dissolved completely.

Table 6 Accessions selected for the DNA isolation by the modified Kobayashi Method.

#	Accession No.	Taxon
1	EI185	*R. christianae*
2	EI153	*R. jasminiflorum*
3	EI154	*R. jasminiflorum*
4	EI155	*R. jasminiflorum*
5	EI156	*R. maxwellii*
6	EI157	*R. majus*
7	EI158	*R. majus*
8	EI159	*R. majus*
9	EI160	*R. verticillatum*
10	EI161	*R. verticillatum*
11	EI162	*R. verticillatum*
12	EI163	*R. sp*
13	EI164	*R. yelliotii*
14	EI165	*R. sp*
15	EI166	*R.* 'Felicitas'
16	EI167	*R. sp*
17	EI168	*R. robinsonii*
18	EI169	*R. gardenia* 'Odyssey'
19	EI170	*R. goodenoughii*
20	EI171	*R. goodenoughii*
21	EI172	*R. goodenoughii*
22	EI173	*R. orbiculatum*

Due to the tough leaves, and the presence of scales and polysaccharides, in some of the species of vireyas, the amount of DNA isolated was insufficient and laden with impurities and thus discoloured. To overcome this, the ingredients and some steps were modified: (1) The percentage of PVP used was increased to 2%. (2) An additional wash with Buffer 1 was used to reduce the polysaccharide content and the scales found on some of the species. A second set of accessions were selected to carry out the modified Kobayashi method, and are shown in Table 6.

4.3.2 DNA Quantitation and Quality Assessment

The DNA was quantified and its purity determined using the two methods: (i) spectrometry, and (ii) gel electrophoresis. The performance of the DNA was further assessed using the RAPD protocol.

4.3.2.1 Spectrometric Method

The DNA used for the sequencing reactions were quantified using spectrometry. The machine used was a NanoDrop® 2000 (Thermo Fisher Scientific, Wilmington, Delaware, USA). This instrument directly measured the concentration and sample purity of the DNA sample within 5 seconds and required a minimum of only 0.5 µl. For this study 1 µl of the DNA samples were tested, and the data was exported to a Microsoft Excel spread sheet. The DNA accessions selected for sequencing was quality checked and quantified using the NanoDrop® 2000.

4.3.2.2 Gel Electrophoresis Method

DNA concentration was estimated using agarose gel electrophoresis of samples with a standard DNA sample in a 0.9% agarose 1×TAE (40 mM Tris-HCl pH 8.0, 20 mM acetic acid, 1 mM EDTA pH 8.0) gel. Two aliquots of extracted DNA (1 µl and 2.5 µl) were mixed with 1× loading dye (416 µg/ml bromophenol blue, 416 µg/ml xylene cyanol, 66.6 mg/ml sucrose) before loading onto the agarose gel. For analysis, up to three λ DNA (Invitrogen™ New Zealand Limited, Auckland) standards (equating to 50 ng each) were loaded at intervals along the row of samples. Electrophoresis was carried out for approximately one hour at 70 volts and the gel was stained in 0.5 µg/ml ethidium bromide solution for 30 min and then examined under ultra-violet light (UV). DNA concentrations were estimated by visual comparison of sample band intensity with the λ standard.

4.3.2.3 Preliminary Assessment of DNA Performance

The performance of the extracted DNA from the modified Kobayashi method was tested using the RAPD protocol described in Section 4.3.5. This method has the added advantage of being able to check whether the samples are of high quality for wider

fingerprinting protocols. The RAPD primer OPAX12 (Operon Technologies Inc., Alameda, CA) was used in the analysis.

4.3.3 DNA Sequencing and Analysis

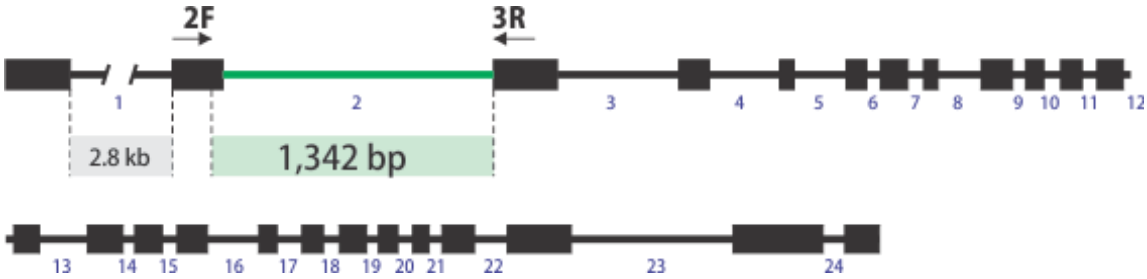

Figure 32 The *rpb*2i gene. Intron (thin line) and exon (solid bar) lengths are shown to scale, with the exception of intron 1 (2.8kb in length). Region sequenced is shown in green (~1 kb in length). The approximate position and polarity of the PCR primers used are shown by an arrow (23F – forward primer, 24R – reverse primer). Adapted from Goetsch et al. (2005).

A total of 87 taxa were selected for DNA sequencing. Taxa were selected based on conservation importance, and additional accessions related to taxa of conservation interest. The number of accessions available for sequencing was also limited due to financial and time constraints.

Goetsch et al. (2005) have shown that the *rpb*2i region (Figure 32) is phylogenetically informative and have successfully used in *Rhododendron*. The 23rd intron of the *rpb*2i region was therefore selected for this study, as this region is approximately 1 kb long, which can be amplified in a single sequencing reaction, but long enough to be used for the phylogenetic analysis to reveal basic taxonomic relationships. Usage of this region also allows the inclusion of published *rpb*2i sequences of *Rhododendron* in this study, enabling comparison of the New Zealand sequences with those previously obtained.

A trial set of samples were analysed by the sequencing reaction, to ensure that the selected primers were amplifying. The annealing temperatures of the primers were initially estimated mathematically, and also analytically determined by running a gradient PCR with the sequencing primers.

The *rpb*2i region was amplified using the primers *rpb*2i-23F (forward – 5' AATTGAGGGCATCTGTCCAGACATC 3') and *rpb*2i-24R (reverse – 5' TCGTATAAGTCA-GCGGACGCCCTG 3') (Goetsch et al. 2005). The PCR was carried out in 20 µl with 10–20 ng of genomic DNA, 20 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 100 µM dNTPs, 2.5 pmol of each primer, and 1.5 units Taq polymerase (Invitrogen, Carlsbad, California, USA). The reactions were carried out on an Eppendorf MasterCycler Pro (Eppendorf AG, Hamburg, Germany) under the following conditions: (1) initial denaturation at 94°C for 4 min; (2) 35 cycles of denaturation at 94°C for 45 s, annealing at 57°C for 45 s, heating ramp of 1°C per 5 s, and extension at 72°C for 45 s to 2 min; (3) final extension at 72°C for 10 min. The sequencing reaction was prepared by adding 5 µl of the PCR product to 8 µl double distilled water and 2 µl of the forward primer for a total volume of 15 µl. The PCR fragments were sequenced directly without purification. Sequence analysis was performed on an ABI 3730XL (PE Applied Biosystem, USA) automated sequencer.

The DNA sequences were assembled, aligned and edited within MEGA version 5 (Tamura et al. 2011). The initial alignment was performed using the ClustalW (Thompson et al. 2002) and MUSCLE (Edgar 2004), which produced very accurate alignments. The resulting alignments were then manually adjusted and fine-tuned before the final genetic analysis. Ambiguity codes were used as required and taken into account in subsequent analyses. Initial alignment of the sequences was performed using the program. The phylogenetic analyses (excluding the Bayesian Inference) were carried out using MEGA version 5. Bayesian Inference was carried out using MrBayes.

4.3.4 Microsatellite Analysis

Microsatellites were used to study the genetic diversity of the selected accessions of vireya taxa. The taxa were selected based on the phylogenetic analyses using the sequence data. The amplification of the microsatellites was carried out using a modified version of the fluorescent M13 universal primer system (Schuelke 2000). The microsatellite forward primers were fluorescently-labelled using FAM, HEX, or NED (Operon Biotechnologies Inc., Huntsville, AL, USA). A touchdown PCR programme with an annealing temperature of 60–55ºC for all primer pairs were carried out. The PCR reactions contained per 10 µl total volume, 1× PCR buffer, 2 mM $MgCl_2$, 0.2 mM dNTPs, 0.3 µM

of each primer, 0.25 units of Taq DNA polymerase (Invitrogen® Platinum™), and 2.5 ng genomic DNA. The concentrations of each component in the PCR reaction were adjusted accordingly when the final volume of the reaction was 15 µl. The PCR protocol consisted of one cycle of initial denaturation at 94°C for 3 min, followed by 35 cycles of denaturation at 93°C for 40 s, annealing at optimum temperature of the primer (60–55°C) for 40 s, and extension at 72°C for 40 s. A final extension cycle at 72°C for 30 min followed. The DNA was amplified in an Eppendorf MasterCycler Pro (Eppendorf AG, Hamburg, Germany).

The PCR reactions were carried out separately for each primer pair and three PCR products (one per microsatellite primer set) were pooled in a multiplex. Multiplexed products were then separated and sized using an ABI 377 sequencer (PE Applied Biosystem, USA) with the software GeneScan (PE Applied Biosystem, USA).

4.3.5 RAPD Analysis

The RAPD analysis was carried out using the amplification programme and reaction mixtures as described in Dehghan-Shoar et al. (1997) except that the annealing temperature was changed to 37°C. The reaction mixture of volume 15 µl consisted of 50 ng of template DNA, 0.17 µl oligonucleotide primer, 10× PCR Buffer, 50 mM $MgCl_2$, 2 mM dNTPs and Taq DNA polymerase (Invitrogen® Platinum™). The PCR amplifications were performed on a Hybaid MBS Satellite 0.5 G Thermal Cycler.

The PCR products were electrophoresed on a 0.9% agarose gel in 1× TBE Buffer (consisting of **T**ris base, **B**oric acid and **E**DTA) at 70 V for 1 hour 45 mins with a 1 kb ladder in the outer lanes of the gel. When the electrophoresis process was completed, the gel was stained with ethidium bromide (1.5 µg/ml) for ~30 minutes. Amplified fragments were visualized under UV light and photographed.

4.4 Data Analysis Methods

Three datasets were used to derive the phylogenetic trees. The first dataset consisted of sequence data obtained for this study from 86 accessions (hereafter referred to as Dataset 1). The second dataset additionally includes sequence data available in the public domain, making a total of 171 sequences (hereafter referred to as Dataset 2). Dataset 3 consists of the data obtained from the microsatellite analyses.

The Datasets 1 and 2 were first analysed to determine the genetic and taxonomic relationships between the taxa. Taxa were selected from the resulting phylogenetic analyses of Dataset 1 & 2, to form the basis of Dataset 3.

4.4.1 Phylogenetic Analyses

Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 5 (Tamura et al. 2011). The Dataset 1 was analysed using four commonly used phylogenetic analysis methods, while the Dataset 2 was analysed using the Maximum Parsimony method only: (i) Maximum Likelihood (ML), (ii) Neighbour Joining (NJ), (iii) Minimum-Evolution (ME), (iv) Maximum Parsimony (MP). The UPGMA method was only used for preliminary analyses for quickly generating trees and testing the datasets.

The initial trees for the Maximum Likelihood trees were determined using the BIONJ method with Maximum Composite Likelihood (MCL) distance matrix. The initial trees for the Neighbour Joining trees were determined using the Maximum Composite Likelihood (MCL) distance matrix.

4.4.1.1 Maximum Likelihood (ML)

Maximum Likelihood (ML) was performed using MEGA version 5 using the Tamura-Nei model evolutionary model. The phylogeny was tested using the Bootstrap method with 1000 replications. Uniform rates among sites were assumed and gaps were treated as complete deletions. The tree inference options ML Heuristic Method was set as Nearest-Neighbour-Interchange (NNI) and automatic initial ML tree generation. The percentage of replicate trees in which the associated taxa clustered together in the

bootstrap test (1000 replicates) >50% are shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by using the following methods: when the number of common sites was <100 or less than one fourth of the total number of sites, the maximum parsimony method was used; otherwise BIONJ method with Maximum Composite Likelihood (MCL) distance matrix was used.

4.4.1.2 Neighbour Joining (NJ)

Neighbour Joining (NJ) was performed using MEGA version 5 using the nucleotide substitution model. The phylogeny was tested using the Bootstrap method with 1000 replications. Uniform rates among sites were assumed and gaps were treated as complete deletions.

4.4.1.3 Minimum Evolution (ME)

Minimum Evolution (ME) was performed using MEGA version 5. The phylogeny was tested using the Bootstrap method with 1000 replications. Uniform rates among sites were assumed and gaps were treated as complete deletions. The substitution model used was Maximum Composite Likelihood (MCL). The tree inference options of ME Heuristic Method were set as Close-Neighbour-Interchange (CNI) and the initial tree generated using the NJ method.

4.4.1.4 Maximum Parsimony (MP)

Maximum Parsimony (MP) was performed using MEGA version 5 using the nucleotide substitution model. The phylogeny was tested using the Bootstrap method with 1000 replications. Uniform rates among sites were assumed and gaps were treated as complete deletions. The tree inference options MP Search Method was set as Close-Neighbour-Interchange (CNI) on Random Trees, with 10 initial trees and MP Search Level 1.

4.4.2 Evaluating the Reliability of Inferred Trees

The jackknifing method will not be used in this study due to its unavailability in the main statistical analysis software packages selected.

4.4.2.1 Bootstrap Analysis

The Bootstrap Analysis was performed using MEGA version 5 with 1,000 replicates for all the analyses based on nucleotide sequence data. The branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed and the percentage of replicate trees in which the associated taxa clustered together in the bootstrap test are shown above the branches.

4.4.2.2 Bayesian Inference

Bayesian Inference (BI) was employed to sample the phylogenetic trees and was performed using MrBayes (version 3.2) with the Markov Chain Monte Carlo chains (MCMC) model (Huelsenbeck et al. 2001; Ronquist et al. 2012). The analysis was started using a random tree and employing 4 simultaneous MCMC chains. After a burn-in period of 2,500 iterations, the analysis was run for 1 million generations of MCMCs and sampled at intervals of 10,000. Average standard deviations of split frequencies were verified to have reached a value lower than 0.01 for all runs. Posterior probabilities of the nodes were computed across the sampled phylogenetic trees after the one million generations.

4.4.3 Genetic Diversity Analyses

This section describes the genetic diversity analyses using microsatellite, RAPD and DNA sequence data. The phylogenetic analyses described in Section 4.4.1 were used to select taxa for the genetic diversity analyses. While the phylogenetic analysis was based on a limited number of accessions of each taxon, more accessions were studied for the genetic diversity analyses. Taxa with multiple accessions were selected to determine the genetic diversity within these taxa.

A total of 192 accessions of *Rhododendron* representing all the sections and subsections of *Rhododendron* Subgenus *Vireya* Argent were selected for the microsatellite analysis. The main criteria for the selection of the accessions, in decreasing order of importance, are:

(a) Conservation interest (based on the IUCN Red List).

(b) Taxa with taxonomic issues related to those selected in (a).

(c) Availability of multiple accessions for a selected taxon.

(d) Multiple accessions of taxa appearing clustered in the phylogenetic analyses.

(e) Representative taxa from sections/subsections of Subgenus *Vireya* for which the DNA samples have amplified.

4.4.3.1 Microsatellite Data

PeakScanner v1.0 software (PE Applied Biosystem, USA) was used to analyse the peaks and the data was entered into Microsoft® Excel (Microsoft Corporation, USA) to generate the data tables required for further analysis. Two different types of data tables were generated for analysis by two different methods:

(a) **binary dataset** – peaks scored as present (1) or absent (0).

(b) **fragment length dataset** – lengths of fragments were recorded as pairs. If a single peak is observed, the fragment length is treated as homozygous and recorded as a repeated pair.

The fragment length dataset was used to compute a distance matrix using Microsatellite Analyser (MSA) 4.05 software (Dieringer & Schlötterer 2003). The resulting distance matrix was used to generate the dendrograms by clustering with the Neighbour Joining method using NTSYSpc software (Rolf 2009).

The NTSYSpc software was also used to generate dendrograms for the binary dataset. A distance matrix was initially generated using Nei's Genetic Distance (Nei 1972). This distance matrix was then clustered using the Neighbour Joining method to generate the dendrograms.

The dataset was refined to include only the accessions that amplified for 95% of the microsatellite markers and preliminary dendrograms were drawn. These dendrograms were further improved by removing accessions of hybrid origin and unrelated taxa.

Accessions of hybrid and unrelated taxa were identified by drawing preliminary dendrograms including all the taxa and subsequently removing accessions that gave rise to extraordinarily high genetic distances (where $(\delta\mu)^2 > 200$).

4.4.3.2 DNA Sequence Data

DNA sequences of taxa with multiple accessions were analysed using MEGA version 5 (Tamura et al. 2011). Pairwise distance matrices were generated using the Tamura-Nei model (distance) (Tamura & Nei 1993), with the substitutions Transitions and Transversions (number of nucleotide substitutions per site). Uniform rate of change among sites and homogenous pattern among lineages were assumed. Gaps within the sequences were treated as partial deletions with the average site cut-off set at 95%. The resulting distance matrices were then exported into NTSYSpc software and clustered with the Neighbour Joining method to generate the dendrograms. The final dendrograms were annotated using CorelDraw® X6 and exported into Microsoft™ Word 2010 as Portable Network Graphics (PNG) files. The PNG files are desirable as they are vector-based and the file sizes are kept at very small sizes.

4.4.3.3 RAPD Data

A small subset of taxa was selected for genetic diversity analysis using RAPD analysis. Clearly resolved polymorphic RAPD bands generated using the primers were scored with Analyze® One 1-D software (Bio-Rad Laboratories, Inc., USA) as present (1) or absent (0). A binary dataset was then produced from this data, which was then analysed with the software NTSYSpc (v. 2.11a; Exeter Software, Setauket, NY, USA) to generate genetic distance dendrograms.

The NTSYSpc software was also used to generate dendrograms for the binary dataset. A distance matrix was initially generated using Nei's Genetic Distance (Nei 1972). This distance matrix was then clustered using the Neighbour Joining method to generate the final dendrograms.

Chapter 5

Results and Analyses

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5 Results and Analyses

This chapter details the results of the experiments that were outlined in Chapter 4. The results arranged into 5 sections, are discussed in detail with respect to the taxonomic and conservation questions raised in Chapter 3. The first section describes the collection and physical examination of the plant material required for this study. In the second section the results and analyses of the DNA extraction methods are outlined which lead to the phylogenetic analyses of vireya taxa. The third section describes the results and analyses of the DNA sequence data. The fourth section describes the results and analyses of the microsatellite data which provide the genetic diversity of selected accessions. This section also utilises the DNA sequence data described in the third section to obtain additional support for the genetic diversity analyses. The fifth section is the culmination of the previous sections which provide the taxonomic and conservation questions raised in Chapter 3. Repetitive and large datasets are provided in the appendices at the end of the document.

5.1 Plant Material Collection

The plant material was collected from 352 accessions which composed of mainly mature plants, but some from seedlings or plants that had not yet flowered. The identities of the majority of plants collected from the Pukeiti collection appear to be accurate, while the accessions from the other collections were questionable, the results of which are detailed in the Appendix A1 The physical examination of the questionable samples revealed that some of these accessions have been mislabelled and the others could be of hybrid or garden origin.

5.2 DNA Isolation & Quantitation

This section details the results of the DNA isolation methods evaluated, and analysis of the final yield and the quality of the isolated DNA. Three common methods were assessed, the CTAB method, the Qiagen Miniprep method and the Kobayashi method. Of these methods assessed, the Kobayashi method proved the most suitable for use in this research.

5.2.1 CTAB Method

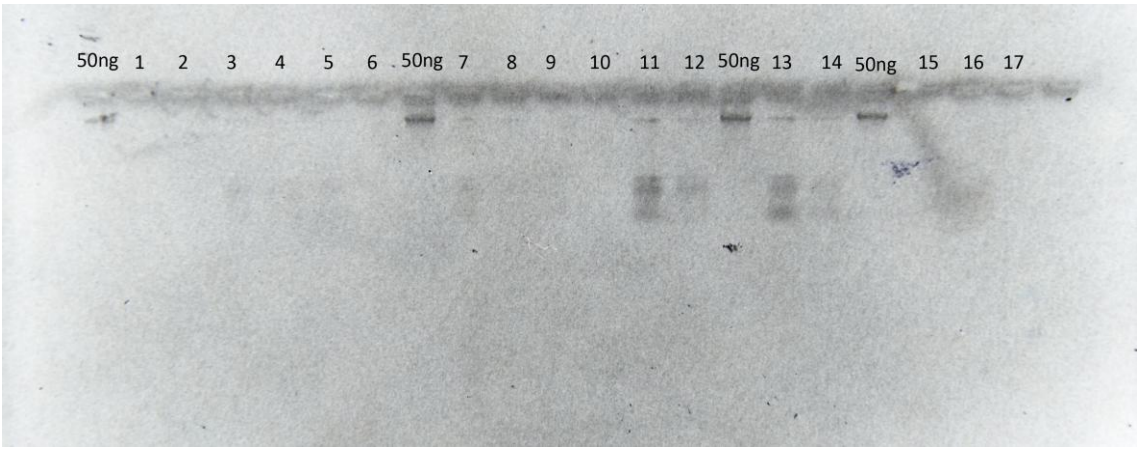

Photo 9 Gel electrophoresis of the DNA isolated from the CTAB method. The lanes marked with 50 ng indicate the λ DNA standards of 50 ng/µl. Lanes 1–7 represents the accessions listed in Table 7 with corresponding numbers (#).

Table 7 Quantity of DNA isolated using the CTAB method.

#	Acc. No.	Taxon	Approx. DNA Conc.
			Dilution 1:2
1	EI135	*R. jasminiflorum*	nil
2	EI136	*R. jasminiflorum*	nil
3	EI137	*R. jasminiflorum*	nil
4	EI138	*R. maxwellii*	nil
5	EI139	*R. bryophilum* (plant does not match the name; possibly a garden hybrid)	nil
6	EI140	*R. loranthiflorum*	nil
7	EI141	*R. luraluense*	trace
8	EI142	*R. beyerinckianum* × *culminicola*	trace
9	EI143	*R. quadrasianum*	nil
10	EI144	*R. quadrasianum*	nil
11	EI145	*R. quadrasianum*	15 ng/µl
12	EI146	*R. goodenoughii*	trace
13	EI147	*R. christi*	15 ng/µl
14	EI148	*R. gardenia* ‘Odyssey’	10 ng/µl
15	EI149	*R. retivenium* var. *gracilentum* (invalid name, collected as is for this trial)	nil
16	EI150	*R. majus*	nil
17	EI152	*R. christianae*	nil

Using the CTAB method, a high proportion of polysaccharides was extracted with the DNA, which made the DNA pellets large and difficult to re-suspend. The gel picture of the DNA samples resulted from the CTAB method is shown in Photo 9, and the results are summarized in Table 7. From Table 7, it can be seen that 11 accessions (~65%) failed to produce any DNA, three accessions produced only trace amounts of DNA (<5 ng/µl), and three accessions produced low quantities of DNA (10–15 ng/µl). The CTAB method is therefore unsuitable to isolate DNA from vireya species, due to the low quality and quantity of DNA the method produces.

5.2.2 Qiagen DNeasy Plant Mini Kit Method

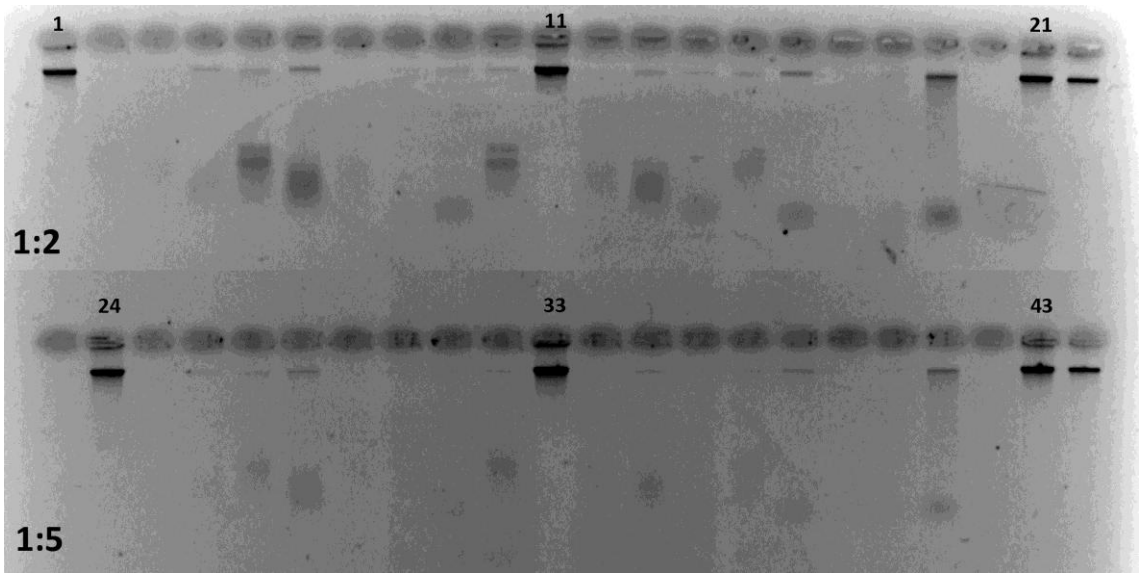

Photo 10 Gel electrophoresis of DNA samples obtained from the Qiagen DNeasy™ Plant Mini Kit Method. Lanes 1–22 correspond to the DNA dilution of 1:2, and lanes 23–44 correspond to the DNA dilution of 1:5. Lanes 1, 11, 21, 24, 33 and 43 are 50 ng/µl DNA standards, while lanes 22 and 44 represent 25 ng/µl DNA standards. Lanes 2–10 represent EI135–EI143, and lanes 23 and lanes 25–32 represent EI135–EI143. Lanes 12–19 represent EI144–EI152, and lanes 34–41 represent EI144–EI152. Lanes 20 and 42 are blank.

The Qiagen DNeasy™ Plant Mini Kit produced very low yields of DNA, and the results are shown in Photo 10 and Table 8. The quality of DNA yielded using this method is relatively high compared with the CTAB method, with very low quantities of impurities such as polysaccharides. The quantity of DNA yielded is also relatively high compared with the CTAB method, with nine accessions (~53%) producing significant quantities. This method of DNA isolation is a better alternative to the CTAB method for vireyas,

however, the quantities of DNA produced are very low. The multiple molecular studies (RAPD, microsatellite and DNA sequencing) carried out in this study require several PCR reactions, as in the case of the microsatellites where each plant will be analysed with over 20 markers, with each marker requiring a quantity of DNA. If however only DNA sequencing is carried out on the samples, the quantities of DNA extracted using this method would suffice.

Table 8 DNA yielded using the Qiagen DNeasy™ Plant Mini Kit. The 'trace' amounts indicate insignificant quantities of DNA (<5 ng/µl).

#	Acc. No.	Taxon	Approx. DNA Conc.	
			Dilution 1:2	Dilution 1:5
1	EI135	*R. jasminiflorum*	trace	trace
2	EI136	*R. jasminiflorum*	nil	nil
3	EI137	*R. jasminiflorum*	5 ng/µl	trace
4	EI138	*R. maxwellii*	5 ng/µl	trace
5	EI139	*R. bryophilum* (plant does not match the name; possibly a garden hybrid)	5 ng/µl	trace
6	EI140	*R. loranthiflorum*	10 ng/µl	5 ng/µl
7	EI141	*R. luraluense*	trace	trace
8	EI142	*R. beyerinckianum* × *culminicola*	trace	trace
9	EI143	*R. quadrasianum*	trace	trace
10	EI144	*R. quadrasianum*	nil	nil
11	EI145	*R. quadrasianum*	5 ng/µl	trace
12	EI146	*R. goodenoughii*	5 ng/µl	trace
13	EI147	*R. christi*	5 ng/µl	trace
14	EI148	*R. gardenia* 'Odyssey'	10 ng/µl	5 ng/µl
15	EI149	*R. retivenium* var. *gracilentum* nom. inval. (collected as is for this trial)	nil	nil
16	EI150	*R. majus*	trace	trace
17	EI152	*R. christianae*	25 ng/µl	10 ng/µl

5.2.3 Kobayashi Method

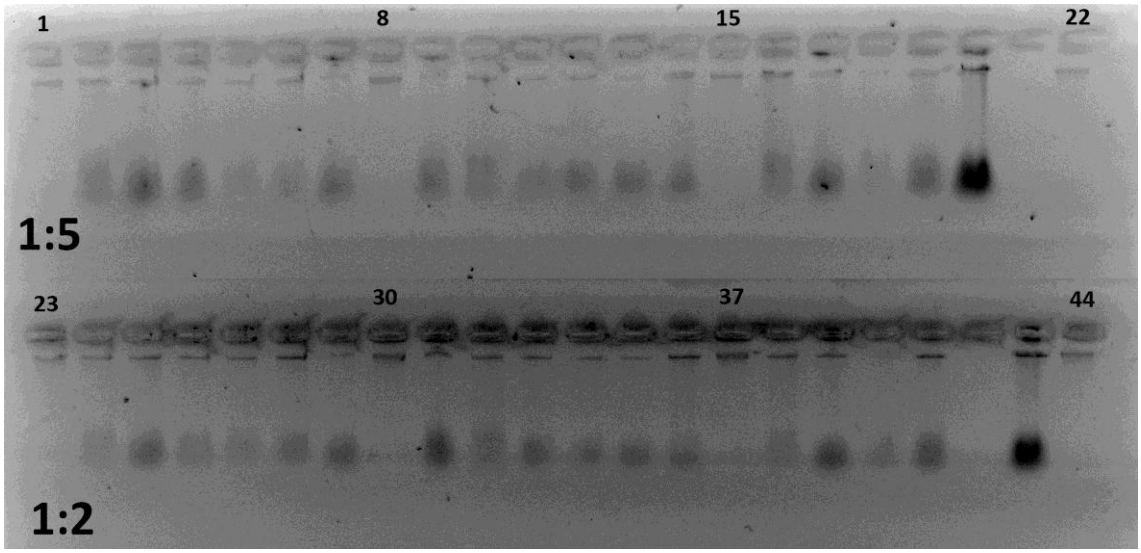

Photo 11 Gel electrophoresis of DNA samples yielded using the Kobayashi et al. (1998) method. Lanes 1, 8, 15, 22, 23, 30, 37 and 44 are 50 ng/µl DNA standards, while lanes 22 and 44 represent 25 ng/µl DNA standards. Lanes 1–22 correspond to the DNA dilution of 1:5, and lanes 23–44 correspond to the DNA dilution of 1:2. Lanes 2–7, 9–14, 16–21 represent EI135–150 and EI152. Lanes 24–29, 31–36 and 38–43 represent EI135–150 and EI152. Lanes 21 and 42 are blank.

Table 9 DNA yielded from the Kobayashi method.

#	Acc. #	Taxon	Approx. DNA Conc.	
			Dilution 1:5	Dilution 1:2
1	EI135	*R. jasminiflorum*	25 ng/µl	30 ng/µl
2	EI136	*R. jasminiflorum*	25 ng/µl	40 ng/µl
3	EI137	*R. jasminiflorum*	25 ng/µl	40 ng/µl
4	EI138	*R. maxwellii*	20 ng/µl	30 ng/µl
5	EI139	*R. bryophilum* (mislabelled garden hybrid)	20 ng/µl	50 ng/µl
6	EI140	*R. loranthiflorum*	15 ng/µl	25 ng/µl
7	EI141	*R. luraluense*	15 ng/µl	30 ng/µl
8	EI142	*R. beyerinckianum* × *culminicola*	20 ng/µl	30 ng/µl
9	EI143	*R. quadrasianum*	20 ng/µl	30 ng/µl
10	EI144	*R. quadrasianum*	20 ng/µl	20 ng/µl
11	EI145	*R. quadrasianum*	15 ng/µl	20 ng/µl
12	EI146	*R. goodenoughii*	20 ng/µl	50 ng/µl
13	EI147	*R. christi*	30 ng/µl	40 ng/µl
14	EI148	*R. gardenia* ‘Odyssey’	20 ng/µl	40 ng/µl
15	EI149	*R. retivenium* var. *gracilentum* nom. inval.	trace	5 ng/µl
16	EI150	*R. majus*	20 ng/µl	40 ng/µl
17	EI152	*R. christianae*	30 ng/µl	20 ng/µl

An example of the results using the Kobayashi et al. (1998) method is shown in Photo 11 and summarized in Table 9. All the accessions except EI149, yielded DNA >20 ng/µl (at 1:2 dilution).

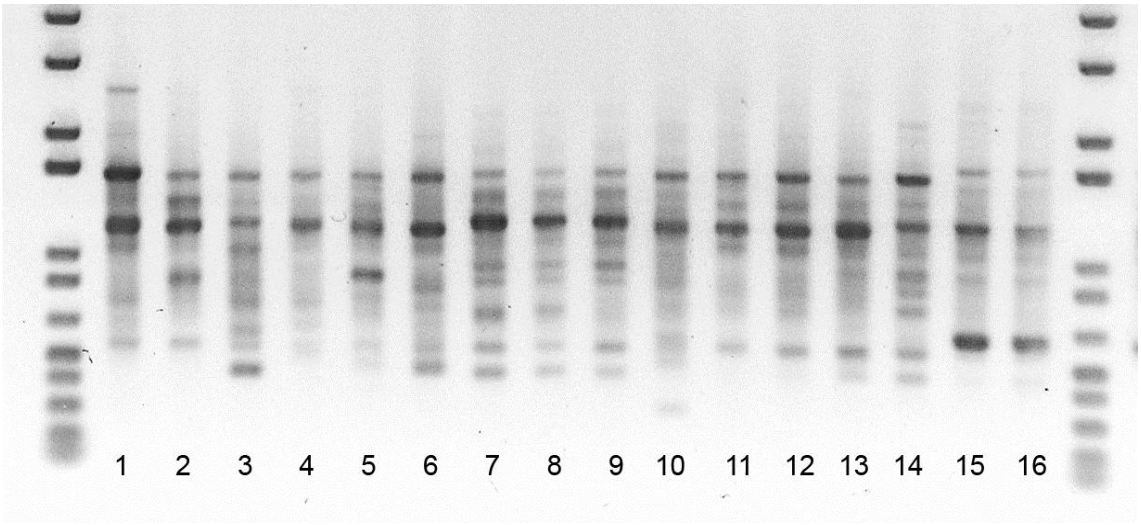

Photo 12 RAPD analysis of DNA from a selection of *Rhododendron* sect. *Vireya* species using the RAPD primer OPAX12 with DNA extracted using the Kobayashi method. Lane 1: *Rhododendron christianae* (EI152), Lane 2: *R. majus* (EI150), Lane 3: *R. retivenium* var. *gracilentum* nom. inval. (EI149), Lane 4: *R. gardenia* 'Odyssey' (EI148), Lane 5: *R. christi* (EI147), Lane 6: *R. goodenoughii* (EI146), Lanes 7–9: *R. quadrasianum* (EI144, EI143), Lane 10: *R. beyerinckianum* × *R. culminicola* (EI142), Lane 11: *R. luraluense* (EI141), Lane 12: *R. loranthiflorum* (EI140), Lane 13: *R. bryophilum* (a wrongly labelled hybrid) (EI139), Lane 14: *R. maxwellii* (EI138), Lanes 15–16: *R. jasminiflorum* (EI137, EI136). The outside lanes contain a 1 kb+ DNA ladder.

The performance of the extracted DNA from the Kobayashi method was determined by RAPD analysis utilizing the primer OPAX12 (Operon Technologies Inc., Alameda, California, USA) as shown in Photo 12. The RAPD fingerprint indicated that the quality of the DNA isolated is of high quality and suitable for molecular studies.

The young and tender leaves of vireyas are often covered with hairs and scales which are sometimes persistent in the final DNA samples, and to overcome this problem a modification of the Kobayashi method was tested. When PVP is added along with CTAB in the extraction buffer, it binds to the polyphenol compounds by forming a complex with hydrogen bonds and helps in removal of impurities. Chloroform:octanol removed chlorophyll and other colouring substances, such as pigments, dyes, etc. In some species the colour was persistent; however this did not affect the performance of PCR reactions.

The use of 2% of PVP during DNA extraction in the modified Kobayashi method significantly reduced the amount of resulting polysaccharides. The additional wash step also removed the polysaccharides along with other impurities that coloured the final DNA pellet.

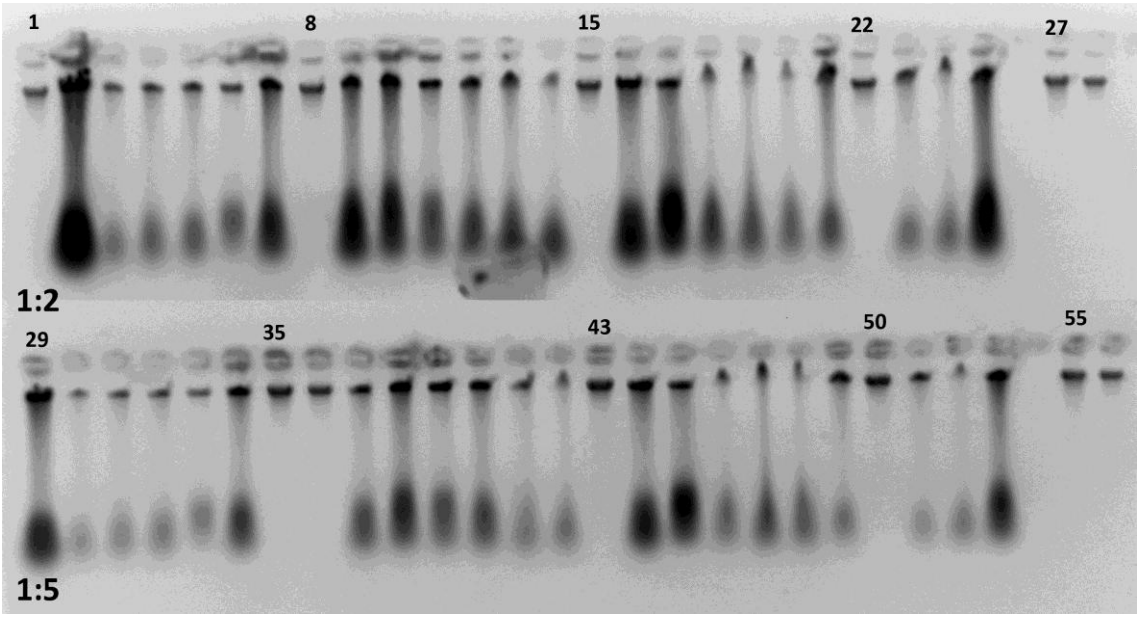

Photo 13 Gel electrophoresis of DNA samples obtained from the modified Kobayashi method. The method used PVP 2% and an additional wash step. Lanes 1, 8, 15, 22, 27, 35, 43, 50 and 55 are 50 ng/µl DNA standards, while lanes 28, 36 and 56 represent 25 ng/µl DNA standards. Lanes 1–25 correspond to the DNA dilution of 1:2, and lanes 29–53 correspond to the DNA dilution of 1:5. Lanes 2–7, 9–14, 16–21 and 23–25 represent EI185, EI153–173. Lanes 29–34, 37–42, 44–49 and 51–53 represent EI185, EI153–173.

Table 10 DNA yielded from the modified Kobayashi method, with 2% PVP and an additional wash step.

#	Acc. #	Taxon	Approx. DNA Conc.	
			Dilution 1:5	Dilution 1:2
1	EI185	*R. christianae*	50 ng/µl	100 ng/µl
2	EI153	*R. jasminiflorum*	10 ng/µl	50 ng/µl
3	EI154	*R. jasminiflorum*	15 ng/µl	50 ng/µl
4	EI155	*R. jasminiflorum*	15 ng/µl	50 ng/µl
5	EI156	*R. maxwellii*	15 ng/µl	50 ng/µl
6	EI157	*R. majus*	50 ng/µl	100 ng/µl
7	EI158	*R. majus*	50 ng/µl	100 ng/µl
8	EI159	*R. majus*	80 ng/µl	100 ng/µl
9	EI160	*R. verticillatum*	80 ng/µl	100 ng/µl
10	EI161	*R. verticillatum*	80 ng/µl	100 ng/µl

11	EI162	*R. verticillatum*	25 ng/µl	50 ng/µl
12	EI163	*R. sp.*	nil	25 ng/µl
13	EI164	*R. yelliotii*	80 ng/µl	100 ng/µl
14	EI165	*R. sp.*	50 ng/µl	100 ng/µl
15	EI166	*R.* 'Felicitas' (garden hybrid)	nil	nil
16	EI167	*R.* 'Red Rover' (*R. viriosum* × *javanicum*)	nil	nil
17	EI168	*R. robinsonii*	nil	nil
18	EI169	*R. gardenia* 'Odyssey'	50 ng/µl	25 ng/µl
19	EI170	*R. goodenoughii*	50 ng/µl	50 ng/µl
20	EI171	*R. goodenoughii*	nil	nil
21	EI172	*R. goodenoughii*	80 ng/µl	100 ng/µl
22	EI173	*R. orbiculatum*	nil	nil

The results of the modified Kobayashi method are shown in Photo 13 and Table 10. The DNA yielded with this method is nearly double the amount yielded with the unmodified Kobayashi method, the majority of the accessions yielding 40–100 ng/µl of DNA (at 1:2 dilution). Five accessions however failed to yield any DNA. This was in part due to the mature leaves that were used for the DNA isolation. The modified Kobayashi method therefore yielded the highest quantity and quality of DNA and was used in subsequent DNA isolations.

5.2.4 Summary

The three DNA extraction methods assessed had their individual useful aspects, but the modified Kobayashi method yielded DNA with high quality and quantity. The quality of the DNA was markedly higher using the DNeasy Kit method, however, the quantity of the DNA isolated was relatively low for subsequent multiple molecular analyses. This method may perhaps be used for sequencing reactions, where high quality and small quantities of DNA are desired. The CTAB method yielded larger quantity DNA compared to that of the DNeasy Kit method but insufficient for the proposed multiple molecular analyses. The quality of the DNA was diminished compared with the DNeasy Kit method, as significantly large quantities of impurities were also extracted with the DNA.

The Kobayashi method produced higher concentrations of DNA compared with the CTAB and the DNeasy Kit method, but with diminished quality. However, the RAPD analysis of the DNA samples showed (Photo 12) that the quality of the samples were

suitable for subsequent molecular analyses. The modified Kobayashi method produced the largest quantities of DNA among all the methods assessed, and the quality of the DNA was at an acceptable level for the subsequent molecular analyses (Photo 13, Table 10).

5.3 Phylogenetic Analyses

This section encompasses the phylogenetic analyses using the DNA sequence data employing various analysis and presentation methods, each providing alternative hypotheses to the species relationships of the vireya taxa.

The phylogenetic analyses were carried out based on sequencing of 100 accessions, out of which 86 (86%) produced sequences with good resolution suitable to be used in subsequent analyses. Fourteen accessions had poor quality sequences due to the following factors: (i) low quality of DNA, since many of the samples were stored in the fridge during the various molecular analyses of this study over a longer time period, (ii) the PCR products of the sequencing reaction were not cleaned (to separate the amplified fragments from other debris) prior to sequencing (due to financial limitations), causing erroneous sequences, eventually leading to base calling errors, (iii) the sequencing was carried out only in one direction (forward) due to financial limitations, and since the intron 23 of *rpb*2i is ~1 kb long, the resolution decreased after 500–600 bases. The fragment lengths of the selected 86 good quality sequences varied from 884–910 bases.

The aligned matrix of the intron 23 of *rpb*2i region therefore consisted of 86 nucleotide sequences with a total of 937 sites (characters or positions), of which 782 (83.5%) are conserved. This matrix represents 84 accessions from New Zealand and two accessions from USA (RSF collection), hereinafter referred to as Dataset 1 (Appendix 0). The base composition of the *rpb*2i intron region 23F–24R was relatively constant: 61.5–63.2% AT and 36.8–38.5% GC. A total of 148 (15.8%) sites were variable, with 63 (42.6%) of these parsimony-informative, and 85 (57.4%) singletons.

A second dataset (Dataset 2) was formed using the sequences of Dataset 1 and sequences from published literature (85 sequences), for a total of 171 nucleotide sequences. The sequence lengths varied from 875–912 bases. The base composition of the *rpb*2i intron region 23F–24R was relatively constant: 60.9–63.3% AT and 36.7–39.1% GC. The final

aligned matrix for the Dataset 2 (Appendix 0) consisted of 945 sites, 644 (68.1%) sites of which were conserved and 149 (15.5%) sites were singletons. A total of 286 (29.7%) sites were variable, with 137 (47.9%) of these parsimony-informative. A condensed view of the sequenced data is shown in Figure 33, which can be used to easily visualize the indels which are summarized in Table 11.

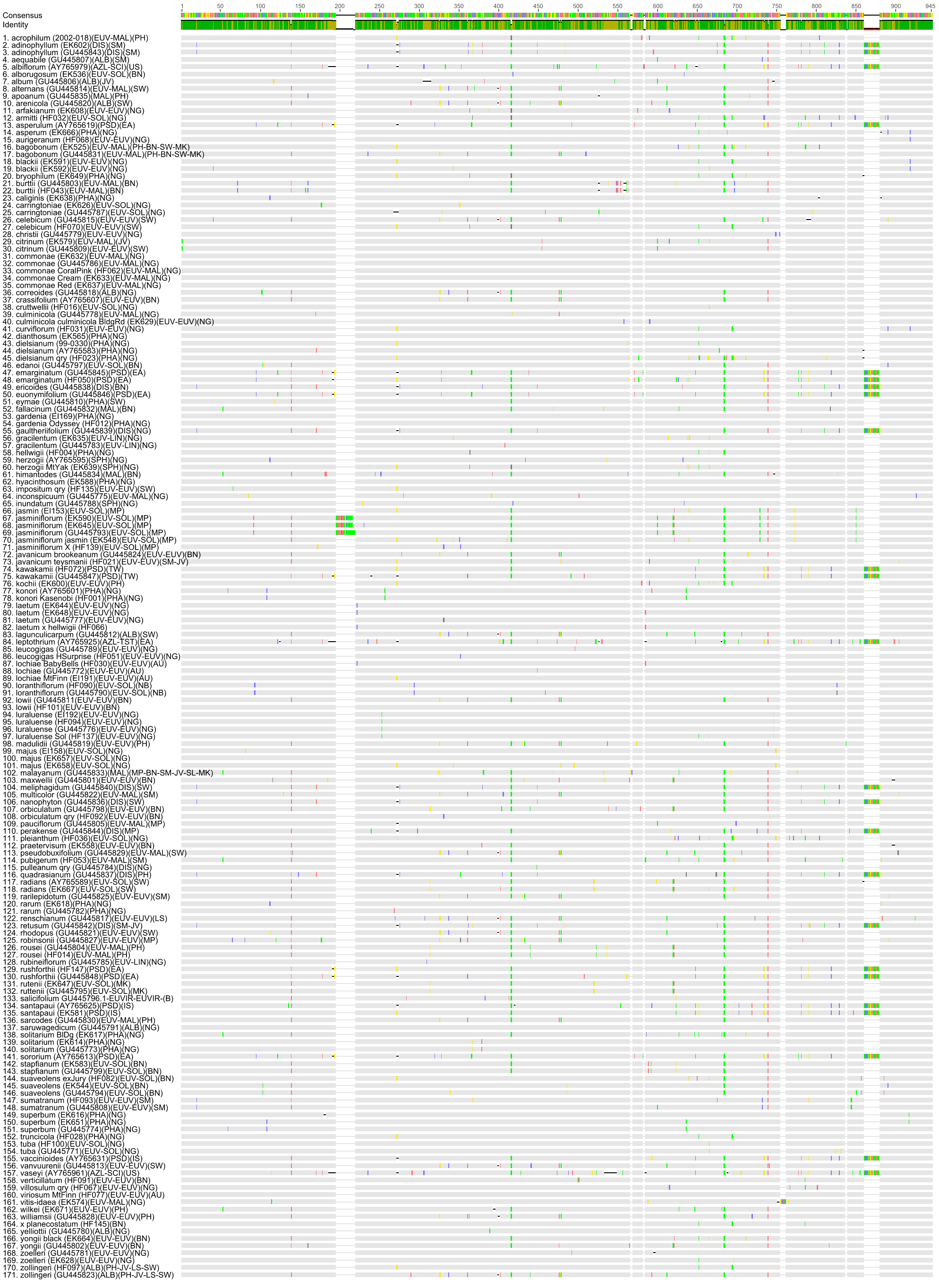

Figure 33 Condensed view of the sequenced data of Dataset 2 for the intron 23 of the *rpb*2i region. The indels seen in this figure are summarized in Table 11.

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Table 11 Indel list for intron 23 of the *rpb*2i region (Dataset 2).

#	Site Range	Type	Taxa
1	186-195	Deletion	Subgenus *Azaleastrum* (sections *Tsutsusi* and *Sciadorhodion*).
2	196–219	Insertion	*R. jasminiflorum* ssp. *oblongifolium* (EK590, EK645 and GU445793).
3	272–274	Deletion	Section *Pseudovireya* and Section *Discovireya*. Exceptions are an accession of *R. carringtoniae* and an accession of *R. pauciflorum*.
4	289–291	Deletion	Section *Pseudovireya*, Section *Discovireya*, Subgenus *Azaleastrum* (sections *Tsutsusi* and *Sciadorhodion*).
5	558–560	Deletion	*R. burtii* (HF043, GU445803).
6	566–568	Insertion	*R. malayanum* (GU445833).
7	583–584	Insertion	*R. albiflorum* (AY765979) (Subgenus *Azaleastrum* Section *Sciadorhodion*).
8	585–586	Deletion	*R. leptothrium* (AY765925) (Subgenus *Azaleastrum* Section *Tsutsusi*) and *R. vaseyi* (AY76596) (Subgenus *Azaleastrum* Section *Sciadorhodion*).
9	751–753	Deletion	*R. vitis-idaea* (EK574)
10	755–761	Insertion	*R. vitis-idaea* (EK574)
11	860–879	Insertion	Section *Pseudovireya*, Section *Discovireya*, Subgenus *Azaleastrum* (sections *Tsutsusi* and *Sciadorhodion*).

From the indels 1 and 7 are unique features of the outgroup taxa Subgenus *Azaleastrum*. Indel 2 is unique to the taxon *R. jasminiflorum* ssp. *oblongifolium* and therefore can be used to identify this subspecies from (perhaps) other subspecies of *R. jasminiflorum*.

The Indel 3 is unique to the sections *Pseudovireya* and *Discovireya* with the exceptions of the accessions of *R. carringtoniae* and *R. pauciflorum*. The sequence of *R. carringtoniae* from published data (GU445787) has a deletion between 268–274 which was not found in the accession from the Pukeiti collection (EK626). This may be due to these accessions being collected from geographically separate localities. The deletion in the accession of *R. pauciflorum* from Malayan Peninsula may in part be due to the geographic proximity of this species to the range of the sections *Pseudovireya* and *Discovireya*.

The indels 4 and 11 are shared by the sections *Pseudovireya* and *Discovireya* and also by the outgroup taxa (outgroup) Subgenus *Azaleastrum* (sections *Tsutsusi* and *Sciadorhodion*) predominantly temperate. This supports the close relationship of the temperate rhododendrons to the sections *Pseudovireya* and *Discovireya* suggested in the

traditional classifications (Argent 2006; Sleumer 1966b). This also supports the placement of the sections *Pseudovireya* (mainland Asia) and *Discovireya* (predominantly Malayan Peninsula to SE Asia) sister to the predominantly Malesian core vireyas. These characteristic indels can be therefore be used to identify the sections *Pseudovireya* and *Discovireya* by just sequencing this intron.

Phylogenetic trees derived from the Dataset 1 of *rpb*2i sequences are shown in Figure 34–Figure 38. Phylogenetic trees derived from the Dataset 2 of *rpb*2i sequences are shown in Figure 41–Figure 46.

5.3.1 Dataset 1

This section describes the results and discussions surrounding the phylogenetic analyses based on the DNA sequences of Dataset 1.

5.3.1.1 Maximum Likelihood

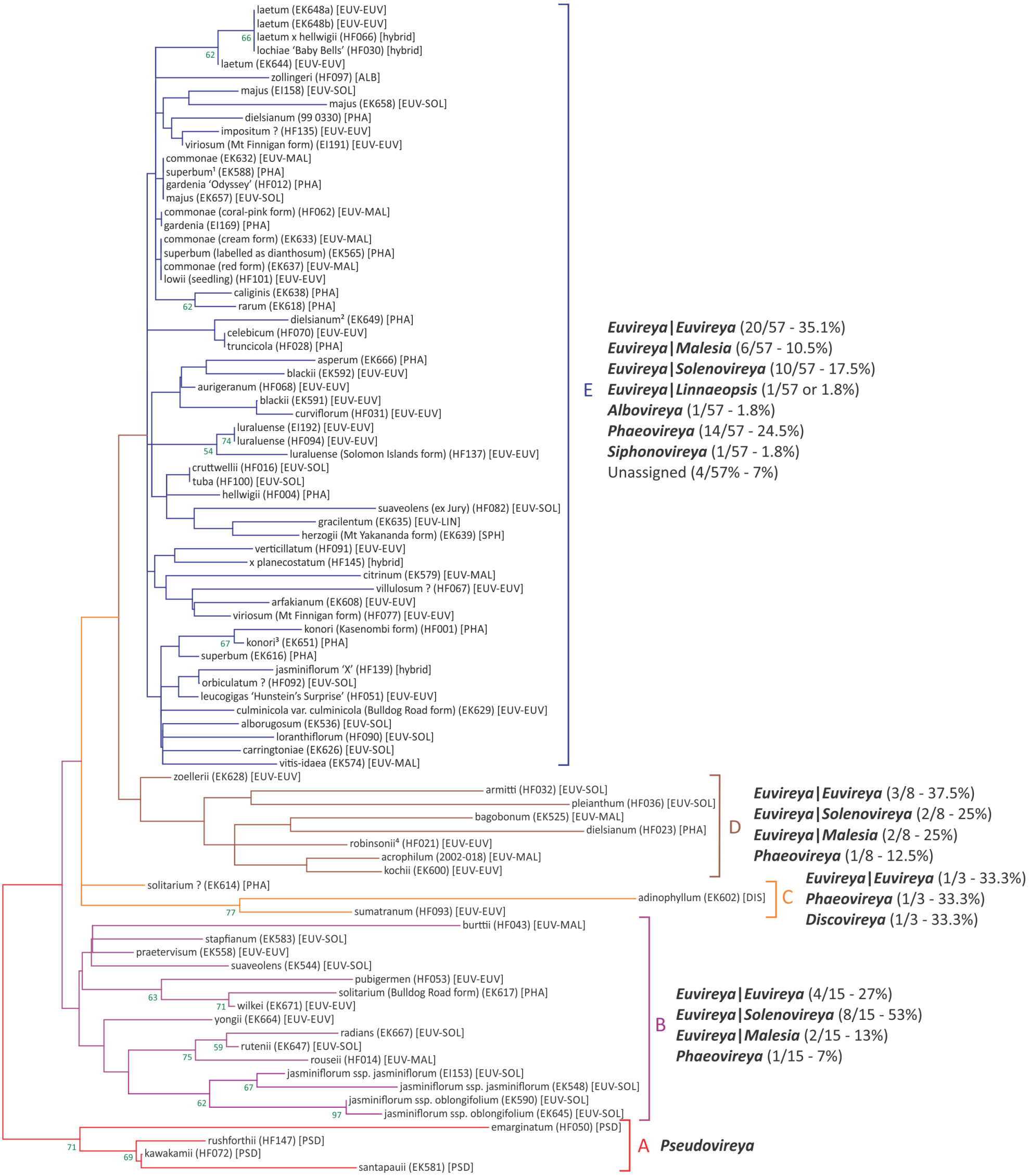

Figure 34 Maximum Likelihood consensus tree using *rpb*2i nucleotide sequence data (Dataset 1). The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model (Tamura & Nei 1993). The bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the taxa analysed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) >50% are shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically as follows. When the number of common sites was < 100 or less than one fourth of the total number of sites, the maximum parsimony method was used; otherwise BIONJ method with MCL distance matrix was used. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 87 nucleotide sequences, with a total of 962 positions in the final dataset. The text within the brackets shows the accession number of the sample. Accessions marked with numbers were labelled in the collection as: (1) *R. hyacinthosmum* (EK588) (2) *R. bryophilum* (EK649), (3) *R. superbum* (EK651), (4) *R. javanicum* ssp. *teysmannii* (HF021).

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The phylogenetic tree in Figure 34 shows the analysis by the Maximum Likelihood method using *rpb*2i nucleotide sequence data (Dataset 1). The evolutionary history was inferred based on the Tamura-Nei model (Tamura & Nei 1993) and the bootstrap support was determined from 1,000 replicates. Five major clades can be identified from the tree loosely corresponding to taxonomic groups. Clade A corresponds to the Section *Pseudovireya*, which is geographically isolated from the majority of the Malesian taxa, and has moderate bootstrap support (71%). This clade also forms a sister group to the rest of the taxa (Clades B–E). The distinctness of Section *Pseudovireya* from the rest of the vireyas is supported by the absence of any of its taxa in the other clades (B–E).

Clade B consists of taxa predominantly belonging to the Section *Euvireya* (14/15 or 93%). The Section *Euvireya* is represented by the subsections *Solenovireya* (8/15 or 53%), *Euvireya* (4/15 or 27%) and *Malesia* (2/15 or 13%). A single representative of the Section *Phaeovireya* is found in this cluster (7%). The remainder of the *Solenovireya* taxa are mostly found scattered in Clade E without forming a significant cluster. This clade mainly consists of taxa exhibiting *jasminiflorum*-type flowers (long-tubular), with all the accessions of *R. jasminiflorum* clustering together with moderate bootstrap support (62%). Two subclades corresponding to the two subspecies (*jasminiflorum* and *oblongifolium*) can be observed within *R. jasminiflorum*. The ssp. *oblongifolium* has very strong bootstrap support (97%) supporting the subspecies status. Clade B also contains several taxa that are not in the Subsection *Solenovireya* Argent. *R. praetervisum* (Subsection *Euvireya* Argent) and *R. solitarium* (Section *Phaeovireya* Argent) in this clade has long corolla tubes akin to *R. jasminiflorum*. *R. rousei*, *R. burttii*, *R. wilkiei* and *R. yongii* also belonging to other sections of Subgenus *Vireya* but do not have the long corolla tubes that are characteristic of the Subsection *Solenovireya*. Their placement within this clade cannot be easily explained and need further study involving multiple accessions of these taxa and other taxonomically related taxa.

Clade C consists of *R. solitarium*, *R. adinophyllum* and *R. sumatranum*, and these taxa are sister to the Clades D and E. There are no shared morphological features between these three taxa, geographically however *R. adinophyllum* and *R. sumatranum* are from the same region in N Sumatra and a hybrid between them was once collected (Argent 2006). The position of the *R. solitarium* accession (EK614) in this clade is questionable

since *R. adinophyllum* belongs to the Section *Discovireya* and *R. sumatranum* belongs to Subsection *Euvireya*, while *R. solitarium* belongs to Section *Phaeovireya* and native to Papua New Guinea (geographically distant) and very distinct from *R. adinophyllum* and *R. sumatranum*. This position of *R. solitarium* accession (EK614) could be due to several factors such as base calling errors or the accession being of hybrid origin. The latter is highly probable, however this accession of *R. solitarium* has never flowered and its identity cannot be adequately verified.

Clade D is sister to the remainder of the taxa (Clade E) and consists of a dissimilar ensemble of taxa, belonging to several subsections of Subgenus *Vireya* Argent. However, the subclades within Clade D show shared morphological characteristics. For example *R. armitii* and *R. pleianthum* belongs to the Subsection *Solenovireya*, and share some similar morphological characters. These two taxa have relatively similar flower shapes and originate from Papua New Guinea (W New Guinea), but they have no known taxonomic relationships between them. Other floral morphology similarities between the taxa include *R. zoelleri*, *R. acrophilum* and *R. robinsonii* sharing similar floral shape. *R. bagobonum* and *R. dielsianum* (HF023, identity not confirmed) even though clustering together, do not share any common morphological features.

Clade E consists of the majority of the taxa of the phylogenetic tree, and belongs primarily to the Section *Euvireya*. The majority of the taxa of this clade are native to E Malesia, mainly distributed throughout New Guinea. Due to the relatively short sequence of DNA used for the study, the taxa within this clade were well not resolved. However, small clusters with shared morphological characters are discernible: (i) *R. konori* and *R. superbum* accessions cluster together, and have very similar morphological characters very often confused with each other, (ii) the three accessions of *R. luraluense* cluster together with moderate bootstrap support (54%), (iii) *R. superbum* (EK588, labelled as *R. hyacinthosmum*) and *R. gardenia* ‘Odyssey’ (HF012) cluster together, and have very similar floral characteristics and have known taxonomic issues (Argent 2006). The two accessions of *R. gardenia* ‘Odyssey’ (EI169 and HF012) does not cluster together. This taxon is thought to be a hybrid between *R. zoelleri* and *R. gardenia*, (iv) *R. laetum* accessions cluster together with moderate bootstrap support (62%).

The accessions of *R. carringtoniae* (EK626) and *R. tuba* (HF100) does not cluster together refuting the hypothesis that these two are related, where *R. tuba* is thought to be an intermediate taxon between *R. carringtoniae* and *R. rhodoleucum* both of which originate from the Maneau Range (Papua New Guinea)(Argent 2006). All the accessions of *R. commonae* are in a subclade within Clade 3, but they do not cluster together and supports the observed level of physical variation seen within this species.

The accessions of the vegetatively similar taxa *R. luraluense* (Subsection *Euvireya* Argent) and *R. loranthiflorum* (Subsection *Solenovireya* Argent) do not cluster together. *R. luraluense* differs from *R. loranthiflorum* by having shorter funnel-shaped corolla and larger lobes (Argent 2006).

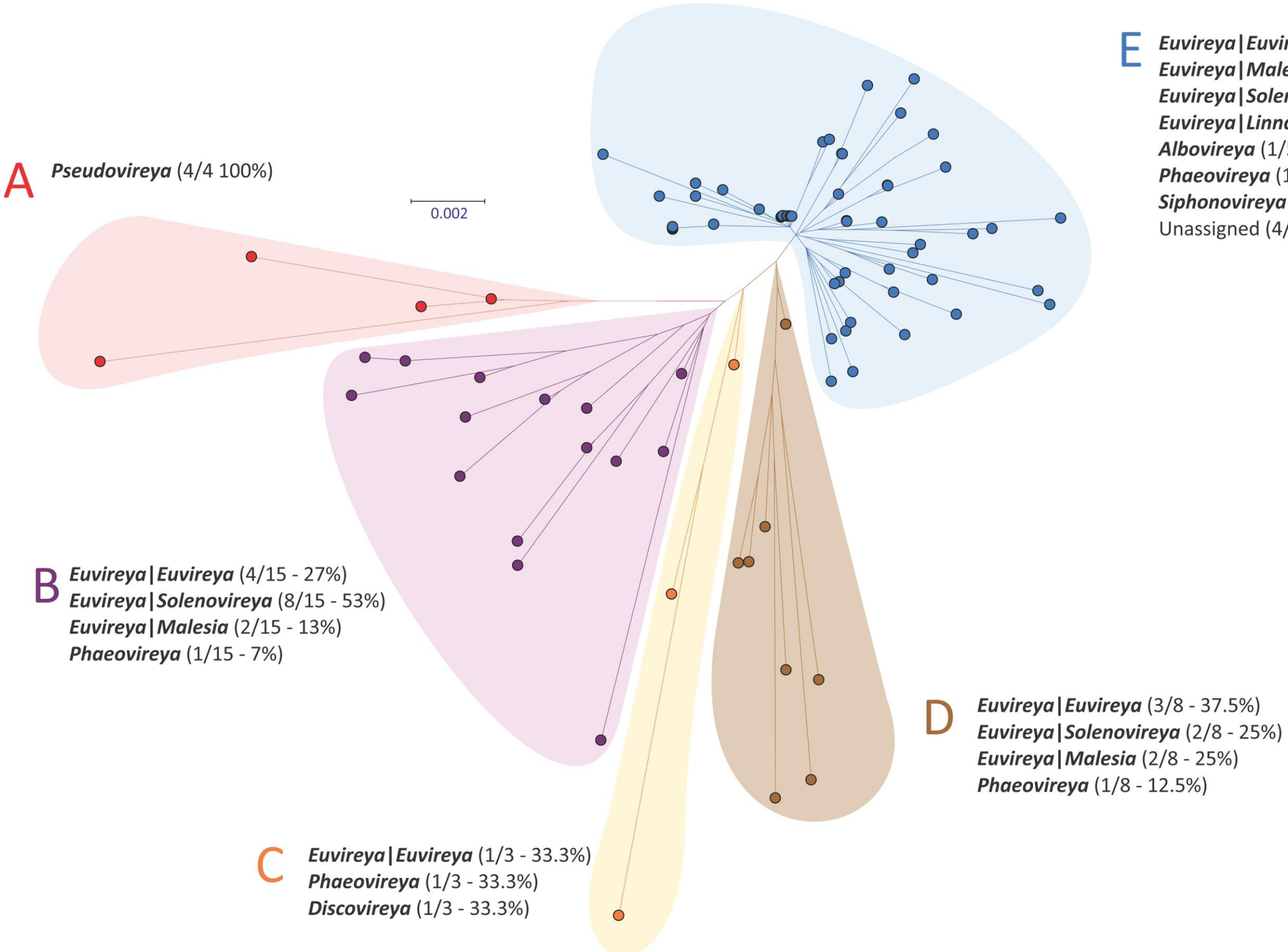

Figure 35 Maximum Likelihood consensus tree using *rpb*2i nucleotide sequence data (Dataset 1). The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model. The bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the taxa analysed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) >50% are shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically as follows. When the number of common sites was < 100 or less than one fourth of the total number of sites, the maximum parsimony method was used; otherwise BIONJ method with MCL distance matrix was used. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 87 nucleotide sequences, with a total of 962 positions in the final dataset.

Figure 35 shows a radial phylogenetic that summarizes the phylogenetic tree in Figure 34, enabling the five major clades identified (A–E) to be easily visualized. Generally, the relative genetic separation of these clades is also easily visualised in this type of trees. Clade A corresponds solely to the taxa of Section *Pseudovireya*, which is sister to the rest of the vireyas or core vireyas (Clades B–E) supporting the classical placement of this group and also in agreement to recent molecular studies. The Clade B consists of taxa belonging to Section *Euvireya* Subsection *Euvireya* (4/15 or 27%), Section *Euvireya* Subsection *Solenovireya* (8/15 or 53%), Section *Euvireya* Subsection *Malesia* (2/15 or 13%) and Section *Phaeovireya* (1/15 or 7%). Notably taxa of Section *Pseudovireya* is absent in Clade B suggesting a significant separation of them from the rest of the vireyas. The Clade C consists of three taxa each representing the Section *Euvireya* Subsection *Euvireya*, Section *Phaeovireya* and Section *Discovireya*, with each taxon contributing 1/3 (33.3%).

Clade D consists of Section *Euvireya* Subsection *Euvireya* (3/8 or 37.5%), Section *Euvireya* Subsection *Solenovireya* (2/8 or 25%), Section *Euvireya* Subsection *Malesia* (2/8 or 25%) and Section *Phaeovireya* (1/8 or 12.5%). The Clade E contains the bulk of the taxa of vireyas and consists predominantly of Section *Euvireya* (37/57 or 65%). Clade E consists of Section *Euvireya* Subsection *Euvireya* (20/57 or 35.1%), Section *Euvireya* Subsection *Malesia* (6/57 or 10.5%)Section *Euvireya* Subsection *Solenovireya* (10/57 or 17.5%), Section *Euvireya* Subsection *Linnaeopsis* (1/57 or 1.8%), Section *Albovireya* (1/57 or 1.8%), Section *Phaeovireya* (14/57 or 24.5%), Section *Siphonovireya* (1/57 or 1.8%) and unassigned taxa (4/57% or 7%). Notably Clade E does not contain the sections *Pseudovireya* and *Discovireya* suggesting a significant genetic demarcation between Clade E and these two sections.

The overall arrangement of the clades suggests that the Section *Pseudovireya* is a genetically separate taxon and sister to the rest of the taxa of Subgenus *Vireya*. The taxa contained in Clade B could be referred to as a separate section, perhaps Section *Solenovireya* as suggested by Sleumer (1966a) where 53% of the taxa belong to this section. The clades C and D could be combined as a single taxon or treated as separate taxonomic groups, if further studies utilising additional taxa and molecular loci also consistently support these clades. The Clade E corresponds to the bulk of the taxa of

Subgenus *Vireya* and predominantly from East Malesia with the majority of the taxa (65%) belonging to the Section *Euvireya*. The subsections of neither Argent (2006) nor Sleumer (1966a) could be recovered, as the taxa are very closely related to each other, and further studies employing additional taxa and sequence data are needed to resolve them. The overall topology of the tree is congruent with that seen in the recent molecular studies where the sections *Pseudovireya* and *Discovireya* are sister to the rest of the vireyas (Brown et al. 2006a, 2006b; Brown et al. 2006c; Craven et al. 2011; Craven et al. 2008; Goetsch et al. 2011). These molecular studies also failed to recover the subsections within the *Euvireya* group.

5.3.1.2 Neighbour Joining

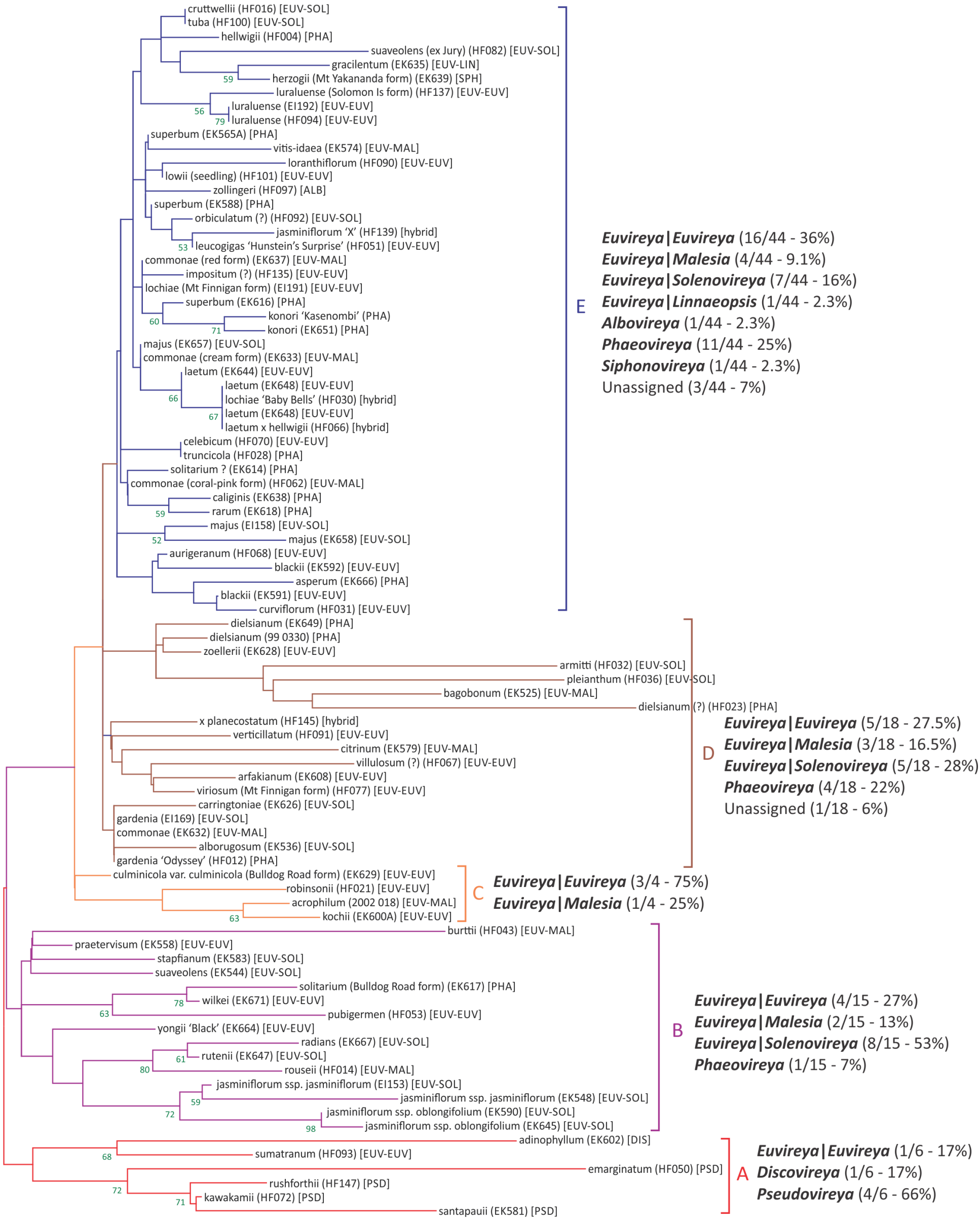

Figure 36 Neighbour-Joining consensus tree using *rpb*2i nucleotide sequence data (Dataset 1). The bootstrap consensus tree inferred from 1,000 replicates to represent the evolutionary history of the taxa analysed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) >50% are shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood (MCL) method and are in the units of the number of base substitutions per site. The analysis involved 87 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 863 positions in the final dataset. The text within the brackets shows the accession number of the sample.

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Figure 36 shows the molecular phylogenetic analysis by Neighbour-Joining method using *rpb*2i nucleotide sequence data (Dataset 1). The bootstrap consensus tree was inferred from 1,000 replicates to represent the evolutionary history of the taxa analysed and the percentage of replicate trees in which the associated taxa clustered together >50% are shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. The analysis involved 87 nucleotide sequences. All positions containing gaps and missing data were eliminated giving a total of 863 nucleotide positions in the final dataset.

The Neighbour-Joining phylogenetic tree exhibits five discernible clusters (Clades A–E) and has a similar topology to the phylogenetic tree based on the Maximum Likelihood method (Figure 34). Clade A with poor bootstrap support (<50%) is sister to Clades B–E, consisting of the outgroup taxa (*R. santapaui*, *R. kawakamii*, *R. rushforthii* and *R. emarginatum*) with moderate bootstrap support (72%), and a cluster consisting of *R. adinophyllum* and *R. sumatranum* (HF093) with moderate bootstrap support (68%). In the Neighbour-Joining tree the taxa *R. adinophyllum* and *R. sumatranum* clusters within the ingroup. The position of *R. adinophyllum* is justifiable as taxonomy based on scale type (Argent 2006) and molecular characters (Craven et al. 2011; Goetsch et al. 2011) suggest that *R. adinophyllum* is sister to pseudovireyas. The position of *R. sumatranum* however may be due to base-calling errors in the DNA sequencing or due to the hypothesis that *R. sumatranum* is related to *R. adinophyllum*, as a likely hybrid of these two was collected on Mt Kemiri (Sumatra) by David Binney (Argent 2006). The accession HF093 therefore could well be a hybrid of *R. sumatranum* and *R. adinophyllum*.

Clade B consists mainly of taxa belonging to the Section *Euvireya* Subsection *Solenovireya* (53% of the taxa) mixed with Section *Euvireya* Subsection *Euvireya* (40% of the taxa) and Section *Phaeovireya* (7%) of the taxa. The majority of the taxa (10/15 or 67%) within this clade exhibit the long-tubular corolla shape reminiscent of *R. jasminiflorum*. Within Clade B, the accessions of *R. jasminiflorum* form a cluster with moderate bootstrap support (72%), and these accessions in turn form two distinct clusters corresponding to the subspecies *jasminiflorum* and *oblongifolium* with 59% and 98% bootstrap support respectively. *R. radians*, *R. rutenii* and *R. rousei* clusters with strong

bootstrap support (80%). Among these three taxa, *R. radians* and *R. rutenii* belong to the Subsection *Solenovireya* and have similar long-tubular white flowers (bootstrap support 61%), while *R. rousei* has broader white flowers and belong to the Subsection *Malesia*. *R. suaveolens* and *R. stapfianum* in Clade B also has long-tubular white flowers and belong to the Subsection *Solenovireya*.

Clade C is a small group of taxa sister the remainder of the taxa (Clades D–E) and has poor bootstrap support (<50%). The only common attribute of the taxa of this clade is that they all belong to the Section *Euvireya*. Clade D has very poor bootstrap support (<50%) and consists of taxa belonging to mainly Section *Euvireya* and a few to Section *Phaeovireya*. Common morphological features or geographic origin among the taxa of this clade cannot be ascertained.

Clade E consists mainly of Section *Euvireya* (72%) represented by Subsection *Euvireya* (44%) and Subsection *Solenovireya* (28%) with the balance of the taxa is made up of Section *Phaeovireya* (22%) and unassigned taxa (6%). The Clade E however has very poor bootstrap support (<50%), and compared to the clades A–D, has lower genetic variation, suggesting that the taxa within this clade are relatively closely related. Within this clade several small clusters of taxa can be observed, notably: (i) *R. laetum* accessions with moderate bootstrap support (66%), (ii) *R. superbum* and its relatives with moderate bootstrap support (60%), and (iii) *R. luraluense* accessions with moderate bootstrap support (56%).

5.3.1.3 Minimum Evolution

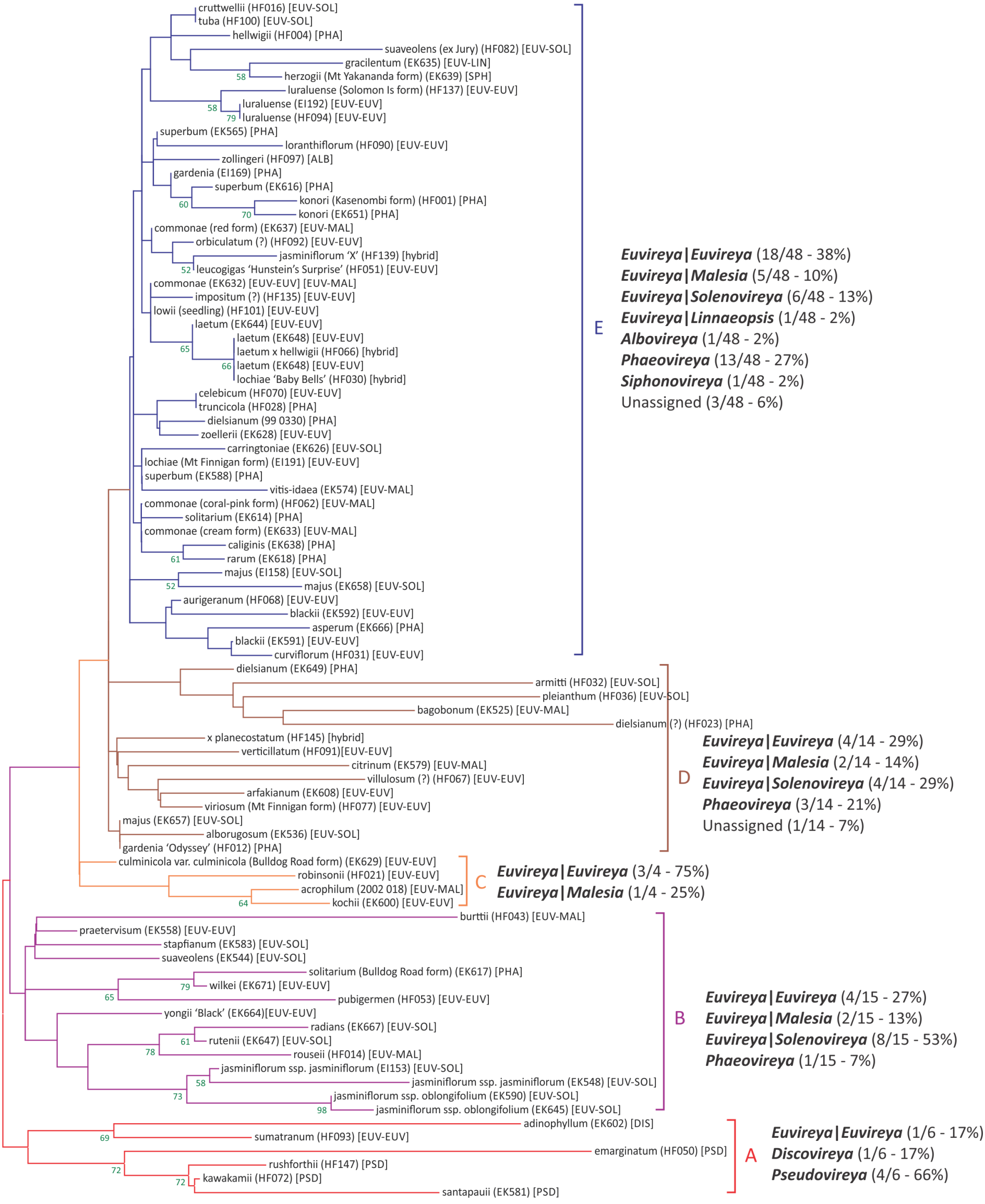

Figure 37 Minimum Evolution consensus tree using *rpb*2i nucleotide sequence data (Dataset 1). The bootstrap consensus tree inferred from 1,000 replicates is taken to represent the evolutionary history of the taxa analysed. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) >50% are shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. The ME tree was searched using the Close-Neighbour-Interchange (CNI) algorithm at a search level of 0. The Neighbour-Joining algorithm was used to generate the initial tree. The analysis involved 87 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 863 positions in the final dataset. . The text within the brackets shows the accession number of the sample.

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Figure 37 shows the molecular phylogenetic analysis by Minimum Evolution method using *rpb*2i nucleotide sequence data (Dataset 1). The bootstrap consensus was tree inferred from 1,000 replicates taken to represent the evolutionary history of the taxa analysed. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed, and the percentage of replicate trees in which the associated taxa clustered together in the bootstrap test >50% are shown next to the branches. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. The Minimum Evolution tree was searched using the Close-Neighbour-Interchange (CNI) algorithm at a search level of 0. The Neighbour-Joining algorithm was used to generate the initial tree. The analysis involved 87 nucleotide sequences, and all positions containing gaps and missing data were eliminated giving a total of 863 nucleotide positions in the final dataset.

The Minimum Evolution phylogenetic tree is very similar to that of the Neighbour-Joining Tree (Figure 36) with similar trends seen in the number of well-marked clades and constituent taxa.

5.3.1.4 Maximum Parsimony

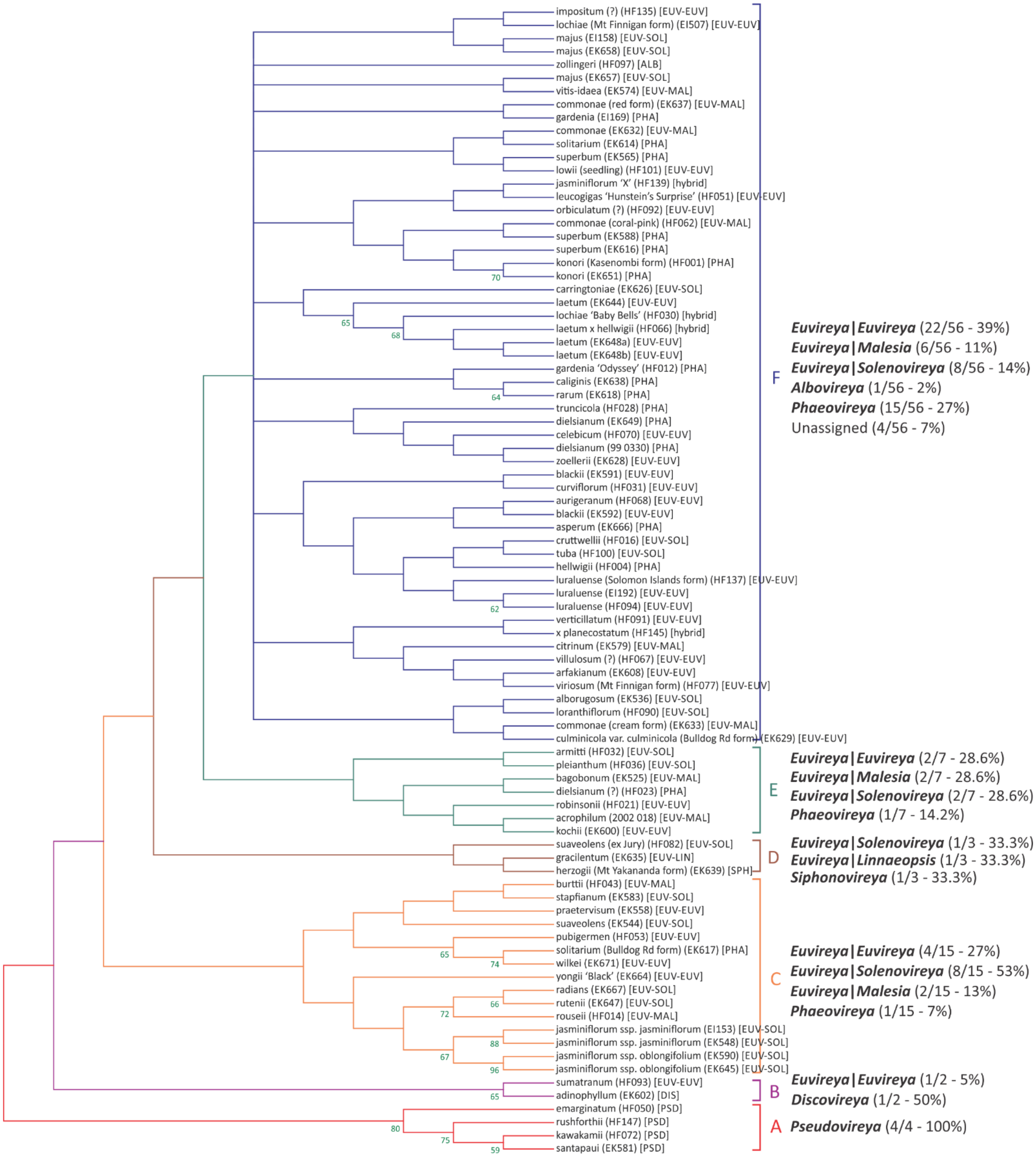

Figure 38 Maximum Parsimony strict consensus tree based on *rpb*2i data (Dataset 1). The bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the taxa analysed. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. The MP tree was obtained using the Close-Neighbour-Interchange (CNI) algorithm with search level 1 in which the initial trees were obtained with the random addition of sequences (10 replicates). The analysis involved 87 nucleotide sequences. There were a total of 937 positions in the final dataset. . The text within the brackets shows the accession number of the sample.

Figure 38 shows the Maximum Parsimony strict consensus tree based on *rpb*2i data (Dataset 1). The bootstrap consensus tree was inferred from 1,000 replicates taken to represent the evolutionary history of the taxa analysed. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed and the percentage of replicate trees in which the associated taxa clustered together is shown below the branches. The Maximum Parsimony tree was obtained using the Close-Neighbour-Interchange algorithm (CNI) with search level set at 1 in which the initial trees were obtained with the random addition of sequences (10 replicates). The analysis involved 87 nucleotide sequences with a total of 937 aligned nucleotide positions in the final dataset.

The Clade A correspond to taxa belonging solely to the Section *Pseudovireya* (the outgroup), which is sister to the rest of the taxa of Subgenus *Vireya* Argent. This clade has very good bootstrap support (80%), additionally the subclade containing *R. rushforthii*, *R. kawakamii* and *R. santapaui* also have good bootstrap support (75%). This result thus supports the placement of *Pseudovireya* with respect to the rest of the vireyas as proposed by Argent (2006) using morphological characters and also the recent molecular studies (Brown et al. 2006a, 2006b; Brown et al. 2006c; Craven et al. 2011; Craven et al. 2008; Goetsch et al. 2011).

The Clade B consists of *R. adinophyllum* (Section *Discovireya*) and *R. sumatranum* (Section *Euvireya* Subsection *Euvireya*) with moderate bootstrap support (65%). These two species originate from the Atjeh province of Northern Sumatra, and have been known to hybridize with each other (Argent 2006). A possible hybrid was reported by David Binney on Mt Kemiri (Sumatra). However, this relationship cannot be observed in the recent molecular studies such as Goetsch et al. (2011) in which these two species were studied. Morphologically these two species are very distinct, especially in their habit and foliage (Photo 14). Further, *R. sumatranum* is also known to hybridize with another Section *Discovireya*, *R. retusum* forming the hybrid *R.* × *epilosum* (Syn: *R. retusum* var. *epilosum*). The placement of *R. sumatranum* (HF093) in this clade can be explained in two ways: (i) an error in the DNA sequence due to base-calling errors, and since the sequence used for this study was from a single locus of ~1 kb long, or (ii) that the accession of *R. sumatranum* (HF093) that have been collected for this study may be akin to the hybrid that David Binney collected from Mt Kemiri.

a *b*

Photo 14 A selection of taxa from the Clade B of Figure 38. (a) *R. adinophyllum* (EK602), (b) *R. sumatranum* (Photo: Chris Callard; plant cultivated at the RBGE, UK).

Clade C consists of taxa belonging to Section *Euvireya* Subsection *Euvireya* (5/15 or 33%), Section *Euvireya* Subsection *Solenovireya* (8/15 or 53%), Section *Euvireya* Subsection *Malesia* (1/15 or 7%) and Section *Phaeovireya* (1/15 – 7%). Clade D consists of taxa belonging to Section *Euvireya* Subsection *Solenovireya* (1/3 or 33.3%), Section *Euvireya* Subsection *Linnaeopsis* (1/3 or 33.3%) and Section *Siphonovireya* (1/3 or 33.3%). Clade E contains taxa belonging to the Section *Euvireya* Subsection *Euvireya* (4/7 or 57%), Section *Euvireya* Subsection *Solenovireya* (2/7 or 29%) and Section *Phaeovireya* (1/7 or 14%). Clade C represents a significant fraction of the Subsection *Solenovireya*, while the clades D and E do not have any discernible taxonomic patterns.

Clade F contains the bulk of the taxa studied, and dominated by the Section *Euvireya* (36/56 or 64%) represented by subsections *Euvireya* (28/56 or 50%) and *Solenovireya* (8/56 or 14%). The balance of the taxa of Clade F is made up by Section *Albovireya* (1/56 or 2%), Section *Phaeovireya* (15/56 or 27%) and unassigned taxa (4/56 or 7%). This tree topology is seen in most of the recent molecular studies of vireyas (Brown et al. 2006a, 2006b; Brown et al. 2006c; Craven et al. 2011; Craven et al. 2008; Goetsch et al. 2011). Neither this study nor the recent molecular studies have been able to recover the subsections within *Euvireya* proposed by Argent (2006) and Sleumer (1966a).

5.3.1.5 Summary

The phylogenetic analyses of the Dataset 1 show similar trends with a topology consisting of more or less the same taxa. Five major clades can be identified that correspond to the Section *Pseudovireya* (outgroup), a clade consisting of significant number of taxa belonging to Section *Euvireya* Subsection *Solenovireya*, two clades with a mixture of sections of Subgenus *Vireya* and a large clade consisting of the bulk of the taxa of Subgenus *Vireya* and predominantly Section *Euvireya* and a mixture of other sections. Comparing the taxonomic groups of Argent (2006), Section *Pseudovireya* is recovered as monophyletic while all the other sections are paraphyletic or polyphyletic.

5.3.2 Dataset 2

This section describes the results and discussions surrounding the phylogenetic analyses based on the DNA sequences of Dataset 2. This dataset includes all the sequences contained in the Dataset 1 and additional sequences for the same locus available in the public domain. For the analysis of the Dataset 2, only Maximum Likelihood and Maximum Parsimony methods are chosen, since the former produces similar results to that from Neighbour Joining and Minimum Evolution as seen in the analyses for the Dataset 1.

5.3.2.1 Maximum Likelihood

Figure 39 shows the molecular phylogenetic analysis by the Maximum Likelihood method showing the consensus tree based on *rpb*2i sequence data including those available in the public domain (Dataset 2). The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model (Tamura & Nei 1993). The tree with the highest log likelihood (-4675.0753) is shown for 171 nucleotide sequences with all ambiguous positions removed for each sequence pair, resulting in 962 positions in the final dataset.

The topology of the tree is vastly improved with better resolution compared with the ML tree produced using the Dataset 1, however a similar overall trend in the arrangement of the clades are perceived. Seven major clades (Outgroup and Clades A–F) can be seen in this tree. The outgroup consisted of *R. vaseyi*, *R. leptothrium* and *R. albiflorum*, and has excellent bootstrap support (96%). When the tree is rooted with the outgroup they form a monophyletic clade sister to the Subgenus *Vireya*, and the ingroup Subgenus *Vireya* is monophyletic.

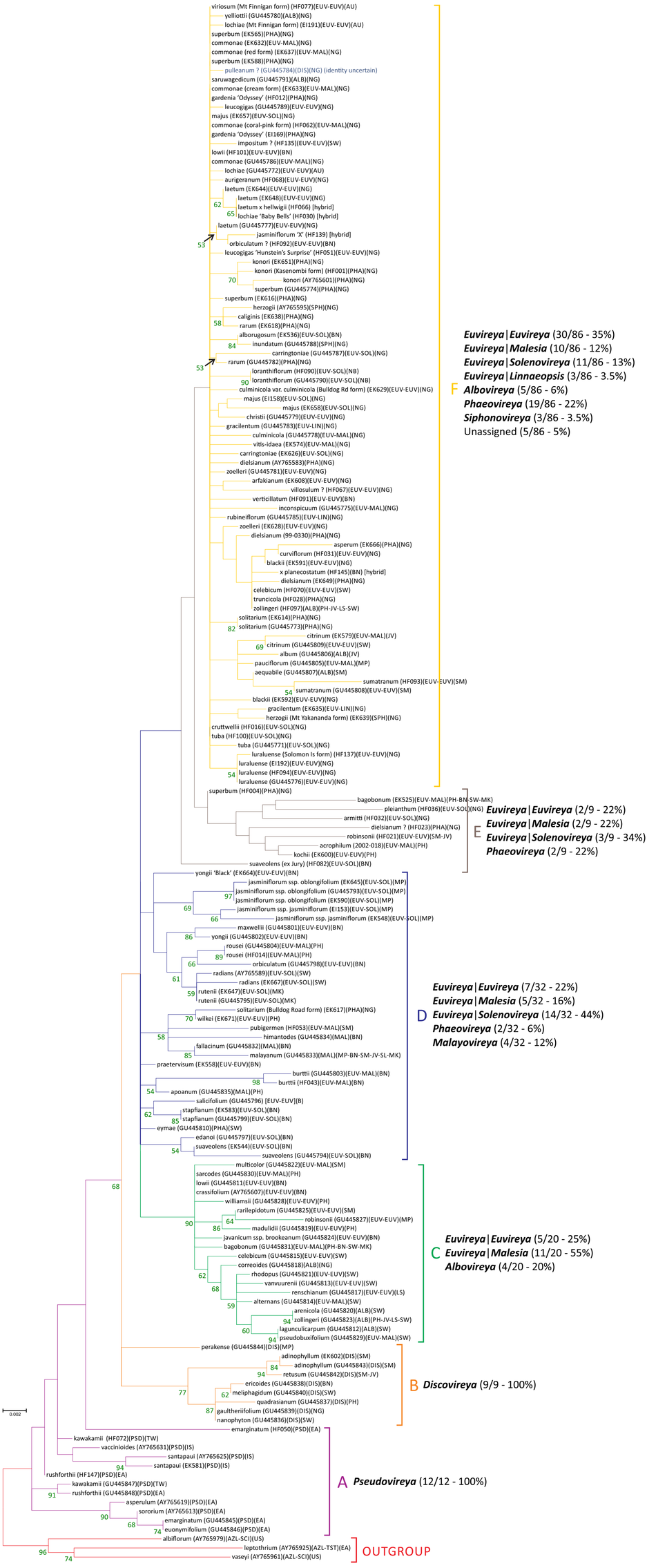

Figure 39 Molecular phylogenetic analysis by Maximum Likelihood method showing the consensus tree based on *rpb*2i data including sequence data available in the public domain (Dataset 2). The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model(). The tree with the highest log likelihood (-4675.0753) is shown. Initial tree(s) for the heuristic search were obtained automatically as follows. When the number of common sites was < 100 or less than one fourth of total number of sites, the maximum parsimony method was used; otherwise BIONJ method with MCL distance matrix was used. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 171 nucleotide sequences. All ambiguous positions were removed for each sequence pair. There were a total of 962 positions in the final dataset. The text within the first brackets shows the accession number of the sample, while the second brackets show the geographic origin of the taxon (Figure 31).

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Clade A consist of the taxa *R. euonymifolium* (Syn: *R. emarginatum*), *R. emarginatum, R. sororium, R. asperulum, R. rushforthii, R. kawakamii, R. santapaui* and *R. kawakamii*, all belonging to the Section *Pseudovireya*. This clade is paraphyletic and sister to the remainder of the vireyas. Within this clade *R. emarginatum* (GU445845) and *R. euonymifolium* (GU445846) cluster together with good bootstrap support (74%), suggesting that these two taxa may not be a single species as suggested by Argent (2006). Further studies with more accessions representing these two taxa need to be carried out to ascertain their taxonomic status. The two accessions of *R. emarginatum* do not cluster together, and could be attributed to a minor base-calling error. Also, accessions of *R. rushforthii* and *R. kawakamii* do not cluster together and may be due to the same reason.

The cluster containing *R. euonymifolium* (Syn: *R. emarginatum*) (GU445846), *R. emarginatum* (GU445845) and *R. sororium* corresponds to taxa from East Asia (EA) with strong bootstrap support (90%). The two accessions of *R. santapaui* cluster together with excellent bootstrap support (94%) suggesting that these may have originated from a single population in the wild. The cluster containing the accessions of *R. santapaui* and *R. vaccinioides* correspond to the taxa from the Indian Subcontinent (IS), but has poor bootstrap support (<50%).

The placement of Section *Pseudovireya* (Clade A) in this study does not agree with some of the recent molecular studies on vireyas. In this study, Section *Pseudovireya* is sister to the rest of the taxa of Subgenus *Vireya*, suggesting that pseudovireyas are the common ancestors to the rest of the vireyas. In some recent molecular studies on vireyas, Section *Discovireya* is sister to the rest of the vireyas (including Section *Pseudovireya*) (Craven 2011; Craven et al. 2008; Goetsch et al. 2011). However in other similar studies Section *Pseudovireya* is seen as sister to the rest of the vireyas (Brown et al. 2006a, 2006b; Brown et al. 2006c).

Clade B represents the taxa belonging to the Section *Discovireya*, and consists of well-supported cluster and a single disjunct accession of *R. perakense*. When *R. perakense* is excluded, the Section *Discovireya* is monophyletic with moderate bootstrap support (77%). This trend is also seen in some of the recent molecular studies on vireyas (Craven et al. 2011; Goetsch et al. 2011). The taxon *R. perakense* could well represent a distinct

group of species, and further studies including its closest relatives (*R. scortechinii*, *R. seimundii* and *R. spathulatum* from Peninsular Malaysia) could shed light on the placement of *R. perakense*.

Clade C is monophyletic with strong bootstrap support (80%) but contains a mixture of taxa, mainly from Section *Euvireya* and a few from Section *Albovireya*. This clade is strongly supported in the recent molecular study by Goetsch et al. (2011) from which the additional sequence data for the Dataset 2 was obtained. The Clade C is sister to the rest of the vireyas and the Section *Euvireya*.

Relationships within Clade C of taxonomic and conservation interest include the relationship between *R. rarilepidotum* and *R. robinsonii*, which cluster together with moderate bootstrap support (64%). Sleumer (1966a) suggested that *R. rarilepidotum* and *R. robinsonii* were closely related, differing mainly in their corolla colour Photo 15a–b), however, *R. robinsonii* flowers are never red in colour unlike *R. rarilepidotum*. Common features uniting the majority of the taxa in this clade are the funnel-shaped yellow to orange-red corollas and the large elliptic leaves (Photo 15).

Photo 15 Examples of taxa belonging to Clade C of Figure 39. (a) *R. rarilepidotum* (red-form) (Sumatra) (b) *R. robinsonii* (Peninsular Malaysia) (c) *R. lowii* (d) *R. javanicum* ssp. *brookeanum* (e) *R. crassifolium* (f) *R. multicolor*.

Clade D consists of a mixture of mainly Section *Euvireya*, with the Subsection *Solenovireya* well-represented (44% of the taxa). This clade also appears in most of the recent molecular studies on vireyas, and were placed sister to the remainder (and bulk) of the Section *Euvireya* (Brown et al. 2006a, 2006b; Brown et al. 2006c; Craven et al. 2011; Craven et al. 2008; Goetsch et al. 2011). Some of the taxa within this clade exhibit the typical *Solenovireya*-type corollas reminiscent of *R. jasminiflorum* and its relatives (Photo 16).

Sleumer (1966a) placed *Solenovireya* as a group sister to the Section *Euvireya* while Argent (2006) placed them as a subsection within Section *Euvireya*. According to the this study, the placement by Sleumer (1966a) seems to be more probable, and is also strongly supported by the geographic origin of the majority of the taxa within this group which is W Malesia. Further studies including more taxa representing Subsection *Solenovireya* may confirm the placement and status of this group.

Photo 16 A selection of taxa belonging to Clade D in Figure 39. (a) *R. jasminiflorum* ssp. *jasminiflorum* (b) *R. jasminiflorum* ssp. *oblongifolium* (c) *R. suaveolens* (d) *R. stapfianum* (e) *R. edanoi* ssp. *edanoi* (Photo: Chris Callard, www.vireya.net) (f) *R. radians* (Photo: Richard Currie, www.vireya.net).

Clade E is a small group of containing a mixture of taxa from Section *Euvireya* (Subsection *Solenovireya* – 34%, Subsection *Malesia* – 22% and Subsection *Euvireya* – 22%) and Section *Phaeovireya* (22%). If the accession of *R. suaveolens* (HF082, collected in a private collection in New Plymouth, of unknown origin) is removed from this group, Clade E forms a weakly supported (bootstrap <50%) monophyletic group. The taxa within this clade display a variety of corolla shapes and colours, and a common morphological character linking these taxa is not apparent (Photo 17). However, *R. armitii* and *R. pleianthum* show similarities in floral morphology.

Photo 17 A selection of taxa belonging to the Clade E of Figure 39. (a) *R. kochii* (Photo: Richard Currie, ww.vireya.net) (b) *R. armitii* (Photo: H Helm, www.vireya.net) (c) *R. pleianthum* (Photo: F Danet, www.vireya.net) (d) *R. acrophilum*.

Clade F consists of the bulk of Section *Euvireya* taxa and is not very strongly supported (<50%). However, recent molecular studies on vireyas has shown a strongly supported

broad *Euvireya* clade (Brown et al. 2006a, 2006b; Brown et al. 2006c; Craven et al. 2011; Craven et al. 2008; Goetsch et al. 2011). The majority of the taxa within this clade are distributed in E Malesia, with a large number of taxa being endemic to New Guinea. Section *Euvireya* represents 63.5% of this clade with the Subsection *Euvireya* contributing 35% to the total. Other subsections of Section *Euvireya* represented in this clade include *Solenovireya* (13%), *Malesia* (12) and *Linnaeopsis* (3.5). The clade is not well-resolved, but interesting groups of taxa similar to those found for the analyses of the Dataset 1 are seen here. *R. superbum* and its relatives cluster together with moderate bootstrap support (70%). All the accessions of *R. laetum* cluster together with moderate bootstrap support (62%). All the accessions of *R. luraluense* cluster together with low bootstrap support (54%). Overall, Clade F represents a very closely related group of taxa and further studies targeted on them with more accessions and DNA sequences may establish the subdivisions within this large group.

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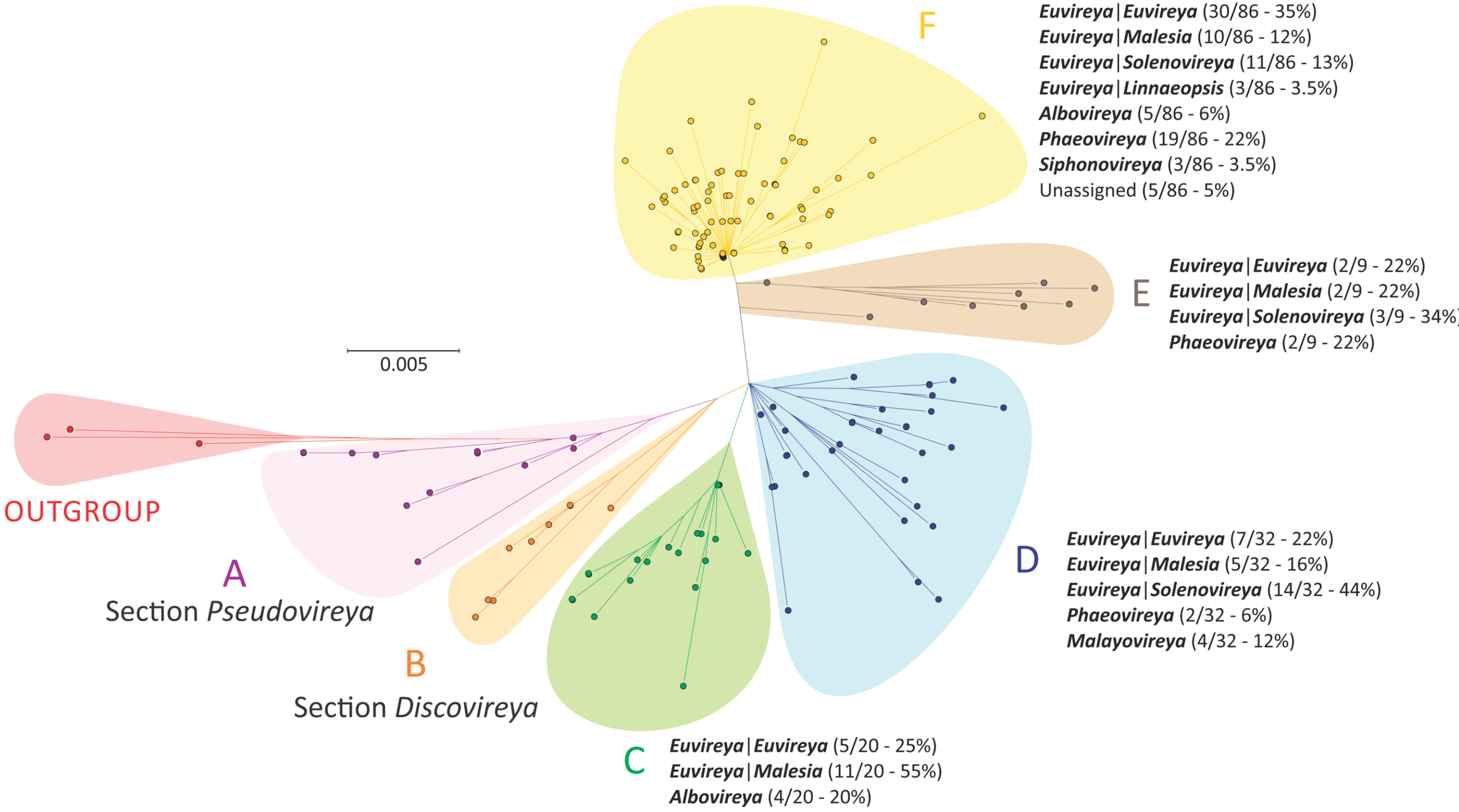

Figure 40 Maximum Likelihood consensus tree based on *rpb*2i data including sequence data available in the public domain (Dataset 2). The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model (Tamura & Nei 1993). The tree with the highest log likelihood (-4675.0753) is shown. Initial tree(s) for the heuristic search were obtained automatically as follows. When the number of common sites was < 100 or less than one fourth of the total number of sites, the maximum parsimony method was used; otherwise BIONJ method with MCL distance matrix was used. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 171 nucleotide sequences. All ambiguous positions were removed for each sequence pair. There were a total of 962 positions in the final dataset.

Figure 40 shows a radial tree of the ML phylogenetic analysis shown in Figure 39. This figure enables easier visualization of the genetic separation of the clades with respect to each other. The outgroup is well-separated from the ingroup and thus suggests that the Subgenus *Vireya* is a monophyletic group. Sections *Pseudovireya* (Clade A) and *Discovireya* (Clade B) form genetically separate clades sister to the bulk of the taxa of Subgenus *Vireya*. As discussed earlier in this section, the placement of *Pseudovireya* and *Discovireya* sister to the core vireyas is supported by the recent molecular studies of vireyas.

Clade C containing a large number of taxa from W Malesia, 80% contributed by the Section *Euvireya* (Subsection *Euvireya* – 25% and Subsection *Malesia* – 55%). The clade also has four accessions belonging to the Section *Albovireya*, representing 20% of the total taxa.

Clade D consists of mainly taxa belonging to the Section *Euvireya* representing 82% of the total. Section *Euvireya* is represented by the subsections *Euvireya* (22%), *Malesia* (16%) and *Solenovireya* (44%). The clade also contains taxa belonging to the Section *Phaeovireya* (6%) and Section *Malayovireya* (12%).

Clade E consists of mainly taxa belonging to the Section *Euvireya* represented by the subsections *Euvireya* (22%), *Malesia* (22%) and *Solenovireya* (34%). The clade also consists of two accessions belonging to the Section *Phaeovireya* (22%).

Clade F represents a monophyletic group of taxa representing the bulk of the Section *Euvireya* which makes up 63.5% of the total taxa of the clade. The Section *Euvireya* is represented by the subsections *Euvireya* (35%), *Malesia* (12%), *Solenovireya* (13%) and *Linnaeopsis* (3.5%). Other accessions contained in this clade include those belonging to the sections *Albovireya* (6%), *Phaeovireya* (22%) and *Siphonovireya* (3.5%). Five accessions in this clade have no sectional assignment as they are hybrids (natural or garden) and those with unverified identities.

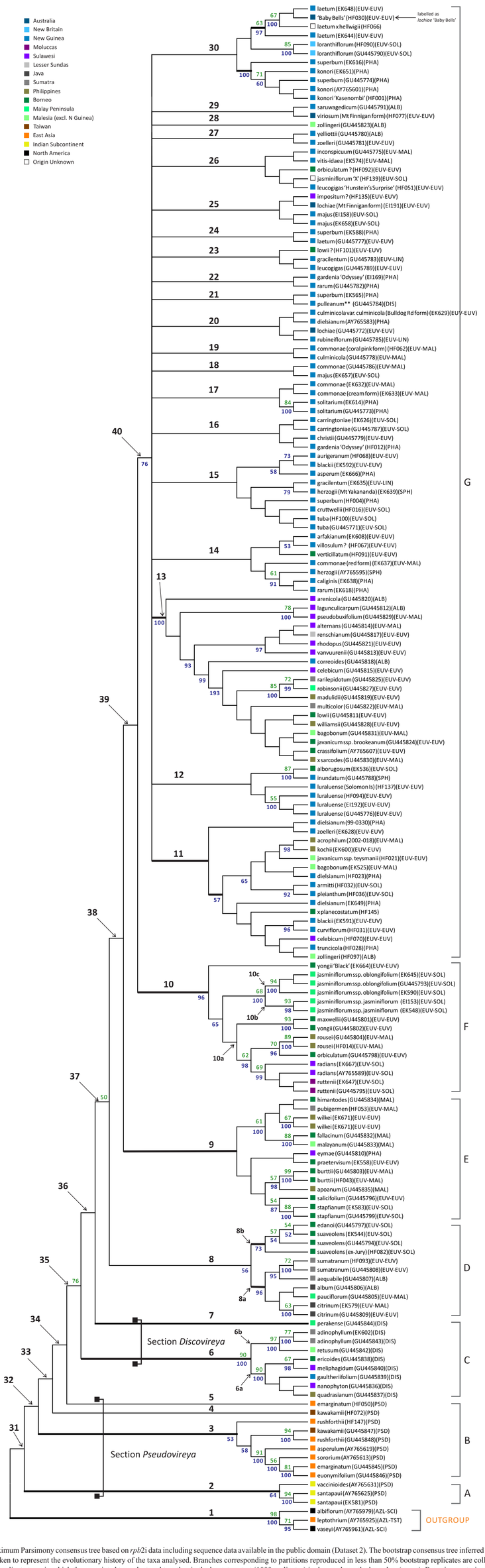

Figure 41 Maximum Parsimony consensus tree based on *rpb*2i data including sequence data available in the public domain (Dataset 2). The bootstrap consensus tree inferred from 1,000 replicates is taken to represent the evolutionary history of the taxa analysed. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown above the branches (green). Bayesian posterior probability values (× 100) from the Bayesian analysis are shown below the nodes they support (blue). The MP tree was obtained using the Close-Neighbour-Interchange algorithm with search level 1 in which the initial trees were obtained with the random addition of sequences (10 replicates). The analysis involved 171 nucleotide sequences. There were a total of 962 positions in the final dataset. The colour codes denote the geographic range of the taxa. The text within the first brackets shows the accession number of the sample, while the second brackets show the geographic origin of the taxon (Figure 31).

Figure 41 shows the Maximum Parsimony consensus tree based on *rpb*2i data including sequence data available in the public domain (Dataset 2). The bootstrap consensus tree was inferred from 1,000 replicates representing the evolutionary history of the taxa analysed, and the percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) is shown above the branches (green). Bayesian posterior probability values (× 100) from the Bayesian analysis are shown below the nodes they support (blue). The MP tree was obtained using the Close-Neighbour-Interchange algorithm with search level 1 in which the initial trees were obtained with the random addition of sequences (10 replicates). The analysis involved 171 nucleotide sequences, with a total of 962 positions in the final dataset. The outgroup is strongly supported with 98% bootstrap support and 100% Bayesian posterior probability (Node 1), thus Subgenus *Vireya* is monophyletic.

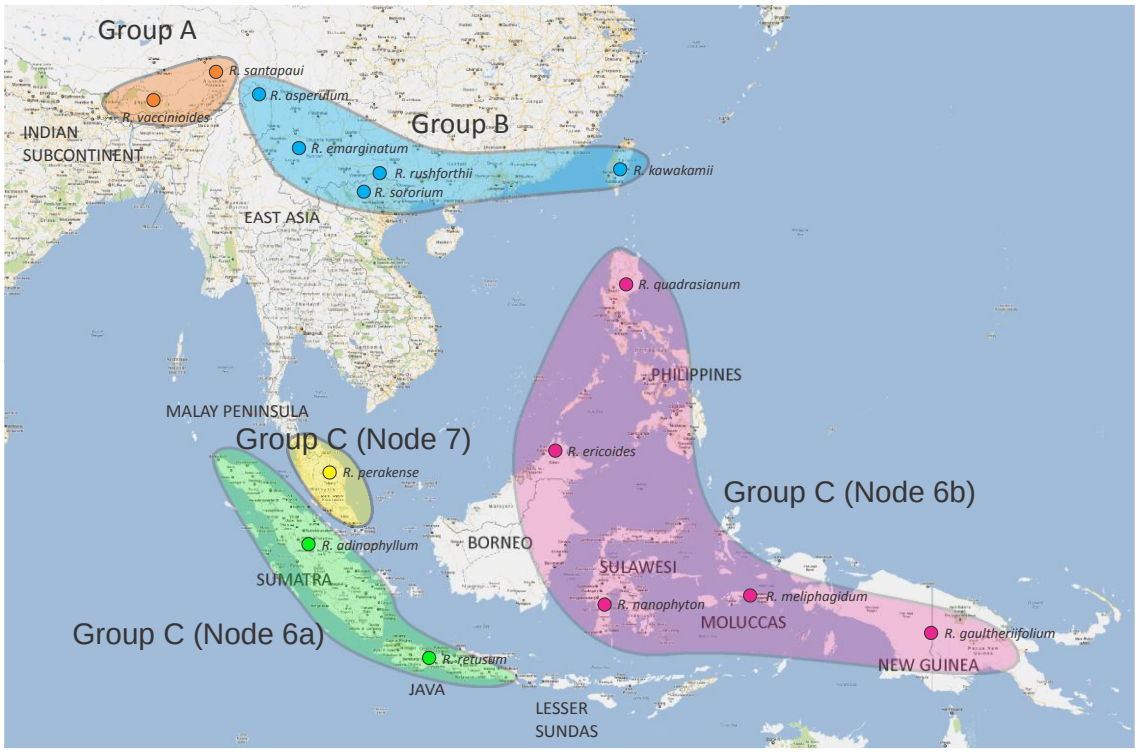

Figure 42 Distribution of taxa of Groups A–C from Figure 41. The positions of the taxa are approximate and are based on the original collection locality (where available). (Map: 2012 © Google Maps).

Group A and B correspond to the Section *Pseudovireya* and consist of small distinct clades (Nodes 2–5), thus making this section paraphyletic. Node 2 represents taxa from the Indian Subcontinent (Figure 42) and is genetically distant from the remainder of

Section *Pseudovireya* taxa, and has moderate Bayesian posterior probability (64%). Nodes 3–5 represent Section *Pseudovireya* taxa from E Asia (mainly Indochina through S China to Taiwan) (Figure 42) and has poor bootstrap support (<50%) and poor Bayesian posterior probability (<53%). However, the taxa of Section *Pseudovireya* are sister to the rest of the taxa of Subgenus *Vireya* and represent the mainland Asia taxa.

Group C consists of taxa belonging to the Section *Discovireya*, and is monophyletic if the accession of *R. perakense* (Node 7) is excluded. Node 6 is monophyletic with strong bootstrap support (90%) and very high Bayesian posterior probability (100%). The taxa in Node 6a is restricted to Sumatra and Java while those in Node 6b is found in a wider distributed in E Malesia (Figure 42). Node 7 is restricted to the Malay Peninsula (Figure 42).

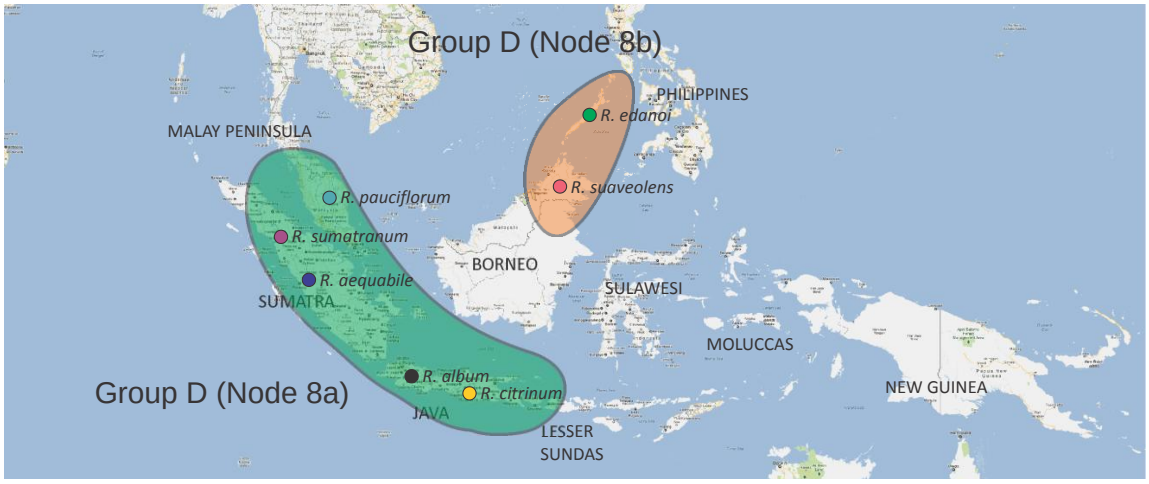

Figure 43 Distribution of taxa of Group D from Figure 41. The positions of the taxa are approximate and are based on the original collection locality (where available). (Map: 2012 © Google Maps).

Group D consist of a single monophyletic clade (Node 8) with low Bayesian posterior probability (56%). Two distinct subclades can be seen within this group (Nodes 8a and 8b). Node 8a represents a group of species from W Malesia with trumpet-shaped corollas, while Node 8b represents taxa from Borneo with long tubular corollas belonging to the Subsection *Solenovireya* (Figure 43).

Group E consists of a single clade with poor bootstrap support (<50%) and low Bayesian posterior probability (<50%). This group consist of taxa mainly from W Malesia and represents several taxa from Section *Malayovireya* (Figure 44).

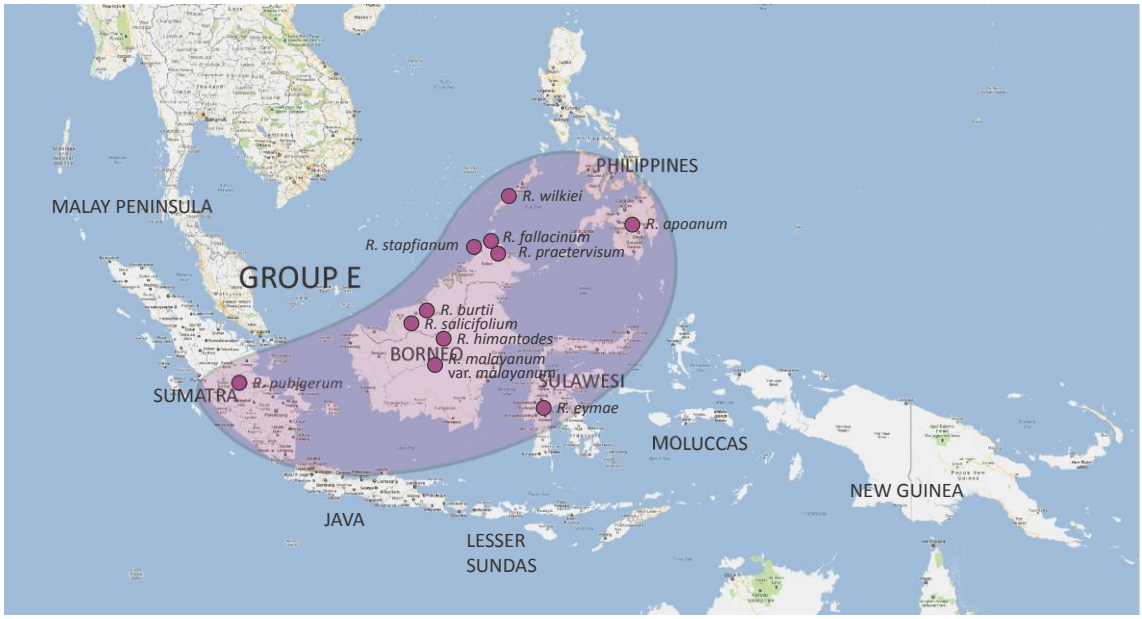

Figure 44 Distribution of taxa of Group E from Figure 41. The positions of the taxa are approximate and are based on the original collection locality (where available). (Map: 2012 © Google Maps).

Group F consists of a single monophyletic clade with high Bayesian posterior probability (96%). The majority (10 out 14) of the taxa within this group exhibit the solenovireya-type corolla shapes (usually white), typical of *R. jasminiflorum*. Node 10a is a poorly supported subclade (bootstrap <50%) containing a mixture of taxa widely distributed in W Malesia. Within Node 10a is a very strongly supported subclade (bootstrap 62% and Bayesian posterior probability 98%) containing taxa with very similar corolla shapes. All the accessions of *R. jasminiflorum* cluster together with moderate bootstrap support (68%) and high Bayesian posterior probability (100%). Within *R. jasminiflorum* two subclades corresponding to two subspecies can be identified (Nodes 10b and 10c). Node 10b represents the subspecies *oblongifolium* with strong support (bootstrap 93%, Bayesian posterior probability 98%), while Node 10c correspond to the subspecies *oblongifolium* with strong support (bootstrap 94%, Bayesian posterior probability 100%).

Group G consists of a single large monophyletic clade (Node 40) with poor bootstrap support (<50%) but good Bayesian posterior probability (76%). Group G consists of the

bulk of Subgenus *Vireya* and also the bulk of Section *Euvireya*. Group G consist of several subclades (Nodes 11–30), where taxa of Nodes 11–13 are widely distributed from W Malesia to C Malesia, while taxa of Nodes 14–30 are predominantly from New Guinea. Within Group G several subgroups with taxonomic and conservation interest can be highlighted. In Node 13 *R. rarilepidotum* and *R. robinsonii* cluster together with good bootstrap support (72%) and high Bayesian posterior probability (99%).

In Node 30 *R. superbum* and its relatives appears as a strongly supported clade (Bayesian posterior probability 100%), and could well represent a single variable species. All the accessions of *R. laetum* cluster together with moderate bootstrap support (63%) and Bayesian posterior probability (97%).

The overall topology of the tree is in agreement with the recent molecular studies on vireyas (Brown et al. 2006a, 2006b; Brown et al. 2006c; Craven et al. 2011; Craven et al. 2008; Goetsch et al. 2011). The sections *Phaeovireya*, *Siphonovireya* and the subsections of Section *Euvireya sensu* Argent (2006) could not be recovered, however sections *Pseudovireya*, *Discovireya* and *Euvireya* (to a large extent) was recovered.

By overlaying the geographic origin of the taxa (coloured squares next to the taxon name), the tree fits loosely into geographic regions (Figure 21). Group A and B representing taxa from mainland Asia, Group C–F representing taxa predominantly from W Malesia and Group G representing taxa predominantly from E Malesia.

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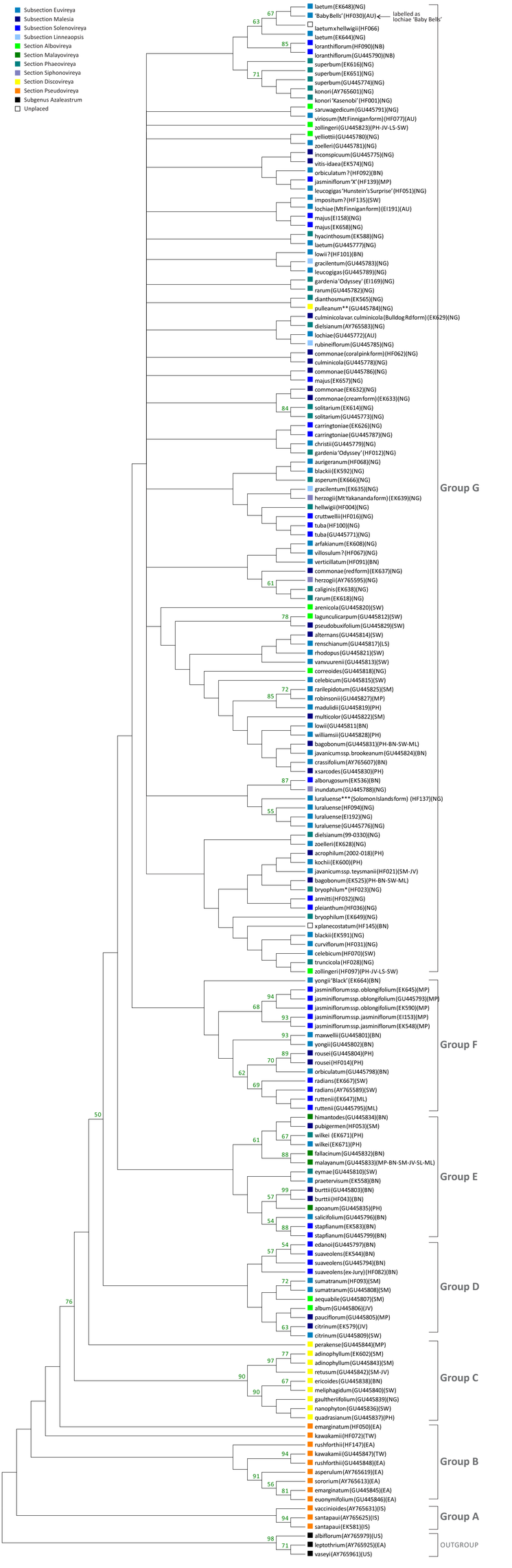


Figure 45 Sections of Argent (2006) overlaid on Maximum Parsimony consensus tree based on *rpb*2i data including sequence data available in the public domain (Dataset 2). The bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the taxa analysed. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown above the branches (green). Bayesian posterior probability values (× 100) from the Bayesian analysis are shown below the nodes they support (blue). The MP tree was obtained using the Close-Neighbour-Interchange algorithm with search level 1 in which the initial trees were obtained with the random addition of sequences (10 replicates). The analysis involved 171 nucleotide sequences with a total of 962 positions in the final dataset. The colour codes denote the section/subsection corresponding to the classification of Argent (2006).

Figure 45 shows the Maximum Parsimony analysis shown in Figure 41 with the taxonomic groups of Argent (2006) overlaid (coloured squares next to the taxon names). The sections *Pseudovireya* (Group A and B) and *Discovireya* (Group C) fit well on the tree supporting the status of these sections. The other sections do not fit well on the tree and thus suggests a revision of the current taxonomic groups, especially the subgroups within Section *Euvireya*.

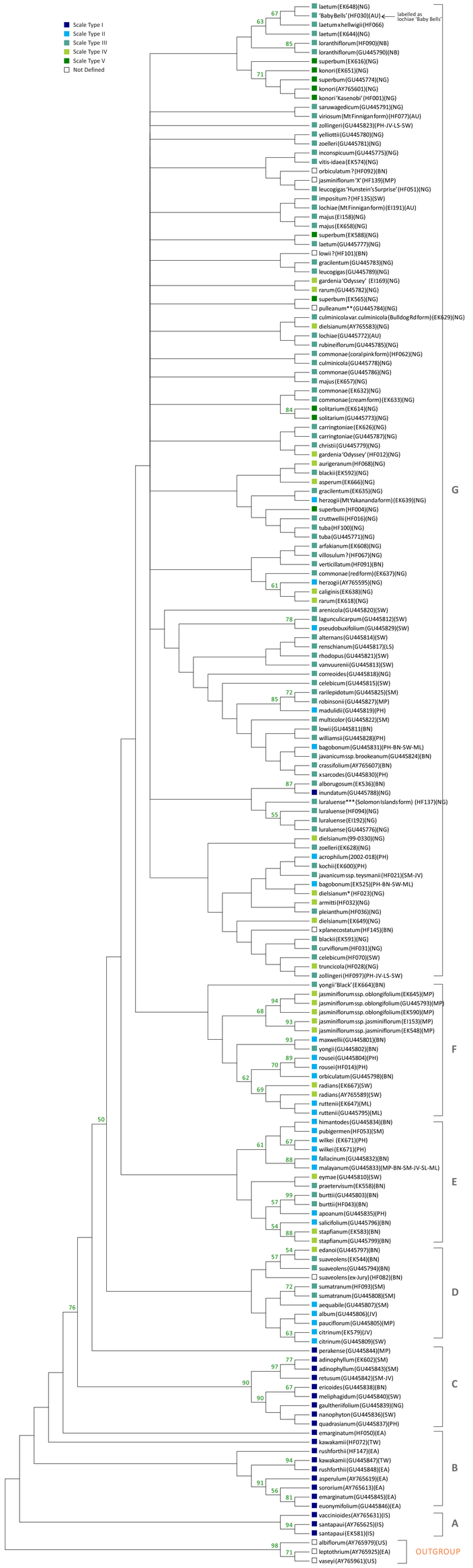


Figure 46 Scale type of vireyas overlaid on Maximum Parsimony consensus tree based on *rpb*2i data including sequence data available in the public domain (Dataset 2). The bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the taxa analysed. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown above the branches (green). Bayesian posterior probability values (× 100) from the Bayesian analysis are shown below the nodes they support (blue). The MP tree was obtained using the Close-Neighbour-Interchange algorithm with search level 1 in which the initial trees were obtained with the random addition of sequences (10 replicates). The analysis involved 171 nucleotide sequences with a total of 962 positions in the final dataset. The colour codes correspond to the scale type exhibited in the plants.

Figure 46 shows the Maximum Parsimony analysis shown in Figure 41 with the scale types (Figure 47) overlaid (coloured squares next to the taxon names). Groups A–C exhibits the scale type I and corresponds to the sections *Pseudovireya* and *Discovireya*. Scale type II–V are found in groups D–G. This shows that the scale type can be most effectively applied to higher level classifications, i.e. at sectional level and not subsectional level.

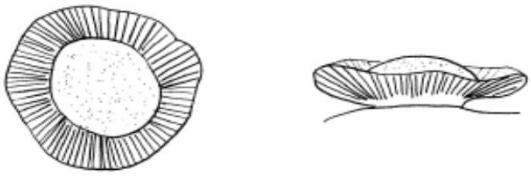
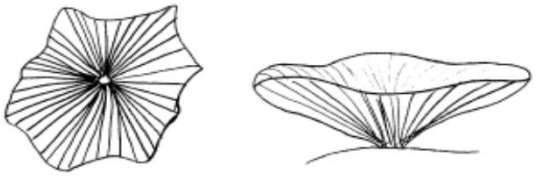
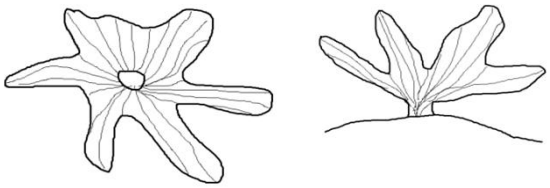
I		Circular with large dome-shaped centres and narrow flange; margin entire or almost so, and sessile. Exhibited in sections *Pseudovireya*, *Discovireya* and *Siphonovireya*.
II		Lobed; swollen centre (large ones coloured); variously lobed with a dark coloured centre, found in two different size classes on one plant. Exhibited in sections *Albovireya* and *Malayovireya*.
III		Moderately substellate or dented, and sessile.
IV		Deeply-lobed; borne on a short epidermal tubercle.
V		Dendroid borne on epidermal tubercles; deeply stellate-incised and dendroid to various degrees. Exhibited in Section *Phaeovireya*.

Figure 47 Scale types of Subgenus *Vireya* (Argent 2006).

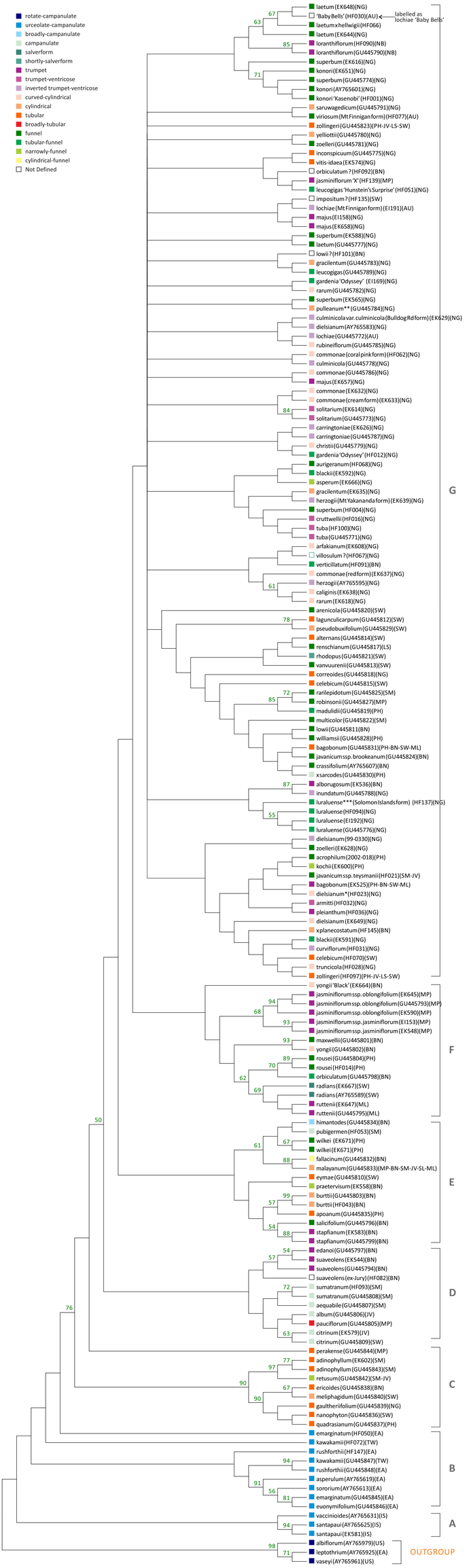


Figure 48 Corolla shapes overlaid on Maximum Parsimony consensus tree based on *rph*2i data including sequence data available in the public domain (Dataset 2). The bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the taxa analysed. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown above the branches (green). Bayesian posterior probability values (× 100) from the Bayesian analysis are shown below the nodes they support (blue). The MP tree was obtained using the Close-Neighbour-Interchange algorithm with search level 1 in which the initial trees were obtained with the random addition of sequences (10 replicates). The analysis involved 171 nucleotide sequences with a total of 962 positions in the final dataset. The colour codes correspond to the corolla shape of the plants.

Figure 48 shows the Maximum Parsimony analysis shown in Figure 41 with the corolla shapes (Figure 49) overlaid (coloured squares next to the taxon names). The corolla shapes fit well with the outgroup taxa and the taxa of Section *Pseudovireya*. The corolla shapes for the groups C and D loosely fit, while corolla shapes have no correlation with the groups E–G.

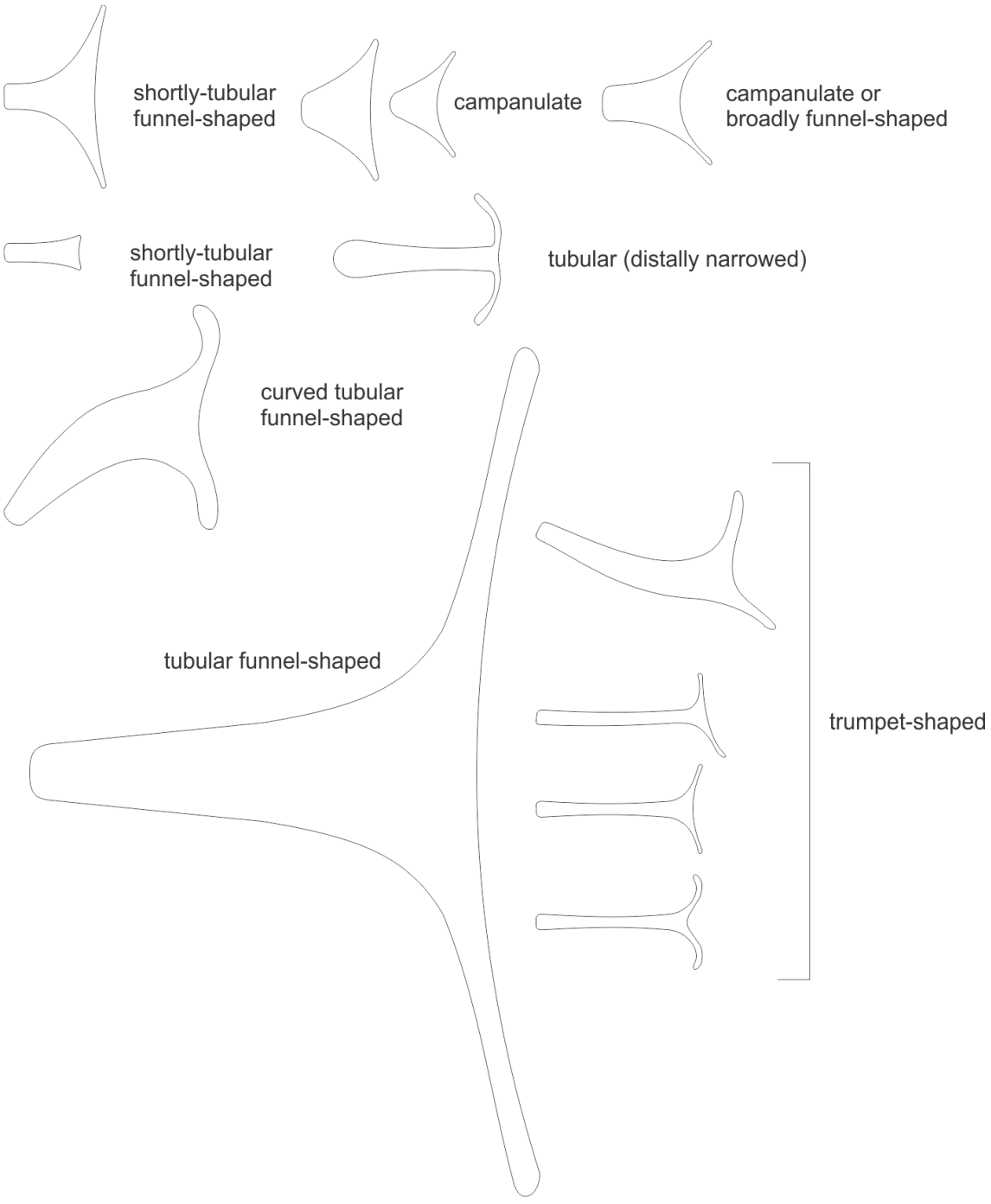


Figure 49 Simplified corolla shapes of Subgenus *Vireya* (drawn to scale, relative to each other).

5.3.2.3 Summary

The Dataset 2 was analysed using two phylogenetic analysis methods: Maximum Likelihood and Maximum Parsimony. Neighbour Joining and Minimum Evolution analysis methods were not carried out as they show very similar tree topology to that seen in the Maximum Likelihood analysis, as seen for the analyses of Dataset 1. The topology of the trees produced by both ML and MP trees showed very similar trends, and in many instances composed of more or less the same taxa.

The ML tree exhibited six discernible clades, with the basal clades (A and B) corresponding to the sections *Pseudovireya* and *Discovireya*. These two sections were restricted to the clades A and B only. The clades C–F consisted of a mixture of the other remaining sections of the Subgenus *Vireya sensu* Argent. The bulk of the taxa found in the clade F representing the majority of the taxa belonging to the Section *Euvireya sensu* Argent (2006).

The MP tree showed a similar topology to that of the ML tree, however with seven distinguishable clades. The clades A and B correspond to the sections *Pseudovireya* and *Discovireya* respectively, and are sister to the rest of the vireya taxa. Clades C–G consisted of a mixture of the remaining sections of the Subgenus *Vireya sensu* Argent. Clade G consisted of the bulk of the taxa analysed and belonging mostly to the Section *Euvireya sensu* Argent (2006).

All phylogenetic trees showed a similar overall pattern with the taxa of the sections *Discovireya* and *Pseudovireya* forming sister to the rest of the vireyas (hereafter referred to as the core vireyas). The core vireyas consist of mainly the Section *Euvireya* with other sections (*Malayovireya*, *Siphonovireya*, *Phaeovireya* and *Albovireya*), forming paraphyletic subgroups.

The MP tree showed a strong correlation between the clades and the geographic origin of the taxa. However, scale type and corolla shape showed only a very weak correlation with the clades. The sections *sensu* Argent (2006) correlates well with the basal clades (corresponding to the sections *Pseudovireya* and *Discovireya*), while the other clades consisted of a mixture of the remaining sections.

5.4 Genetic Diversity Analyses

The genetic diversity analyses were carried out on selected groups of accessions using microsatellite data, nucleotide sequence data and RAPD data. The following sections describe the results obtained for each of these three methods.

A total of 27 microsatellite markers were examined, 16 of which were found to be polymorphic. Of the remaining 11 markers, five were found to be monomorphic and six did not amplify as shown in Table 12.

Table 12 Microsatellite markers used in the genetic diversity analysis of *Rhododendron* accessions. Markers 1–24 were designed by Dunemann et al. (1999), and markers 25–27 by Naito et al. (1998).

#	Primer	Primer Sequence (5' to 3')		Variability	Range/bp	T_m/°C
1	GA211	**F**	GCA CCA GAA GGT GGA AAG ACT C	Polymorphic	289–???	
		R	TGC TGG AGC AGC TTA TGG CTA G			
2	GA512	**F**	GCA GTC CTT ATC AGT TTA CAC CG	Monomorphic		
		R	GAG CAT GCA AGA TGA AGG ACA TG			
3	GA758	**F**	GTC TAT CCA ATG ATA TTC TCT TTC C	Polymorphic	235–268	
		R	CTG AAG TGC TTG CAG GAA TAC TC			
4	GA102	**F**	CAT TGG AGT GTT GCT TAA TTC AGC	Monomorphic	178	
		R	CAA AAC GTG CTT ATA CAT TTC CCG			
5	GA106	**F**	TGT AAG ATG GCC CCG ATA GTG TG	Did not amplify	–	
		R	GGT CGC CAA TGG GGT ATT AGA GG			
6	GA108	**F**	CCG CAT CTA CTC ACT CAA TCC TG	Polymorphic	145–179	
		R	TGA AGC CCA AAA ACC AAA CGA CC			
7	GA110	**F**	CAA TTC TTC TTC TTC GCT TCT G	Did not amplify	–	
		R	CTT TCT GGT TGT AGA TGG GTT TTC			
8	GA111	**F**	GAT TAG AAG TCC GCA CGC AGA G	Did not amplify	–	
		R	CGG CAA TCA TAT CAG ACA AAA AGG			
9	GA117	**F**	CTT ATC CGA GAG ACC AAA CAA GT	Polymorphic		
		R	CGT TGA TCC TAT TGC TCC TC			
10	DC011	**F**	AGA CGA TCC CAT TAG AGT ATT C	Polymorphic	148-157	
		R	AAG TTA TTG CTT GTG AAG TCC T			
11	DC019	**F**	GAT GGT TGC TGG GGA TT	Polymorphic		
		R	TGG ACG GAA TGA ATA AAA AGA C			
12	DC022	**F**	GCC TTA GTT AGT TAA TTT TTG C	Did not amplify	–	
		R	TCC TAA ACT AAT CAA GGG AGA			
13	DC024	**F**	ACA ACT TAC CTA GGC ACC AAG C	Did not amplify	–	
		R	GCG AGT ACC CTT AAA ATC ACG			
14	DC027	**F**	GGC AAA TAG TTC CAT CAA AAG C	Polymorphic	174–189	
		R	GAA CTC CAT TCC GAG AGG GTA			
15	DC044	**F**	TCT TCT CCG GAA CTT CTC AAA C	Monomorphic	150–183	
		R	TCT CAA AAC CCA AAC CGA ATA G			
16	DC045	**F**	AGA GGT ACA CAA ATA CAA ATG G	Monomorphic		

#	Primer	Primer Sequence (5' to 3')		Variability	Range/bp	T_m/°C
		R	GAA GTC ATA GGC TCA AGG TT			
17	DC046	**F**	AGA AGC TGT ACC GAG AGA AAC	Polymorphic		
		R	TCA GGA AAA AGT ATT GGA AGA C			
18	DC047	**F**	TCC ACT TCT CAA ATC CCT AGC	Monomorphic		
		R	CAA CAC CGT TTG ATC TTT TAG C			
19	DC048	**F**	CCG CAT CTA CTC ACT CAA TCC T	Polymorphic	231–264	
		R	TTT CAC GTA AAA GCC CAA TGT C			
20	DC049	**F**	GAT GAT CGA TTC TGG GAG T	Polymorphic	175–211	
		R	ACA ACA ATA TTG GCA CAA AAC T			
21	DC055	**F**	AAT GCC TTA ATG AGA GTA AT	Polymorphic		
		R	CTA GAC ATA CAA ACA TAG ATG C			
22	DD042	**F**	AGT TTT CAG CAC CCA ATA CCA G	Polymorphic		
		R	CCC AGT CGC CAC TTT AGA GAC			
23	DD095	**F**	GCT TAA CAC CCC TCA TTC TTA T	Polymorphic		
		R	AAT ACT ACC TCT AGG CCC TTC C			
24	DD113	**F**	AAC CAC CGA TTT TCC TCC AC	Polymorphic		
		R	CGT TTT GAT CTG GCT CTC TGA C			
25	RM2D2	**F**	ATG TGT TTC GTT GCT ACT GT	Polymorphic	126–143	
		R	ATG GTT GGT TTG TTT TCC TA			
26	RM3D2	**F**	TCA ACA CAT AAT AAA CAA AC	Polymorphic		
		R	GAA AAG AAG GGC AAG TAA GT			
27	RM9D6	**F**	CTC GCC TCC CAA AAG CAA T	Polymorphic	188–219	
		R	CGT GTC CTC ACC CCC GTA AC			

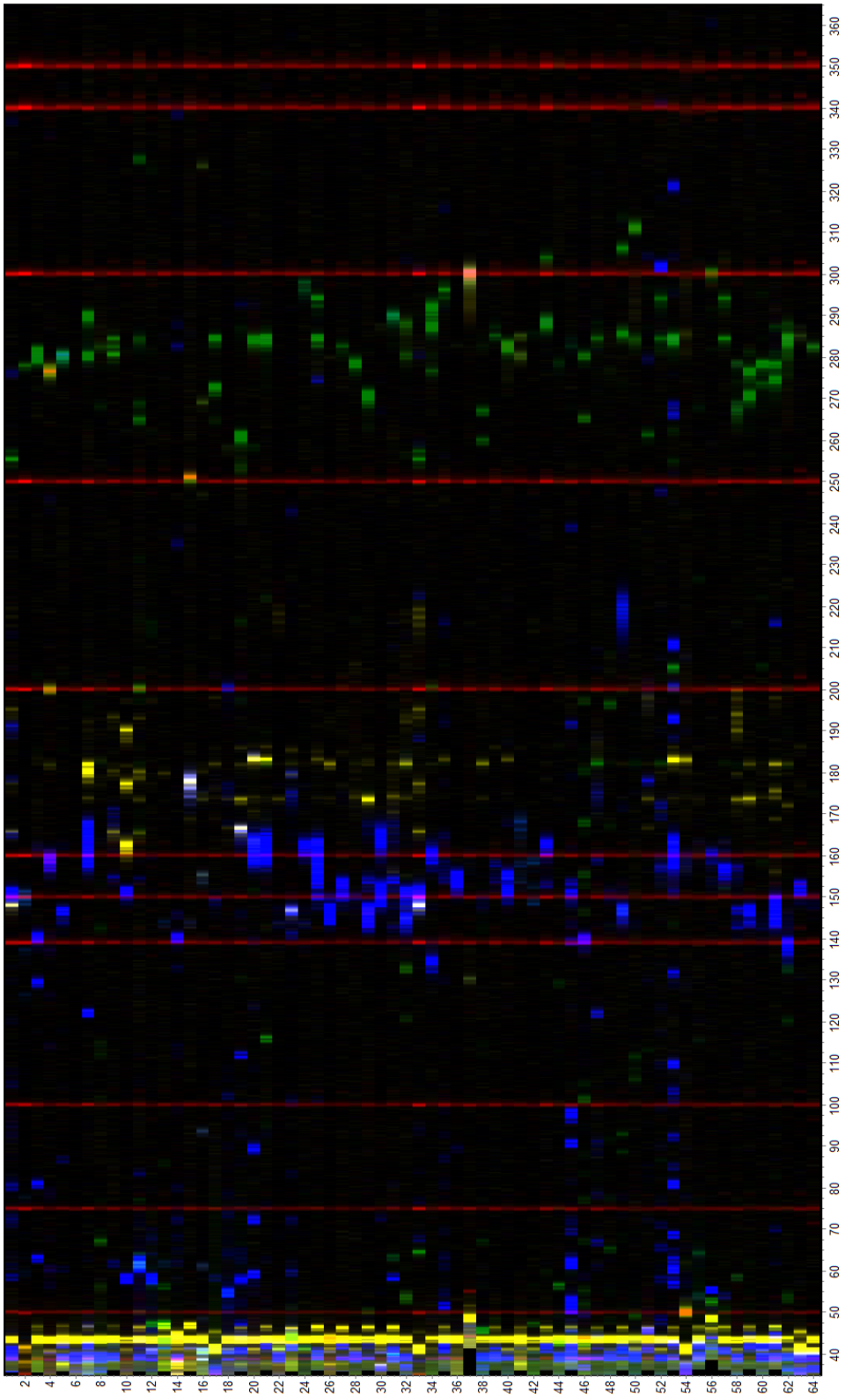


Photo 18 Polyacrylamide gel electrophoresis of three microsatellite markers. The gel represents the markers DC046 (blue), DC048 (green) and DC055 (yellow) carried out on 64 accessions of *Rhododendron*. The red lines indicate the size standards; the scale at the bottom shows the accessions (1–64), and the scale on the right indicates to the size of the fragments (number of bases). The direction of flow of the amplicons is from the top to the bottom (i.e. smaller fragments at the bottom and larger fragments at the top).

Photo 18 shows a sample image of the separation by polyacrylamide gel electrophoresis of three polymorphic markers (DC046, DC048 and DC055) for 64 *Rhododendron* accessions. A master table of all the generated results from the microsatellite analyses is provided in the Appendix 5. In the microsatellite analysis, some markers did not amplify for all of the accessions. This may be due to the fact that these primers were originally designed for the geographically distinct temperate *Rhododendron* species.

Taxa with multiple accessions were selected from the nucleotide sequence dataset (Dataset 2) of Section 5.3.2 and were analysed using MEGA version 5 (Tamura et al. 2011). A total of 38 taxa (or groups of related taxa) were selected, of which 27 taxa had only two accessions. The remaining 11 taxa (or groups) had more than three accessions.

Table 13 List of accessions used for the RAPD analyses. The accession EK612 in lane 36 was a duplicate and was not used in subsequent analyses.

Lane	PFR #	Taxon	Source
1	EK548	*R. jasminiflorum* ssp. *jasminiflorum*	Pukeiti Gardens
2	EK590	*R. jasminiflorum* ssp. *oblongifolium*	Pukeiti Gardens
3	EK612	*R. jasminiflorum* ssp. *jasminiflorum*	Pukeiti Gardens
4	EK645	*R. jasminiflorum* ssp. *oblongifolium*	Pukeiti Gardens
5	EK656	*R. jasminiflorum* ssp. *jasminiflorum*	Pukeiti Gardens
6	EI153	*R. jasminiflorum* ssp. *jasminiflorum*	Victoria Esplanade
7	EI154	*R. jasminiflorum* ssp. *jasminiflorum*	Victoria Esplanade
8	EK657	*R. majus*	Pukeiti Gardens
9	EK658	*R. baenitzianum*	Pukeiti Gardens
10	EI157	*R. majus*	Victoria Esplanade
11	EI158	*R. majus*	Victoria Esplanade
12	EK591	*R. blackii*	Pukeiti Gardens
13	EK592	*R. blackii*	Pukeiti Gardens
14	EK593	*R. blackii*	Pukeiti Gardens
15	EK613	*R. konori* (Edie Creek form)	Pukeiti Gardens
16	EK619	*R. konori* (white form)	Pukeiti Gardens
17	EK618	*R. rarum*	Pukeiti Gardens
18	EK655	*R. rarum*	Pukeiti Gardens
19	EK572	*R. viriosum*	Pukeiti Gardens
20	EK589	*R. viriosum* (Mt Finnigan form)	Pukeiti Gardens
21	EK604	*R. viriosum*	Pukeiti Gardens
22	EK620	*R. viriosum*	Pukeiti Gardens
23	EK630	*R. viriosum*	Pukeiti Gardens
24	EK507	*R. viriosum* (Mt. Finnigan form)	Victoria Esplanade
25	EK569	*R. viriosum*	Pukeiti Gardens
26	EK574	*R. vitis-idaea*	Pukeiti Gardens
27	EK575	*R. vitis-idaea*	Pukeiti Gardens
28	EK609	*R. christi* (Mt Miap form)	Pukeiti Gardens
29	EK610	*R. christi*	Pukeiti Gardens
30	EK616	*R. superbum*	Pukeiti Gardens
31	EK651	*R. superbum*	Pukeiti Gardens
32	EK603	*R. gracilentum*	Pukeiti Gardens
33	EK621	*R. gracilentum* (Mt Miap form)	Pukeiti Gardens
34	EK614	*R. solitarium*	Pukeiti Gardens
35	EK617	*R. solitarium* (Bulldog Rd form)	Pukeiti Gardens
36	EK612	*R. jasminiflorum* ssp. *jasminiflorum*	Pukeiti Gardens

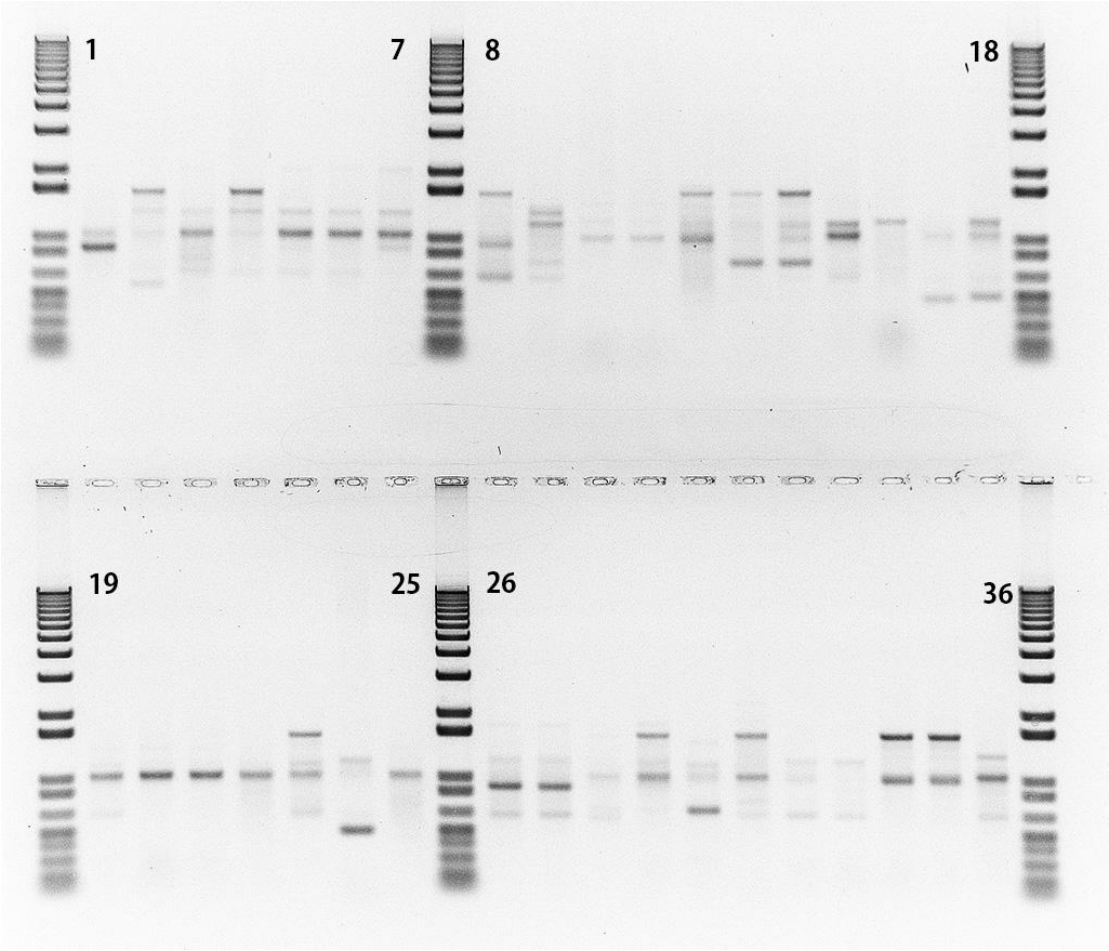


Photo 19 RAPD gel electrophoresis of 36 vireya samples. The RAPD primer used was OPAT-15.

Table 14 Sequences of 11 primers and the number of amplified bands of selected vireya accessions.

Primer	Sequence	Bands	Polymorphic	% Polymorphic
OPAB-13	CCTACCGTGG	6	6	100%
OPAN-14	AGCCGGGTAA	18	18	100%
OPAN-18	TGTCCTGCGT	8	8	100%
OPAN-20	GAGTCCTCAC	18	18	100%
OPAR-08	GTGAATGCGG	9	9	100%
OPAR-09	GGGGTGTTCT	9	9	100%
OPAR-10	TGGGGCTGTC	5	5	100%
OPAT-15	TGACGCACGG	11	11	100%
OPAV-16	GACAAGGACC	4	4	100%
OPAX-12	GGTCGGGTCA	10	10	100%
OPAX-20	ACACTCGGCA	21	21	100%

The RAPD analysis was carried out on 35 accessions representing 11 taxa (or groups of taxa) (Table 13). A total of 119 bands were amplified using 11 primers, for which all of the bands (100%) were polymorphic with respect to the accessions in the dataset. The average number of bands amplified by a primer was ten. A sample gel picture is shown in Photo 19 and the results are summarized in Table 14.

The following sections describe in detail, taxa or groups of taxa analysed for genetic diversity using microsatellite data, DNA sequence data and RAPD data. The subsets were chosen based on those taxa for which data for multiple accessions were available. The numbers above the branches indicate the length of the branches (which is the genetic distance used in the respective dendrogram).

5.4.1 *R. lochiae* and *R. viriosum*

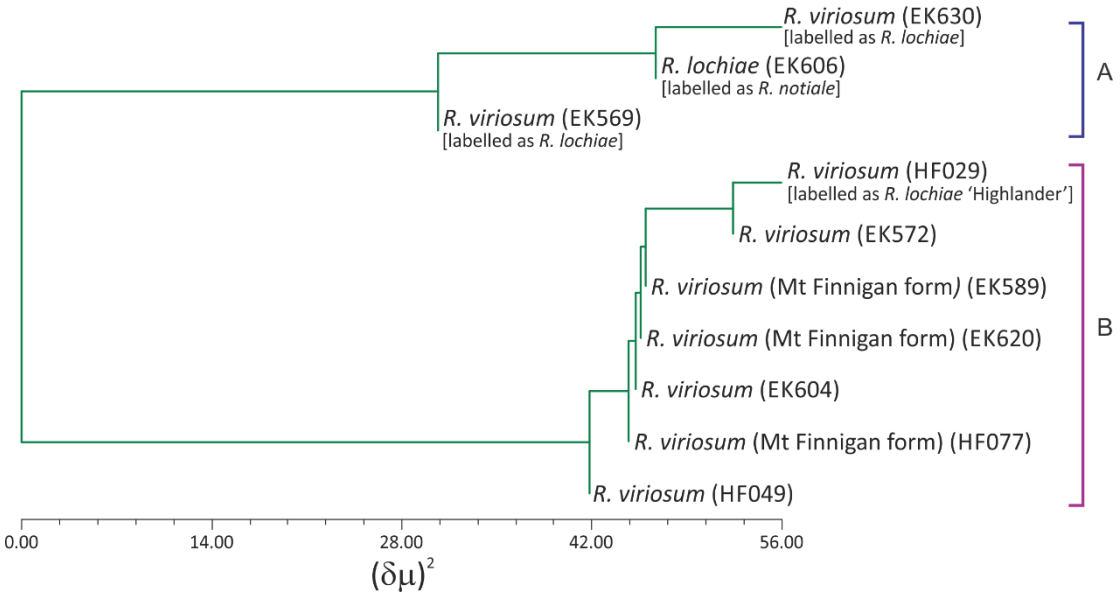


Figure 50 Genetic diversity among ten accessions of Australian *Rhododendron* taxa using microsatellite data. NJ dendrogram using fragment length dataset using three microsatellite markers, based on $(\delta\mu)^2$ genetic distance and assuming the Stepwise Mutation Model (SMM).

Figure 50 illustrates the dendrogram derived from the microsatellite dataset using the fragment length dataset for the Australian *Rhododendron* taxa. The genetic distances were calculated using the $(\delta\mu)^2$ method assuming the Stepwise Mutation Model (SMM), and the dendrogram was inferred using the Neighbour-Joining method.

Two genetically distinct groups (A and B) with significant genetic distance (72.48) can be observed from the Figure 50. The genetic distance between the accessions within Group A varied from 9.23–16.03, while that within Group B varied from 0.37–6.43.

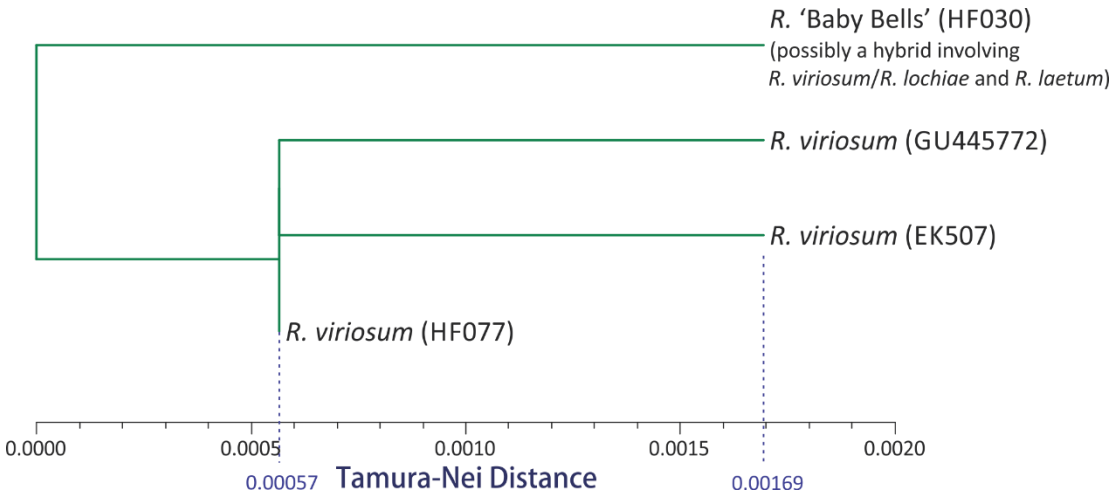


Figure 51 Genetic diversity among four accessions of Australian *Rhododendron* taxa using sequence data. NJ-based dendrogram showing the evolutionary divergence between *rpb*2i DNA sequences inferred from Tamura-Nei distance.

Figure 51 illustrates the Neighbour-Joining dendrogram for the evolutionary divergence between *rpb*2i DNA sequences of the Australian *Rhododendron* accessions using the Tamura-Nei distance. The genetic distance between the accessions GU445772 and EK507 is 0.00226 (0.23%) suggesting that these two accessions are genetically very similar. The genetic distance between GU445772, HF077 and EK507 is 0.00113 (0.11%) suggesting a very close genetic relationship. The genetic distance between the accession HF030 and the rest of the accessions is 0.00226 (0.23%), and is thus relatively genetically distant from the other accessions.

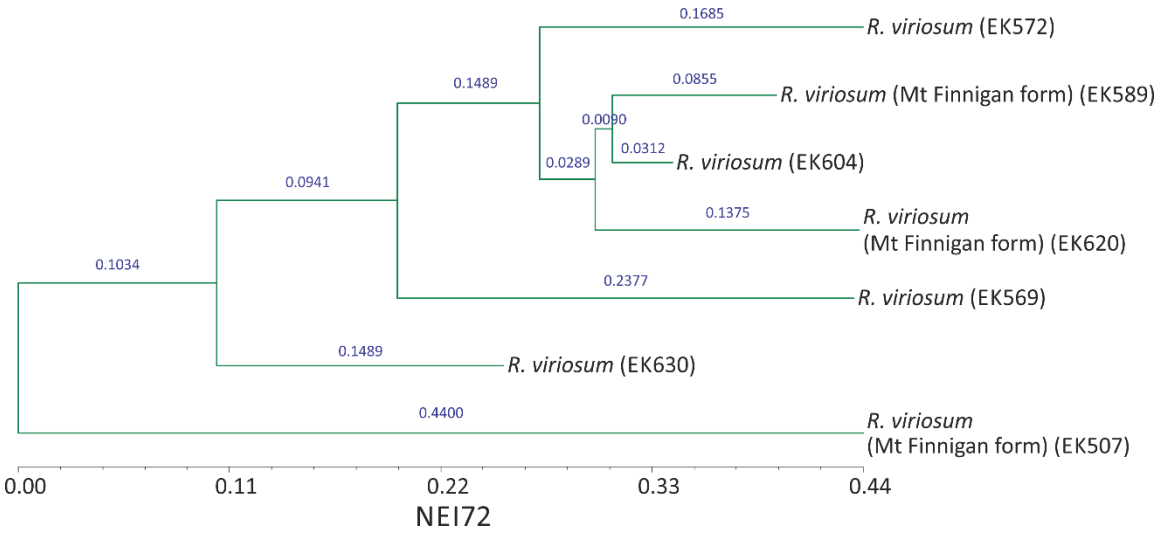


Figure 52 Genetic diversity among seven accessions of *R. viriosum* using RAPD data. Dendrogram based on Nei's unbiased genetic distances and NJ clustering using 11 primers.

Figure 52 illustrates the dendrogram generated from RAPD data based on Nei's unbiased genetic distances and NJ clustering among seven accessions of *R. viriosum* and 11 primers. The lowest genetic distance is 0.1166 between EK589 and EK604, and the highest genetic distance is 0.9688 between EK507 and EK569. The accession EK507 appears to be genetically distant from the rest of the accessions.

5.4.2 *R. jasminiflorum*

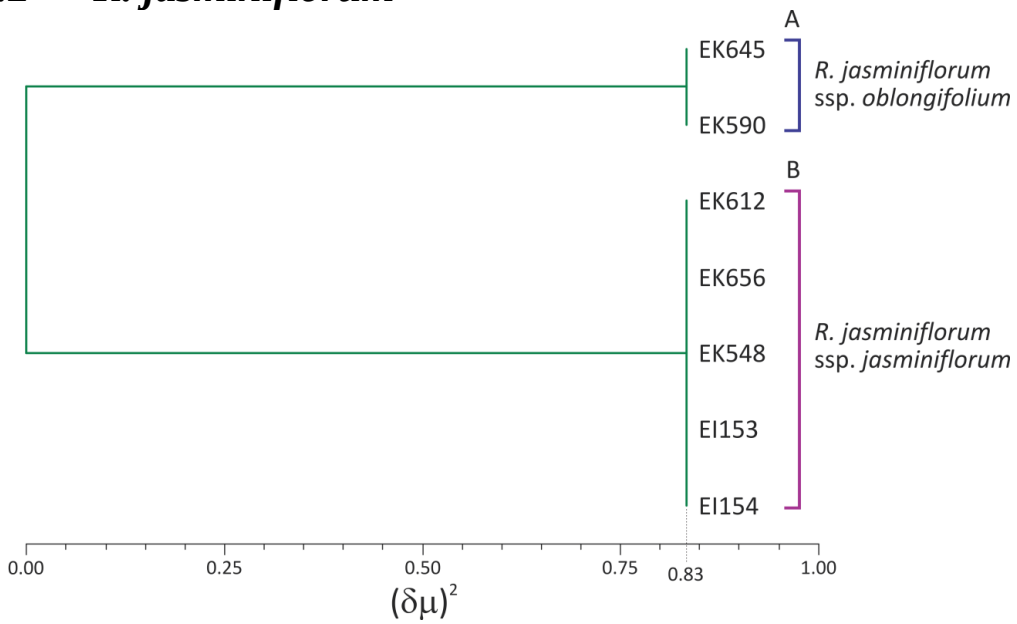


Figure 53 Genetic diversity among seven accessions of *R. jasminiflorum* using microsatellite data. NJ-based dendrogram of the microsatellite fragment length dataset using the microsatellite markers DC027, DC046 and DC049. The distance measure used is the $(\delta\mu)^2$ genetic distance assuming the Stepwise Mutation Model (SMM).Figure 53 illustrates the dendrogram derived

from the microsatellite fragment length dataset for the *R. jasminiflorum* accessions. The genetic distance measure used was $(\delta\mu)^2$ and the dendrogram was inferred using the Neighbour-Joining method. The $(\delta\mu)^2$ genetic distance between the two subspecies (A and B) is 1.667. The genetic diversity among the accessions within each group (A and B) was constantly 0.

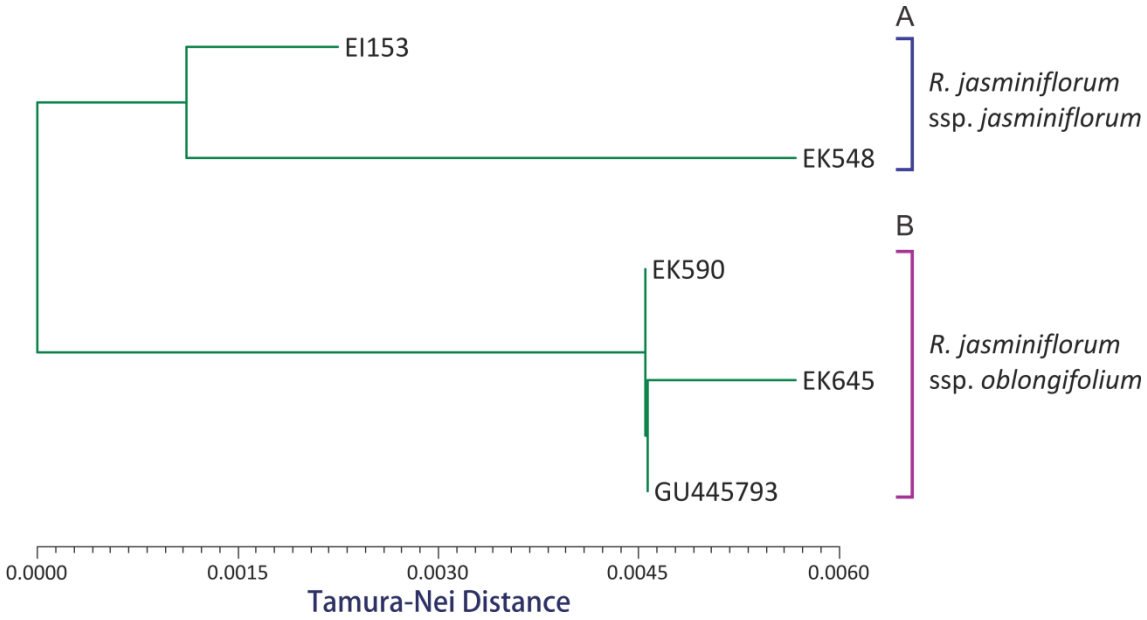


Figure 54 Genetic diversity among five accessions of *R. jasminiflorum* using sequence data. NJ-based dendrogram showing the evolutionary divergence among *rpb*2i DNA sequences inferred from the Tamura-Nei distance.

Figure 54 illustrates the Neighbour-Joining dendrogram of the evolutionary divergence between *rpb*2i DNA sequences of *R. jasminiflorum* accessions based on the Tamura-Nei genetic distance. The accessions formed two distinct clades corresponding to the subspecies *jasminiflorum* and *oblongifolium*, similar to the dendrogram obtained from microsatellite data (Figure 53). The genetic distance between these two subspecies is 0.056 (5.6%). The genetic distance between the accessions of ssp. *jasminiflorum* is 0.057 (5.7%), while that between the accessions of ssp. *oblongifolium* varied from ~0–0.0011 (0–1.1%). The accessions EK590 and GU445793 are almost genetically identical, however these two accessions are genetically distinct from EK645 by a distance of 0.0011 (1.1%).

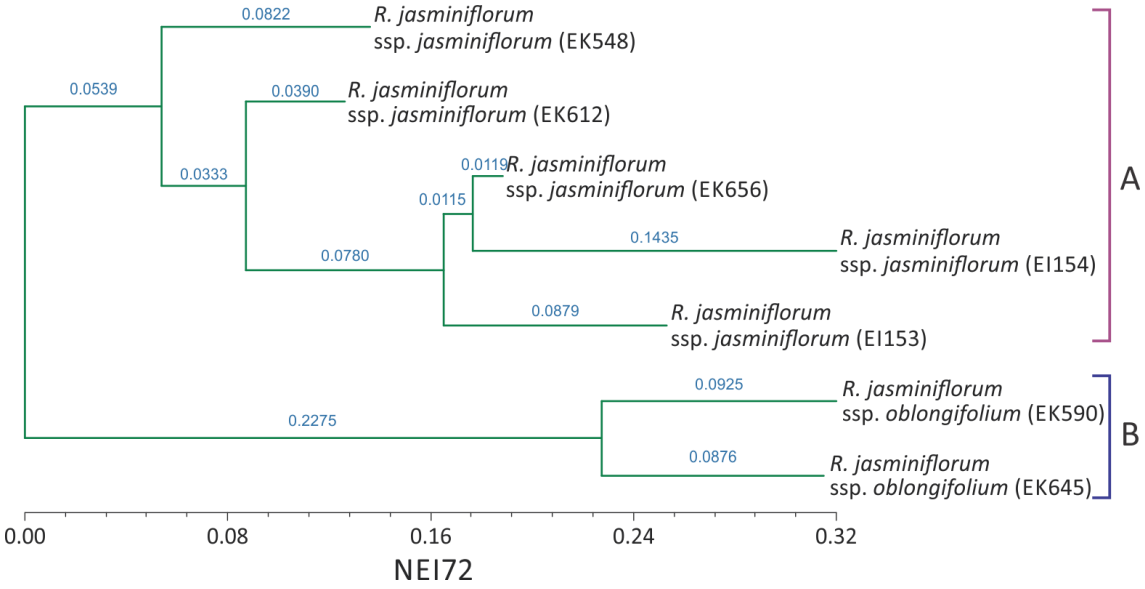


Figure 55 Genetic diversity among seven accessions of *R. jasminiflorum* using RAPD data. Dendrogram generated from RAPD data based on Nei's unbiased genetic distances and NJ clustering using 11 primers.

Figure 55 illustrates the dendrogram generated from RAPD data based on Nei's unbiased genetic distances and NJ clustering among seven accessions of *R. jasminiflorum* and 11 primers. The dendrogram is partitioned into two discernible clusters, A and B. The cluster A consists of accessions belonging to the subspecies *jasminiflorum* and the cluster B consists of accessions belonging to the subspecies *oblongifolium*. The lowest genetic distance observed is 0.11740 between the accessions EK612 and EK656, while the largest genetic distance is 0.7075 between EK590 and EI154. The genetic distance between the clusters A and B is 0.2814.

5.4.3 *R. luraluense* ssp. *luraluense*

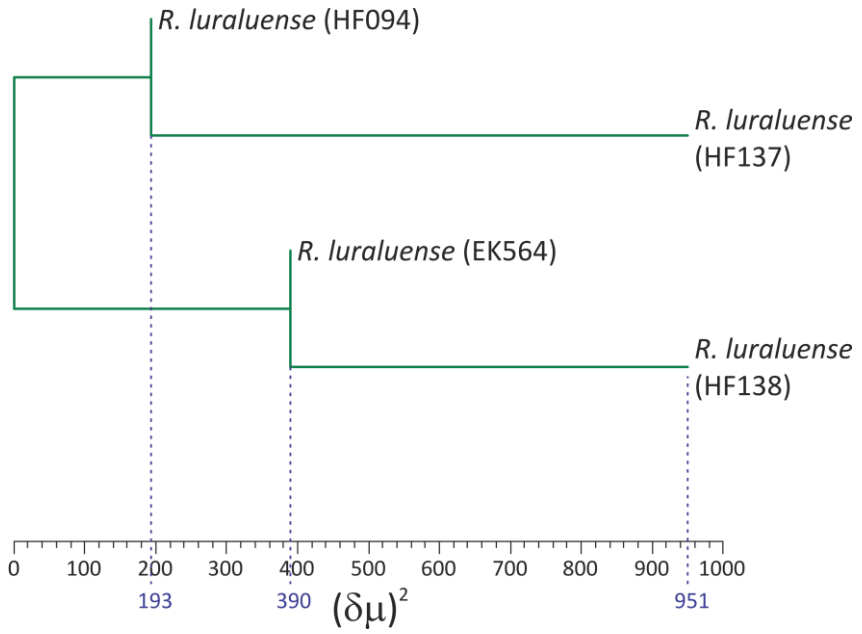


Figure 56 Genetic diversity among four accessions of *R. luraluense* ssp. *luraluense* using microsatellite data. NJ dendrogram for the microsatellite fragment length dataset based on $(\delta\mu)^2$ genetic distance using four microsatellite markers (DD042, DC046, GA211 and DC027A1).

Figure 56 shows the NJ-based dendrogram for the microsatellite fragment length dataset, for four accessions of *R. luraluense* ssp. *luraluense* based on $(\delta\mu)^2$ genetic distance and four microsatellite markers. The genetic distances among the accessions varied from 560–1,902.

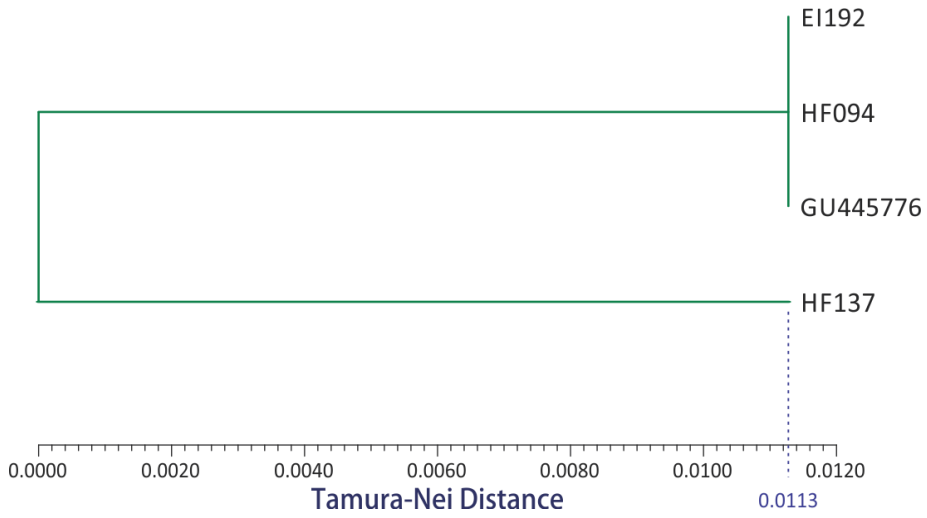


Figure 57 Genetic diversity among four *R. luraluense* ssp. *luraluense* accessions using sequence data. NJ-based dendrogram showing the evolutionary divergence between *rpb*2i DNA sequences inferred from the Tamura-Nei distance. All accessions assumed to belong to the taxon *R. luraluense* ssp. *luraluense*.

Figure 57 illustrates the NJ dendrogram of the evolutionary divergence between *rpb*2i DNA sequences of *R. luraluense* ssp. *luraluense* accessions inferred using the Tamura-Nei genetic distance, showing a similar trend as seen in the genetic diversity analysis using microsatellites. The accessions formed two distinct clades with one clade containing EI192, HF094 and GU445776, while the other clade consisting of only the accession HF137. The two clades are genetically separated by a distance of 0.0226 (2.26%). The accessions EI192, HF094 and GU445776 are genetically identical with a genetic distance of 0.

5.4.4 *R. gracilentum*

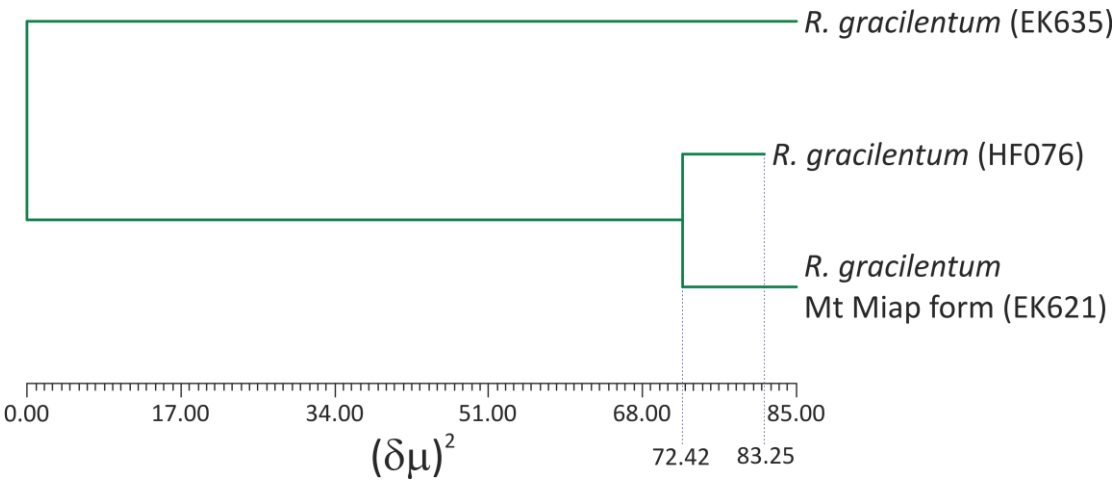


Figure 58 Genetic diversity among three accessions of *R. gracilentum* using microsatellite data. NJ dendrogram for the fragment length dataset from five microsatellite markers based on $(\delta\mu)^2$ genetic distance and assuming the Stepwise Mutation Model (SMM).

Figure 58 illustrates the genetic relationships between the accessions of *R. gracilentum* based on the fragment length dataset using the $(\delta\mu)^2$ genetic distance. The accessions HF076 and EK621 are closely related with a genetic distance of 23.41. The accession EK635 is relatively distant from the accessions HF076 and EK621 with a genetic distance of 157.42.

5.4.5 *R. macgregoriae*

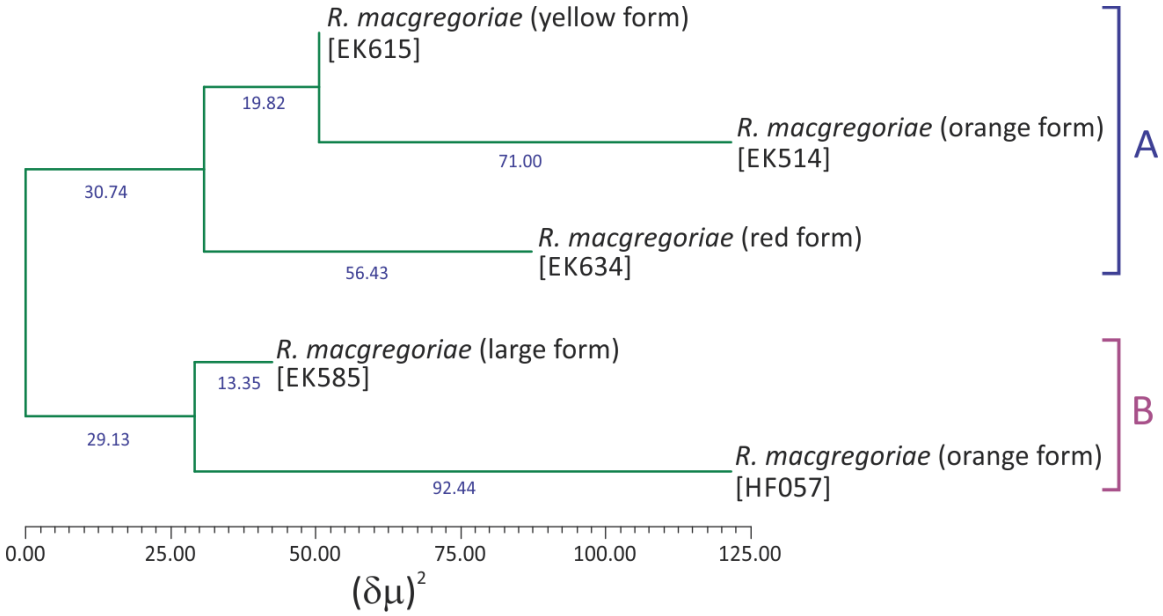


Figure 59 Genetic diversity among five accessions of *R. macgregoriae* using microsatellite data. NJ dendrogram for the fragment length dataset from five microsatellite markers based on $(\delta\mu)^2$ genetic distance and assuming the Stepwise Mutation Model (SMM).

Figure 59 illustrates the NJ dendrogram of five accessions of *R. macgregoriae* using five microsatellite markers, based on the $(\delta\mu)^2$ genetic distance and assuming the Stepwise Mutation Model (SMM). The genetic diversity among the accessions is relatively high (71–247), suggesting that the accessions may have been collected from geographically separate populations. The accessions form two distinct groups (A and B) with a genetic distance of 59.87. The genetic distance within Group A ranged from 71–147.25, and the genetic distance between the two accessions of Group B was 105.79.

5.4.6 *R. javanicum*

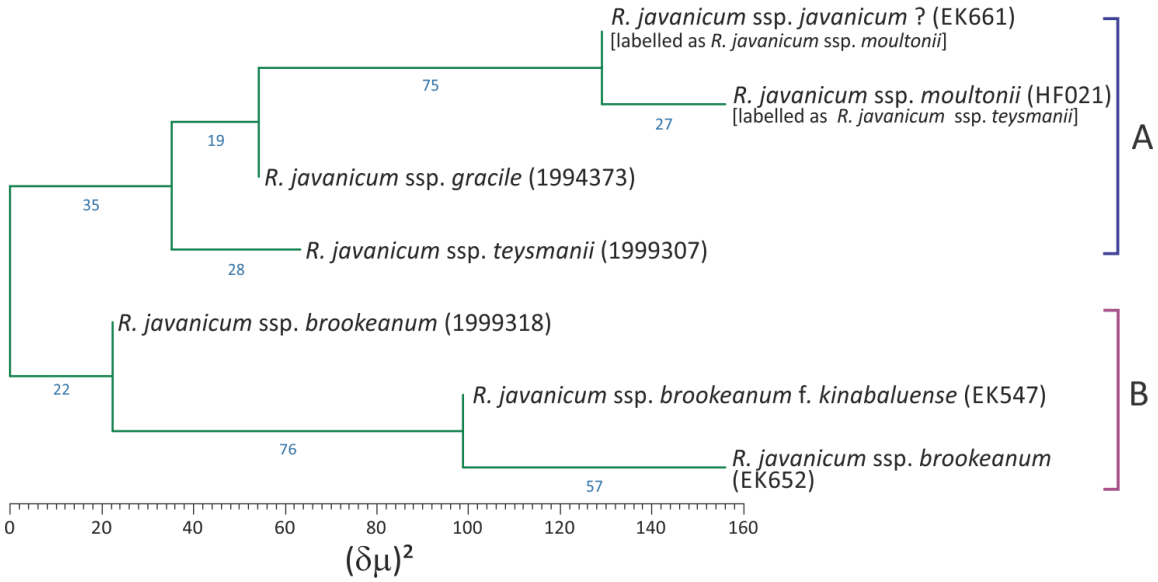


Figure 60 Genetic diversity among seven accessions of *R. javanicum* using microsatellite data. NJ dendrogram for the microsatellite fragment length dataset, based on $(\delta\mu)^2$ genetic distance and assuming the Stepwise Mutation Model (SMM).

Figure 60 illustrates the NJ dendrogram for the analysis of the microsatellite fragment length dataset, showing relationships between seven accessions of *R. javanicum* based on $(\delta\mu)^2$ genetic distance and assuming the Stepwise Mutation Model (SMM). The accessions formed two distinct groups: (A) containing the subspecies *moultonii*, *teysmannii* and *gracile*, and (B) containing only the subspecies *brookeanum*. The genetic distance between the accessions varied from 27–311. The genetic distance among the accessions within Group A ranged from 27–129, and that within Group B ranged from 57–133.

5.4.7 *R. superbum* and *R. konori*

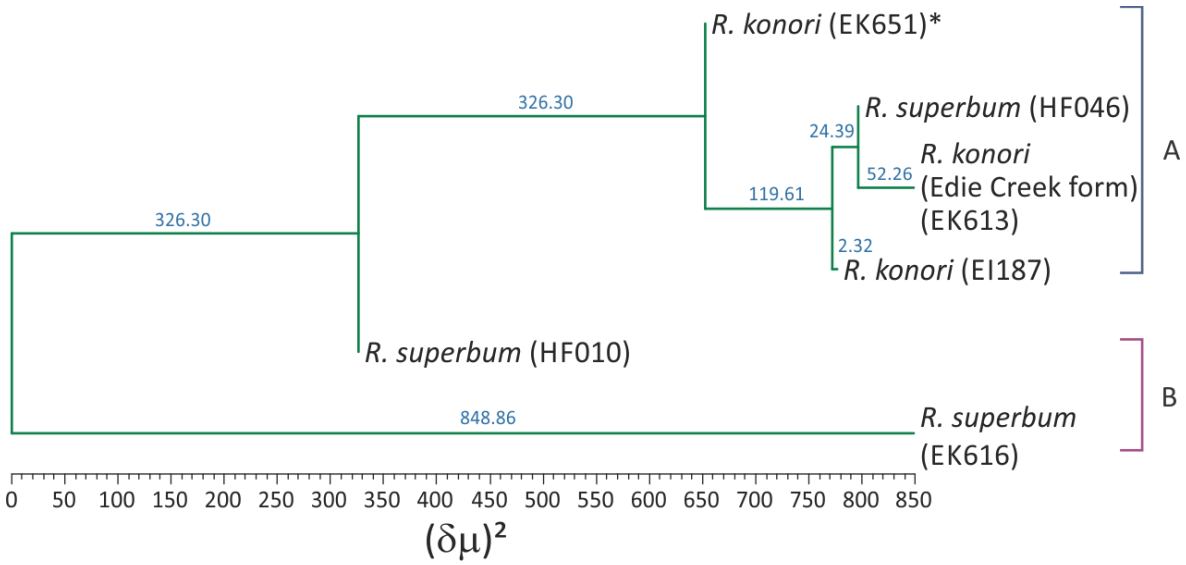


Figure 61 Genetic diversity among accessions of *R. konori* and *R. superbum* using microsatellite data. NJ dendrogram for the fragment length dataset from five microsatellite markers based on $(\delta\mu)^2$ genetic distance. Accession marked with * was collected labelled as *R. superbum*.

Figure 61 illustrates the NJ-based dendrogram for the fragment length dataset of five microsatellite markers, showing relationships between six accessions of *R. superbum* and *R. konori* based on $(\delta\mu)^2$ genetic distance. The genetic diversity analysis showed that the $(\delta\mu)^2$ genetic distances varied from 2.32–1,697.72, suggesting very high genetic diversity among the accessions. However, the genetic distances among the accessions of Group A are relatively small, 2.32–196.26. In Group B, the genetic diversity among the accessions is significantly high.

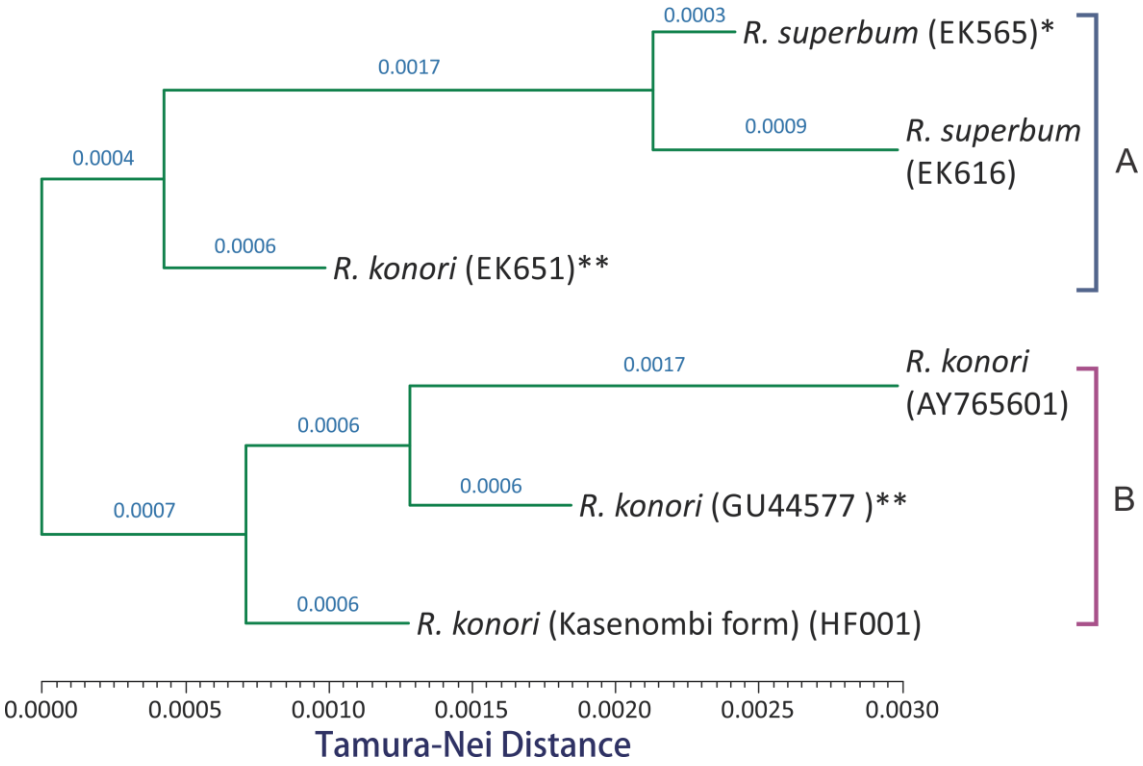


Figure 62 Genetic diversity among accessions of *R. konori* and *R. superbum* using sequence data. NJ-based dendrogram showing the evolutionary divergence between *rpb*2i DNA sequences inferred from the Tamura-Nei distance. Accessions marked with * were originally labelled as *R. dianthosmum*, while those marked with ** were labelled as *R. superbum*.

Figure 62 illustrates the NJ-based dendrogram showing the evolutionary divergence between *rpb*2i DNA sequences of *R. superbum* and *R. konori* accessions inferred from the Tamura-Nei distance. The genetic diversity analysis of this group showed that the genetic distances between the accessions are very low, suggesting a very closely related ensemble of accessions. The accessions cluster into two distinct groups (A and B) with a genetic distance of 0.0011. The genetic distances within Group A varied from 0.003–0.0032, and that of Group B varied from 0.0006–0.0029.

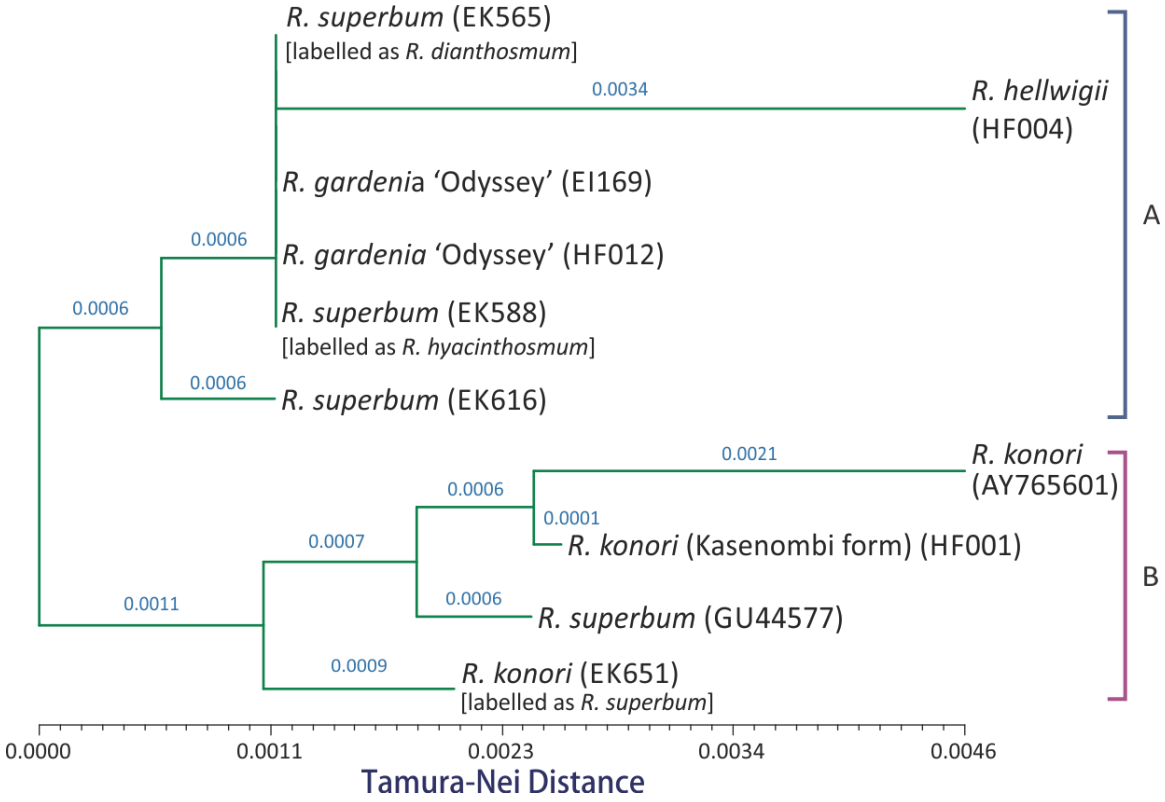


Figure 63 Genetic diversity among accessions of *R. superbum* and its relatives using sequence data. NJ-based dendrogram showing the evolutionary divergence between *rpb*2i DNA sequences inferred from the Tamura-Nei distance.

Figure 63 illustrates the NJ-based dendrogram generated for the evolutionary divergence between *rpb*2i DNA sequences of *R. superbum* and related taxa inferred from the Tamura-Nei distance. This analysis showed that the accessions formed two distinct groups (A and B) containing a mixture of taxa. The Group A contains *R. superbum*, *R. hellwigii* and *R. gardenia* 'Odyssey', while the Group B contained *R. superbum* and *R. konori* accessions. The genetic distance between the two groups is 0.0017. The genetic variation between the accessions of Group A is 0.0006–0.0046. The genetic variation between the accessions of Group B is 0.0001–0.0043. The overall genetic diversity among all the accessions is relatively low.

5.4.8 *R. orbiculatum*

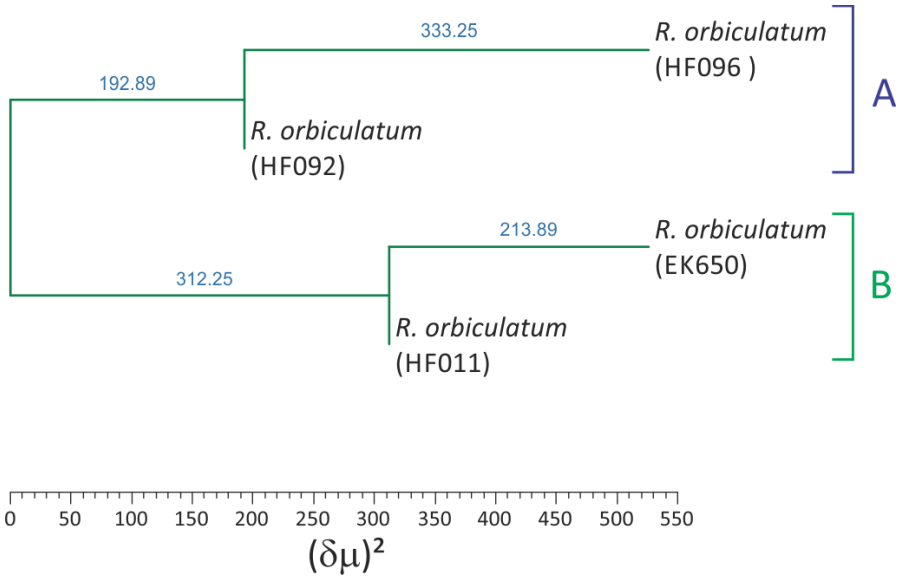


Figure 64 Genetic diversity among four accessions of *R. orbiculatum* using microsatellite data. NJ-based dendrogram for the microsatellite fragment length dataset using four microsatellite markers, based on $(\delta\mu)^2$ genetic distance and assuming the Stepwise Mutation Model (SMM).

Figure 64 illustrates the genetic diversity analysis of four *R. orbiculatum* accessions using microsatellite data based on the $(\delta\mu)^2$ genetic distance and assuming the SMM model. The accessions formed two distinct clusters (A and B) separated by a genetic distance of 505.14. The genetic distance within Group A is 333.25, while that of Group B is 213.89. The overall genetic distance among the accessions is relatively high.

5.4.9 *R. laetum*

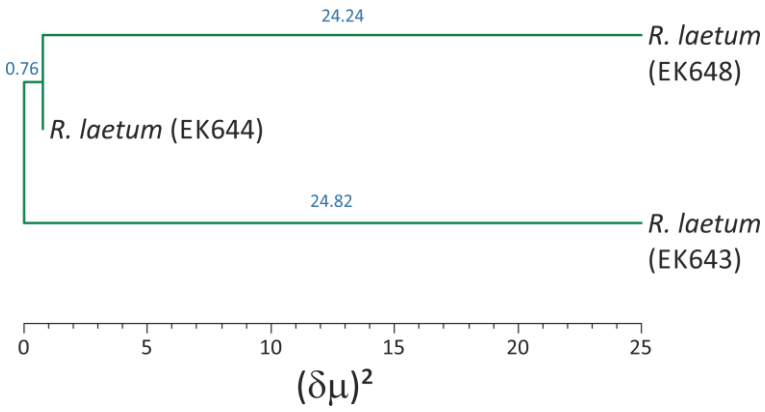


Figure 65 Genetic diversity among three accessions of *R. laetum* using microsatellite data. NJ dendrogram for the fragment length dataset based on $(\delta\mu)^2$ genetic distance and assuming the Stepwise Mutation Model (SMM).

Figure 65 illustrates the genetic diversity analysis of the *R. laetum* accessions using microsatellite data and the genetic distance $(\delta\mu)^2$ assuming SMM. The genetic diversity is relatively low among the accessions, suggesting that these accessions are very closely related, and perhaps collected from a single wild population.

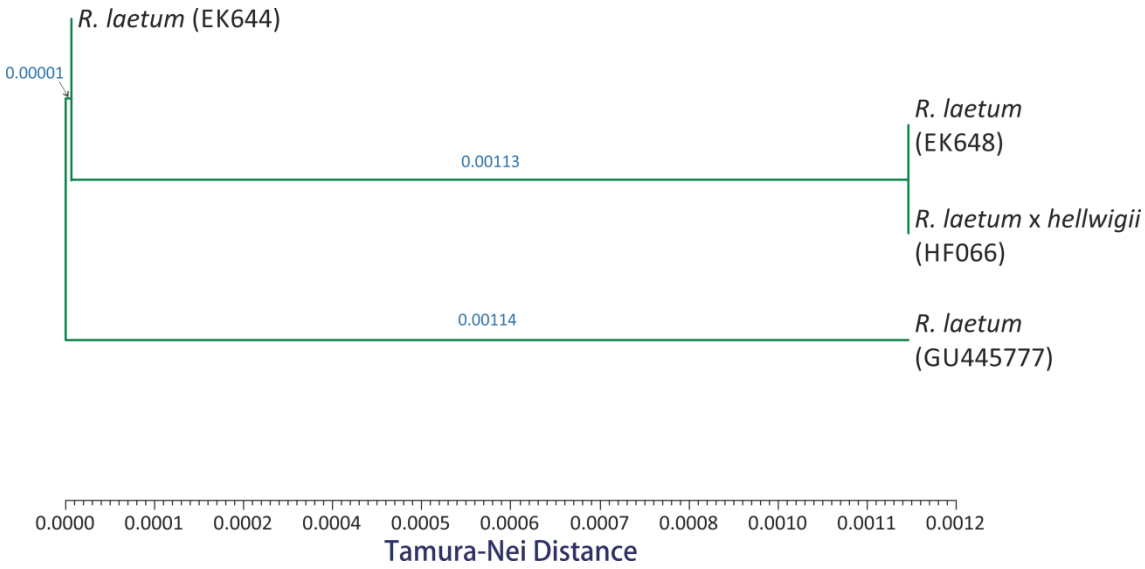


Figure 66 Genetic diversity among *R. laetum* accessions using sequence data. NJ-based dendrogram showing the evolutionary divergence among *rpb*2i DNA sequences inferred using the Tamura-Nei model.

Figure 66 illustrates the evolutionary divergence between *rpb*2i DNA sequences of the *R. laetum* complex using the Tamura-Nei model. The genetic differentiation between the accessions is very low (<0.2%), suggesting that these accessions may have originated from a single wild population. The accessions EK648 and HF066 cluster together and are genetically identical, thus HF066 could be a vegetative clone of EK648.

5.4.10 *R. quadrasianum*

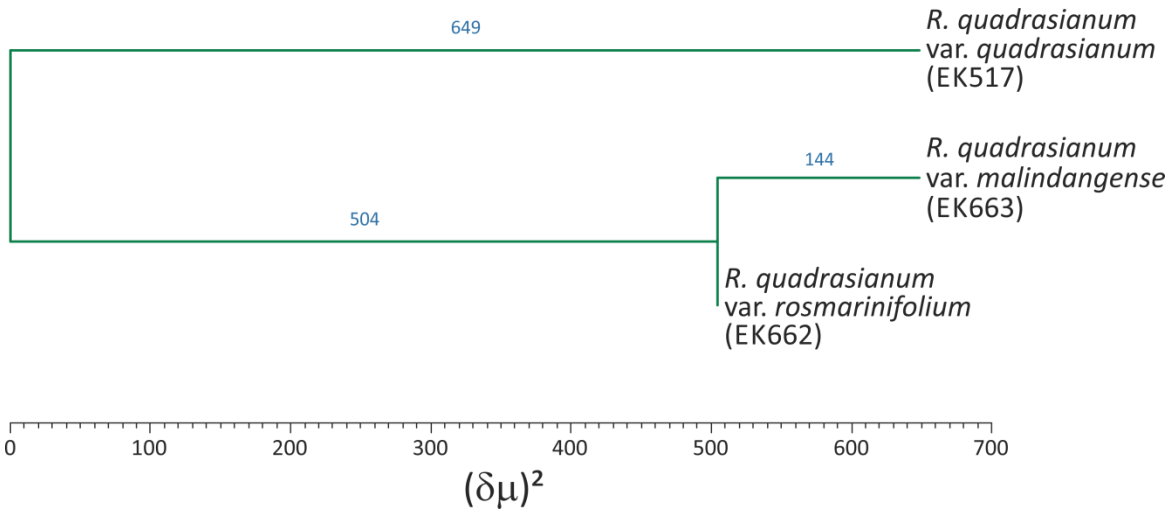


Figure 67 Genetic diversity among three *R. quadrasianum* taxa using microsatellite data. NJ dendrogram for the fragment length dataset based on $(\delta\mu)^2$ genetic distance and assuming the Stepwise Mutation Model (SMM).

Figure 67 illustrates the NJ-based dendrogram for the fragment length dataset, showing relationships between three accessions of *R. quadrasianum* based on $(\delta\mu)^2$ genetic distance and assuming the Stepwise Mutation Model (SMM). The genetic diversity among the accessions is relatively high (144–1,297) suggesting collections from geographically separated localities. The accessions EK662 and EK663 cluster together with a genetic difference of 144. The genetic distance between the accession EK517 and the cluster containing the accessions EK662 and EK663 is 1,153.

5.4.11 *R. christi*

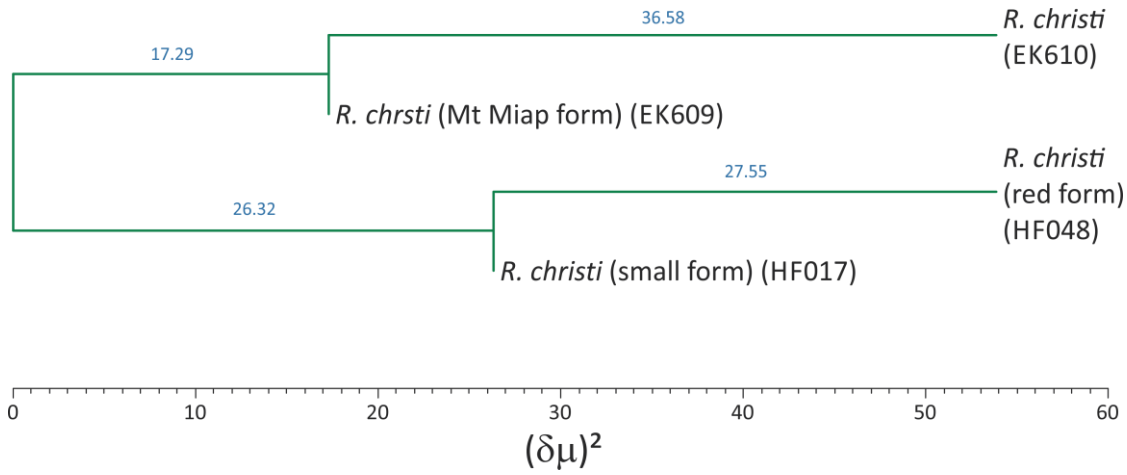


Figure 68 Genetic diversity among four accessions of *R. christi* using microsatellite data. NJ dendrogram for the fragment length dataset based on $(\delta\mu)^2$ genetic distance and assuming the Stepwise Mutation Model (SMM).

Figure 68 illustrates the NJ-based dendrogram for the fragment length dataset, showing relationships between four accessions of *R. christi* based on $(\delta\mu)^2$ genetic distance and assuming the Stepwise Mutation Model (SMM). The low genetic diversity among the accessions suggest that these are very closely related and further studies including multiple accessions of each morphological form could reveal the taxonomic limits of these forms.

5.4.12 *R. culminicola*

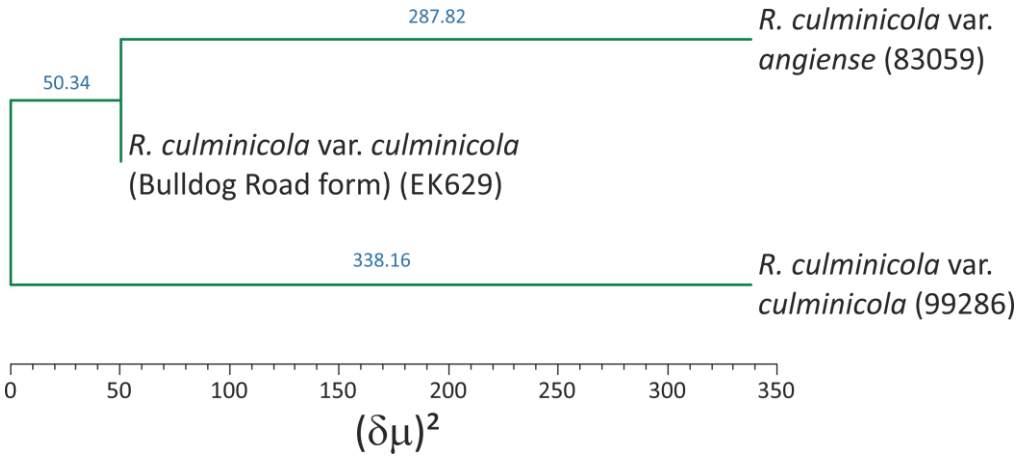


Figure 69 Genetic diversity among three accessions of *R. culminicola* using microsatellite data. NJ dendrogram for the fragment length dataset based on $(\delta\mu)^2$ genetic distance and assuming the Stepwise Mutation Model (SMM).

Figure 69 illustrates the NJ-based dendrogram for the fragment length dataset, showing relationships between three accessions of *R. culminicola* based on $(\delta\mu)^2$ genetic distance and assuming the Stepwise Mutation Model (SMM). The genetic distances among the accessions varied between 287.82– 676.32. The two accessions (99286 from USA and EK629 from New Zealand) belonging to the subspecies *culminicola* do not cluster together, and are separated by a genetic distance of 388.5. The single accession (83059 from USA) belonging to the subspecies *angiense* with a genetic distance of 287.82.

5.4.13 *R. dielsianum*

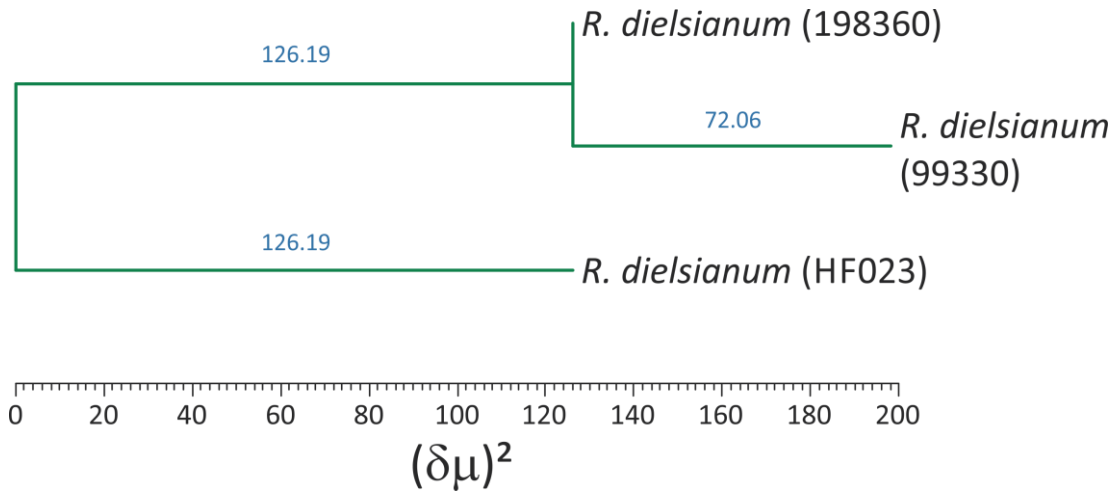


Figure 70 Genetic diversity among three accessions of *R. dielsianum* using microsatellite data. NJ dendrogram for the fragment length dataset based on $(\delta\mu)^2$ genetic distance and assuming the Stepwise Mutation Model (SMM).

Figure 70 illustrates the NJ-based dendrogram for the fragment length dataset, showing relationships between three accessions of *R. dielsianum* based on $(\delta\mu)^2$ genetic distance and assuming the Stepwise Mutation Model (SMM). The overall genetic distances among the accessions are moderate (72.06–324.44). The accessions 198360 and 99330 cluster together with a genetic distance of 72.06. The accession HF023 is relatively distant from the accessions 198360 and 99330, with a genetic distance of 252.38.

5.4.14 *R. commonae*

The evolutionary divergence between *rpb*2i DNA sequences of the five *R. commonae* accessions inferred from the Tamura-Nei distance showed that the accessions are

genetically identical. Due to the lack of genetic diversity a dendrogram cannot be produced for this dataset. The accessions examined were EK632, EK633, EK637, HF062 and GU445786.

5.4.15 *R. emarginatum*

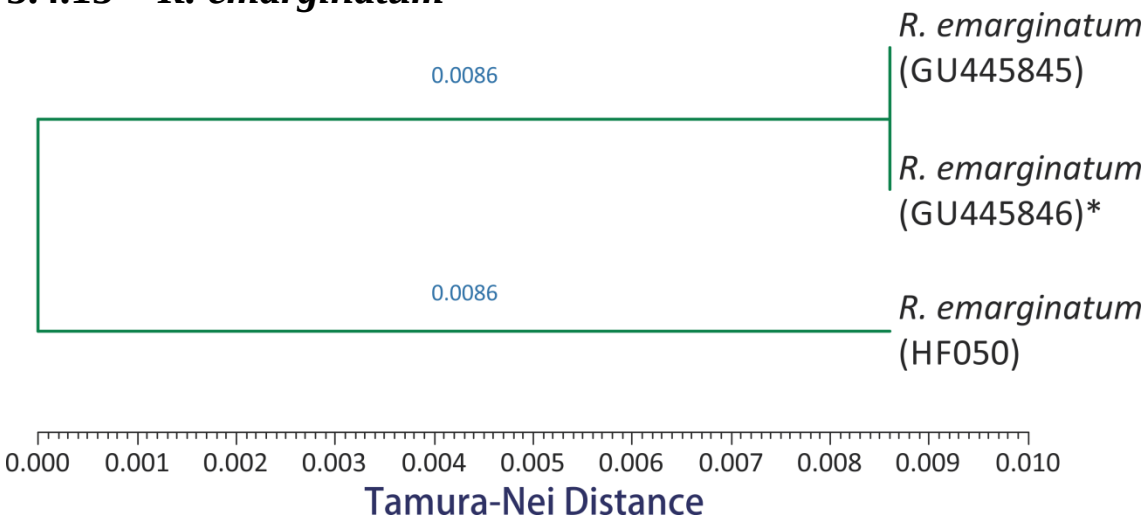


Figure 71 Genetic diversity among three accessions of *R. emarginatum* using sequence data. NJ-based dendrogram inferred from the Tamura-Nei distance. All accessions assumed to belong to a single variable taxon. The accession marked with * was published as *R. eunonymifolium* (now reduced to a synonym of *R.emarginatum*).

Figure 71 illustrates the NJ-based dendrogram showing the evolutionary divergence between *rpb*2i DNA sequences of three *R. emarginatum* accessions inferred from the Tamura-Nei distance. The accessions GU445845 and GU445846 cluster together with no genetic differentiation between them. The accession HF050 is significantly distant from the accessions GU445845 and GU445846 with a genetic distance of 0.0172. The genetic distances between the accessions are relatively low, suggesting very closely related accessions.

5.4.16 *R. majus*

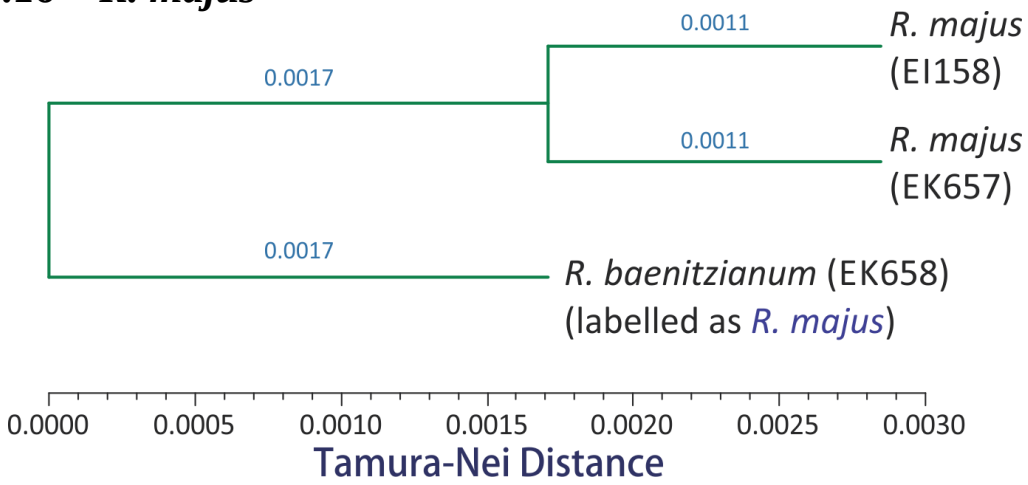


Figure 72 Genetic diversity among three accessions of *R.majus* using sequence data. NJ-based dendrogram inferred from the Tamura-Nei distance.

Figure 72 illustrates the NJ-based dendrogram showing the evolutionary divergence between *rpb*2i DNA sequences of *R. majus* accessions inferred from the Tamura-Nei distance. The accessions EI158 and EK657 appears to be closely related (genetic distance 0.0022) to each other than to EK658. The genetic distance between the accession EK658 and the cluster containing EI158 and EK657 is 0.0034.

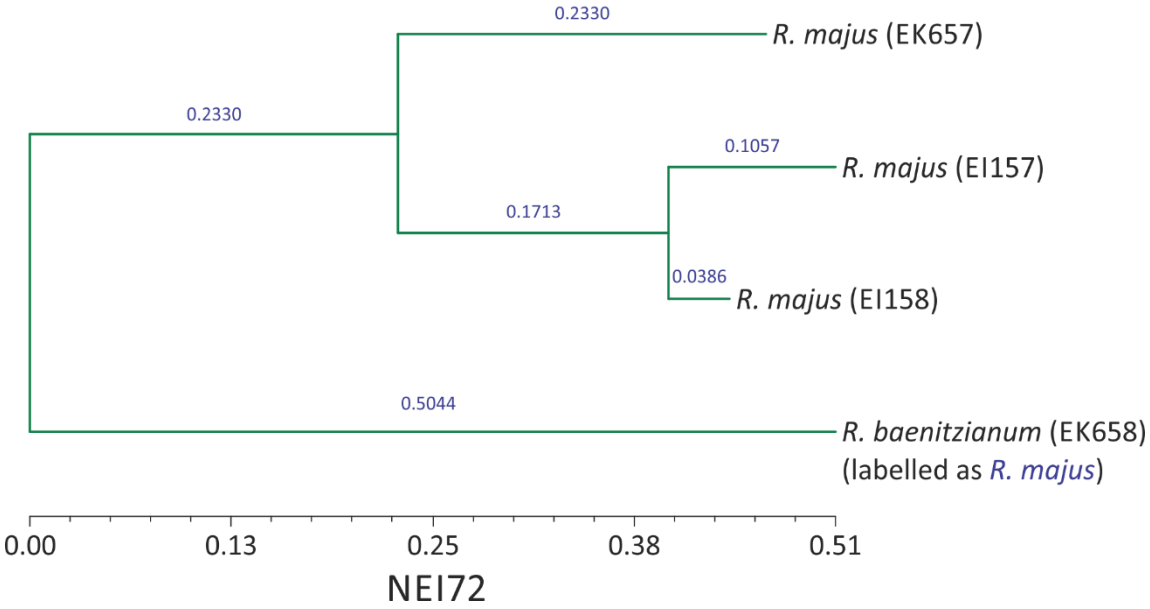


Figure 73 Genetic diversity among four accessions of *R. majus* using RAPD data. Dendrogram generated from RAPD data based on Nei's unbiased genetic distances and NJ clustering using 11 primers.

Figure 73 illustrates the dendrogram generated from RAPD data based on Nei's unbiased genetic distances and NJ clustering among four accessions of *R. majus* using 11 primers.

The lowest genetic distance is 0.1430 between the two accessions EI157 and EI158, and the largest genetic distance is 1.053 between EI157 and EK658. The accessions EI157 and EI158 cluster together with very low genetic differentiation (0.1430), and these two accessions cluster together with the accession EK657. The accession EK658 is genetically significantly distinct from the rest of the accessions. On physical examination of EK658, the accession keys out to *R. baenitzianum*.

5.4.17 *R. solitarium*

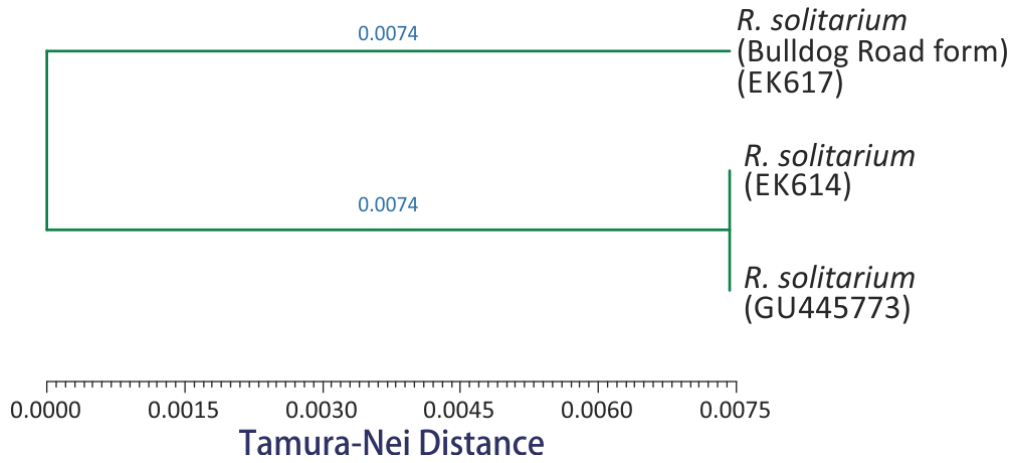


Figure 74 Genetic diversity among three accessions of *R. solitarium* using sequence data. NJ-based dendrogram inferred from the Tamura-Nei distance.

Figure 74 illustrates the NJ-based dendrogram showing the evolutionary divergence between *rpb*2i DNA sequences of three *R. solitarium* accessions inferred from the Tamura-Nei distance. The accessions EK614 and GU445773 cluster together with no genetic difference between them. The accession EK617 is significantly different from the accessions EK614 and GUI445773 with a genetic distance of 0.0148. The genetic distances between the accessions are relatively low, suggesting very closely related accessions.

5.4.18 *R. suaveolens*

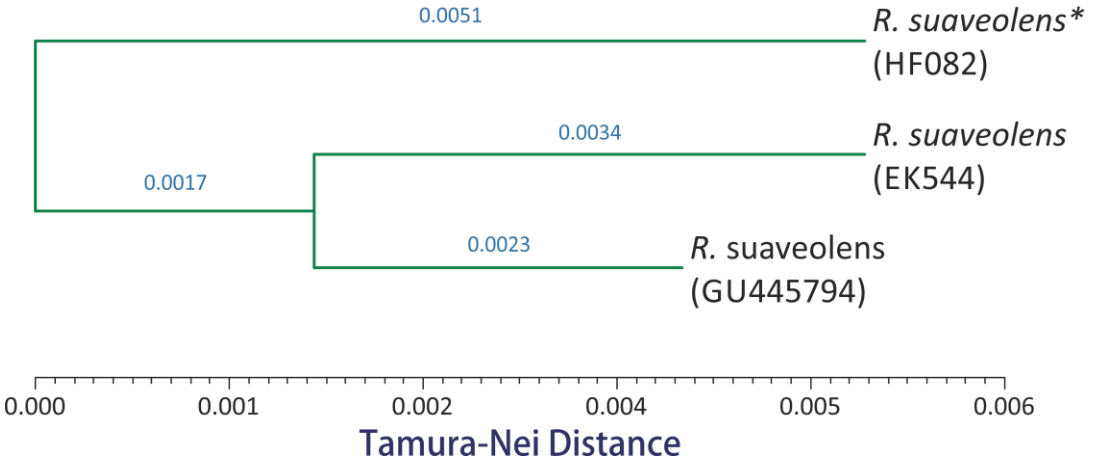


Figure 75 Genetic diversity among three accessions of *R. suaveolens* using sequence data. NJ-based dendrogram showing the evolutionary divergence between *rpb*2i DNA sequences inferred from the Tamura-Nei distance. The accession marked with * was obtained from a private collection in New Zealand.

Figure 75 illustrates the NJ-based dendrogram showing the evolutionary divergence between *rpb*2i DNA sequences of three *R. suaveolens* accessions inferred from the Tamura-Nei distance. The accessions EK544 and GU445794 cluster together with relatively low genetic difference between them (0.0058). The accession HF082 is significantly distant from the accessions EK544 and GU445794 with a genetic distance of 0.0068. The genetic distances between the accessions are relatively low, suggesting very closely related accessions.

5.4.19 *R. fallacinum*

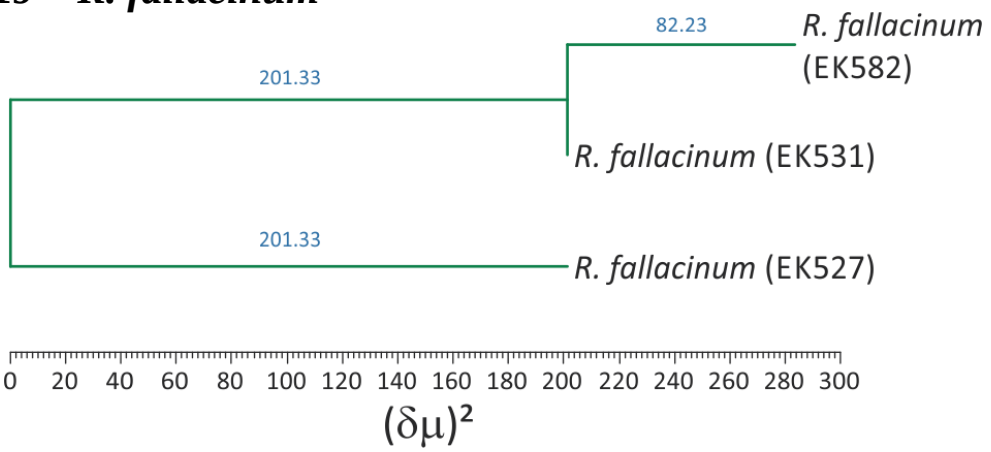


Figure 76 Genetic diversity among three accessions of *R. fallacinum* using microsatellite data. NJ dendrogram for the fragment length dataset based on $(\delta\mu)^2$ genetic distance and assuming the Stepwise Mutation Model (SMM).

Figure 76 illustrates the NJ-based dendrogram for the fragment length dataset, showing relationships between three accessions of *R. fallacinum* based on $(\delta\mu)^2$ genetic distance and assuming the Stepwise Mutation Model (SMM). The accessions EK582 and EK531 cluster together with a genetic difference of 82.23 between them. The accession EK527 is significantly distant from the other two accessions with a genetic distance of 402.66.

5.4.20 *R. stenophyllum* & *R. crassifolium*

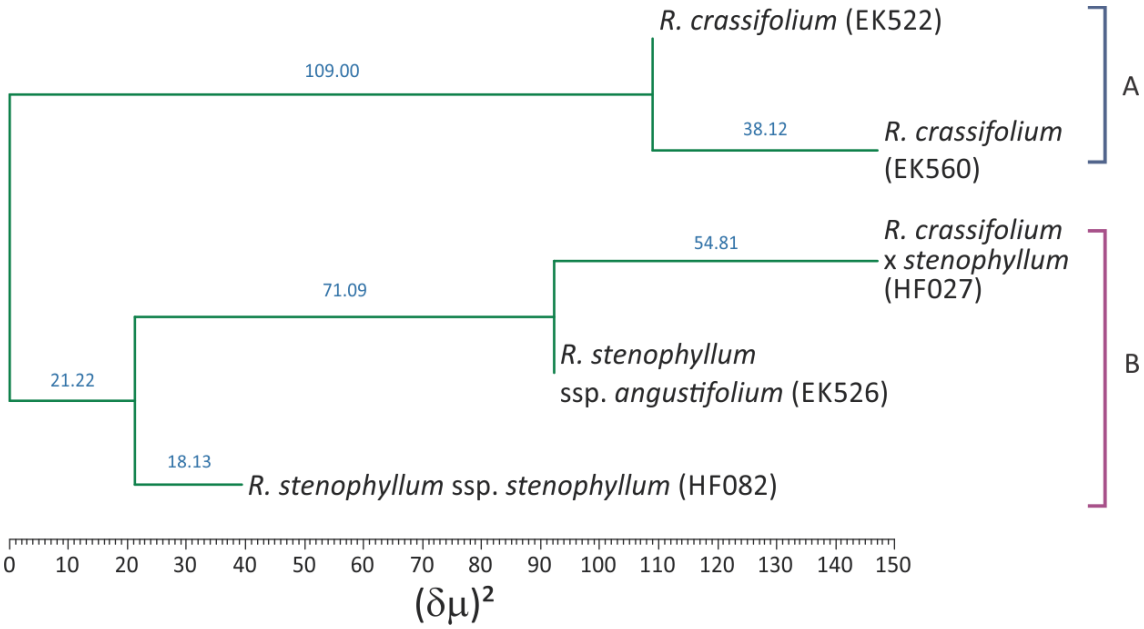


Figure 77 Genetic diversity among accessions of *R. crassifolium*, *R. stenophyllum* and their hybrid. NJ dendrogram for the fragment length dataset based on $(\delta\mu)^2$ genetic distance and assuming the Stepwise Mutation Model (SMM). The numbers above the branches indicate the length of the branches.

Figure 77 illustrates the NJ-based dendrogram for the fragment length dataset, showing relationships between accessions of *R. crassifolium*, *R. stenophyllum* and their hybrid, based on $(\delta\mu)^2$ genetic distance and assuming the Stepwise Mutation Model (SMM). The genetic distances among the accessions varied from 18.13–294.24. The accessions formed two distinct groups (A and B), with a genetic distance of 130.22. The genetic variation within Group A is 38.12, while that of Group B is 18.13–144.03.

5.4.21 *R. blackii*

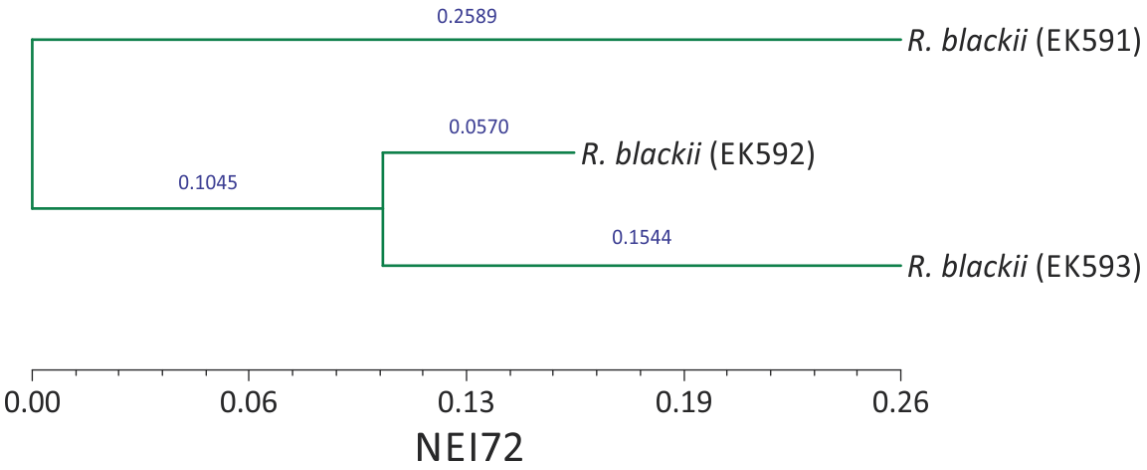


Figure 78 Genetic diversity among three accessions of *R. blackii* using RAPD data. Dendrogram generated from RAPD data based on Nei's unbiased genetic distances and NJ clustering using 11 primers.

Figure 78 illustrates the dendrogram generated from RAPD data based on Nei's unbiased genetic distances and NJ clustering between three accessions of *R. blackii* using 11 primers. The smallest genetic distance is 0.2114, observed between the accessions EK592 and EK593, while the largest genetic distance is 0.5178, observed between the accessions EK591 and EK593. The accessions EK592 and EK593 are more closely related than any of these to the accession EK591. The overall genetic distance between the accessions is relatively low, suggesting very closely related accessions.

5.4.22 Taxa with Only Two Accessions

This section describes the genetic diversity between accessions of taxa for which only two accessions were available for study.

Table 15 Genetic Distance between accessions of vireyas with only two accessions using microsatellite data. The distances are based on the $(\delta\mu)^2$ genetic distance assuming the Stepwise Mutation Model (SMM). **a** – *R. stenophyllum* ssp. *stenophyllum*, **b** – *R. stenophyllum* ssp. *angustifolium*.

#	Taxon	Accession 1	Accession 2	# of markers	Genetic Dist. $(\delta\mu)^2$
1	*R. kawakamii*	HF059	HF072	4	0.00
2	*R. rugosum*	EK540	HF005	4	6.66
3	*R. rarilepidotum*	EK584	EK665	4	16.25
4	*R. crassifolium*	EK522	EK560	5	18.81
5	*R. archboldianum*	HF002	HF003	8	20.20
6	*R. polyanthemum*	94/333	994/336	5	27.50
7	*R. acrophilum*	EK669	2002/018	6	31.33
8	*R. phaeochitum*	HF019	HF022	2	32.00
9	*R. perakense*	EK553	HF026	4	38.66
10	*R. suaveolens*	EK544	HF081	4	45.00
11	*R. stenophyllum*	HF082[a]	EK526[b]	3	58.50
12	*R. rhodopus*	EK577	EK597	4	63.00
13	*R. blackii*	HF056	EK591	4	86.50
14	*R. taxifolium*	EK578	EK580	3	98.50
15	*R. arenicola*	EK596	EK660	5	143.25
16	*R. inundatum*	EK654	HF042	6	168.00
17	*R. longiflorum*	EK668	HF047	4	441.00

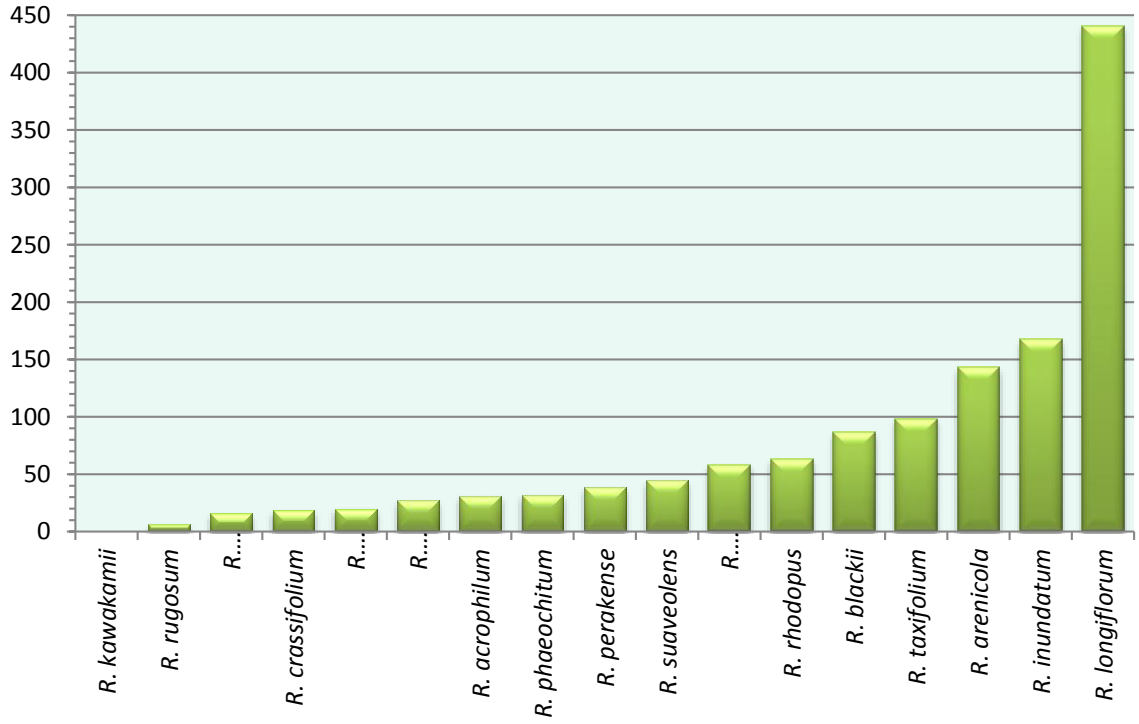


Figure 79 Graphical representation of genetic distance between vireya taxa with only two accessions using microsatellite data. The distances are based on the $(\delta\mu)^2$ genetic distance assuming the Stepwise Mutation Model (SMM). **a** – *R. stenophyllum* ssp. *stenophyllum*, **b** – *R. stenophyllum* ssp. *angustifolium*.

Table 15 lists the genetic distance between accessions of vireya taxa with only two accessions, using microsatellite data. The taxa are arranged in ascending order of genetic distance ($(\delta\mu)^2$, assuming the Stepwise Mutation Model (SMM)) between each pair of accessions and are illustrated in Figure 79. The genetic distance between the pair of accessions of the taxon *R. kawakamii* is 0, suggesting that these two accessions are genetically similar with respect to the microsatellite loci examined. The remaining taxa show genetic distances varying from 6.66–441. The highest genetic distance is observed between the accessions of *R. longiflorum*.

Table 16 Estimates of evolutionary divergence between DNA sequences of *Rhododendron* taxa with only two accessions. The distances are based on the Tamura-Nei Genetic Distance. Accessions beginning with EK and HF are New Zealand accessions, while those starting with AY and GU are from published data. **a** – *R. culminicola* Bulldog Road form, **b** – *R. gardenia* 'Odyssey', **c** – *R. herzogii* Mt Yakananda form, **d** – *R. javanicum* ssp. *teysmannii*, **e** –*R. javanicum* ssp. *brookeanum*, **f** – *R. leucogigas* 'Hunstein's Surprise', **g** – *R. saruwagedicum* (now a synonym of *R. yelliotii*). Note: The taxon descriptions in square brackets '[]' correspond to accessions from New Zealand collections only.

#	Taxon	Accession 1	Accession 2	Tamura-Nei Dist.
1	*R. gardenia* ['Odyssey']	HF012[b]	EI169	0.000000
2	*R. loranthiflorum*	HF090	GU445790	0.000000
3	*R. rousei*	HF014	GU445804	0.000000
4	*R. stapfianum*	EK583	GU445799	0.000000
5	*R. adinophyllum*	EK602	GU445843	0.001133
6	*R. yelliotii*	GU445780	GU445791[g]	0.001133
7	*R. leucogigas* ['Hunstein's Surprise']	HF051[f]	GU445789	0.002269
8	*R. tuba*	HF100	GU445771	0.002269
9	*R. citrinum*	EK579	GU445809	0.003407
10	*R. rarum*	EK618	GU445782	0.003413
11	*R. zoelleri*	EK628	GU445781	0.003416
12	*R. burtii*	HF043	GU445803	0.003422
13	*R. rutenii*	EK647	GU445795	0.004546
14	*R. radians*	EK667	AY765589	0.004566
15	*R. culminicola* [Bulldog Rd form]	EK629[a]	GU445778	0.005688
16	*R. sumatranum*	HF093	GU445808	0.005688
17	*R. blackii*	EK591	EK592	0.005691
18	*R. santapaui*	EK581	AY765625	0.005694
19	*R. kawakamii*	HF072	GU445847	0.006837
20	*R. carringtoniae*	EK626	GU445787	0.006863
21	*R. yongii*	EK664	GU445781	0.008008
22	*R. herzogii* [Mt Yakananda form]	EK639[c]	AY765595	0.008021
23	*R. lowii*	HF101	GU445811	0.009138
24	*R. javanicum* [ssp. *teysmannii*]	HF021[d]	GU445824[e]	0.012597
25	*R. celebicum*	HF070	GU445815	0.013903
26	*R. zollingeri*	HF097	GU445823	0.019542
27	*R. bagobonum*	EK525	GU445831	0.019546

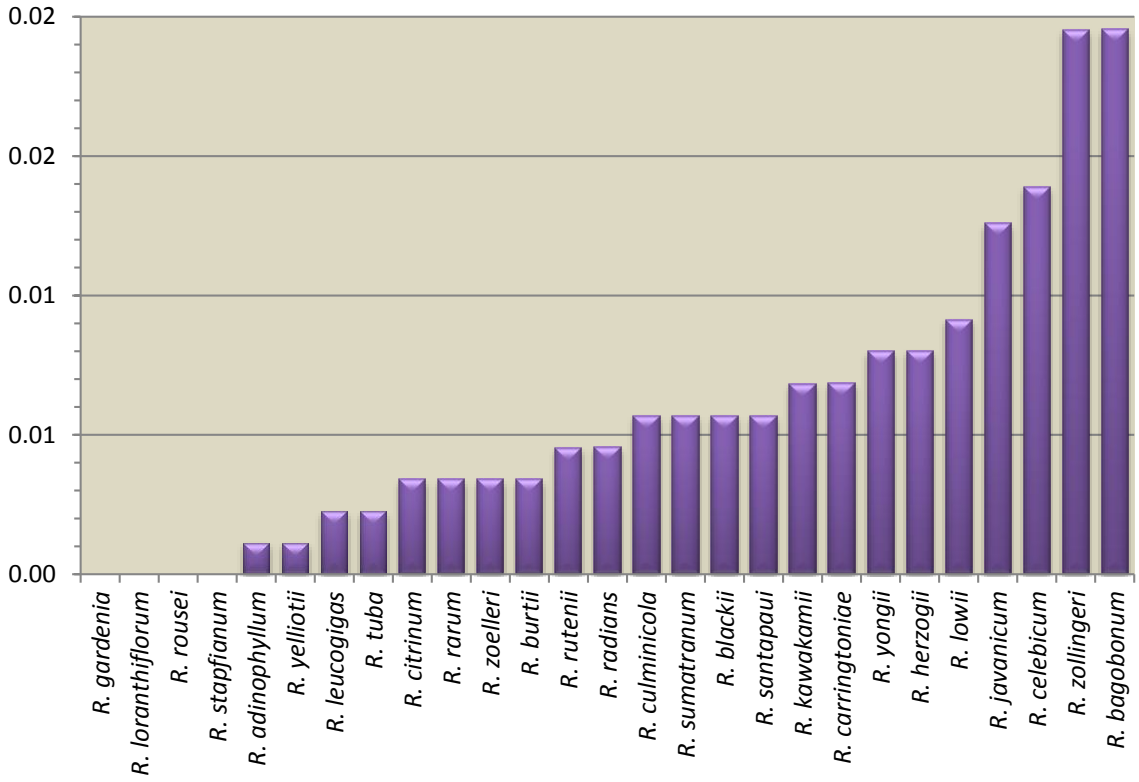


Figure 80 Graphical representation of estimates of evolutionary divergence between DNA sequences of *Rhododendron* taxa with only two accessions. The distances are based on the Tamura-Nei Genetic Distance.

Table 16 lists the estimates of evolutionary divergence between DNA sequences of *Rhododendron* taxa with only two accessions. The taxa are arranged in ascending order of genetic distance (Tamura-Nei) between each pair of accessions and are illustrated in Figure 80. The genetic distance between the pair of accessions of the taxa *R. gardenia* 'Odyssey', *R. loranthiflorum*, *R. rousei* and *R. stapfianum* are all 0, suggesting that the accessions of these taxa are genetically similar with respect to the *rpb*2i locus examined. For the remaining taxa, the genetic distances varied from 0.001133–0.019546. The highest genetic distances were observed between the accessions of *R. bagobonum* (0.019546) and the accessions of *R. zollingeri* (0.019542). Significant genetic differentiation is observed between the New Zealand accessions (with prefixes EK and HF) and the published data (with prefixes GU and AY).

5.4.23 Summary

The genetic diversity analyses described in this section showed that several taxa exhibit significant genetic diversity sufficient for them to be selected for conservation. Analyses based on both the microsatellite data and the sequence data showed similar trends in the genetic diversity among accessions of the majority of taxa examined. The results also suggest that both of these methods could therefore be applied in the genetic diversity analyses.

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Chapter 6

Discussion

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6 Discussion

This chapter describes the findings and discussion of the results and analyses of the molecular work described in Chapter 4 of this study. The chapter is divided into two major sections corresponding to the major areas of study contained in this thesis. The first section describes the phylogenetic analyses based on the nucleotide sequence data and their relationship on the systematics (both classical and modern) of the vireyas and implications for conservation planning. The second section describes the genetic diversity analyses and its impact on the selection and prioritization of vireya taxa for conservation.

6.1 Phylogenetic Analyses

The phylogenetic analysis based on the nucleotide sequence of the *rpb*2i intron region has revealed several new pieces of information that prompt the revision of the classification of vireyas previously suggested, based on morphological data. The phylogenetic analyses were carried out on two separate datasets, with the first dataset (Dataset 1) composed of the 87 accessions that were sequenced for this study. The second dataset (Dataset 2) combines the data of Dataset 1 with the currently publicly available sequence data for the same nuclear region to form a larger dataset containing 171 accessions. Dataset 2 represents approximately 110 vireya taxa (~35% of vireyas), and thus forms the largest study to date of vireya using nucleotide sequence data.

Dataset 1 was analysed using four phylogenetic analysis methods: Maximum Likelihood (ML) (Figure 34), Neighbour Joining (NJ) (Figure 36), Minimum Evolution (ME) (Figure 37) and Maximum Parsimony (MP) (Figure 38). The analyses showed an overall similarity in the tree topology, with the majority of the clades consisting of more or less the same taxa, and forming five discernible clades. The MP tree showed a slightly different topology with six discernible clades. It was noteworthy that the basal clade (A) in all the analyses contains the taxa belonging to the Section *Pseudovireya*, and was sister to the rest of the vireyas.

All phylogenetic trees showed a similar overall pattern with the taxa of the sections *Discovireya* and *Pseudovireya* sister to the rest of the vireyas (hereafter referred to as the

core vireyas). The core vireyas consist of mainly the Section *Euvireya* with other sections (*Malayovireya*, *Siphonovireya*, *Phaeovireya* and *Albovireya*), forming paraphyletic subgroups.

These results are consistent with the two recent studies by Goetsch et al. (2011) and Craven et al. (2011) which revealed relationships within the vireyas and its placement within the genus *Rhododendron*. These two studies also proposed the restoration of the correct name for the vireyas, Section *Schistanthe*. However, for the current study Subgenus *Vireya* has been chosen for two main reasons: (i) the most recent monograph of vireyas by Argent (2006) referred to them as Subgenus *Vireya*, and (ii) the proposal for the use of Section *Schistanthe* for vireyas by Goetsch et al. (2011) and Craven et al. (2011) was published towards the end of the current study and the name has not been established well at present.

The present molecular study and previous studies showed that *R. emarginatum* and *R. euonymifolium* are very closely related to each other with mostly 100% bootstrap support (Goetsch et al. 2011). This supports the notion of Sleumer (1966a) and Argent (2006) to combine these two taxa into the taxon *R. emarginatum*. *R. emarginatum*, however consists of two varieties (*emarginatum* and *eriocarpum*), and further studies need to be carried out to determine which variety (and the validity of the status of variety) *R. euonymifolium* belongs to. These studies should include multiple accessions of the two varieties of *R. emarginatum* and multiple accessions of *R. euonymifolium*.

The Dataset 2 was analysed using two phylogenetic analysis methods: Maximum Likelihood (Figure 39) and Maximum Parsimony (Figure 41), since Neighbour Joining and Minimum Evolution analysis methods produced very similar tree topologies to that of the Maximum Likelihood analysis for the Dataset 1. The topology of the trees produced by both ML and MP trees showed very similar trends. Although, the ML tree had six recognizable clades, while the MP tree had seven, the constituent taxa within the clades of both the trees were comparable.

As the MP tree had more resolved taxa than in the other trees of this study, it was analysed further by mapping physical characters (scale type and corolla shape) (Figure 46 and Figure 48 respectively), geographic origin (Figure 41) and sectional assignments (Figure

45) of the taxa *sensu* Argent (2006) to it. The MP tree showed a strong correlation between the observed clades and the geographic origin of the taxa. However, the physical characters (scale type and corolla shape) showed very weak correlation with the observed clades. The sections *sensu* Argent (2006) correlate well with the basal clades (corresponding to the sections *Pseudovireya* and *Discovireya*), while the other clades consisted of a mixture of the remaining sections (*Malayovireya*, *Siphonovireya*, *Phaeovireya* and *Albovireya*) which were not recovered as monophyletic clades.

All phylogenetic trees (using Dataset 1 and Dataset 2) showed a similar overall pattern, with the taxa of the basal clades representing the sections *Discovireya* and *Pseudovireya* being sister to the rest of the vireyas (or core vireyas). The core vireyas consisted of mainly taxa belonging to the Section *Euvireya*, with representatives of other sections (*Malayovireya*, *Siphonovireya*, *Phaeovireya* and *Albovireya*), forming paraphyletic clades. From this point forward the discussions are based around the MP tree using the Dataset 2.

In one of the earliest known classifications of vireyas, Schlechter (1919) divided the vireyas into two major groups (A and B) based on floral and foliar characteristics. Each group was further divided into two and three sections respectively. Group A is composed of the sections *Schistanthe* and *Linnaeopsis*, while Group B is composed of the sections *Zygomorphanthe*, *Hapalanthe* and *Hadranthe*. The current study does not contain any taxa belonging to Group A, however the Group B was well-represented. All the taxa of Group B are found in the Clade G of the MP tree; suggesting that the sections *Zygomorphanthe*, *Hapalanthe* and *Hadranthe* are equivalent to the clade representing the Section *Euvireya*.

The subsequent classification by Copeland (1929) represented 20 vireya taxa of which 17 are recognized at present. Mapping these on to the MP tree of this study (where the taxon was available for study), the taxa in Section *Lepipherum* appear in both Clade C (*R. quadrasianum*) and Clade E (*R. apoanum*). The taxa of Section *Vireya* are found in Clade G only (*R. bagobonum*, *R. kochii* and *R. williamsii*). The subsections of Copeland (1929) therefore were not recovered in the MP tree. Accessions of the taxa *R. bagobonum* and *R. williamsii* are found in the Cluster 13 (Clade G) which has very high Bayesian posterior probability (100%), suggesting a very close relationship between these taxa.

The comprehensive classification system proposed by Sleumer (1949) consisted of a larger number of taxa compared with the previous classifications. In that classification the vireyas are classified as the Section *Vireya* encompassing ten subsections and approximately 67 taxa. Considering only those subsections that were studied with more than one taxon, the Subsection *Pseudovireya* (C. B. Clarke) Sleumer of this classification was recovered from the MP tree as Clade A with the representative taxa *R. vaccinioides* and *R. asperulum*. The Subsection *Solenovireya* H. F. Copeland was not recovered as a monophyletic clade, but formed a cluster containing *R. jasminiflorum*, *R. orbiculatum*, *R. radians* and *R. rutenii* (MP tree – Clade F). The Subsection *Discovireya* Sleumer was not recovered as a monophyletic clade, and the constituent taxa are scattered around the tree. The Subsection *Linearanthera* H. F. Copeland was partially recovered, since the only taxa available for this study were *R. emarginatum* and *R. kawakamii*. The Section *Euvireya* was recovered as a monophyletic clade (MP tree – Clade G) if only the taxa used for this study are considered. Taxa of Subsection *Leiovireya* H. F. Copeland are scattered around Clade G, thus they cannot be recovered as a monophyletic clade. To summarize, only the Subsection *Pseudovireya* can be recovered from the MP tree as a monophyletic clade, and the Subsection *Euvireya* can be partially recovered as a monophyletic clade (when taxa not available for this study are excluded).

The current understanding of the classification of vireyas stemmed from the hallmark study by Sleumer (1966a). The vireyas were classified under the Section *Vireya* (Blume) H. F. Copeland, and consisted of seven subsections (based on the scale type). The Subsection *Euvireya* was further subdivided into seven series. The classification included 276 named species and several intraspecific taxa. The Subsection *Pseudovireya sensu* Sleumer (1966a) could not be recovered as a monophyletic clade from the MP tree, instead is paraphyletic and composed of the subsections *Pseudovireya* and *Discovireya sensu* Sleumer (1966a). The remaining subsections also cannot be recovered as monophyletic clades, but contain corresponding taxa.

The most current classification system (based on morphological characters) in use is that of Argent (2006). In this classification the vireyas are classified as the Subgenus *Vireya* composed of seven sections (based mainly on the scale type). The Section *Euvireya* is further divided into five subsections (mostly based on floral and foliar characteristics). The Section *Pseudovireya* cannot be recovered as a monophyletic clade from the MP tree,

however consists of several monophyletic clades relating strongly to geographic origin of the taxa. The Section *Discovireya* can be recovered as a monophyletic clade, if the taxon *R. perakense* is excluded. The remaining sections *Malayovireya*, *Siphonovireya*, *Phaeovireya*, *Albovireya* and *Euvireya* could not be recovered as monophyletic clades, and are paraphyletic.

A number of molecular phylogenetic studies emerged within the last decade proposing new classification systems for the vireyas (Brown et al. 2006a, 2006b; Brown et al. 2006c; Craven et al. 2011; Craven et al. 2008; Goetsch et al. 2011). In all these studies *Pseudovireya* and *Discovireya sensu* Argent (2006) were recovered, while the remaining groups were mixed together. Another significant feature is the formation of a large clade containing the majority of the vireya taxa, and most of which belonging to Section *Euvireya sensu* Argent (2006). The sections *Phaeovireya*, *Malayovireya*, *Albovireya* and *Siphonovireya* were not recovered as monophyletic groups in any of these studies.

Brown et al. (2006a) was one of the first molecular phylogenetic studies focussed on vireyas. That study showed that *Pseudovireya sensu* Sleumer (1966a) was paraphyletic, and formed two clusters: a small cluster corresponding to mainland Asian species and a larger cluster with two subclusters corresponding to Taiwanese and Malesian species respectively. *Pseudovireya* was also shown to be sister to the rest of the vireyas, labelled as '*Euvireya*'. This *Euvireya* clade consists of a mixture of all the subgroups within *Vireya* excluding *Pseudovireya*, and these subgroups were shown to be paraphyletic. This topology is observed in both the ML and the MP trees. The subclades formed within the *Euvireya* clade of Brown et al. (2006a) showed strong correlation with specific geographic regions and this correlation with geographic regions can also be seen in the present study.

A second study by Brown et al. (2006b) based on the sequences of the non-coding regions of cpDNA (*psb*A-*trn*H and *trn*T-*trn*L) revealed a well-resolved phylogeny of vireyas. The study representing 75 vireya taxa showed that the Section *Vireya* (Blume) H. F. Copeland was monophyletic and very similar to that formulated in the study of Brown et al. (2006a). Once again the *Pseudovireya sensu* Sleumer (1966a) was shown to be paraphyletic, and formed two clades corresponding to mainland Asian species, and Malesian and Taiwanese species. The taxa belonging to the subsections *Euvireya*, *Siphonovireya* and

Malayovireya formed a large monophyletic clade, while the individual subsections were shown to be paraphyletic. The clades supported by the cpDNA analyses strongly correlate to geographic regions rather than taxonomic boundaries. The present study also supports this segregation of taxa into clades with geographical correlations.

The study by Craven et al. (2008) based on *rpb*2i sequences that included 25 vireya taxa showed that the Subgenus *Vireya* (*sensu* Argent) is polyphyletic and embedded within Subgenus *Rhododendron* (*sensu* Craven), and therefore cannot be a sister taxon to it. The maximum parsimony strict consensus phylogenetic tree showed that *Vireya* (*sensu* Argent and Sleumer) was paraphyletic, and Section *Pseudovireya* was sister to the Section *Vireya*. Section *Discovireya* was not sister to the clade formed by the sections *Vireya* and *Pseudovireya*, but more closely related to the temperate sections *Rhododendron* and *Pogonanthum*. This is in contrast to the findings of the present study, where the temperate taxa were sister to the vireyas and the Section *Pseudovireya* was sister to the rest of the vireyas. The bulk of the vireya taxa formed a well-supported cluster similar to the Clade G of the MP tree. A clade containing *R. fallacinum* and *R. malayanum* with excellent bootstrap support can be seen and represents the Section *Vireya* Subsection *Malayovireya* of the new proposed classification. These two taxa appear together in Clade E of the MP tree of the present study supporting the status of the group Subsection *Malayovireya*.

Goetsch et al. (2011) is the most comprehensive molecular study of the *Vireya* group published to date, representing 54 vireya taxa. The study consisted of a phylogeny derived from the analysis of sequences from multiple nuclear genes, *rpb*2i, *rpb*2d and *rpc*1. Reinstating the rank Section *Schistanthe* as the collective group name for the vireyas, this section is shown to be monophyletic (when one accession of *R. santapaui* was excluded). The tree has well-defined clades corresponding to the subsections *Euvireya*, *Malayovireya*, *Pseudovireya*, and *Discovireya*. Within the subsection *Euvireya*, the subclades follow geography more closely than traditional taxonomic groupings based on morphology. The phylogeny seen in the MP tree of the present study is congruent with that of Goetsch et al. (2011), except for the fact that the Subsection *Discovireya* is the basal clade sister to the rest of the vireyas.

The present study included relatively more taxa than in the previous studies, and thus presented additional taxonomic groupings. The topology of the phylogenetic analyses,

especially that of the MP tree is congruent with the majority of the recent phylogenetic studies using molecular data (Brown et al. 2006a, 2006b; Brown et al. 2006c; Craven et al. 2011; Craven et al. 2008; Goetsch et al. 2011). As suggested in these studies, a new classification is warranted, which take into account the geographical correlations and evolutionary relationships.

6.1.1 Proposal for a New Classification of Vireyas

Figure 81 shows a proposed classification system based on the present study. The taxon Section *Schistanthe* Schltr. is reinstated to represent the vireyas and the clades (A–G) represent the subsections (*Pseudovireya*, *Discovireya*, *Albovireya*, *Malayovireya*, *Solenovireya* and *Euvireya*). Further study including more vireya taxa may reveal subdivisions within Subsection *Euvireya*. The other subsections can also be further refined by using more taxa closely related to those sections.

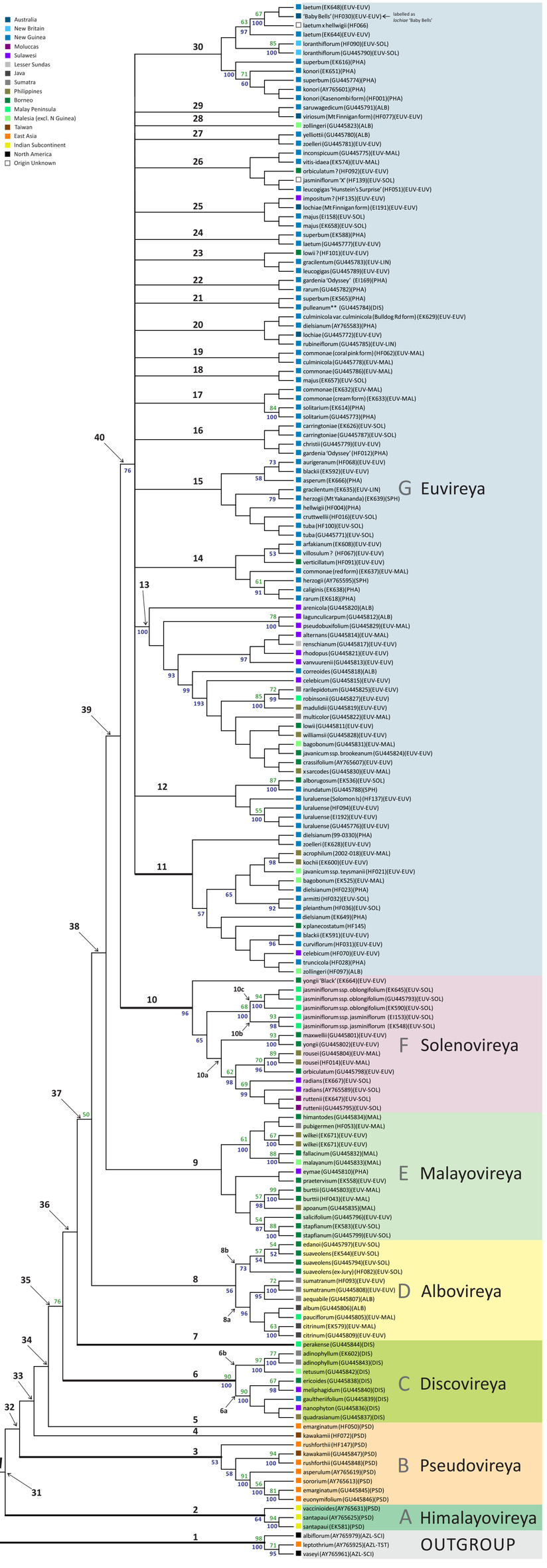

Figure 81 A new classification of the vireyas (Section *Schistanthe* Schltr.) showing the proposed arrangement of the subsections. Figure based on the tree shown in the Maximum Parsimony tree ofFigure 41.

In the proposed classification (Figure 81), the Section *Pseudovireya sensu* Argent is divided into two groups. The first group Subsection *Himalayovireya* Fayaz *subsect*. *nov*. (Clade A) represents *pro parte* Section *Pseudovireya sensu* Argent (2006) including the type species *R. vaccinioides*. The taxa of this group are genetically distinct from the rest of the Section *Pseudovireya sensu* Argent (2006), and have white or pink flowers, and inflorescences are few-flowered or flowers solitary. The range of the taxa is from the Himalayas eastward to Yunnan (China). The remaining taxa of *Pseudovireya sensu* Argent (2006) are assigned to Subsection *Pseudovireya* (Clarke) Sleumer *stat*. *nov*. (Clade B) *pro parte* Section *Pseudovireya sensu* Argent (2006) excluding the type species *R. vaccinioides* is therefore assigned. The range of the taxa within this subsection extends from the Eastern Himalayas through S China to Taiwan. The taxa usually has yellow or orange flowers, and inflorescences are mostly 1–2-flowered or in umbels of 3 or more.

Subsection *Discovireya* Sleumer *stat. nov*. (Clade C) corresponds to Section *Discovireya sensu* Argent (2006). The taxa differentiated from the subsections *Himalayovireya* and *Discovireya* by having relatively longer corolla tubes. The range of the taxa extends from Sumatra eastwards through Malay Peninsular and Borneo to the Philippines and southward through Sulawesi to New Guinea.

Clade D represents a group of taxa belonging to the sections *Euvireya* and *Albovireya*, characterized by deeply-lobed scales. The taxa are widely distributed from Sumatra eastwards through Malaysia to the Philippines. Subsection *Albovireya* Sleumer *stat*. *nov*. excluding the type, can be designated to this group.

Clade E represents a group of taxa belonging to the sections *Euvireya*, *Malayovireya* and *Phaeovireya*. Subsection *Malayovireya* Sleumer *stat*. *nov*. can be designated to this group. Further studies are needed to ascertain the common physical characters uniting the taxa. The majority of the taxa of this group are distributed in Borneo, with a few outliers in New Guinea and the Philippines.

Clade F represents a group of taxa mostly originating from Borneo, extending eastwards to the Philippines and southwards to the Moluccas. Several taxa within this clade exhibit long corolla tubes and the flowers are usually white. Subsection *Solenovireya* H. F. Copeland *stat*. *nov.* can be assigned to this clade.

Table 17 Comparison of classification systems proposed by Sleumer (1966), Argent (2006) and this study.

Sleumer (1966)	Argent (2006)	This Study
Subsect. *Pseudovireya*	Sect. *Pseudovireya* Sect. *Discovireya pro parte*	Subsect. *Himalayovireya* Subsect. *Pseudovireya*
Subsect. *Discovireya*	Sect. *Discovireya pro parte*	Subsect. *Discovireya*
Subsect. *Albovireya*	Sect. *Albovireya*	Subsect. *Albovireya*
Subsect. *Malayovireya*	Sect. *Malayovireya*	Subsect. *Malayovireya*
Subsect. *Solenovireya*	Subsect. *Solenovireya*	Subsect. *Solenovireya*
Subsect. *Euvireya*	Sect. *Euvireya*	Subsect. *Euvireya*

Clade G represents the majority of the taxa of vireyas and the majority of the taxa belonging to Section *Euvireya sensu* Argent (2006). Common physical characters uniting this clade are hard to ascertain given the large number of morphologically diverse taxa contained within the clade. The clade consists of a mixture of all the vireya sections *sensu* Argent (2006), excluding those belonging to the sections *Pseudovireya* and *Discovireya*. The phylogenetic analyses suggest that this clade consists of taxa that are very closely related to each other, and therefore warrants a revision of the current classification. This clade is therefore assigned the Subsection *Euvireya* H. F. Copeland *stat. nov.* inclusive of the type species. Further research including more taxa and additional nucleotide sequence loci will perhaps reveal the intricate relationships within this group. Table 17 summarises and compares the classification systems proposed by Sleumer (1966), Argent (2006) and this study.

6.2 Genetic Diversity Analyses

The genetic diversity analyses have shown very interesting results, and are moderately comparable between the different methods used. Genetic diversity has traditionally been investigated using RAPDs, RFLPs, microsatellites, etc. In this study, sequence data was also used in addition to microsatellites and RAPDs. Liu et al. (2012) is the latest of these studies in which *rpb2* sequence data was used in the genetic diversity of cultivated mushrooms in China. The sequence analyses of that study showed the genetic relationships between the studied strains of the mushroom (*Pleurotus ostreatus*), providing valuable information on the relationships among the strains. The study showed that the sequence data was useful in examining genetic diversity among the mushroom strains.

In the current study the three methods (microsatellites, *rpb*2i sequence data and RAPDs) showed that there is significant genetic diversity among the accessions of the selected taxa that were studied. Except for *R. commonae*, for which the genetic differences were not revealed, despite the fact that they had different flower colours. One of the limitations of the study was that a common set of accessions was not available for analysis with the microsatellites, sequence data and RAPDs, except for *R. jasminiflorum* and *R. lochiae*. Analysis of these two taxa thus allowed a comparison of the analysis methods, and showed a very similar topology in the dendrograms.

The microsatellite markers used in this study were originally developed for temperate *Rhododendron* species (Dunemann et al. 1999; Naito et al. 1998; Tan et al. 2009), and were the first of their kind for *Rhododendron*. To date microsatellite markers specific to vireyas have not been developed, and thus accounts for the limited amplifications of the markers used for this study. The study by Naito et al. (1999) was among the earliest genetic diversity studies on *Rhododendron* using microsatellites, in which population structures were examined in the morphologically variable *R. metternichii* var. *hondoense* that had implications on their conservation. A more recent study by Caser et al. (2010) showed that accessions can be uniquely identified using microsatellite markers, and the variation was shown to be consistent, for germplasm conservation and restoration of historical genetic resources. The studies by Kameyama et al. (2000) and Kondo et al. (2009) utilized microsatellites in the genetic diversity and characterization of gene flow in temperate *Rhododendron* taxa. The studies confirmed the effectiveness of microsatellites in genetic diversity of *Rhododendron*.

The genetic diversity observed among the accessions of the taxa used in the current study confirms the effectiveness of using microsatellite markers in uniquely identifying individual plants. The drawback of this marker system is that it is time-consuming and expensive compared to some of the newer methods such as nucleotide sequence data. Since the microsatellite markers used for the current study were originally designed for temperate taxa, a large number of accessions failed to amplify for the majority of the loci.

Genetic diversity studies using nucleotide sequence data are uncommon for any organism and rare in *Rhododendron* (Chung et al. 2007; Huang et al. 2011). Chung et al. (Chung et al. 2007) is one of these studies where chloroplast DNA (cpDNA) was used to reveal the

origin and evolutionary history of a *Rhododendron* species complex in Taiwan. Huang et al. (2011) was a study carried out to investigate the genetic population structure of the alpine species *Rhododendron pseudochrysanthum sensu lato*, using chloroplast DNA (cpDNA) and nuclear DNA (nrDNA) sequences. The potential utility of the non-coding regions (introns) of the nuclear gene *rpb*2i in phylogenetic applications and especially in *Rhododendron* have extensively been tested (Craven et al. 2011; Goetsch et al. 2011; Goetsch et al. 2005; Hall et al. 2006), and these have been discussed in detail in Sections 2.1.3 and 2.2.3. Diploid *Rhododendron* genomes contain only a single copy of *rpb*2i, which makes the sequencing of this region very easy (Goetsch et al. 2005).

In the current study, a single intron region of the *rpb*2i was utilized to study the genetic variation of *Rhododendron* taxa, and the results were comparable with those obtained for the microsatellite studies. In cases where the same accessions were used in both the sequence data and microsatellite analyses, the results were congruent for several taxa such as *R. jasminiflorum*.

The following subsections discuss the results of the molecular study in the light of physical examination of the accessions, and their implications on the conservation of Red-Listed taxa.

6.2.1 *R. lochiae* and *R. viriosum*

The Australian rhododendron taxa represent the southernmost extent of the range of the genus. It was originally believed that Australia had only a single variable native *Rhododendron* species, *R. lochiae* F. Muell. (*Vict. Nat*. 3:157, 1887), which occurred over a limited range in N Queensland. However, recent research into the taxonomy of specimens collected in that area revealed that there were in fact two physically distinctive species, *R. lochiae* F. Muell. (*Vict. Nat*. 3:157, 1887) and *R. viriosum* Craven (*Edinburgh Journal of Bot*any 59(3): 448, 2002) (Craven & Withers 1996b). This discovery led to the confusion in taxonomy in recent years between the taxa *R. lochiae* and *R. viriosum*.

R. lochiae F. Muell. was originally described as *Rhododendron* ***lochae*** by F.Muell. in *Vict. Natural*. iii:157, 1887. The name was also published as *R. lochae* in *Gard. Chron.* i:543, 1887 and *Bot. Centralbl.* xxx:277, 1887. The taxon was later described as *Azalea*

lochae by Kuntze in *Rev. Gen. Pl.* 387, 1891. A century later, with the discovery of a second species (Craven & Withers 1996b), Craven described the taxon as *R. notiale* Craven in *Edinb. J. Bot.* 53(1): 33, 1996. Craven (Craven & Withers 1996b) further indicated that the name '*lochiae'* had been originally applied in 1887 to the less common of the two Australian species, thus the name *R. lochiae* had been incorrectly applied to most of the plants that are commonly in cultivation. In an attempt to overcome the confusion Craven (Craven & Withers 1996b) applied the name *Rhododendron notiale* to the less common plant so that *R. lochiae* could be retained as the name for the more commonly grown species, adding more confusion to the taxonomy. This name change required a change to the 'type' specimen, and therefore was later rejected. The more common species was later described as a new species *R. viriosum* Craven and published in *Edinburgh Journal of Botany* 59(3):448, 2002. The name *R. lochiae* was retained for the less common species as it was originally described, and the name *R. notiale* is reduced to a synonym of *R. lochiae*. Thus the status quo of the two taxa is *R. lochiae* F. Muell. corresponding to the less common species and *R. viriosum* Craven corresponding to the more common species.

Both species are found in Queensland, where the less common *R. lochiae* grows as a terrestrial species known from only two localities: Bellenden Kerr Range and Bell Peak in Malbon Thompson Range (altitude 1,200–1,520 m) (Argent 2006; Gibbs et al. 2011). *R. viriosum* is also from Queensland, but much more widespread and found in the localities: Mt Finnigan, Thornton Peak (Mt Lewis), Mt Windsor Tableland and the Main Coast Range (altitude 910–1,330 m) (Argent 2006). In the wild *R. viriosum* grows clinging to cliffs by sending roots down into crevices in the rocks. The species has also been described as growing as an epiphyte on trees. Due to its rarity, *R. lochiae* has been Red-listed by IUCN as VU D2 (Vulnerable), while *R. viriosum* is listed as LC (Least Concern). The most obvious difference between the two Australian species is the shape of the corolla tube of the flower – 'curved' in *R. lochiae* and 'straight' in *R. viriosum* (Craven 2003; Craven & Withers 1996a).

Table 18 A selection of morphological differences between *R. lochiae* and *R. viriosum*. Characters based on the descriptions of Argent (2006).

Character	***R. lochiae***	***R. viriosum***
Habit	Shrub or small tree to **6.5 m** high.	Shrub to **3 m** high.
Leaves	**3–5** together in tight pseudowhorls.	**2–6** together in tight pseudowhorls.
Leaf lamina	Broadly elliptic, occasionally sub-obovate, **5–9 × 3–5 cm**; base **broadly tapering to rounded**, apex **acute to rounded or sometimes minutely emarginate**, **with a small pale gland which rarely slightly protrudes**.	Elliptic to broadly elliptic or obovate, **2.5–11 × 1–7 cm**; base **broadly tapering to tapering**, apex **shortly acuminate to obtuse**.
Petiole	Densely brown scaly.	Scaly, usually **red**.
Corolla	**2.5–5.5 × 3.5–4.5 cm**, red or pink; tube **curved**, scaly outside, **glabrous inside**.	**5–5.8 × 5–5.8 cm**, red or deep pink; tube **straight**, laxly scaly and **sparsely hairy outside**, **hairy inside**.
Stamens	**Loosely clustered on the upper side of the mouth**; filaments **glabrous**.	**Irregularly spread all around the mouth**, or round the lower ⅔ of the mouth; filaments **hairy**.
Ovary	Densely scaly and **sometimes hairy**; style held on the **upper side** of the corolla tube.	Densely scaly and **densely hairy**; style appressed to the **lower side** of the corolla tube.
Fruit	**Fusiform**.	**Ellipsoid**.

According to Argent (2006), all early records of *R. lochiae* can be referred to *R. viriosum*, and hybrids formed from this species used *R. viriosum* as parent. Although superficially similar, careful morphological examination shows that these two taxa differ from each other, as shown in Table 18. The curvature of the corolla tube is the most easily distinguished character between *R. lochiae* and *R. viriosum*; however plants with both curved- and straight-tubed flowers have been reported from Mt Lewis[48] (Andrew Small *pers. comm.*). Further studies with more accessions collected from verified geographic localities would help to resolve the taxonomic issues among the Australian *Rhododendron* taxa.

[48] The sample from Mt Lewis (Accession #: HF045, labelled as *R.* lochiae Mt Lewis form) used in this study amplified for only a single microsatellite marker. The size of the amplicon however suggests that this accession would possibly belong to *R. viriosum* group (see Appendix 0).

Photo 20 Accessions of Australian *Rhododendron* taxa. Taxa with curved corolla tube: (a) EK606. Taxa with straight corolla tube: (b) EK589 (c) EK620 (d) EK604 (e) HF049.

Photo 20 illustrates the accessions of Australian *Rhododendron* taxa. The corolla shape and the arrangement of the stamens do not match the groupings of the dendrogram in Figure 50. The taxon shown in Photo 20*a* correspond to *R. lochiae* with curved corolla tubes and stamens clustered at the upper side of the mouth, while Photo 20*b*–*e* correspond to *R. viriosum* with straight corolla tubes and the stamens clustered around the corolla mouth.

The taxonomic status of *R. lochiae* (phenotype with curved corolla tubes) and *R. viriosum* (phenotype with straight corolla tubes) can only be confirmed by carrying out further study using more accessions of these two phenotypes. A phylogenetic analysis with DNA sequence data can reveal the genetic differentiation between the two taxa, suggesting whether the taxa be maintained as species, both taxa as a single species or both taxa at

subspecies (or variety) status. The relatively high genetic distance between these two groups confirms the status of the two described taxa from Australia.

The phylogenetic analyses described in this study (Chapter 4) failed to coalesce the accessions of *R. lochiae* and *R. viriosum*, instead the accessions were scattered among the taxa of Section *Euvireya*. There are thus no discernible patterns in their position along the phylogenetic trees. However, this confirms the taxonomic complexity of these two taxa, and also indicates that there may be incorrectly identified accessions or accessions of hybrid origin in the collections selected for this study.

Regardless of the failure to cluster the accessions of *R. lochiae* and *R. viriosum* in the phylogenetic analyses, they were treated as a single variable taxon and genetic diversity analyses were carried out. The genetic distance analysis showed that the accessions formed two distinct groups, A and B (Figure 50). The genetic distance between the accessions within Group A were significantly higher (9.23–16.03) than that was observed within Group B (0.37–6.43). These results suggest that genetic diversity within Group A is relatively higher than that within Group B, and from a conservation point of view, the accessions within Group A are more suitable candidates for conservation.

The genetic diversity analysis using the nucleotide sequence data revealed (Figure 51) very low genetic diversity between these accessions and maybe as a result of them being collected from the same locality. Since all the accessions used for the microsatellite analysis were not available for the nucleotide sequence study, and therefore could not be compared. The accessions HF077 and EK507 key out to *R. viriosum* while the accession HF030 is believed to be of hybrid origin. HF030 was collected from the Pukeiti collection and was labelled as *R. viriosum* 'Baby Bells'[49], and possibly a hybrid involving *R. laetum* and *R. viriosum* (or *R. lochiae*) (as they cluster together in Figure 41 – Clade 30). Physical examination of this accession showed that it is superficially very similar to *R. viriosum*, thus the rationale for including it in this data subset.

The genetic diversity analysis using RAPD data showed that the accessions are significantly genetically diverse (Figure 52). All the accessions studied keys to

[49] Not to be confused with *R.* 'Baby Bells', a widely cultivated hybrid involving *R. saxifragoides*, with distinctive erect flowers.

R. viriosum and therefore a demarcation between *R. viriosum* and *R. lochiae* cannot be established. The accession EK507 and EK630 appear to be genetically distant from the rest of the accessions, perhaps due to collection from geographically separate populations.

Further studies including multiple accessions of the Red-Listed true *R. lochiae* in addition to *R. viriosum* accessions are needed to determine the genetic differentiation between these two taxa and the genetic diversity among the accessions of these two taxa. Without further study, a premise for the conservation of the *R. lochiae* cannot be created.

6.2.2 *R. jasminiflorum*

R. jasminiflorum is a highly variable species with five described subspecies, distributed from the Malayan Peninsula south to Sumatra, eastwards through Borneo to the Philippines. The typical subspecies is restricted to the Malayan Peninsula while the subspecies *oblongifolium* is distributed widely in the Malayan Peninsular with disjunct populations in Sarawak (W Borneo). The subspecies *chaemaepitys* is restricted to the summit area of Mt Lambir (Sarawak, Borneo), while the subspecies *heusseri* is restricted to Sumatra. The subspecies *copelandii* is restricted to the summit area of Mt Apo (Mindanao, Philippines), and is Red-Listed as VU D2, while all the other subspecies are categorized as LC (Argent 2006; Gibbs et al. 2011).

There are several taxonomic issues related to this species, mainly due to the superficial similarity among the subspecies, and also the similarities among several related taxa such as *R. radians*, *R. rutenii* and *R. edanoi*. The MP phylogenetic analysis of this study showed that the taxa *R. radians*, *R. rutenii* and *R. jasminiflorum* are very closely related, and forms a strongly supported clade (Figure 41). The MP phylogenetic analysis also revealed a clade containing the accessions of *R. jasminiflorum* is very strongly supported, suggesting a very close relationship among them. Additionally, the status of the subspecies *jasminiflorum* and *oblongifolium* are strongly supported. However, due to the close genetic relationship shown in the phylogenetic analyses all the accessions assigned to *R. jasminiflorum* were treated as a single taxon for the genetic diversity analysis.

The microsatellite fragment length analysis of the *R. jasminiflorum* accessions showed that the accessions formed two distinct groups, but with very low genetic distance $((\delta\mu)^2$

= 1.667) between the groups (A and B) (Figure 53). This result suggests that these two groups contain taxa that are very closely related. Physical examination of the accessions confirmed the presence of the two subspecies, corresponding to *R. jasminiflorum* ssp. *oblongifolium* (Group A) and *R. jasminiflorum* ssp. *jasminiflorum* (Group B). The genetic diversity among the accessions within each group (A and B) was constantly 0, suggesting that these accessions are genetically identical with respect to the three microsatellite markers used. Further study employing more microsatellite markers may reveal genetic diversity between the accessions within each group (A and B).

The genetic distance determined using the nucleotide sequence data (Figure 54) also revealed two distinct groups (A and B) corresponding to the subspecies *jasminiflorum* and *oblongifolium*, similar to the dendrogram obtained from microsatellite data (Figure 53). The genetic distance between these two subspecies is 0.056 (5.6%). The genetic distance between the accessions of ssp. *jasminiflorum* is 0.057 (5.7%), while that among the accessions of ssp. *oblongifolium* varied from ~0–0.0011 (0–1.1%). The accessions EK590 and GU445793 are almost genetically identical, however these two accessions are genetically distinct from EK645 by a distance of 0.0011 (1.1%). These results suggest that the genetic diversity within the subspecies *oblongifolium* (0–1.1%) is relatively lower than that within the subspecies *jasminiflorum* (5.7%).

Photo 21 A selection of morphologically different *R. jasminiflorum* accessions. (a) EK548 *R. jasminiflorum* ssp. *jasminiflorum* (b) EK 645 *R. jasminiflorum* ssp. *oblongifolium* (c) EI153 *R. jasminiflorum* ssp. *jasminiflorum* (d) Claimed as *R. jasminiflorum* ssp. *copelandii* from Java (published on the internet[50]). The orange arrows indicate the pedicels, while the blue arrows point at the style.

Photo 21 shows a selection of accessions of *R. jasminiflorum* used in this study, compared with an image of an accession posted on the internet (Photo 21*d*). The true *R. jasminiflorum* ssp. *jasminiflorum*, even though variable, have very distinct features, such as green pedicels, short white (or very pale pink) styles, and flared corolla lobes (Photo 21*c*). *R. jasminiflorum* ssp. *oblongifolium* is also a distinct phenotype with distinctly longer and oblong leaves, semi-pendent flowers, red pedicels, and conspicuous,

[50] http://www.asianflora.com/Ericaceae/Rhododendron-jasminiflorum-copelandii.htm

long-exserted, pinkish-red styles. The accession EK548, reportedly collected from Sarawak (Borneo) is phenotypically distinct from the other accessions of *R. jasminiflorum* ssp. *jasminiflorum* to which the accession was assigned to. Unlike the true ssp. *jasminiflorum*, EK548 does not have flared (or later recurved) corolla lobes or green pedicels, instead the corolla lobes are slightly flared (with the lobes more or less straight and not recurved) and the pedicels distinctly red. These results warrant further investigation of all the accessions closely resembling the phenotype EK548, and a re-evaluation of the key separating the subspecies of *R. jasminiflorum*.

As the nucleotide sequence data analysis revealed that there is genetic distance between the accessions within each group (A and B), a physical examination was performed. This showed that the accession EK548 is phenotypically distinct from EI153 as seen in the dendrogram (Figure 54). The accession EK548 (a form of *R. jasminiflorum* ssp. *jasminiflorum* labelled in the garden as *R. jasminiflorum* ssp. *punctatum*, which is presently a synonym of ssp. *jasminiflorum sensu* Argent (2006)) exhibits distinct physical characteristics rarely seen in literature and in cultivation, and may correspond to a subspecies not yet described (Photo 21*a*). The physical differences include reddish pedicels, smaller corolla lobes that are recognizably flared, flowers less pendent and hairy floral parts. These differences further suggest that this accession may be an intermediate taxon between the subspecies *jasminiflorum* and *oblongifolium*. The genetic differentiation of this accession from the other subspecies is not demonstrated from the microsatellite studies (Figure 53).

The genetic distance analysis using RAPD data (Figure 55) showed that the two subspecies *jasminiflorum* and *oblongifolium* can be identified. The genetic separation between these two subspecies (0.2814) is significantly higher than that observed among the accessions within a subspecies. This suggests that although these accessions are very closely related, the results of the RAPD analysis support the status of the two subspecies. The genetic diversity observed within each subspecies varied, with the highest variation among the accessions of the subspecies *jasminiflorum*. The accession EK548 discussed above appears to fall between the two subspecies and allied more closely to the subspecies *jasminiflorum*. This result also supports the hypothesis that this may represent a genetically distinct taxon compared to the other accessions. The accessions EI153 and EI154 are plants grown together and physically identical, however they do not appear to

be clones, instead show low genetic variation. This may have resulted from collection from the same population or perhaps grown from the same seed stock.

The only taxon of current conservation interest within the *R. jasminiflorum* complex is the point endemic *R. jasminiflorum* ssp. *copelandii* from the Philippines. This taxon has been categorized in the IUCN Red List as VU D2 (Vulnerable), but was not available for this study. The inclusion of this taxon in further studies of the *R. jasminiflorum* complex may reveal the genetic diversity between the different subspecies and thus the justification for the conservation of *R. jasminiflorum* ssp. *copelandii*, and also whether it should be promoted to status of a species. The results described above however, support the notion of differences among the subspecies of *R. jasminiflorum*, which in turn supports the case for conservation of all subspecific taxa.

6.2.3 *R. luraluense*

R. luraluense has two described subspecies, namely ssp. *luraluense* and ssp. *whitmorei*. The subspecies *whitmorei* differs from the typical subspecies by having a hairy style, that is scaly for only c. 4 mm (about a fifth of its length) proximally, compared to 12–15 mm (¾ the length of the style) in the typical subspecies (Argent 2006). The subspecies *luraluense* is Red-Listed by IUCN as VU D2 while the ssp. *whitmorei* is not yet evaluated (NE). The phylogenetic analysis in Figure 41 showed that the accessions of *R. luraluense* form a well-resolved clade with 53% bootstrap support.

The genetic diversity study based on microsatellite fragment length data showed that the four accessions of *R. luraluense* had extremely high (560–1,902) genetic distances between the accessions (Figure 56). These extremely high genetic distances may be due to the low number of microsatellite markers used and that some of these did not amplify for all the accessions.

A similar trend is seen in the genetic diversity analysis using DNA sequence data (Figure 57) which showed that the accessions formed two distinct groups with one containing EI192, HF094 and GU445776, while the other consisting of the accession HF137. The two clades have a very low genetic separation (2.26%), suggesting that the two clades are

genetically very similar. The accessions EI192, HF094 and GU445776 are genetically identical with a genetic distance of 0.

The genetic diversity within *R. luraluense* accessions suggested by microsatellite and DNA sequence data is significant to support conservation of this species. Further research using more accessions representing the two subspecies, additional microsatellite markers and sequencing of more DNA loci may further reveal the genetic diversity between the accessions and perhaps establish the status of the subspecies *whitmorei*.

6.2.4 *R. gracilentum*

R. gracilentum is a terrestrial, erect or prostrate shrub, usually with spreading branches, found in the Central District (Mt Musgrave) and the Morobe District (Mt Kaindi and Edie Creek) of Papua New Guinea (Argent 2006). The plant can grow to a maximum height of 60 cm, and due to its spreading habit and the profusion of its ± pendent cylindrical red flowers, it is very popular among growers and hybridisers.

R. gracilentum is categorized as LC and thus of low conservation interest, and is not related to or confused with taxa of conservation interest. *R. gracilentum* is analysed here due to the availability of multiple accessions for study and to provide analytical support for the data analysis methods.

The genetic diversity study using microsatellite data showed that the genetic relationships between the accessions of *R. gracilentum* were significant (Figure 58). The accessions HF076 and EK621 are closely related with a genetic distance of 23.41, suggesting that these accessions may have been collected from a single population. The accession EK635 showed a markedly significant genetic differentiation from the accessions HF076 and EK621 (genetic distance 157.42). These results suggest that the accession EK635 may have been collected from a geographically separate population from that of the accessions HF076 and EK621.

6.2.5 *R. macgregoriae*

R. macgregoriae is a variable species, often hybridizing with other vireyas forming hybrid swarms in the wild mostly with *R. herzogii*, *R. zoelleri*, *R. dielsianum* and *R. inconspicuum* (Argent 2006). There are several forms of this species in cultivation: orange (the most commonly cultivated form), yellow, red and large. In addition to these forms and the natural hybrids, there are over 200 garden hybrids in cultivation around the world exhibiting numerous colour forms. These garden hybrids are often hardy enough to be grown outside in subtropical to warm temperate regions. *R. macgregoriae* is of low conservation interest, but was included here as there were multiple accessions of this taxon available for study, and to provide analytical support for the data analysis methods.

Accessions of *R. macgregoriae* in several collections (e.g. Pukeiti, NZ) consist of true species, natural hybrids and garden hybrids involving this species. The genetic diversity based on microsatellite markers showed that the genetic diversity among the accessions is relatively high (71–247) (Figure 59), suggesting that the accessions may have been collected from geographically separate populations and/or the presence of hybrid taxa. The different forms of *R. macgregoriae* does not cluster together, this may in part be due to the low number of microsatellite markers used.

The accessions form two distinct groups (A and B) with a genetic distance of 59.87. The genetic distance within Group A (71–147.25) is higher than that within Group B (105.79). The results suggest that the accessions of *R. macgregoriae* consist of genetically distant and variable individuals, and further studies including a phylogenetic analysis could reveal if there are genetic demarcations between the various forms seen in cultivation.

6.2.6 *R. javanicum*

R. javanicum is a highly variable species with 11 subspecies described, and are widely distributed from Sumatra and Java eastward through Borneo to Philippines and southward to Sulawesi. The greatest diversity of *R. javanicum* is found in Borneo with six subspecies described (Argent 2006; Argent et al. 2007). The subspecies *cockburnii* is the only IUCN Red-Listed taxon of this group, and is found in only two locations in Borneo (Sabah).

The genetic diversity analysis based on microsatellite fragment length data showed that the accessions form two distinct groups: (A) containing the subspecies *moultonii*, *teysmannii* and *gracile*, and (B) containing only the subspecies *brookeanum*. The genetic distance between the accessions varied from 0.63–494.08 (Figure 60) suggesting that the accessions may have originated from geographically separated populations. The results suggest that *R. javanicum* is highly variable and the subspecies *brookeanum* is genetically distinct from the other subspecies. There are still taxonomic uncertainties remaining within this species and subspecies that further research including a larger ensemble of accessions need to be examined. A phylogenetic study including a similar large number of accessions of *R. javanicum* may also reveal the intraspecific status of many of the taxa.

With regard to conservation, none of the examined accessions belong to the Red-Listed subspecies *cockburnii*, and thus are of no conservation interest. However, these accessions will be invaluable for further studies involving the subspecies *cockburnii* and to determine the genetic distance between this taxon and the other subspecies.

6.2.7 *R. superbum* and its relatives

The species *R. konori* and *R. superbum* are highly variable, and belong to the Section *Phaeovireya*. *R. konori* is represented by two subspecies *konori* and *phaeopeplum*, and are distributed in New Guinea. *R. superbum*, also from New Guinea is represented by two subspecies *superbum* and *ibele*. *R. superbum* and *R. konori* form part of the group relatively large-flowered species that include *R. hyacinthosmum*, *R. dianthosmum*, *R. gardenia* and *R. hellwigii*. Amongst these taxa, *R. superbum* ssp. *ibele* is of conservation interest as it has limited known distribution.

According to the phylogenetic analyses using DNA sequences, the accessions of *R. superbum* and *R. konori* cluster together with moderate support (Figure 41), but the accessions do not form distinct clades corresponding to these two taxa. This suggests that these accessions are very closely related and perhaps represent individuals of a single variable taxon.

The genetic diversity analysis using microsatellite markers showed that there is high genetic diversity among the accessions (2.32–1,697.72) (Figure 61). The genetic

distances among the accessions of Group A however is relatively small (2.32–196.26) compared to that of Group B, suggesting that there is high genetic variability within Group B. In Group A, the high genetic diversity among the accessions suggests that these accessions may have been collected from geographically separate populations. The large distances between the accessions of Group B suggest that some of these accessions may have been of hybrid origin (natural or garden).

One of the distinctive features between *R. konori* and *R. superbum* is the morphology of the style. In *R. konori*, the style is white to reddish, densely hairy and laxly stellate-scaly in the proximal half, gradually more scaly and less hairy in the following ¼, then exclusively scaly, and the remainder glabrous. In *R. superbum*, the completely glabrous style is red or green, lying on the lower side of the corolla tube but curving upwards as the flower ages (Argent 2006). According to the morphology of the style, EK613 should be *R. superbum*, as it has a glabrous style which is curved upwards (Photo 22).

Figure 63 illustrates the dendrogram generated for the evolutionary divergence between *rpb*2i DNA sequences of *R. superbum* and related taxa inferred from the Tamura-Nei distance. This analysis showed that the studied accessions form two separate groups (A and B).

EK613 *R. konori* (Edie Creek form)

EK613 *R. konori* (Edie Creek form) details

EK651 *E. konori*

EK651 *E. konori* style details

EI187 *R. konori*

EI187 *R. konori* (style details)

Photo 22 Accessions of the *R. superbum* and relativesdepicted in Figure 61.

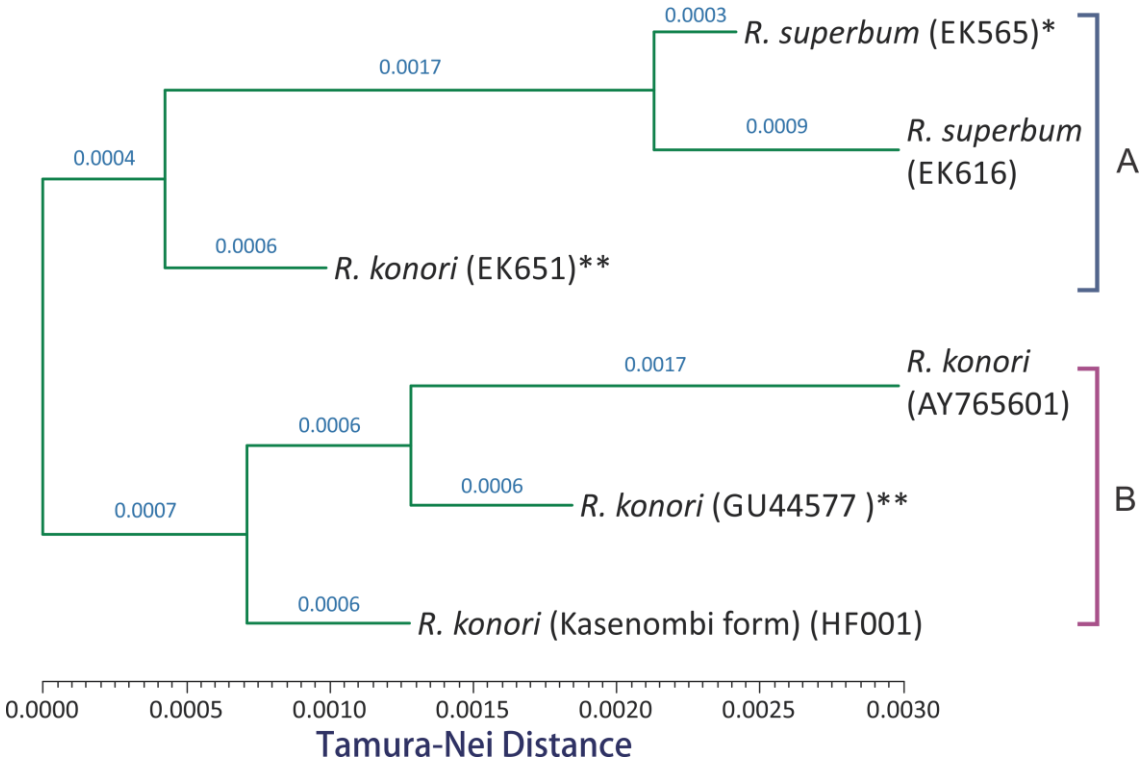

Figure 82 Genetic diversity among accessions of *R. superbum*, *R. konori* and *R. dianthosmum* using sequence data. NJ-based dendrogram inferred from the Tamura-Nei distance. Accessions marked with * were originally labelled as *R. dianthosmum*, while those marked with ** were labelled as *R. superbum*.

The genetic diversity analysis using the *rpb*2i DNA sequences of the *R. superbum* and its relatives showed that the genetic distances between the accessions are very low, suggesting a very closely related ensemble of accessions Figure 82. The accessions however cluster into two discernible clades, where the Group A loosely correspond to the taxon *R. superbum* while the Group B corresponding to *R. konori*.

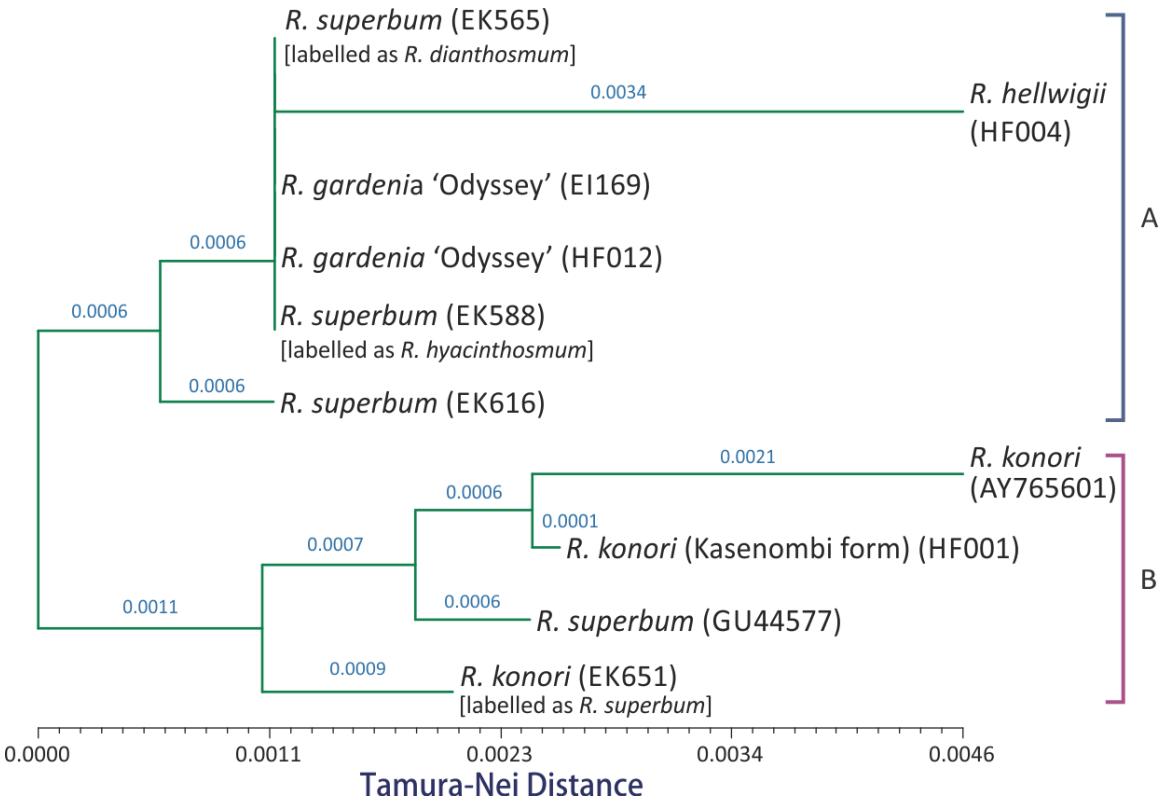

Figure 83 Genetic diversity among *R. superbum* and its relatives using sequence data. NJ-based dendrogram inferred from the Tamura-Nei distance.

The genetic diversity analyses and the phylogenetic analyses suggest that *R. konori* and *R. superbum* are very closely related. However, to establish the status of these taxa, further studies using accessions from multiple populations displaying the various phenotypes must be carried out. In view of the genetic closeness of the accessions studied, the subspecies *ibele* may also turn out to be a very closely related entity (Figure 83).

6.2.8 *R. orbiculatum*

R. orbiculatum is a widespread species ranging from Borneo to Sulawesi, usually growing as a shrub or a small tree to 4 m high. Even though *R. orbiculatum* is not Red-Listed by IUCN, it is often confused with Red-Listed (VU D2) species *R. edanoi* ssp. *edanoi* (endemic to Philippines, but geographically close to Borneo) and *R. lambianum* (endemic to Sabah and Sarawak, Borneo). Based on phylogenetic analyses *R. suaveolens* was not found to be related to *R. orbiculatum*, and *R. lambianum* was not available for this study. *R. orbiculatum* was previously placed by Sleumer (1966) in the Section *Solenovireya*, but have now been moved to the Section *Euvireya* Subsection *Euvireya* by Argent (2006).

However, the phylogenetic analyses described in Section 6.1 show that *R. orbiculatum* belongs with *R. jasminiflorum*, despite the several macromorphological differences such as the orbicular leaves and relatively large flowers of the former.

Due to the close relationships of *R. orbiculatum* with species of conservation interest, this species was examined for genetic diversity. The genetic diversity analysis using microsatellite data showed there was relatively high genetic diversity among the accessions (Figure 64). This suggests that the accessions may have been collected from geographically isolated populations. The high genetic distances also suggest that these individuals may have belonged to outbreeding populations.

The accession HF092 was further analysed using DNA sequence data and compared with published sequences of *R. orbiculatum* (GU445798). However the analysis showed there is very low genetic differentiation between these two accessions (0.01488 or ~1.5%). The relatively low genetic differentiation suggests that these two accessions may have originated from the same wild population.

6.2.9 *R. laetum*

R. laetum is a small shrub from W New Guinea, and grows up to a height of 3 m in the wild. *R. laetum* is not Red-Listed by IUCN, however this species cluster with species of conservation interest in the phylogenetic analyses, usually with *R. lochiae* (VU D2), *R. superbum* (VU D2 assigned to the ssp. *ibele*) and *R. loranthiflorum* (DD). *R. laetum* is known to hybridise with *R. hellwigii* (LC) and this hybrid clusters within the clade containing *R. laetum*.

Figure 65 illustrates the genetic diversity analysis of the *R. laetum* accessions using microsatellite data and the genetic distance $(\delta\mu)^2$ assuming SMM. The genetic diversity is relatively low among the accessions, suggesting that these accessions are very closely related, and perhaps collected from a single wild population.

Figure 66 illustrates the evolutionary divergence between *rpb*2i DNA sequences of the *R. laetum* complex using the Tamura-Nei model. The genetic differentiation between the accessions is very low (max. 0.2%), suggesting that these accessions may have originated

from a single wild population. The accessions EK648 and HF066 cluster together and are genetically identical. This confirms that the wild collected hybrid *R. laetum* × *hellwigii* is indeed a progeny of *R. laetum*.

6.2.10 *R. quadrasianum*

R. quadrasianum is a highly variable species from the Philippines, with six varieties described to date. However, none of these varieties is Red-Listed by IUCN, and thus of no conservation value at present. *R. quadrasianum* belongs to the Section *Discovireya*, which contains similar small stature plants with very small leaves and flowers. This complex species is included here due to the availability of multiple accessions representing some of the varieties and to test the analysis methods of this study.

Figure 67 shows the NJ-based dendrogram for the fragment length dataset, showing relationships between three accessions of *R. quadrasianum* based on $(\delta\mu)^2$ genetic distance and assuming the Stepwise Mutation Model (SMM). The genetic diversity among the accessions is relatively high suggesting geographically separate origins. The typical variety *quadrasianum* is genetically very distinct from the other two varieties. Further similar studies using more accessions representing all the varieties may reveal status of the varieties and provide genetic support for these taxonomic groups.

6.2.11 *R. christi*

R. christi is a variable species from New Guinea with a wide distribution. This species is not very well studied to date, as evident from the lack of varietal or subspecific names for the various morphological forms (most of which are widely cultivated). Commonly, *R. christi* grows as a shrub to 1.2 m high, but trailing forms are also found, such as those from Mt Miap. Other common forms include the red form (with bright red flowers) and the small form (with relatively small flowers). *R. christi* is not Red-Listed by IUCN and is not of conservation interest at present. This species is included here due to the availability of multiple accessions and to provide support for the genetic diversity analysis methods.

Figure 68 shows the NJ-based dendrogram for the fragment length dataset, showing relationships between four accessions of *R. christi* based on $(\delta\mu)^2$ genetic distance and assuming the Stepwise Mutation Model (SMM). The low genetic diversity among the accessions suggest that these are very closely related and further studies including multiple accessions of each morphological form could reveal the taxonomic limits of these forms.

6.2.12 *R. culminicola*

R. culminicola is a shrub or a tree to 8 m high, New Guinea. a variable species from New Guinea (Argent 2006). The NJ-based dendrogram (Figure 69) for the fragment length dataset showed relationships between three accessions of *R. culminicola* based on $(\delta\mu)^2$ genetic distance and assuming the Stepwise Mutation Model (SMM). The significantly large genetic distances among the accessions (287.82– 676.32) suggest high genetic variation among the accessions. The two accessions (99286 from USA, and EK629 from New Zealand) belonging to the subspecies *culminicola* did not cluster together, and are separated by a genetic distance of 388.5 suggesting a significant genetic separation between the accessions. The single accession from USA (83059) belonging to the subspecies *angiense* was separated from the others with a genetic distance of 287.82 suggesting a significant genetic differentiation between this subspecies and the subspecies *culminicola*.

6.2.13 *R. dielsianum*

R. dielsianum is a small shrub to 1.5 m high, originating from Papua New Guinea. This species is categorized by IUCN as LC. However, this species is often confused with *R. bryophilum* which has been categorized as DD, and thus *R. dielsianum* is of low conservation interest. This species is also included here due to the availability of multiple accessions representing some of the varieties and to test the analysis methods of this study.

The genetic diversity analysis using microsatellite data showed that the overall genetic distances among the accessions are moderate (72.06–324.44) (Figure 70). The accessions 198360 and 99330 analysed here using DNA obtained from RSF (USA) clustered

together with a genetic distance of 72.06, indicating that these two accessions may have been collected from a single population. However, the New Zealand accession HF023 is relatively distant from the RSF accessions (genetic distance 252.38), suggesting that this accession may have originated from a geographically separate population.

6.2.14 *R. commonae*

R. commonae is a compact shrub to 6 m high from New Guinea, with an IUCN category of LC. *R. commonae* is thus of low conservation interest, but was included here as there were multiple accessions of this taxon available for study, and to provide analytical support for the data analysis methods.

The genetic diversity analysis using *rpb*2i DNA sequence data of the four *R. commonae* accessions showed the absence of any genetic diversity among all the accessions. The analysis contained three accessions from New Zealand and data from a single published accession. The three New Zealand accessions has three different flower colours, while the flower colour of accession from published data is of unknown flower colour. Despite the differences in flower colour and representing accessions from different data sets, no genetic differentiation can be observed among the accessions. This result also suggests that the flower colour has no significance to the genetic diversity of this species when analysed using the selected intron region of the *rpb*2i gene.

6.2.15 *R. emarginatum*

R. emarginatum is a small shrub to 2 m high, from China. There are two described varieties of this species, var. *emarginatum* from China and Vietnam, and the var. *eriocarpum* from China only. The taxon *R. eunonymifolium* H. Lév. Has been reduced to a synonym of *R. emarginatum* in the latest monograph of vireyas (Argent 2006), however *R. eunonymifolium* is still being used as a different taxon by some recent molecular studies on vireyas. This suggests that there are still taxonomic uncertainties around these two taxa. However, the present study and other recent molecular studies that included these two taxa showed that they cluster together in the phylogeny with very good statistical support (Craven et al. 2011; Goetsch et al. 2011). The taxon *R. eunonymifolium* is also not recognized in the latest Red List of Rhododendrons (Gibbs et al. 2011).

R. emarginatum has been assigned the IUCN category of LC, thus of no direct conservation interest. However, this species is known to be closely related to the two threatened species, *R. santapaui* (EN B2ab(ii,iii,v)) and *R. rushforthii* (VU D2). Due to these relationships, *R. emarginatum* is included here for genetic diversity analysis.

The genetic diversity analysis using *rpb*2i DNA sequences of three *R. emarginatum* accessions showed that there is very low genetic differentiation between these accessions (Figure 71). The accessions from published data, GU445845 and GU445846 (referred to as *R. eunonymifolium*) clustered together with no genetic differentiation between them, thus supporting the taxonomic status of *R. eunonymifolium* as a synonym. The New Zealand accession of *R. emarginatum*, HF050 is significantly distant from the accessions GU445845 and GU445846, thus suggesting a slight genetic difference, perhaps due to all of these accessions being collected from a single population.

6.2.16 *R. majus*

Rhododendron majus (J. J. Sm.) Sleumer is a small shrub up to 2 m high (Photo 23) and found in New Guinea, and belongs to the Subsection *Solenovireya* within Section *Euvireya sensu* Argent (2006). This species was previously known as *R. carringtoniae* var. *majus* J. J. Sm., which has now been reduced to a synonym. This species has a wide distribution, but are restricted to high altitudes (Argent 2006), and there are no known taxonomic or conservation issues related to this species (IUCN code is LC). *R. majus* is thus of low conservation interest, but was included here as there were multiple accessions of this taxon available for study, and to provide analytical support for the data analysis methods. The accession EK658 was included in the analyses as it was originally labelled as *R. majus* and thus needed to verify its identity.

Photo 23 *Rhododendron majus* (EI158) The plant was growing at the Victoria Esplanade Gardens (Palmerston North, New Zealand).

The genetic analysis using the *rpb*2i data (Figure 72) showed that the accessions EI157 and EI158 are genetically very similar (genetic distance 0.0022). The MP phylogenetic analysis using Dataset 2 (Figure 41) in contrast showed that accessions EI158 and EK658 cluster together, while the accession EK657 clusters with an accession of *R. commonae* (GU445786).

The genetic analysis using RAPD data showed that the accessions EI157 and EI158 are very closely related together. These two accessions are grown side by side at the Victoria Esplanade, and may have originated from a single wild population. The accession from the Pukeiti collection EK657 also cluster together with the two accessions EI157 and EI158, suggesting that these may have originated from a single population. The accession EK658 is significantly genetically distant from the rest of the accessions, as also seen in the genetic analysis using *rpb*2i data. The physical characters of this accession keys out to *R. baenitzianum.*

6.2.17 *R. solitarium*

R. solitarium is an erect or sprawling shrub to 1.5 m high, originating from Papua New Guinea. Presently known only from a very small area (Argent 2006), thus of conservation interest. However, the IUCN category assigned to this species at present is LC (Gibbs et al. 2011), and the status of this species needs to be re-evaluated with more field data. This species is included here due to its limited distribution and the availability of multiple accessions for genetic diversity analyses.

The genetic diversity analysis based on *rpb*2i DNA sequences of three *R. solitarium* accessions showed that the genetic distances between the accessions are relatively low (Figure 74), suggesting very closely related accessions. The accessions EK614 (New Zealand) and GU445773 (published data) are genetically identical, while the accession EK617 (New Zealand) is significantly different from the other accessions. The low genetic distances between the accessions suggests that the accessions of *R.solitarium* may have originated from a single wild population.

6.2.18 *R. suaveolens*

R. suaveolens is a shrub or a small tree to 3 m high, originating from Borneo (Sabah). There are two described forms of this species, *f. suaveolens* and *f. roseum*, the latter differing from the typical form in having uniformly pink-coloured flowers (Argent 2006). The IUCIN category assigned for this species is LC, however this species is often confused with the related species *R. alborugosum* which has the IUCN endangered category EN D assigned. *R. suaveolens* is therefore of moderate conservation interest being related to a threatened taxon.

The genetic diversity analysis using *rpb*2i DNA sequences of three *R. suaveolens* showed that there is relatively low genetic variation among the accessions (Figure 75). The accessions EK544 (from New Zealand collections) and GU445794 (from published data) cluster together with relatively low genetic difference between them (0.0058), suggesting that they are genetically very similar and perhaps collected from the same wild population. The accession HF082 from New Zealand collections is however significantly distant from the accessions EK544 and GU445794 (genetic distance – 0.0068),

suggesting that HF082 may have been collected from a geographically separate population or from a population with significant genetic diversity. The results suggest that both the New Zealand accessions EK544 and HF082 are suitable for conservation.

6.2.19 *R. fallacinum*

R. fallacinum is a shrub or a small tree to 6 m high, originating from Borneo (Sabah and Sarawak). There are no known taxonomic or conservation issues related to this species (IUCN code LC), and thus of no conservation interest, but has been included here as there were multiple accessions of this taxon available for study, and also to provide analytical support for the data analysis methods.

The genetic diversity analysis using microsatellite data showed that there is significant genetic diversity among these accessions (Figure 76). The accessions EK582 and EK531 has a smaller genetic distance between them (82.23), while the accession EK527 is significantly genetically distant from the other two accessions (402.66). These results suggest that the three accessions of *R. fallacinum* analysed here may have been collected from three geographically different populations or from a population with significant genetic diversity.

6.2.20 *R. stenophyllum* and *R. crassifolium*

R. stenophyllum is a shrub to 3 m high originating from Borneo. There are two described subspecies, ssp. *stenophyllum* (restricted to Sabah) (Photo 24a) and ssp. *angustifolium* (widely distributed in Sabah, Brunei and Kalimantan) (Photo 24b). The subspecies *angustifolium* differs from the type species mainly by having longer and narrower leaves among other morphological features (Argent 2006). Neither of the two subspecies is of conservation interest, as they have been assigned IUCN LC category (Gibbs et al. 2011).

R. crassifolium is a shrub to 2.5 m high, originating from Borneo. There are two described varieties of this species, var. *crassifolium* (Photo 24c) and var. *pseudomurudense*. The variety *pseudomurudense* differs from the typical variety by having glabrous filaments (Argent 2006). Neither of these varieties have been Red Listed, and been assigned the IUCN LC category (Gibbs et al. 2011).

Both *R. stenophyllum* and *R. crassifolium* are known to hybridise in the wild. Plants referred to as *R. nervulosum* may be a hybrid between *R. crassifolium* and *R. stenophyllum*, and such a hybrid is cultivated at the Pukeiti Gardens (HF027) (Argent 2006). This hybrid accession displays linear foliage typical of *R. stenophyllum* (Photo 24d).

(a) *R. stenophyllum* ssp. *stenophyllum* [HF082] (b) *R. stenophyllum* ssp. *angustifolium* [EK526]

(c) *R. crassifolium* [EK560] (d) *R. crassifolium* × *stenophyllum* [HF027]

Photo 24 *Rhododendron stenophyllum, R. crassifolium* and their suspected hybrid. (a) *R. stenophyllum* ssp. *stenophyllum* [HF082] (b) *R. stenophyllum* ssp. *angustifolium* [EK526] (c) *R. crassifolium* [EK560] (d) *R. crassifolium* × *stenophyllum* [HF027].

Genetic diversity analysis based on microsatellite data showed that the ensemble of the accessions representing *R. crassifolium*, *R. stenophyllum* and their hybrid formed two distinct groups (Figure 77). The range of genetic diversity within this group (18.13–294.24) suggests that this ensemble consists of very closely related accessions (or taxa).

6.2.21 *R. blackii*

Rhododendron blackii is a small shrub to 3 m high from Papua New Guinea (Western & Southern Highlands), and are found at an altitude of 2,500–3,300 m. This species is known to be vegetatively very similar to *R. carrii* (VU), although without the down-turned basal lobes to the leaves and with much more distinct reticulation. Also, the flowers, being long, tubular and pink, are quite different. The species is found in a single location, but have been introduced into cultivation by Paul Kores in 1976 (Argent 2006). Sleumer (1973) recorded this species as being in cultivation at the time of publication of the species both at Michael Black's garden in Grasmere in the UK and in Australia from the seeds from a Vink collection (17041) of 1966 (Argent 2006). As there are no significant taxonomic or conservation issues related to *R. blackii*, this species have been assigned the LC category by IUCN. *R. blackii* is thus of no conservation interest at present, but was included here as there were multiple accessions of this taxon available for study, and also to provide analytical support for the data analysis methods.

The genetic diversity analysis using RAPD data (Figure 78) showed that the three accessions studied (EK591, EK592 and EK593) are genetically distinct. The relatively low genetic distances between the accessions suggest that these accessions may have originated from a single but genetically diverse population. The phylogenetic analysis of the accessions EK591 and EK592 showed that these two accessions does not cluster together, suggesting a significant genetic distance between these two accessions. The genetic diversity analysis using the *rpb*2i data (Table 16) showed that the genetic distance (Tamura-Nei) between these two accessions was 0.005691.

The results of the genetic analyses using *rpb*2i and RAPD data showed that there is genetic diversity among the accessions of *R. blackii*, but relatively low compared to the genetic diversity seen among the accessions of other taxa. There are three additional accessions of *R. blackii* that have not been studied (EK594, EK625 and HF056), and

future studies including these accessions may reveal the true genetic diversity of this species.

6.3 Summary

The various phylogenetic analysis methods evaluated here showed that different methods reveal different aspects of the phylogeny. However, the Maximum Likelihood method inferred very similar phylogenies to those produced by the Minimum Evolution and Neighbour Joining methods (using nucleotide sequence Dataset 1). Thus, only the Maximum Likelihood and Maximum Parsimony methods were applied to the Dataset 2. The Dataset 2, which contains a combination of data from this study and published data revealed a more resolved phylogeny than that obtained for the Dataset 1. Thus, larger number or taxa produce increased phylogenetic resolution.

The phylogenetic analyses described in this study contained relatively larger number of more taxa than in previous studies of vireyas, and thus revealed additional taxonomic information previously not seen. The overall topology of the phylogenetic analyses, especially that of the MP tree is congruent with the majority of the recent phylogenetic studies using molecular data (Brown et al. 2006a, 2006b; Brown et al. 2006c; Craven et al. 2011; Craven et al. 2008; Goetsch et al. 2011). The various clades seen in the phylogenetic analyses of this study also support the premise that they correspond to geographic regions rather than previous taxonomic groups. As suggested in the recent molecular studies, a new classification is warranted which take into account the geographical correlations of the clades and evolutionary relationships. A possible classification based on the phylogenetic analyses was therefore suggested (Figure 81).

The application of microsatellites in determining the genetic diversity of selected taxa and taxa complexes showed that several of them have relatively high genetic diversity suitable for conservation planning. The major limitation during the genetic diversity analyses was the low number of accessions available for the taxa studied. Another limitation was that microsatellites specific to vireyas were not available for this study, and resulted in several taxa not amplifying for the majority of the markers. A larger selection of accessions and more specific microsatellite markers could be used to improve these results. The genetic diversity analyses using microsatellites showed that the

majority of the taxa studied displayed significant genetic diversity, and therefore are suitable for utilization in *ex situ* conservation.

Genetic diversity analyses using *rpb*2i and RAPD data also showed significant genetic diversity among the accessions of the majority of the taxa studied. However, only limited taxa were evaluated using these methods and also had fewer accessions in the analyses. Utilization of nucleotide sequence data in the genetic diversity analysis of *Rhododendron*, and in particular, vireyas present a novel method. Further study utilizing more accessions of vireyas will greatly improve the present results.

The following subsections summarize the discussions and findings of this chapter applied to the vireyas, and are arranged according to the classification of Argent (2006).

6.3.1 Section *Pseudovireya* (Clarke) Sleumer

This section summarizes the results and findings of the genetic analyses carried out on the taxa of Section *Pseudovireya* (Clarke) Sleumer. The results and its implications on taxonomy and conservation are summarized in Table 19. Taxa belonging to this section are predominantly distributed in mainland Asia with a representative taxon in offshore island Taiwan (*R. kawakamii*).

Photo 25 shows a selection of taxa belonging to the Section *Pseudovireya* that were used in the present study. The floral characters (among other characters) of this section are significantly different from the rest of the sections of Subgenus *Vireya*. As shown in the phylogenetic analyses described in the previous chapters, they are shown to be sister to all the other sections. This section is therefore less derived than the other sections and very closely related to the temperate rhododendrons than the rest of the vireyas. The table below summarises the taxonomic and conservation answers for the queries raised for Section *Pseudovireya* (Appendix A2.1).

Photo 25 A selection of taxa belonging to Section *Pseudovireya*. (a) *R. santapaui* growing at the Pukeiti Garden (NZ) (b) *R. vaccinioides* growing in Lava, Kalimpong (W Bengal, India) (Photo: D Scherberich) (c) *R. emarginatum* growing at Pukeiti Garden (NZ) (d) *R. rushforthii* (Photo: W Moyles) (e) *R. kawakamii* (Photo: F Muller).

Table 19 Summary of answers to the taxonomic and conservation queries of Section *Pseudovireya*, raised in Appendix A2.1. The taxa in **boldface** denote those analysed in this study.

#	Taxon	Range	Questions Answered	IUCN Code
1	***R. vaccinioides***	IS	(i) *R. vaccinioides* and *R. asperulum* does not cluster together. *R. vaccinioides* is closely related to *R. santapaui* which cluster together (Group A). *R. asperulum* is more closely related to *R. emarginatum*, *R. euonymifolium*, *R. sororium*, *R. rushforthii*, and *R. kawakamii*, all of which cluster together. The differentiation between the species *R. vaccinioides* and *R. asperulum* can be attributed to their geographic separation.	LC

#	Taxon	Range	Questions Answered	IUCN Code
			(ii) The cluster containing *R. vaccinioides* (Group A) is sister to the rest of the taxa of Section *Pseudovireya* (Group B) (Figure 41). *R. vaccinioides* thus belong to *Pseudovireya* as suggested by morphological studies.	
2	***R. santapaui***	IS	(i) *R. santapaui* (Group A) is not closely related to *R. kawakamii* (Group B) (Figure 41). This differentiation can be attributed to their vast geographic separation, in which *R. santapaui* is found in the Indian Subcontinent, while *R. kawakamii* is endemic to the island Taiwan. (ii) The cluster containing *R. santapaui* (Group A) is sister to the rest of the taxa of Section *Pseudovireya* (Group B) (Figure 41). *R. santapaui* thus belong to *Pseudovireya* as suggested by morphological studies. (iii) The two accessions of this species (AY765631 and EK581) cluster together with very good bootstrap support (94%) and very high Bayesian posterior probability (97%) (Figure 41). This indicates that these two accessions are nearly identical and may have originated from the same population.	DD
3	***R. asperulum***	EA	(i) *R. asperulum* and *R. vaccinioides* are not closely related, and are found on genetically distant clusters (B and A respectively) (Figure 41). This differentiation can be attributed to their geographic separation, in which *R. asperulum* is found in East Asia, while *R. vaccinioides* is found in the Indian Subcontinent. (ii) *R. asperulum* clusters together with the majority of taxa belonging to the Section *Pseudovireya* (Group B) (see Figure 41). Thus this species belong to the core of Section *Pseudovireya*.	VU D2
4	*R. insculptum*	EA	No known taxonomic issues.	DD
5	*R. rupivalleculatum*	EA	No known taxonomic issues.	DD
6a	***R. emarginatum* var. *emarginatum***	EA	(i) *R. emarginatum* var. *emarginatum* is closely related to *R. kawakamii*, forming a cluster within Group B (Figure 41). However, *R. emarginatum* var. *emarginatum* is much closely related to *R. euonymifolium*, forming a cluster with	LC

#	Taxon	Range	Questions Answered	IUCN Code
			strong bootstrap support (81%) and high Bayesian posterior probability (97%). (ii) *R. emarginatum* var. *emarginatum* belongs within the cluster containing the majority of the taxa of Section *Pseudovireya* (Group B) (Figure 41). (iii) The two accessions of this species (GU445848 and HF050) do not cluster together. This could be a result of a sequencing error (during base calling) in the accession HF050 or due to the limited number of parsimony-informative molecular characters. (iv) *R. emarginatum* & *R. euonymifolium* cluster together with strong bootstrap support (81%) and high Bayesian posterior probability (97%), suggesting that these two accessions are genetically very similar and thus can be treated as a single species as suggested by Argent (2006) and Sleumer (1966a).	
6b	*R. emarginatum* var. *eriocarpum*	EA	No known taxonomic issues.	NE
7	***R. sororium***	EA	(i) *R. emarginatum* and *R. sororium* are closely related to each other and belongs within the same cluster (Group B) (Figure 41). The bootstrap support is weak (56%), however the Bayesian posterior probability is very high (100%) suggesting a very close relationship relative (Figure 41). (ii) *R. sororium* clusters together with the majority of taxa belonging to the Section *Pseudovireya* within Group B (Figure 41). Thus this species belong to the core of Section *Pseudovireya*.	LC
8	*R. densifolium*	EA	No known taxonomic issues.	VU D2
9	***R. rushforthii***	EA	(i) *R. rushforthii* and *R. kawakamii* are very closely related, clustering together with very strong bootstrap support (94%) and high Bayesian posterior probability (100%). However, they are geographically separate, with *R. rushforthii* found on the mainland East Asia, while *R. kawakamii* is restricted to the island Taiwan. The close genetic relationship indicates that these two species shared a recent common ancestor, and further suggests that the Section *Pseudovireya* spread from the	DD

#	Taxon	Range	Questions Answered	IUCN Code
			Indian Subcontinent through East Asia eastwards to Taiwan. (ii) The two accessions of this species (GU445848 and HF147) do not cluster together. This genetic differentiation can be due to a base-calling error in the sequence of the accession HF147 or due to the limited number of parsimony-informative molecular characters. (iii) *R. rushforthii* is also related to *R. euonymifolium*, *R. emarginatum*, *R. sororium* and *R. asperulum* (Group B Figure 41). These three taxa share similar floral morphology (Photo 25) and originate from mainland East Asia.	
10	*R. datiandingense*	EA	Taxon not available for this study.	DD
11	***R. kawakamii***	TW	(i) *R. kawakamii* clusters together with the mainland East Asia taxa *R. rushforthii* and *R. emarginatum* within Group B. *R. kawakamii* is restricted to the geographically separated island Taiwan. However, these three taxa share very similar flora morphology (Photo 25). (ii) *R. kawakamii* clusters together with the majority of taxa belonging to the Section *Pseudovireya* (Group B) (Figure 41). Thus this species belong to the core of Section *Pseudovireya*. (iii) The two accessions of this species (GU445847 and HF072) do not cluster together. This could be a result of a sequencing error in the accession HF072 or due to the lack of parsimony informative molecular characters.	LC

6.3.2 Section *Discovireya* (Sleumer) Argent

In the analyses of the Dataset 1, the sole representative of the Section *Discovireya*, *R. adinophyllum* does not show a very close relationship with the taxa of Section *Pseudovireya*, but clusters with *R. sumatranum* (Section *Euvireya*), with 77% bootstrap support (Figure 34). This relationship is also seen in Figure 36 (with 68% bootstrap support), Figure 37 (with 69% bootstrap support) and Figure 38 (with 65% bootstrap support). According to Argent (2006), a wild hybrid of *R. adinophyllum* with *R. sumatranum* was collected by David Binney on Mt Kemiri (Sumatra) in 1998. The

phylogenetic analysis thus supports the close relationship between these two species. The reason for these two species not appearing together in Figure 38 is in part due to the additional accession of *R. sumatranum* and the presence of additional closely related taxa.

In the MP analysis using the Dataset 2 (Figure 41), the taxa of Section *Discovireya* (Group C) cluster together with very good bootstrap support (90%) and high Bayesian posterior probability (99%), if *R. perakense* and *R. pulleanum* are excluded. The nodes within the bulk of the Section *Discovireya* correspond to their geographic range (Figure 21). *R. nanophyton, R. gaultheriifolium, R. meliphagidum, R. ericoides* and *R. quadrasianum* are restricted to East Malesia (Node 6a corresponding to NE Borneo eastward through Sulawesi and the Moluccas to Central New Guinea). *R. retusum* and *R. adinophyllum* restricted to Java and Sumatra respectively (Node 6b corresponding to Java and Sumatra).

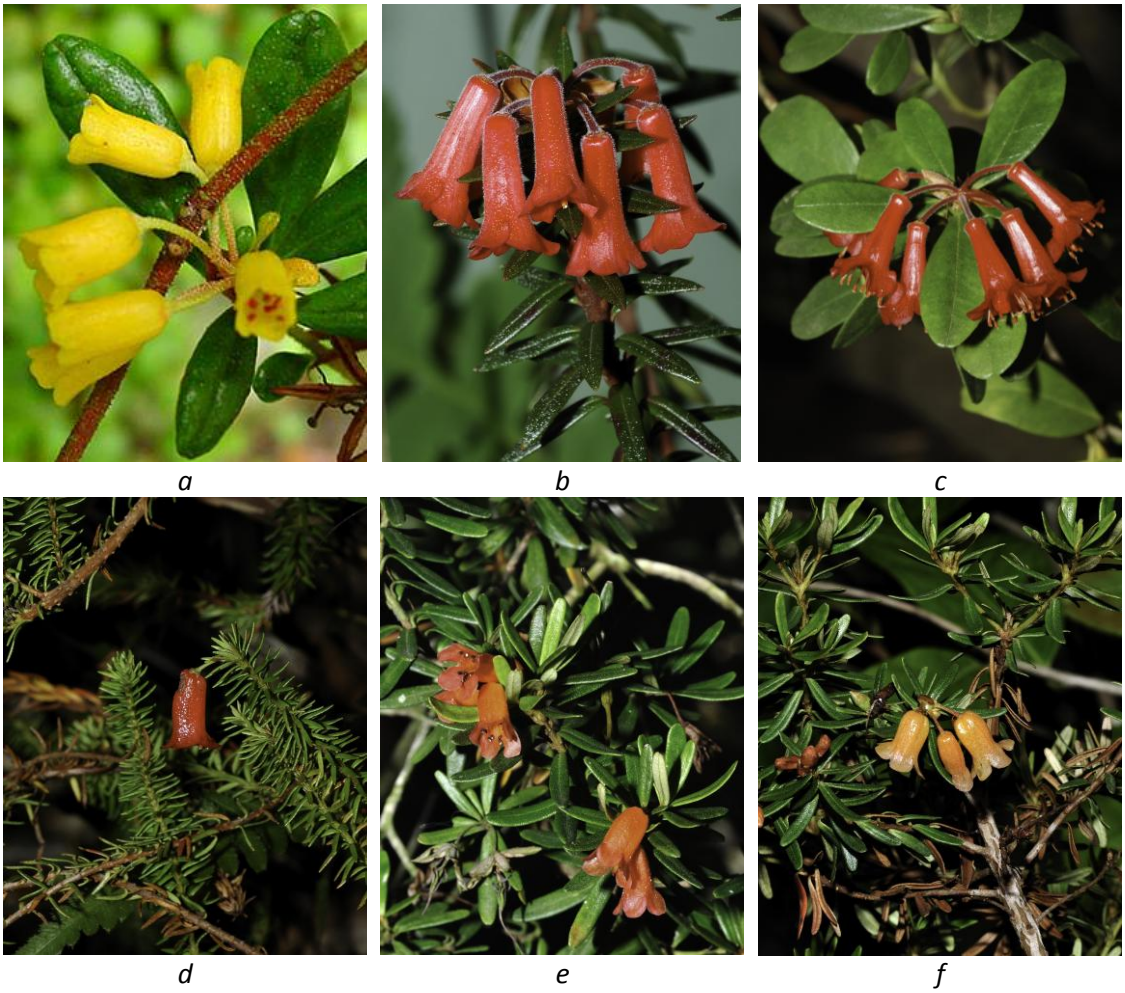

Photo 26 A selection of taxa belonging to Section *Discovireya*. (a) *R. perakense* (Photo: R Currie). (b) *R. adinophyllum* (collected from Sumatra) (EK602). (c) *R. retusum* var. *retusum* (EK571). (d) *R. ericoides* (EK537). (e) *R. quadrasianum* var. *quadrasianum* (EI143). (f) *R. quadrasianum* var. *rosmarinifolium* (EK662).

R. perakense is restricted to Malay Peninsula and geographically separate from the taxa of nodes 2 and 3. *R. perakense* falls outside the rest of the *Discovireya* and are sister to the core vireyas (sections *Malayovireya, Siphonovireya, Phaeovireya, Albovireya* and *Euvireya*). This positioning of *R. perakense* is also seen in recent molecular studies (Goetsch et al. 2011). It appears that this taxon belongs to a disjunct taxonomic group, however, only further studies that includes the other Malay Peninsula taxa *R. scortechinii*, *R. seimundii* and *R. spathulatum*, may reveal the precise placement of *R. perakense*.

R. pulleanum does not cluster or show any relationships with the rest of the taxa of Section *Discovireya*; instead this species allies with the taxa of the Core Vireyas and clustering with *R. dianthosmum* (Section *Phaeovireya*). The association of this species with Section *Euvireya* taxa can also be seen in recent molecular studies (Goetsch et al. 2011). Morphologically, *R. pulleanum* has the physical characteristics of the Section *Discovireya.* Further study employing multiple accessions of the two varieties of this species could resolve the proper placement of this species.

The Subgroup C1 contains the taxa *R. nanophyton, R. gaultheriifolium, R. meliphagidum* and *R. ericoides*, ranging from NE Borneo eastward through Sulawesi and the Moluccas to Central New Guinea. The Subgroup C2 consists of *R. retusum* and two accessions of *R. adinophyllum*, ranging from Peninsular Malaysia through Sumatra eastwards to Java. The two accessions of *R. adinophyllum* have high bootstrap support (81%), suggesting that they are genetically very similar and perhaps collected from the same locality or population. The single accession of *R. perakense* falls outside the rest of the taxa of Group C (Section *Discovireya*), but is sister to (and not clustering with) the Core Vireyas. *R. perakense* is found in Peninsular Malaysia, and its position on the phylogenetic tree could be a result of this geographic separation.

Table 20 Answers to the taxonomic and conservation queries of Section *Discovireya* Appendix A2.20. The taxa in **boldface** denote those analysed in this study.

#	Taxon	Range	Questions Answered	IUCN Code
12	***R. perakense***	MP	(i) This species does not cluster with the rest of the taxa of Section *Discovireya*, and sister to the rest of the taxa of Subgenus *Vireya*. (ii) This species does not show any close relationship to the other taxa of Section *Discovireya* or the other taxa of Subgenus *Vireya*. (iii) Only a single accession avalable. (iv) *R. scortechinii*, *R. seimundii* and *R. spathulatum* not available for this study.	LC
13	*R. scortechinii*	MP	Taxon not available for this study.	LC
14	*R. seimundii*	MP	Taxon not available for this study.	DD
15	*R. spathulatum*	MP	Taxon not available for this study.	LC
16	***R. adinophyllum***	SM	(i) *R. adinophyllum* and *R. sumatranum* clusters together in the ML tree for the Dataset 1, and this relationship do not appear in the other analyses. This suggests that there is a very weak relationship between these two species. (ii) There are no further observed relationships with taxa outside *Discovireya*. (iii) *R. adinophyllum* appears to be very closely related to *R. retusum* with strong bootstrap support (97%) and high Bayesian posterior probability (99%). (iv) The two accessions of this species cluster together with moderate bootstrap support (77%) and very high Bayesian posterior probability (100%). This suggests that these two accessions may have been collected from the same population in the wild.	LC
17a	***R. retusum* var. *retusum***	SM JV	(i) This taxon cluster together with the bulk of the taxa of Section *Discovireya*. (ii) This taxon is very closely related to *R. adinophyllum* with strong bootstrap support (97%) and high Bayesian posterior probability (99%). (iii) Data for only a single accession was available for this study.	LC

#	Taxon	Range	Questions Answered	IUCN Code
17b	*R. retusum* var. *trichostylum*	SM	Taxon not available for this study.	DD
17c	*R.* × *epilosum*	SM	Taxon not available for this study.	NE
18a	*R. borneense* ssp. *borneense*	BN	DNA did not amplify.	LC
18b	*R. borneense* ssp. *villosum*	BN	DNA did not amplify.	LC
18c	*R. borneense* ssp. *angustissimum*	BN	Taxon not available for this study.	LC
19	*R. buxoides*	BN	Taxon not available for this study.	VU
20a	*R. cuneifolium* var. *cuneifolium*	BN SW	Taxon not available for this study.	LC
20b	*R. cuneifolium* var. *microcarpum*	BN	Taxon not available for this study.	VU D2
21a	***R. ericoides***	BN	(i) This species clusters together with the bulk of the taxa of Section *Discovireya*. (ii) *R. borneense* and *R. cuneifolium* were not studied. (iii) This species is very closely related to *R. meliphagidum* with low bootstrap support (67%) and high Bayesian posterior probability (88%). *R. ericoides* is also related to *R. gaultheriifolium*, *R. nanophyton* and *R. quadrasianum* with high bootstrap support (90%) and very high Bayesian posterior probability (100%). (iv) Data for only a single accession was available for this study.	VU
21b	*R.* × *silvicola*	BN	Taxon not available for this study.	NE
22a	***R. nanophyton* var. *nanophyton***	SW	(i) This species clusters together with the bulk of the taxa of Section *Discovireya*. (ii) Data for only a single variety was available for this study.	EN D
22b	*R. nanophyton* var. *petrophilum*	SW	Taxon not available for this study.	DD
23	*R. monodii*	SW	Taxon not available for this study.	DD
24	***R. meliphagidum***	SW, ML	No issues.	LC
25a	***R. quadrasianum* var. *quadrasianum***	PH	(i) This taxon clusters together with the bulk of the taxa of Section *Discovireya*.	LC

#	Taxon	Range	Questions Answered	IUCN Code
			(ii) This taxon clusters together with *R. nanophyton* and *R. gaultheriifolium* but with very low bootstrap support (<50%). (iii) Data for only a single accession was available for this study. (iv) Data for only a single variety was available for this study. (v) Data for only a single variety was available for this study.	
25b	*R. quadrasianum* var. *davaoense*	PH	Taxon not available for this study.	NE
25c	***R. quadrasianum* var. *rosmarinifolium***	PH	No issues.	NE
25d	*R. quadrasianum* var. *malindangense*	PH	DNA did not amplify.	NE
25e	*R. quadrasianum* var. *marivelesense*	PH	Taxon not available for this study.	NE
25f	*R. quadrasianum* var. *intermedium*	PH	Taxon not available for this study.	NE
26	*R. taxoides*	NG	Taxon not available for this study.	VU
27a	***R. pulleanum* var. *pulleanum***	NG	(i) The placement of this taxon is questionable, as it does not cluster with any other taxa of Section *Discovireya*. The phylogenetic analyses show that this taxon is related to *R. dianthosmum* (belonging to the Section *Phaeovireya*) and forms a constituent of the Core Vireyas. Further study is needed to determine the precise placement of this taxon. (ii) Taxon not available for study.	LC
27b	*R. pulleanum* var. *maiusculum*	NG	Taxon not available for this study.	NE
28	*R. nummatum*	NG	Taxon not available for this study.	LC
29a	***R. gaultheriifolium* var. *gaultheriifolium***	NG	(i) This variety is placed within the core of the group containing Section *Discovireya* taxa (Subgroup C1) (see Figure 41). The Subgroup C1 with 94% bootstrap support is distributed in Eastern Malesia. This variety is very closely related to the taxa *R. nanophyton*, *R. ericoides* and *R. meliphagidum*. (ii) (taxon not available for study)	LC

#	Taxon	Range	Questions Answered	IUCN Code
29b	*R. gaultheriifolium* var. *expositum*	NG	Taxon not available for this study.	LC
30a	*R. oreites* var. *oreites*	NG	Taxon not available for this study.	LC
30b	*R. oreites* var. *chlorops*	NG	Taxon not available for this study.	LC
31	*R. erosipetalum*	NG	Taxon not available for this study.	LC
32	*R. detznerianum*	NG	Taxon not available for this study.	DD
33	*R. hameliiflorum*	NG	Taxon not available for this study.	DD
34a	*R. lindaueanum* var. *lindaueanum*	NG	Taxon not available for this study.	LC
34b	*R. lindaueanum* var. *bantaengense*	SW	Taxon not available for this study.	VU D2
35	*R. cyrtophyllum*	NG	Taxon not available for this study.	DD
36	*R. ciliilobum*	NG	Taxon not available for this study.	LC

6.3.3 Section *Siphonovireya* (Sleumer) Argent

The Section *Siphonovireya* (Sleumer) Argent was not recovered in any of the phylogenetic analyses, instead its constituent taxa were mixed among taxa of the other sections, but restricted only to the Group G. *R. inundatum* (single accession) and *R. herzogii* (2 accessions) were only studied, and these two taxa do not cluster together. Also, the two accessions of *R. herzogii* do not cluster together. The relationship between *R. inundatum* and *R. konori* was not established. The relationship between *R. herzogii* and *R. culminicola* was neither established. Further studies that include all the taxa of this section and with additional nucleotide sequences perhaps may reveal the placement of this small group of taxa, and better resolution of the Group G. The outcomes of the queries raised for the Section *Siphonovireya* are summarized in the table below.

Table 21 Answers to the taxonomic and conservation queries of Section *Siphonovireya*, raised in Appendix A2.3. The taxa in **boldface** denote those analysed in this study.

#	Taxon	Range	Questions Answered	IUCN Code
37	*R. agathodaemonis*	NG	Taxon not available for this study.	DD
38	*R. incommodum*	NG	Taxon not available for this study.	LC
39	***R. inundatum***	NG	(i) No relationship with this species and *R. konori* was established. (ii) *R. inundatum* clusters with *R. alborugosum* with good bootstrap	LC

#	Taxon	Range	Questions Answered	IUCN Code
			support of 87% in the MP tree (Figure 41) and 85% in the ML tree (Figure 39), suggesting a close relationship.	
40	*R. protandrum*	NG	Taxon not available for this study.	DD
41	*R. habbemae*	NG	Taxon not available for this study.	LC
42	*R. cinchoniflorum*	NG	Taxon not available for this study.	LC
43	***R. herzogii***	NG	(i) The relationship between this species, *R. archboldianum* and *R. macgregoriae* were not determined, as the DNA for the latter were not sequenced. No relationship between *R. herzogii*, *R. inundatum* and *R. culminicola* was established from any of the phylogenetic analyses.	LC
44	*R. gideonii*	NG	Taxon not available for this study.	DD
45	*R. searleanum*	NG	DNA did not amplify for the sequencing.	LC
NEW	*R. dutartrei*	NG	Taxon not available for this study.	CR C2a(ii)
NEW	*R. kogo*	NG	Taxon not available for this study.	DD

6.3.4 Section *Phaeovireya* (Sleumer) Argent

The Section *Phaeovireya* was not recovered in any of the phylogenetic analyses, instead its constituent taxa were mixed among taxa of the other sections, but restricted only to the Groups E and G. Smaller clusters with Section *Phaeovireya* taxa are seen within these two groups such as the cluster with *R. superbum* and *R. konori* (Node 30 in Figure 41). The table below summarizes the answers to the taxonomic questions raised for Section *Phaeovireya*.

Table 22 Answers to the taxonomic and conservation queries of Section *Phaeovireya* raised in Appendix A2.4. The taxa in **boldface** denote those analysed in this study.

#	Taxon	Range	Questions Answered	IUCN Code
46	*R. eymae*	SW	Taxon not available for this study.	EN D
47	*R. psilanthum*	SW	Taxon not available for this study.	DD
48	*R. asperrimum*	NG	Taxon not available for this study.	DD
49	***R. asperum***	NG	(i) The relationship between this species and *R. laetum* were not observed in any of the phylogenetic analyses.	LC

#	Taxon	Range	Questions Answered	IUCN Code
			(ii) *R. asperum* formed a cluster containing taxa belonging to the sections *Phaeovireya*, *Siphonovireya* and *Euvireya*. *R. asperum* appears to be related *R. blackii* and *R. aurigeranum*, both belonging to Sect. *Euvireya* subsect. *Euvireya*, but with very low bootstrap support (<50%) (Figure 41).	
50	*R. beyerinckianum*	NG	Taxon not available for this study.	LC
51	***R. bryophilum***	NG	(i) *R. dielsianum* accessions cluster in Group G, at Node 11. This cluster has low bootstrap support (<50%), suggesting a very weak relationship.	DD
52	*R. bullifolium*	NG	Taxon not available for this study.	DD
53	***R. caliginis***	NG	(i) *R. hooglandii* was not available for this study.	LC
54a	*R. delicatulum* var. *delicatulum*	NG	Taxon not available for this study.	DD
54b	*R. delicatulum* var. *lanceolatoides*	NG	Taxon not available for this study.	DD
55	***R. dianthosmum***	NG	(i) This species is placed within the core vireyas (Group G), and closely related to the taxa of Section *Euvireya*. (ii) No genetic relationship was found between this species and other similar-flowered taxa.	VU D2
56a	***R. dielsianum* var. *dielsianum***	NG	(i) Only the type variety was available for study.	LC
56b	*R. dielsianum* var. *stylotrichum*	NG	Taxon not available for this study.	LC
57	*R. extrorsum*	NG	Taxon not available for this study.	DD
58	*R. gardenia*	NG	(i) The two accessions of *R. gardenia* 'Odyssey' appear to be distinct taxa in the majority of the phylogenetic analyses. In the NJ tree using the Dataset 1, these two taxa clusters with *R. carringtoniae*, *R. commonae*, and *R. alborugosum* (Figure 36), but with very low bootstrap support (<50%). However, these two taxa belong to the core vireyas. One hypothesis is that *R. gardenia* 'Odyssey' may have resulted from introgressive hybridization. And further study with multiple accessions of these two taxa,	LC

#	Taxon	Range	Questions Answered	IUCN Code
			the true *R. gardenia* and their related taxa can establish their true relationships. (ii) The relationship between *R. gardenia* and *R. superbum* were not established in of the phylogenetic analyses. (iii) *R. gardenia* 'Odyssey' clusters with the similar-flowered *R. superbum* (labelled originally as *R. hyacinthosmum*) in the ML tree (Figure 39), but this relationship was not seen in the other phylogenetic analyses.	
59	*R. haematophthalmum*	NG	Taxon not available for this study.	LC
60	*R. hellwigii*	NG	Taxon not available for this study.	LC
61	*R. hooglandii*	NG	Taxon not available for this study.	DD
62	***R. hyacinthosmum***	NG	(i) This species is related to other similar-flowered species such as *R. leucogigas* and *R. orbiculatum* (Figure 41), *R. gardenia* 'Odyssey' (Figure 41).	LC
63	*R. kerowagiense*	NG	Taxon not available for this study.	VU
64a	***R. konori* var. *konori***	NG	(i) This taxon clusters with accessions of *R. laetum* but with very low bootstrap support (<50%). There is no close relationship between this taxon and *R. asperum*. (ii) Only a single variety was available for study.	LC
64b	*R. konori* var. *phaeopeplum*	NG	Taxon not available for this study.	NE
65	*R. leptanthum*	NG	Taxon not available for this study.	LC
66	*R. melantherum*	NG	Taxon not available for this study.	DD
67	*R. neobritannicum*	NB	Taxon not available for this study.	VU C1
68	*R. neriifolium*	NG	Taxon not available for this study.	DD
69	*R. opulentum*	NG	Taxon not available for this study.	LC
70	*R. phaeochitum*	NG	Taxon not available for this study.	LC
71	*R. phaeochristum*	NG	Taxon not available for this study.	LC
72	*R. phaeops*	NG	Taxon not available for this study.	DD
73	*R. prainianum*	NG	Taxon not available for this study.	LC
74	*R. rappardii*	NG	Taxon not available for this study.	LC

#	Taxon	Range	Questions Answered	IUCN Code
75	***R. rarum***	NG	(i) *R. stelligerum* was not available for study.	LC
76	*R. revolutum*	NG	Taxon not available for this study.	DD
77	*R. rhodochroum*	NG	Taxon not available for this study.	DD
78	*R. rubellum*	NG	Taxon not available for this study.	LC
79	***R. solitarium***	NG	(i) The accessions EK614 and GU445773 of *R. solitarium* cluster together with very good bootstrap support (84%) but with only moderate Bayesian posterior support (63%). This suggests that these two accessions are closely related, and perhaps collected from the same locality in the wild. (ii) See (i) above. (iii) Herbarium sample for the type specimen was not available for physical examination.	LC
80	*R. spondylophyllum*	NG	Taxon not available for this study.	LC
81	*R. stelligerum*	NG	Taxon not available for this study.	LC
82	*R. stolleanum*	NG	Taxon not available for this study.	DD
83a	***R. superbum* ssp. *superbum***	NG	(i) There is no genetic relationship between this subspecies and *R. hellwigii*. (ii) This subspecies is not related to *R. inundatum.* (iii) This subspecies is very closely related to the similar-flowered *R. konori* with 50-71% bootstrap support, suggesting a close relationship. (iv) Only a single subspecies was available for study. (v) Herbarium samples of the type specimen were not available to further examination or comparison.	LC
83b	*R. superbum* ssp. *ibele*	NG	Taxon not available for this study.	NE
84	*R. thaumasianthum*	NG	Taxon not available for this study.	DD
85	*R. truncicola*	NG	Taxon not available for this study.	LC
86	*R. tuberculiferum*	NG	Taxon not available for this study.	DD
87	*R. evelyneae*	NG	Taxon not available for this study.	CR D
88	*R. kawir*	NG	Taxon not available for this study.	DD

#	Taxon	Range	Questions Answered	IUCN Code
89	*R. tintinnabellum*	NG	Taxon not available for this study.	VU D1+2
89a	*R.* × *gilliardii*	NG	Taxon not available for this study.	NE
89b	*R.* × *schoddei*	NG	Taxon not available for this study.	NE

6.3.5 Section *Malayovireya* (Sleumer) Argent

The Section *Malayovireya* was not recovered in any of the phylogenetic analyses; instead its constituent taxa were mixed among taxa of the other sections, but restricted only to the Groups E and G.

Table 23 Answers to the taxonomic and conservation queries of Section *Malayovireya*, raised in Appendix A2.5. The taxa in **boldface** denote those analysed in this study.

#	Taxon	Range	Questions Answered	IUCN Code
90	*R. acuminatum*	BN	Taxon not available for this study.	EN A4a
91	***R. apoanum***	PH	(i) This species clusters with other taxa of Section *Malayovireya* (Node 9: *R. malayanum*, *R. fallacinum* and *R. himantodes*), but with low bootstrap support (<50%) (Figure 41). *R. fallacinum*, *R. malayanum*, *R. himantodes* and *R. apoanum* are strongly supported with 100% bootstrap support in Goetsch et al. (2011) and corresponds to their Subsection *Malayovireya*.	LC
92a	*R. durionifolium* ssp. *durionifolium*	BN	Taxon not available for this study.	LC
92b	*R. durionifolium* ssp. *sabahense*	BN	Taxon not available for this study.	LC
93	***R. fallacinum***	BN	(i) *R. durionifolium* was not available for this study.	LC
94	*R. fortunans*	BN	Taxon not available for this study.	NT
95a	***R. himantodes* var. *himantodes***	BN	(i) *R. stenophyllum*, *R. vinicolor*, *R. lineare* and *R. fortunans* were not studied.	LC
95b	*R. himantodes* var. *lavandulifolium*	BN	Taxon not available for this study.	NE
96a	*R. lamrialianum* ssp. *lamrialianum*	BN	Taxon not available for this study.	VU D2
96b	*R. lamrialianum* ssp. *gunsalamianum*	BN	Taxon not available for this study.	EN D

#	Taxon	Range	Questions Answered	IUCN Code
97	*R. lineare*	BN	Taxon not available for this study.	NT
98a	***R. malayanum* var. *malayanum f. malayanum***	MP, BN, SM, JV, SW, ML	(i) *R. malayanum* and *R. apoanum* cluster together, but with poor bootstrap support (<50%) at Node 9 (Figure 41). However, Goetsch et al. (2011) shows these two accessions clustering together with strong bootstrap support (100%) and this cluster corresponds to their Subsection *Malayovireya*. (ii) *R. micromalayanum* was not used for this phylogenetic study. (iii) *R. malayanum* is not related to *R. jasminiflorum* or *R. javanicum* according to all the phylogenetic analyses.	LC
98b	*R. malayanum* var. *malayanum f. latifolium*	BN	Taxon not available for this study.	NE
98c	*R. malayanum* var. *pubens*	ML	Taxon not available for this study.	DD
98d	*R. malayanum* var. *pilosifilum*	BN, ML	Taxon not available for this study.	LC
99	*R. micromalayanum*	BN	Taxon not available for this study.	LC
100	*R. nortoniae*	PH	Taxon not available for this study.	DD
101	*R. obscurum*	MP	Taxon not available for this study.	DD
102	*R. vinicolor*	SM	Taxon not available for this study.	LC
102a	*R.* × *andersonii*	BN	Taxon not available for this study.	NE
102b	*R.* × *hybridogenum*	MP	Taxon not available for this study.	NE
102c	*R.* × *variolosum*	BN	Taxon not available for this study.	NE
102d	*R.* × *wilhelminae*	JV	Taxon not available for this study.	NE

6.3.6 Section *Albovireya* Sleumer

The Section *Albovireya* was not recovered in any of the phylogenetic analyses, instead its constituent taxa were mixed among taxa of the other sections, but restricted to the Groups D, F and G.

Table 24 Answers to the taxonomic and conservation queries of Section *Albovireya*, raised in Appendix A2.60. The taxa in **boldface** denote those analysed in this study.

#	Taxon	Range	Questions Answered	IUCN Code
103	***R. aequabile***	SM	Taxon not available for this study.	LC
104	*R. lampongum*	SM	Taxon not available for this study.	DD
105	*R. cernuum*	SM	Taxon not available for this study.	EN B2ab(ii)
106	***R. album***	JV	(i) *R. album* is not related to *R. javanicum* according to the phylogenetic analyses, and *R. rubriflorum* was not available for this study. (ii) *R. album* clusters with *R. sumatranum* and *R. aequabile* (with other taxa) at (Node 8, Figure 41), with poor bootstrap support (<50%), but no relationship is seen with *R. culminicola*. This trend is seen in Goetsch et al. (2011) but with moderate bootstrap support (72%). Thus *R. album*, *R. sumatranum* and *R. aequabile* are very closely related species. (iii) Section *Albovireya* appears to be polyphyletic in all the phylogenetic analyses, thus *R. album* does not cluster with the other taxa of Section *Albovireya*.	VU B2ab(v)
107	***R. zollingeri***	JV, LS, SW, PH	(i) *R. lagunculicarpum* and *R. zollingeri* does not appear to be related according to the phylogenetic analyses of this study. However, Goetsch et al. (2011) showed that these two species are closely related, forming a cluster with strong bootstrap support (93%).	LC
108	***R. arenicola***	SW	(i) *R. arenicola* clusters together with the core vireyas at Node 13 (Group G, Figure 41).	DD
109	*R. pudorinum*	SW	Taxon not available for this study.	VU
110	***R. lagunculicarpum***	SW	(i) *R. lagunculicarpum* and *R. correoides* appear to be related according to the phylogenetic analyses but with poor bootstrap support (<50%) at Node 13 (Group G, Figure 41), but they are not related *R. zollingeri*. However, Goetsch et al. (2011) showed that	LC

#	Taxon	Range	Questions Answered	IUCN Code
			R. lagunculicarpum, *R. correoides* and *R. zollingeri* are closely related, forming a cluster with strong bootstrap support (93%).	
111	***R. correoides***	NG	(i) *R. lagunculicarpum* and *R. correoides* appear to be related according to the phylogenetic analyses but with poor bootstrap support (<50%) at Node 13 (Group G, Figure 41). However, Goetsch et al. (2011) showed that *R. lagunculicarpum* and *R. correoides* are closely related, forming a cluster with strong bootstrap support (86%)	LC
112	*R. proliferum*	NG	Taxon not available for this study.	DD
113	*R. giulianettii*	NG	Taxon not available for this study.	DD
114a	*R. comptum* var. *comptum*	NG	Taxon not available for this study.	LC
114b	*R. comptum* var. *trichodes*	NG	Taxon not available for this study.	DD
115	***R. yelliotii*** Syn: ***R. saruwagedicum***	NG	(i) *R. inconspicuum* and *R. yelliotii* does not appear to be closely related to each other, but they appear on the large Group G, with poor bootstrap support (<50%) for the whole group (Figure 41). A similar trend is seen in Goetsch et al. (2011), but with very strong bootstrap support (100%) for the whole group. (ii) *R. saruwagedicum* and *R. yelliotii* does not appear to be closely related to each other in the current study. However, they do belong to the large clade Group G (Figure 41). Goetsch et al. (2011) showed that *R. saruwagedicum* and *R. yelliotii* are very closely related, with strong bootstrap support (91%). Thus, these two taxa can be treated as a single taxon. (iii) *R. yelliotii* (and *R. saruwagedicum*) does not have any relationships with the taxa of Section *Pseudovireya*. However, *R. yelliotii* is belongs within the	LC

#	Taxon	Range	Questions Answered	IUCN Code
			clade that contains the majority of the taxa of Section *Euvireya*.	
116	*R. versteegii*	NG	Taxon not available for this study.	LC

6.3.7 Section *Euvireya* (H F Copeland) Argent

Section *Euvireya* is the largest of all the sections of the vireyas, with approximately 270 taxa. The section is further divided into five subsections, with the Subsection *Euvireya* being the largest and carrying the majority of the taxa. Subsection *Saxifragoidea* is a monotypic with only a single species, *R. saxifragoides*.

6.3.7.1 Subsection *Linnaeopsis* (Schlechter) Sleumer

Out of the 16 taxa of this subsection, only two were available for this study. The answers to the taxonomic questions raised for this subsection are summarized in the table below.

Table 25 Answers to taxonomic and conservation issues of Subsection *Linnaeopsis* and its implications on their conservation. The taxa are arranged according to the classification of Argent (2006), and the taxon numbers only the leftmost column correspond to the taxon number used in Argent's (2006) classification. The column 'Range' shows the geographic region the taxa belong (Figure 21).

#	Taxon	Range	Questions Answered	IUCN Code
117	*R. caespitosum*	NG	Taxon not available for this study.	VU D2
118	*R. schizostigma*	NG	Taxon not available for this study.	LC
119	*R. pusillum*	NG	Taxon not available for this study.	DD
120	*R. microphyllum*	NG	Taxon not available for this study.	DD
121	*R. coelorum*	NG	Taxon not available for this study.	DD
122	*R. muscicola*	NG	Taxon not available for this study.	DD
123	*R. xenium*	NG	Taxon not available for this study.	NE
124	*R. parvulum*	NG	Taxon not available for this study.	DD
125	*R. oxycoccoides*	NG	Taxon not available for this study.	DD
126a	*R. disterigmoides* ssp. *disterigmoides*	NG	Taxon not available for this study.	LC
126b	*R. disterigmoides* ssp. *astromontium*	NG	Taxon not available for this study.	NE
127	*R. anagalliflorum*	NG, NB	Taxon not available for this study.	LC

#	Taxon	Range	Questions Answered	IUCN Code
128	***R. rubineiflorum***	NG	(i) *R. anagalliflorum* was not available for the phylogenetic study.	LC
129	*R. capellae*	NG	Taxon not available for this study.	VU D2
130	***R. womersleyi***	NG	No issues.	LC
131	***R. gracilentum***	NG	No known taxonomic issues.	LC

6.3.7.2 Subsection *Saxifragoidea* (Sleumer) Argent

This monotypic subsection with the sole taxon *R. saxifragoides* has no known taxonomic or conservation issue. DNA was not available for the nucleotide sequencing of this species. Also the accession from which the initial DNA extraction was made has since died. Further study is needed to determine the precise placement of this species within Subgenus *Vireya*. The table below shows the entry for this species.

Table 26 Answers to taxonomic and conservation issues of Subsection *Saxifragoidea* and its implications on their conservation. The taxon number only the leftmost column correspond to the taxon number used in Argent's (2006) classification. The column 'Range' shows the geographic region the taxa belong (Figure 21).

#	Taxon	Range	Questions Answered	IUCN Code
132	***R. saxifragoides***	NG	No issues.	LC

6.3.7.3 Subsection *Solenovireya* H F Copeland

There are 46 taxa in the Subsection *Solenovireya*, and the taxonomic questions answered are summarized in the table below.

Table 27 Answers to taxonomic and conservation issues of Subsection *Solenovireya* and its implications on their conservation. The taxa are arranged according to the classification of Argent (2006), and the taxon numbers only the leftmost column correspond to the taxon number used in Argent's (2006) classification. The column 'Range' shows the geographic region the taxa belong (Figure 21).

#	Taxon	Range	Questions Answered	IUCN Code
133a	***R. jasminiflorum* ssp. *jasminiflorum***	MP	(i) *R. jasminiflorum* ssp. *punctatum* was not sequenced, therefore could not be compared with the available typical subspecies. (ii) A relationship between *R. jasminiflorum* and *R. malayanum*	LC

#	Taxon	Range	Questions Answered	IUCN Code
			is not seen in any of the phylogenetic analyses. (iii) The subspecies status of this taxon can be maintained according to the molecular analyses. The accessions of *R. jasminiflorum* studied falls into two distinct and strongly-supported clusters (Node 10, Figure 41). These two clusters correspond to *R. jasminiflorum* ssp. *jasminiflorum* (Node 10a) and *R. jasminiflorum* ssp. *oblongifolium* (Node 10b). Node 10a has very strong bootstrap support (94%). Node 10b has strong bootstrap support (94%) and high Bayesian posterior probability (93%).	
133b	*R. jasminiflorum* ssp. *chaemaepitys*	BN	Taxon not available for study.	NE
133c	*R. jasminiflorum* ssp. *copelandii*	PH	Taxon not available for study.	VU D2
133d	*R. jasminiflorum* ssp. *heusseri*	SM	Taxon not available for study.	LC
133e	***R. jasminiflorum* ssp. *oblongifolium***	MP	(i) See entry for taxon #133a. (ii) A relationship between *R. jasminiflorum* and *R. malayanum* is not seen in any of the phylogenetic analyses. (iii) See entry for taxon # 133a.	LC
134a	***R. edanoi* ssp. *edanoi***	PH	(i) A relationship between *R. edanoi* and *R. jasminiflorum* could not be established from the phylogenetic analyses.	VU D2
134b	*R. edanoi* ssp. *pneumonanthum*	BN	Taxon not available for this study.	LC
135	***R. stapfianum***	BN	(i) A relationship between *R. stapfianum* and *R. jasminiflorum* could not be established from the phylogenetic analyses.	LC
136	***R. alborugosum***	BN	(i) No relationships can be established between *R. alborugosum*, *R. rugosum*, *R. suaveolens* and *R. orbiculatum*, based on the phylogenetic analyses.	EN D
137a	***R. suaveolens* f. *suaveolens***	BN	(i) *R. suaveolens* f. *suaveolens* is not related to *R. orbiculatum*, based on the phylogenetic analyses.	LC

#	Taxon	Range	Questions Answered	IUCN Code
			R. lambianum was not available for this study. (ii) *R. niveoflorum* was not available for this study.	
137b	*R. suaveolens* f. *roseum*	BN	Taxon not available for this study.	NE
138	*R. lambianum*	BN	Taxon not available for this study.	VU D2
139	*R. niveoflorum*	BN	Taxon not available for this study.	LC
140	*R. pseudotrichanthum*	BN	Taxon not available for this study.	DD
141	*R. mogeanum*	BN	Taxon not available for this study.	VU D1+2
142	*R. amabile*	SW	Taxon not available for this study.	DD
143a	***R. radians* var. *radians***	SW	No issues.	LC
143b	*R. radians* var. *minahasae*	SW	Taxon not available for this study.	LC
143c	*R. radians* var. *pubitubum*	SW	Taxon not available for this study.	DD
144	***R. rutenii***	ML	(i) The cluster containing *R. rutenii* and *R. jasminiflorum* has poor bootstrap support (<50%) and low Bayesian posterior probability (51%) (Node 10, Figure 41). This suggests that there is a relationship between these two taxa, but not a strong one. *R. malayanum* does not cluster with these two species.	LC
145	*R. brachypodarium*	NG	Taxon not available for this study.	LC
146	*R. carstensense*	NG	Taxon not available for this study.	DD
147	*R. cinerascens*	NG	Taxon not available for this study.	DD
148	*R. macrosiphon*	NG	Taxon not available for this study.	LC
149	*R. oreadum*	NG	Taxon not available for this study.	DD
150	*R. rhodosalpinx*	NG	Taxon not available for this study.	DD
151	*R. roseiflorum*	NG	Taxon not available for this study.	DD
152	*R. syringoideum*	NG	Taxon not available for this study.	DD
153	***R. majus***	NG	No issues.	LC
154	*R. archboldianum*	NG	(i) The DNA sample of this species did not produce good quality DNA for sequencing purposes.	DD
155	***R. armitii***	NG	No issues.	LC
156	*R. carrii*	NG	Taxon not available for this study.	VU D2
157	***R. carringtoniae***	NG	(i) The phylogenetic analyses showed that the Section	LC

#	Taxon	Range	Questions Answered	IUCN Code
			Solenovireya is polyphyletic, thus the placement of this species cannot be established within *Solenovireya*. This species however is placed within the core vireyas (Group G) (Node 16, Figure 41).	
158	***R. cruttwellii***	NG	(i) *R. hartleyi* and *R. multinervium* were not available for this study.	LC
159	*R. hartleyi*	NG	Taxon not available for this study.	DD
160	***R. goodenoughii***	GI	Taxon not available for this study.	DD
161	*R. multinervium*	NG	Taxon not available for this study.	LC
162	*R. natalicium*	NG	Taxon not available for this study.	DD
163	*R. retrorsipilum*	NG	Taxon not available for this study.	EX
164	*R. oliganthum*	NG	Taxon not available for this study.	DD
165	***R. pleianthum***	NG	Taxon not available for this study.	LC
166	***R. tuba***	NG	(i) *R. culminicola*, *R. rhodoleucum*, *R. carringtoniae* and *R. tuba* does not cluster together, thus there is no close relationship between these taxa.	LC
167	*R. rhodoleucum*	NG	Taxon not available for this study.	LC
168a	***R. loranthiflorum* ssp. *loranthiflorum***	NG, SI	(i) The phylogenetic studies do not show a close relationship between *R. loranthiflorum* & *R. luraluense*. However, Goetsch et al. (2011) showed that these two species are closely related with good bootstrap support (90%). Further studies using multiple accessions of *R. loranthiflorum* and utilizing more nucleotide sequences may perhaps ascertain their true relationship.	LC
168b	*R. loranthiflorum* ssp. *lakekamuensis*	NG	Taxon not available for this study.	DD

6.3.7.4 Subsection *Malesia* H F Copeland

This subsection was not recovered in any of the phylogenetic studies, and the taxa of this subsection were scattered within the Groups D–G. Thus, this subsection is polyphyletic. The taxonomic questions answered are summarized in the table below.

Table 28 Answers to taxonomic and conservation issues of Subsection *Malesia* and its implications on their conservation. The taxa are arranged according to the classification of Argent (2006), and the taxon numbers only the leftmost column correspond to the taxon number used in Argent's (2006) classification. The column 'Range' shows the geographic region the taxa belong (Figure 21).

#	Taxon	Range	Questions Answered	IUCN Code
169	*R. chevalieri*	EA	Taxon not available for this study.	LC
170	***R. pauciflorum***	MP	No issues.	LC
171	***R. pubigermen***	SM	No issues.	LC
172	*R. frey-wysslingii*	SM	(taxon not available for this study)	DD
173	***R. multicolor***	SM	(i) *R. ripleyi* was not available for this study. According to the phylogenetic analyses, *R. multicolor* and *R. salicifolium* does not appear to be related.	LC
174	*R. pyrrhophorum*	SM	Taxon not available for this study.	DD
175	*R. banghamiorum*	SM	Taxon not available for this study.	VU
176a	*R. ripleyi* var. *ripleyi*	SM	Taxon not available for this study.	DD
176b	*R. ripleyi* var. *basitrichum*	SM	Taxon not available for this study.	LC
176c	*R. ripleyi* var. *cryptogonium*	SM	Taxon not available for this study.	LC
177a	***R. citrinum* var. *citrinum***	JV	No issues.	LC
177b	*R. citrinum* var. *discoloratum*	SM	Taxon not available for this study.	LC
178	*R. meijeri*	BN	Taxon not available for this study.	CR
179a	*R. abietifolium*	BN	Taxon not available for this study.	VU D1
179b	*R.* × *sheilae*	BN	Taxon not available for this study.	NE
180	***R. burttii***	BN	No issues.	LC
181	*R. sugaui*	BN	Taxon not available for this study.	DD
182	*R. buxifolium*	BN	Taxon not available for this study.	VU
183	*R. tuhanensis*	BN	Taxon not available for this study.	CR
184	*R. nieuwenhuisii*	BN	Taxon not available for this study.	LC
185	***R. taxifolium***	PH	(i) Not used in the phylogenetic analysis. (ii) Could not establish a relationship with *R. stenophyllum* as *R. taxifolium* was not used in the phylogenetic analysis. (iii) The genetic diversity between the two accessions examined was	CR B1ab(iii)

#	Taxon	Range	Questions Answered	IUCN Code
			98.50 using microsatellite data, suggesting that the two accessions may have originated from two different populations or from a single highly diverse population.	
186	***R. acrophilum***	PH	(i) The phylogenetic analyses do not show a relationship between *R. acrophilum* and *R. wilkiei*.	CR B1a+2ab(iii)
187	***R. wilkiei***	PH	(i) The phylogenetic analyses do not show a relationship between *R. acrophilum* and *R. wilkiei*.	VU
188	***R. rousei***	PH	(i) *R. vidalii* was not available for this study.	DD
189	*R. whiteheadii*	PH	Taxon not available for this study.	DD
190a	*R. vidalii* ssp. *vidalii*	PH	Taxon not available for this study.	NE
190b	*R. vidalii* ssp. *brachystemon*	PH	Taxon not available for this study.	VU
191	*R. scarlatinum*	SW	Taxon not available for this study.	VU
192	*R. leptomorphum*	SW	Taxon not available for this study.	DD
193	***R. alternans***	SW	No issues.	DD
194	***R. bagobonum***	PH, BN, SW, ML	(i) The phylogenetic analyses do not support *R. bagobonum* to be related to any taxa of Section *Discovireya*. *R. borneense* and *R. cuneifolium* were not used in the phylogenetic study. (ii) The two accessions of this species do not cluster together. (iii) One accession of *R. bagobonum* (EK525) cluster together with *R.* × *planecostatum* (Node 11, Figure 41). This node has poor bootstrap support (<50%). The other accession (GU445831) clusters with *R. crassifolium* (Node 13, Figure 41). This node has poor bootstrap support (<50%), but high Bayesian posterior probability (88%). These results suggest that these taxa are related, but their precise relationships can only be determined by utilizing more accessions and larger nucleotide sequences. (iv) *R. javanicum* ssp. *schadenbergii* was not available for this study. However, *R.* × *sarcodes*, *R. bagobonum* and *R. javanicum*	LC

#	Taxon	Range	Questions Answered	IUCN Code
			cluster together, but with poor bootstrap support (<50%) (Node 13, Figure 41). This suggests that *R.* × *sarcodes* could well be the hybrid of *R. bagobonum* and *R. javanicum*. Goetsch et al. (2011) showed that these taxa cluster together with 79% bootstrap support.	
195	***R. pseudobuxifolium***	SW	(i) *R. pseudobuxifolium* and *R. celebicum* cluster together, but with poor bootstrap support (<50%) (Node 13, Figure 41). The Node 13 has high Bayesian posterior probability (88%). Goetsch et al. (2011) showed that these two taxa cluster together with 88% bootstrap support. These results suggest the two taxa are closely related.	VU D2
196	*R. nubicola*	NG	Taxon not available for this study.	LC
197	*R. vinkii*	NG	Taxon not available for this study.	DD
198	*R. flavoviride*	NG	Taxon not available for this study.	LC
199	***R. vitis-idaea***	NG	No issues.	LC
200	***R. stevensianum***	NG	No issues.	LC
201	*R. hatamense*	NG	Taxon not available for this study.	VU D2
202	*R. cornu-bovis*	NG	Taxon not available for this study.	DD
203	***R. commonae***	NG	(i) *R. womersleyi* and *R. macgregoriae* were not used in the phylogenetic studies. However, *R. commonae* clusters together with *R. culminicola*, but with poor bootstrap support (<50%) (Node 19, Figure 41). These results suggest that *R. commonae* and *R. culminicola* are related.	LC
204	*R. rhodostomum*	NG	Taxon not available for this study.	LC
205	*R. takeuchii*	NG	Taxon not available for this study.	VU
206	*R. helodes*	NG	Taxon not available for this study.	DD
207	*R. psammogenes*	NG	Taxon not available for this study.	DD
208a	*R. brassii*	NG	Taxon not available for this study.	LC
208b	*R.* × *nebulicola*	NG	Taxon not available for this study.	NE
209	*R. porphyranthes*	NG	Taxon not available for this study.	DD
210	*R. rubrobracteatum*	NG	Taxon not available for this study.	LC
211	*R. myrsinites*	NG	Taxon not available for this study.	DD

#	Taxon	Range	Questions Answered	IUCN Code
212	*R. purpureiflorum*	NG	Taxon not available for this study.	DD
213	*R. ultimum*	NG	Taxon not available for this study.	VU B1ab(ii,iv); D2
214	*R. atropurpureum*	NG	Taxon not available for this study.	LC
215	*R. subuliferum*	NG	Taxon not available for this study.	LC
216	***R. inconspicuum***	NG	(i) *R. inconspicuum* does not cluster with *R. yelliotii* in any of the phylogenetic analyses. In Goetsch et al. (2011), these two species appear in a strongly-supported (but not well-resolved) clade with 100% bootstrap support. These results imply that these two species are not closely related. (ii) *R. inconspicuum* does not cluster with taxa from the Section *Albovireya*.	LC
217	*R. lamii*	NG	Taxon not available for this study.	DD
218	*R. simulans*	NG	Taxon not available for this study.	LC
219	*R. papuanum*	NG	Taxon not available for this study.	LC
220a	*R. wrightianum* var. *wrightianum*	NG	Taxon not available for this study.	LC
220b	*R. wrightianum* var. *cyclopense*	NG	Taxon not available for this study.	NE
220c	*R. wrightianum* var. *insulare*	NG	Taxon not available for this study.	LC
221	*R. subcrenulatum*	NG	Taxon not available for this study.	LC
222	*R. calosanthes*	NG	Taxon not available for this study.	VU D2
222a	***R. × sarcodes***	PH	(i) See taxon entry # 194.	NE

6.3.7.5 Subsection *Euvireya* H F Copeland

This subsection was not recovered in any of the phylogenetic studies as monophyletic, and the taxa of this subsection were scattered among the Groups D–G, thus the subsection is polyphyletic. The taxonomic questions answered are summarized in the table below.

Table 29 Answers to taxonomic and conservation issues of Subsection *Euvireya* and its implications on their conservation. The taxa are arranged according to the classification of Argent (2006), and the taxon numbers only the leftmost column correspond to the taxon number used in Argent's (2006) classification. The column 'Range' shows the geographic region the taxa belong (Figure 21).

#	Taxon	Range	Questions Raised	IUCN Code
223	*R. triumphans*	EA	Taxon not available for this study.	EN B1ab(ii,iii,v)
224a	***R. longiflorum* var. *longiflorum***	MP, BN, SM	Taxon not available for the phylogenetic study.	LC
224b	*R. longiflorum* var. *longipetalum*	BN	Taxon not available for this study.	CR B2ab(iii)
224c	*R. longiflorum* var. *bancanum*	SM	Taxon not available for this study.	CR B2ab(iii)
224d	*R. longiflorum* var. *subcordatum*	BN	Taxon not available for this study.	LC
225	***R. robinsonii***	MP	(i) *R. robinsonii* clusters with *R. javanicum* ssp. *brookeanum* (see Node 13 of Figure 41). The sub-cluster with these two taxa have low bootstrap support (<50%), suggesting a weak relationship. (ii) *R. robinsonii* clusters with *R. javanicum* with moderate bootstrap support (72%) and very high Bayes posterior probability (99%). Thus, these two species very closely related.	LC
226	***R. rarilepidotum***	SM	(i) This species is not related to *R. sumatranum*, as there are no close phylogenetic relationships.	LC
226i	*R.* × *ootrichum*	SM	(i) Taxon not available for this study. Its parents, *R. sumatranum* and *R. rarilepidotum* are not genetically related.	NE
227a	***R. javanicum* ssp. *javanicum***	SM	(i) The accessions of *R. javanicum* do not cluster together, however they are restricted to the core vireyas (Group G, Figure 41). (ii) The subspecific status of this taxon cannot be established. Further studies with additional subspecies may perhaps reveal the genetic differentiation among these taxa.	LC
227b	***R. javanicum* ssp. *brookeanum***	BN	(i) This subspecies belong to the core vireyas and clusters with other	LC

#	Taxon	Range	Questions Raised	IUCN Code
			taxa of Subsection *Euvireya* (Node 13, Figure 41). (ii) The subspecific status of this taxon cannot be established. Further studies with additional subspecies may perhaps reveal the genetic differentiation among these taxa.	
227c	***R. javanicum* ssp. *gracile***	BN	No issues.	LC
227d	*R. javanicum* ssp. *cladotrichum*	BN	Taxon not available for this study.	NE
227e	*R. javanicum* ssp. *cockburnii*	BN	Taxon not available for this study.	VU D1
227f	*R. javanicum* ssp. *schadenbergii*	PH, SW	Taxon not available for this study.	LC
227g	*R. javanicum* ssp. *palawanense*	PH	Taxon not available for this study.	NE
227h	***R. javanicum* ssp. *kinabaluense***	BN	No issues.	NE
227i	***R. javanicum* ssp. *moultonii***	BN	No issues.	LC
227j	***R. javanicum* ssp. *teysmannii***	MP, SM, JV	(i) The subspecific status of this taxon cannot be established. Further studies with additional subspecies may perhaps reveal the genetic differentiation among these taxa. (ii) *R. javanicum* ssp. *teysmannii* does not appear to be closely related to *R. javanicum* ssp. *brookeanum*; however they both belong to the core vireyas (Group G, Figure 41). (iii) *R. javanicum* ssp. *teysmannii* does not appear to be related to *R. album*. *R. beccarii* and *R. basirotundatum* were not available for this study. (iv) The phylogenetic studies do not show a close relationship between *R. javanicum* var. *teysmannii* and *R. malayanum*, and *R.* × *wilhelminae* were not available for this study.	NE
228	***R. sumatranum***	SM	(i) *R. ripleyi* was not available for this study. However, the clade containing *R. adinophyllum* (Node 6b, Figure 41) is sister to the clade containing *R. sumatranum* (Node 8a, Figure 41), suggesting a	LC

#	Taxon	Range	Questions Raised	IUCN Code
			relationship. *R. sumatranum* does not appear to be closely related to *R. rarilepidotum*.	
229	*R. perplexum*	SM	Taxon not available for this study.	DD
230	*R. sessilifolium*	SM	Taxon not available for the phylogenetic study.	LC
231	*R. beccarii*	SM	Taxon not available for this study.	DD
232	*R. loerzingii*	JV	Taxon not available for this study.	VU D2
233	***R. renschianum***	LS	No known taxonomic issues.	VU D2
234a	***R. stenophyllum* ssp. *stenophyllum***	BN	Taxon not available for the phylogenetic study.	LC
234b	***R. stenophyllum* ssp. *angustifolium***	BN	Taxon not available for the phylogenetic study.	NE
235	***R. verticillatum***	BN	(i) *R. verticillatum* does not appear to be related to *R. polyanthemum*.	LC
236a	***R. crassifolium* var. *crassifolium***	BN	(i) The varietal status of this taxon cannot be established as only a single accession was available for the phylogenetic analysis. (ii) Need further study to establish the relationship of this taxon to *R. stenophyllum*.	LC
236b	*R. crassifolium* var. *pseudomurudense*	BN	Taxon not available for this study.	DD
237	*R. jiewhoei*	BN	Taxon not available for this study.	NE
238	*R. kemulense*	BN	Taxon not available for this study.	DD
239	*R. monkoboense*	BN	Taxon not available for this study.	CR B1ab(i)
240	*R. apiense*	BN	Taxon not available for this study.	NE
241a	***R. rugosum* var. *rugosum***	BN	Taxon not available for the phylogenetic study.	LC
241b	*R. rugosum* var. *kinabaluense*	BN	Taxon not available for this study.	NE
241x	*R.* × *coriifolium*	BN	Taxon not available for this study.	NE
242	*R. nervulosum*	BN	Taxon not available for this study.	VU D1
243	***R. salicifolium***	BN	(i) *R. salicifolium* is not closely related the *R. javanicum* complex or *R. multicolor*.	LC
244	***R. yongii***	BN	(i) *R. yongii* is not closely related to *R. praetervisum* (*R.* × *keditii* was not available for this study).	LC
245	*R. baconii*	BN	Taxon not available for this study.	EN D

#	Taxon	Range	Questions Raised	IUCN Code
246	***R. praetervisum***	BN	(i) *R. longiflorum* was not available for this study.	LC
247	***R. orbiculatum***	BN, SW	(i) *R. orbiculatum* does not appear to be closely related to *R. edanoi* or *R. suaveolens*. (ii) The placement of this species within Subsection *Euvireya* is not supported by molecular data. This species however, is found in a clade (Node 10, Figure 41) sister to the core vireyas (Group G, Figure 41). (iii) *R. orbiculatum* clusters with taxa of Subsection *Solenovireya* (Node 10, Figure 41), with moderate bootstrap support (62%) and high Bayes posterior probability (98%).	LC
248	*R. lanceolatum*	BN	Taxon not available for this study.	LC
249	*R. exuberans*	BN	No known issues.	LC
250	*R. commutatum*	BN	Taxon not available for this study.	LC
251	*R. intranervatum*	BN	Taxon not available for this study.	VU D1
252	***R. maxwellii***	BN	(i) *R. maxwellii* does not appear to be related to *R. rugosum* or *R. lowii*.	DD
253	***R. retivenium***	BN	(i) *R. retivenium* was not available for the phylogenetic study.	LC
254	***R. polyanthemum***	BN	Taxon not available for the phylogenetic study.	LC
255	***R. lowii***	BN	No known issues.	LC
256	*R. mendumiae*	PH	Taxon not available for this study.	CR B2ab(i)
257	***R. kochii***	PH	(i) *R. kochii* does not appear to be related to *R. williamsii*.	LC
258	***R. williamsii***	PH	(i) *R. williamsii* does not appear to be related to *R. kochii*.	LC
259	*R. mindanaense*	PH	No known issues.	LC
260	*R. reynosoi*	PH	Taxon not available for this study.	CR B2ab(i)
261	*R. brachygynum*	PH	Taxon not available for this study.	DD
262a	*R. leytense* var. *leytense*	PH	Taxon not available for this study.	LC
262b	*R. leytense* var. *loheri*	PH	Taxon not available for this study.	DD
263	*R. loboense*	PH	Taxon not available for this study.	LC
264	*R. xanthopetalum*	PH	Taxon not available for this study.	DD

#	Taxon	Range	Questions Raised	IUCN Code
265	***R. madulidii***	PH	(i) *R. madulidii* does not appear to be related to *R. mendumiae* or *R. acrophilum*.	EN B2ab(i)
266	*R. impressopunctatum*	ML	Taxon not available for this study.	DD
267	*R. seranicum*	ML, SW	Taxon not available for this study.	LC
268	***R. celebicum***	SW	(i) The two accessions of this this species does not cluster together, and further studies involving additional accessions (perhaps with varying morphology) may reveal the intraspecific relations of this species.	LC
269	***R. rhodopus***	SW	(i) *R. rhodopus* is very closely related to *R. vanvuurenii* with high Bayes posterior probability (97%). *R. rhodopus* is however not related to *R. quadrasianum*. *R. seranicum* was not available for this study.	DD
270	*R. bloembergenii*	SW	Taxon not available for this study.	DD
271	*R. poromense*	SW	Taxon not available for this study.	DD
272	*R. leptobrachion*	SW	No known issues.	LC
273	***R. vanvuurenii***	SW	(i) *R. rhodopus* is very closely related to *R. vanvuurenii* with high Bayes posterior probability (97%).	LC
274	*R. stresemannii*	ML	Taxon not available for this study.	DD
275	***R. impositum***	SW	No known issues.	LC
276	*R. buruense*	ML	Taxon not available for this study.	DD
277	*R. toxopei*	ML	Taxon not available for this study.	DD
278	*R. lompohense*	SW	Taxon not available for this study.	DD
279	*R. subulosum*	NG	Taxon not available for this study.	DD
280	*R. glabriflorum*	NG	Taxon not available for this study.	LC
281	*R. pachycarpon*	NG	Taxon not available for this study.	LC
282	*R. pachystigma*	NG	Taxon not available for this study.	LC
283	*R. angulatum*	NG	Taxon not available for this study.	DD
284	*R. alticola*	NG	Taxon not available for this study.	LC
285	*R. sayeri*	NG	Taxon not available for this study.	DD
286a	***R. aurigeranum* ssp. *aurigeranum***	NG	(i) The subspecific status of this taxon cannot be established as only a single accession was available for this study.	LC

#	Taxon	Range	Questions Raised	IUCN Code
286b	*R. aurigeranum* ssp. *hirsutum*	NG	Taxon not available for this study.	NE
287	***R. laetum***	NG	(i) *R. laetum* does not appear to be related to *R. konori* or *R. zoelleri*.	LC
288a	***R. christi***	NG	(i) *R. christi* does not appear to be related to *R. villosulum* or *R. curviflorum*.	NE
288b	***R. christi* (Mt Miap form)**	NG	Taxon not available for the phylogenetic study.	NE
289	***R. villosulum***	NG	(i) *R. villosulum* does not appear to be related to *R. christi*.	LC
290	***R. curviflorum***	NG	(i) *R. curviflorum* does not appear to be related to *R. christi*.	LC
291	*R. milleri*	NG	Taxon not available for this study.	VU D1
292	*R. macgregoriae*	NG	Taxon not available for the phylogenetic study.	LC
293	***R. christianae***	NG	No known issues.	LC
294	*R. rosendahlii*	NG	No known issues.	LC
295a	***R. culminicola* var. *culminicola***	NG	(i) The varietal status of this taxon cannot be established, and further studies involving more accessions may reveal their relationships within this species. (ii) *R. nubicola* was not available for this study. (iii) No phylogenetic support for the relationship between *R. culminicola* var. *culminicola* and *R. herzogii* was found. The sequencing for *R. archboldianum* was not successful to examine the relationship of this species with *R. culminicola* var. *culminicola*.	LC
295b	*R. culminicola* var. *angiense*	NG	(i) The varietal status of this taxon cannot be established, and further studies involving more accessions may reveal their relationships within this species. (ii) *R. culminicola* var. *angiense* was not available for this study.	NE
296	***R. arfakianum***	NG	(i) *R. culminicola* var. *angiense* was not available for this study.	DD
297	***R. blackii***	NG	No known issues.	LC
298	*R. hirtolepidotum*	NG	No known taxonomic issues.	VU D2

#	Taxon	Range	Questions Raised	IUCN Code
299	*R. comparabile*	NG	Taxon not available for this study.	DD
300a	***R. luraluense* ssp. *luraluense***	NG	(i) The subspecific status of this taxon cannot be verified without analysing both subspecies.	VU D2
300b	*R. luraluense* ssp. *whitmorei*	SI	Taxon not available for this study.	NE
301	*R. wentianum*	NG	Taxon not available for this study.	LC
302	*R. glabrifilum*	NG	Taxon not available for this study.	NE
303	*R. schlechteri*	NG	Taxon not available for this study.	LC
304	***R. leucogigas***	NG	(i) *R. leucogigas* was shown to belong to the core vireyas (Group G, Figure 41). (ii) *R. leucogigas* belong to the core vireyas, but does not cluster with other taxa of Section *Phaeovireya* (Group G, Figure 41).	DD
305	*R. brevipes*	NG	Taxon not available for this study.	DD
306	*R. englerianum*	NG	Taxon not available for this study.	NT
307	*R. mollianum*	NG	Taxon not available for this study.	DD
308	*R. cuspidellum*	NG	Taxon not available for this study.	LC
309	*R. baenitzianum*	NG	Taxon not available for this study.	DD
310	*R. scabridibracteum*	NG	No known issues.	LC
311	***R. zoelleri***	NG, ML	(i) The two accessions of this species did not cluster together, and additional accessions may be needed to determine the interspecific relationships. (ii) *R. zoelleri* does not show a close relationship with *R. laetum*. *R. macgregoriae* was not available for the phylogenetic study.	LC
312	***R. lochiae***	AU	(i) Genetic relationship between *R. lochiae* and *R. viriosum* was not established. (ii) The status of the taxon *R. notiale* could not be established due to lack of accessions matching the physical characteristics of the *R. notiale.*	VU D2
313	***R. viriosum***	AU	(i) Genetic relationship between *R. lochiae* and *R. viriosum* could not be established an further studies needed.	LC

Chapter 7
Conservation Plan for Vireyas

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7 Conservation Plan for Vireyas

This chapter describes an *ex situ* conservation plan for threatened vireya taxa cultivated in New Zealand collections and its potential contribution to international conservation of vireyas. The plan proposes strategic conservation of vireya plant genetic resources examining the values and limits of *ex situ* methods and providing definite recommendations to improve and integrate *ex situ* programs into mainstream plant conservation. The plan includes priority setting with regard to the urgency of measures to be taken to save the most severely threatened species.

The main areas examined in the plan include objectives, criteria and the strategic actions required for *ex situ* conservation. The conservation strategies proposed are based on the results of the molecular analyses of this study, which enabled the systematic selection and prioritization of suitable taxa and their representative accessions for conservation. The basic underlying principles of biodiversity conservation have been discussed in Chapter 3 and will not be discussed in detail here. The primary aim of this conservation plan is to provide a framework for the global *ex situ* conservation of vireyas and the sustainable use of the conserved material for *in situ* re-introduction programmes and international *ex situ* programmes.

7.1 Principles of Biodiversity Conservation

Conservation of biodiversity is based on the premise that we need to preserve the extant biodiversity to maintain our life support systems (Lowe et al. 2000). The natural diversity of plant life in particular, forms the basis of nearly all other life on earth and, hence, conservation of plants is seen as high priority everywhere (Hyvärinen et al. 2011). Species are currently being lost 100–1,000 times faster than the natural rate, mostly related human activities, primarily due to changes in land use and resulting in loss of habitats (Wilson 1992). Changes in climate also contribute to this ever-growing destructive trend, and new approaches to adapt to these rapid changes are needed in order to reduce threats (Hulme 2005).

The Convention on Biological Diversity (CBD) is a global agreement addressing all aspects of biological diversity (genetic resources, species, and ecosystems) and aim to stop or reduce the loss of biodiversity by employing a variety of means (CBD 2002). The basic philosophy of the CBD rests not on the need to protect particular species or habitats that might be endangered or threatened, but on the need to protect biological diversity, in all its forms, in its own right (CBD 1992, 2002).

The purpose of a conservation plan is to provide a framework for the effective use and management of genetic resources (germplasm) to prevent their degradation and ensure their sustained availability for future generations. The conservation plan thus becomes an integral part of the conservation process, and follows genetic diversity and phylogenetic analyses, that determine conservation priorities (Given 1994; Guerrant et al. 2004; Koskela & Amaral 2002; Margules & Pressey 2000). To understand the underlying principles of biodiversity conservation and develop a conservation plan, the following need to be addressed: (i) global strategies in biodiversity conservation, (ii) principles of *ex situ* conservation, (iii) taxonomic complexity and conservation, (iv) conservation methods, and (v) strategic planning.

7.1.1 Global Strategies in Biodiversity Conservation

One of the most prolific global strategies for the conservation of biodiversity is the Global Strategy for Plant Conservation (GSPC). GSPC is a cross-cutting programme of the CBD and includes 16 global targets set for 2020 (as outlined in Chapter 3). The aim of the GSPC is to halt the continuing loss of plant diversity and to secure a positive, sustainable future where human activities support the diversity of plant life, and where in turn the diversity of plants support and improve our livelihoods and well-being (CBD 1992, 2002). Target 2 aims to have an assessment of the conservation status of all known plant species, as far as possible, to guide conservation action. For the vireyas (and *Rhododendron* as a whole) Target 2 has been achieved by the publication of the Red List of Rhododendron (Gibbs et al. 2011). The Target 8 of the GSPC aims to have at least 75% of threatened plant species in *ex situ* collections, preferably in the country of origin, and at least 20% available for recovery and restoration programmes (CBD 2002). At present, 65% of all threatened rhododendrons are held at botanic gardens around the world (BGCI 2012), thus 10% away from the required 75% of Target 8.

The two basic methods of conservation of biodiversity and more specifically genetic diversity used in global strategies are *in situ* and *ex situ*. These two methods are complementary to each other and must be carried out together for the effective conservation of genetic variation (Guerrant et al. 2004). Genetic diversity has now become the primary unit of biodiversity and forms the foundation of plant diversity at all other levels. Genetic variability also governs the ability to persist on an evolutionary time-scale (Frankel 1970; Moritz 2002) and a lack of genetic variation can leave species susceptible to extinction from future changes to their ecosystems (Ellstrand & Elam 1993; Huenneke 1991). Genetic considerations have therefore become fundamental to conservation research and this has been internationally recognised through the Convention on Biological Diversity (CBD 2002).

In situ conservation strategies ensure that the future generations of the natural populations of species can evolve and adapt to the changing natural environment and the ecosystem. *In situ* methods also conserve biodiversity at all its levels, genetic species and as intact ecosystems, by setting aside adequate representations of wilderness as 'protected areas' (CBD 1992). However, *in situ* conservation is not always practical or feasible, especially in the preliminary stages of major global biodiversity conservation programmes, and an *ex situ* approach may be more desirable.

7.1.2 Principles of *Ex Situ* Conservation

The science of *ex situ* conservation preserves not only wild species of ornamental value, but also the huge number of varieties and cultivars of domesticated species that humans have developed over millennia, since the beginnings of agriculture (Hammer & Teklu 2008). Unfortunately, many of the most useful plants to humanity are the ones that are most threatened with extinction because of overuse (Guerrant et al. 2004). This is particularly true of medicinal plants, where more than 80% of the developing world still relies on traditional medicines, mainly from plants, for their primary healthcare (Farnsworth & Soejarto 1991). The *ex situ* cultivation of some of these plants can reduce the pressure on wild populations and improve the quality of life for many communities (Guerrant et al. 2004). *Ex situ* conservation cannot afford to be only a process of collection and storage; the release of material for repatriation and reintroduction provides the

ultimate service to the clients of *ex situ* conservation, be they protected area managers, private landowners, or rural communities (Maunder 1992; Sperling 2001).

The main purpose of *ex situ* conservation is to secure and maintain representative samples of the existing genetic diversity of a taxon, by keeping components of biodiversity alive outside of their original habitat or natural environment. The conservation strategy should therefore include the removal of germplasm resources (seed, pollen, cuttings or individuals in case of plants), from their original habitat or natural environment (Towill et al. 1989). Unlike *in situ* conservation, *ex situ* conservation of plants consequentially requires significant human intervention, in the form of germplasm collection, storage and maintenance of the cultivated plants. The other disadvantages of collecting germplasm samples for *ex situ* conservation are: (i) limited coverage of genetic variation, (ii) bias during collection of plant material, and (iii) samples that are too large to deal with. *Ex situ* conservation therefore should target on sampling and maintaining as much genetic variation as possible that is present within and among populations of selected taxa utilising the least number of accessions (Brown & Hardner 2000).

The growing awareness and research in conservation has led to properly managed *ex situ* collections which can make the critical difference between extinction and survival of species (Guerrant et al. 2004). *Ex situ* facilities such as botanic gardens and seed banks, have now become artificial centres of diversity unrivalled by those in the wild in terms of species richness. However, these *ex situ* facilities still remain as largely underused plant conservation resources around the world (Wyse Jackson 2001). *Ex situ* conservation is vitally important and has prevented the extinction of many species of plants and animals in the past (IUCN 2005). Botanic gardens are the predominant *ex situ* conservation facilities and have enabled the survival of many species that have become extinct in the wild. For example, *Franklinia alatamaha* Marshall (Theaceae) was last seen in the wild in 1803, though extinct in the wild (EW), due to *ex situ* conservation, this species have been saved. A more recent example is the Chilean Blue Cross (*Tecophilaea cyanocrocus* Leyb.), which is also extinct in the wild (EW), but fortunately quite common in *ex situ* collections (Maunder et al. 2001a; Maunder et al. 2001b). The temperate rhododendron species *R. kanehirae* E. H. Wilson from Taiwan is categorized as EW (Extinct in the wild) is another example of successful re-introduction. This species was fortunately being in

cultivation at RBGE and re-introduction into the wild was therefore possible (Argent 2006; Gibbs et al. 2011).

7.1.3 Taxonomic Complexity and Conservation

Not all conservation programmes deal with species with definite taxonomic boundaries, and traditional species-based approaches with readily identifiable species may not be appropriate. In certain taxonomically complex groups such as vireyas, it is not possible to classify biodiversity into discrete and unambiguous species. Attempts to impose species-based conservation on such taxonomically complex groups are proving untenable, and may redirect scarce resources and taxonomic expertise from the conservation of other priority groups (Ennos et al. 2005). Ennos et al. (2005) proposed a new approach for taxonomically complex groups in which the evolutionary processes that generate taxonomic biodiversity is conserved, rather than 'the preservation of a limited number of poorly defined taxa arising from this evolution' (Ennos et al. 2005). Molecular fingerprinting methods can therefore play a very useful role in such cases where species limits are either subtle or ambiguous (Weising et al. 2005). Another consequence of taxonomic complexities is the difficulty in making an assessment of the distribution of a species. If the species in question cannot be delineated as a specific entity, assessment of threats that are related to its distribution may be problematic and also the development of appropriate conservation strategies (Hollingsworth 2003).

The genus *Rhododendron* is taxonomically complex and so are the vireyas, with numerous taxa with unclear taxonomic boundaries leading to several of these taxa misidentified in collections (Argent 2006). An example of this is the case with cryptic species[51] *R. bryophilum* (DD), of which all the accessions collected under this name for this study turned out to be *R. dielsianum* (LC) after physical examination. The two species have very subtle morphological differences that are not very conspicuous and need to be properly identified to set appropriate conservation priorities. The taxonomic challenge posed by cryptic species has been recognized for nearly 300 years, but the recent advances in 'relatively inexpensive and rapid DNA sequencing has given biologists a new tool for detecting and differentiating morphologically similar species' (Bickford et al. 2007).

[51] Two or more distinct species classified as a single species.

7.1.4 Conservation Methods

The huge number of tools available to conservation biologists at present has become a means to an end, leading ultimately to survival in the wild, and also as an integral component of larger integrated conservation efforts (Guerrant et al. 2004). Foremost among these tools are molecular techniques that enable the assessment and quantification of genetic variation within populations, which is essential when dealing with small populations, whether *in situ* or *ex situ* (Guerrant et al. 2004). Molecular markers are also increasingly used for screening of germplasm to study genetic diversity, identify redundancies in the collections, test accession stability and integrity, and resolve taxonomic relationships (Rao 2004).

7.1.5 Strategic Planning

Strategic plans can be developed for a single species or for a group of large number of species (Fleishman et al. 2000; IUCN 2008) such as the vireyas. The factors that determine the strategy used will depend on the size of the study group (IUCN 2008). According to IUCN (2008) the factors leading to a multi-species conservation strategy include:

- Limited data are available on the distribution of and threats to each species.
- Multiple species share largely overlapping ranges and habitats.
- A guild of species with similar ecological roles is of concern.
- There are common threats to a group of species.
- There are limited resources for or interest in multiple plans for individual species in a group.

However, in any strategic plan, a structured approach should be used to set out a series of conservation research and actions. They include:

- Examination of the current conservation status.
- Set objectives for the conservation.
- Identification of taxa for conservation assessment.

- Undertake assessment.
- Identify species for conservation action.
- Identify conservation actions appropriate for the selected taxa.
- Employ appropriate conservation management programmes for the selected taxa.

7.2 Rationale for Conservation of Vireyas

The global assessment of the conservation status of rhododendrons by the Global Trees Campaign and the subsequent publication of the Red List of Rhododendrons formed the basis for the development of a strategic conservation programme for rhododendrons. The Red List included 344 taxa of vireyas of out of the 390 described to date (Gibbs et al. 2011; MacKay et al. 2010; Oldfield 2009). Other pivotal issues that warrant the conservation of vireyas include:

1. The Red List identified 63 vireya taxa as threatened (VU, EN and CR) and a further three taxa as NT (Near Threatened).

2. *R. retrorsipilum* Sleumer, a vireya species from Papua New Guinea (Morobe District, mountain range above Markham Point near Lae) has been cited as Extinct (EX). The original forest cover at that locality was completely lost to agriculture and firewood collection and recovery thus eliminating any likelihood recovery of this species in the future. This species is not known to be in cultivation anywhere in the world, thus re-introduction is not possible. The temperate species *R. kanehirae* E. H. Wilson from Taiwan is categorized as EW (Extinct in the wild). Fortunately, this species being in cultivation at RBGE, re-introduction into the wild is possible (Argent 2006; Gibbs et al. 2011).

3. Human-caused habitat loss and degradation and invasive species are accelerating the loss of species (Tilman & Lehman 2001). In addition, many habitats are vulnerable to alteration through human-caused climate change, and these changes are occurring at a pace that is beyond the dispersal ability of many plant species (Crumpacker et al. 2001). The Malesian region, which is home to the majority of the vireyas, is under threat from habitat loss. For example, in Papua New Guinea

which is a hotspot for vireyas with a large number of endemic taxa, have lost (Butler 2008; Wilcove & Koh 2010).

4. A large number of taxa are categorized as Data Deficient (101) or Not Evaluated (46), and with further research many of these are highly likely to fall into the threatened categories thus requiring conservation (Gibbs et al. 2011).

5. Vireyas are found in numerous *ex situ* collections worldwide, and they have the potential for contribution to *in situ* conservation programmes or re-introduction into the wild, if the original provenance is debilitated.

6. Vireyas are becoming increasingly a choice for breeders of ornamental plants, and several commercial producers have been established worldwide (Fairweather 2003).

Rhododendrons in general are now a permanent feature of well-known gardens around the world. Production of rhododendrons for the horticultural trade has increased greatly. For example in Germany, the increase from three million in 1960, to 20 million in 2000 makes rhododendron the most important ornamental woody plant genus for gardens and parks besides the rose genus. Notably the vireya species *R. vaccinioides* is held in this collection along with several other endangered rhododendrons (Spethmann et al. 2010).

In New Zealand, every year a festival centred on the blooming of rhododendrons was held in Taranaki as the Taranaki Rhododendron and Garden Festival. Nearly 50 gardens around Taranaki featuring rhododendrons open their doors for visitors from around the country and overseas during the festival. Rhododendrons are more commonly used as ornamental plants and are commercially produced around the world. Rhododendrons are also being studied for their use in medicine, as they have been shown effective as antibiotics, anti-inflammatories and for the treatment of diarrhoea. Some species of rhododendron have also been used for firewood, timber and honey (usually toxic), development of insecticides and as a potential narcotic, among other domestic uses (Chettri & Sharma 2007; Gibbs et al. 2011; Kerkvliet 1981; Singh et al. 2003; Wang et al. 2010).

Rhododendrons usually grow in areas of high humidity and on acidic soils, which are unsuitable for other plants, and they play an important role in ecosystems by stabilizing slopes and protecting watersheds. Many vireyas in particular have adapted to epiphytic lifestyles and serve as an indicator species for the health of the ecosystem (Gibbs et al. 2011; Heads 2003; Stevens 1976).

The recent conference 'Rhododendrons: Conservation and Sustainable Use' held in 2010 in Sikkim, India (a hotspot for temperate rhododendrons with several endangered taxa) highlights the growing interest in the conservation of rhododendrons in general (Sastry 2010). The paper by Millar (2010) presented at the same conference introduced the importance of the rhododendrons in New Zealand collections.

The above highlights the importance of rhododendron taxa as an important component of their ecosystem and their domestic and commercial value to humans. Being Red-Listed also emphasizes their vulnerability and plight for survival in the wild with dwindling numbers and continuing habitat loss, thus requiring urgent conservation action.

7.3 Objectives for Conservation

Vireyas not being native to New Zealand, the only available mode of conservation for them in New Zealand is through *ex situ* conservation. The *ex situ* collections thus need to have strict guidelines in the management and cultivation and well-defined conservation objectives. To carry out a conservation plan a set of objectives need to be set. According to Engels & Visser (Engels & Visser 2003) and Maunder & Byers (Maunder & Byers 2005) the main objectives of *ex situ* conservation are:

1. To maintain in cultivation at least one accession representing each wild population of the threatened plant taxon.
2. To manage representatives of wild populations in *ex situ* collections as insurance against extinction in the wild.
3. To manage *ex situ* collections to provide material for future species recovery programmes.
4. To facilitate and promote taxonomic and ecological research using *ex situ* collections.

5. To maintain germplasm from wild populations in a long-term storage facility.
6. To increase awareness and educate the public of threatened plants and the importance of *ex situ* collections. Provide outreach opportunities that are appropriate and compatible with the purpose and goals of the particular collection.
7. To encourage the cultivation of threatened plants by propagators and gardeners.

Strict adherence to these objectives would enable sustainable management of the vireyas in collections and maintain the contributions to scientific research and conservation exercises (Engels & Visser 2003; Maunder & Byers 2005). For any threatened taxon, a Conservation Plan must therefore address the following objectives:

- To serve as a crucial backup measure, should existing *in situ* conservation programmes be in peril or are unavailable.
- To ensure that a wide range of the phenotypic and genotypic diversity of a taxon is conserved.
- To manage the regeneration of the taxa outside its original natural provenance (Brown & Hardner 2000; FAO 2010a; Koskela & Amaral 2002; Muller-Starck & Schubert 2001; Stern & Roche 1974).

The establishment of a well-developed conservation plan and adherence to it form the basis of any conservation exercise. The objectives of the conservation plan should initially be laid out to determine the scope of the programme, and stringent criteria must then be applied to ascertain accurate selection of conservation taxa, type of accession, quantity and the mode of conservation (Given 1994; Havens et al. 2006). It must be emphasized, however, that it is difficult to develop a general set of guidelines that can be applied to all conservation programmes. In order to develop an efficient *ex situ* conservation strategy, a number of key questions regarding the conservation objective, availability of essential resources, the origin of the material to be conserved, the present use and the conservation status of the taxa have to be addressed (Engels & Visser 2003).

7.4 Taxa Selection for Conservation Assessment

A major step in the implementation of a conservation plan is the selection of taxa for conservation assessment. The taxa that are selected for *ex situ* conservation must meet very strict criteria, so as to reduce the financial burden on the stakeholders and to elevate the efficiency of these collections (Maunder & Byers 2005). The following are the minimum criteria that need to be met in the selection of vireyas for ex situ conservation:

1. The taxa must be ideally Red-Listed by IUCN or a by similar body, or closely related to Red-Listed taxa, and preferably supported by molecular studies.

2. The taxa held in *ex situ* collections must be of wild origin. Taxa can be recognized as *ex situ* collections if their provenance is well-documented or can be accurately verified.

3. The identity of the taxa must be verified by physical examination and/or by molecular studies and any taxonomic issues within the selected taxon and between other taxa must be kept to a minimum.

4. The genetic identity of the collection population must be maintained during propagation by seeds. Cross-breeding with other plants of the same taxon or close relatives in the garden must be reduced if not excluded.

5. The genetic and/or phenotypic uniqueness of the local collections must be comparative or significant to those in similar international collections.

6. Adequate facilities must be available for the housing of the living specimens and the storage of germplasm.

7. Feasibility of safeguarding the genetic diversity and integrity of the cultivated stock must be addressed.

8. The conservation exercises must have minimal or negligible impact on the local (host) environment, flora and fauna (Engels & Visser 2003; Maunder & Byers 2005).

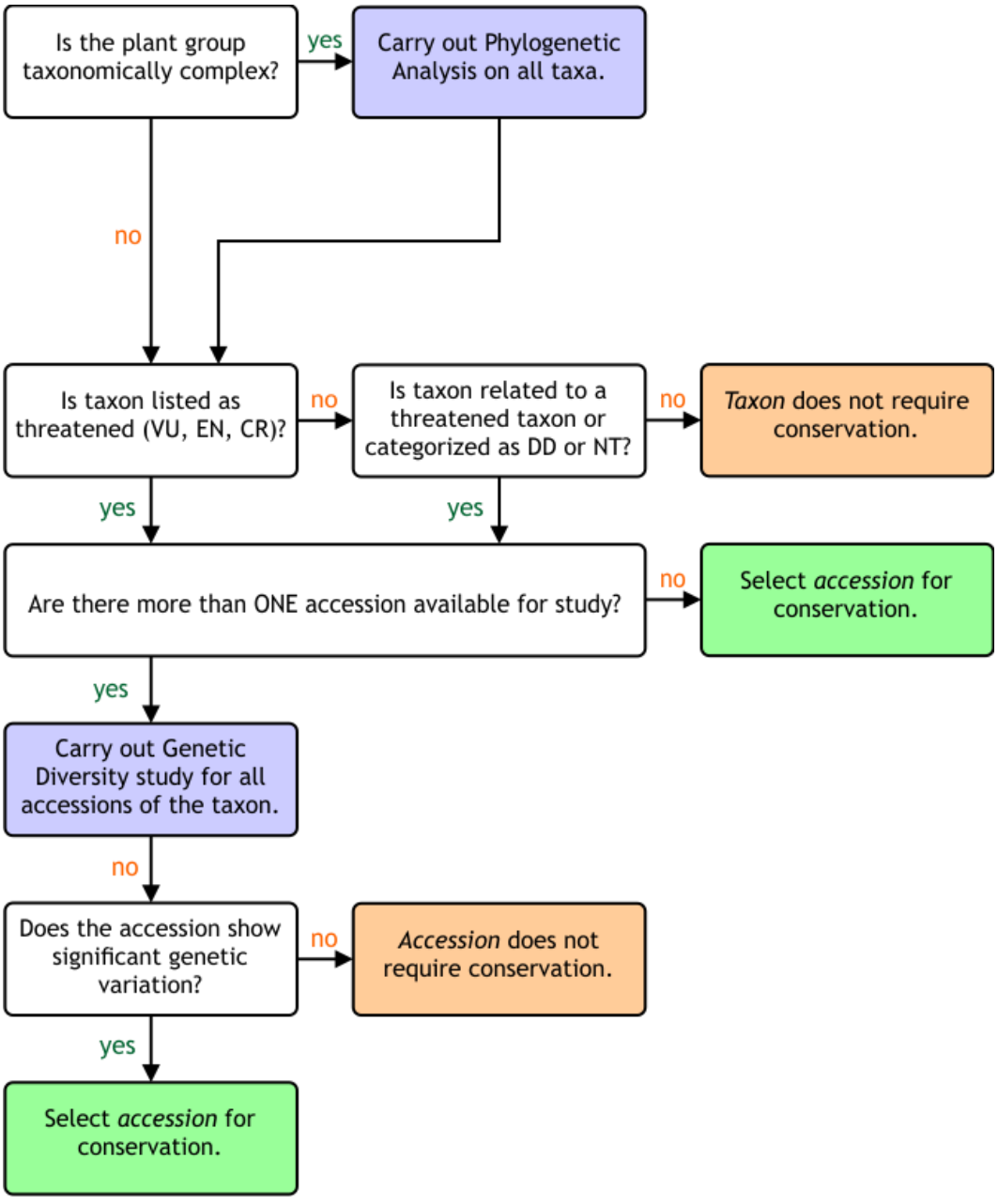

Figure 84 Flowchart for the selection of taxa and accessions for conservation.

Figure 84 shows a simplified flowchart for the selection of taxa and their representative accessions for conservation assessment. If the plant group being studied is taxonomically complex like the vireyas, an initial phylogenetic assessment is necessary to determine

relationships among the taxa. This assessment will also reveal relationships of threatened taxa to those that are of low conservation interest, and therefore to determine whether the taxa selection should be widened to encompass all the related taxa. For the vireyas, the *rpb*2i sequences have shown to be phylogenetically informative in this study and sequence data for a large number of vireyas are available in the public domain. For other plant groups instead of the nuclear region *rpb*2i, sequences of the plastid regions could also be explored (Hollingsworth et al. 2009; von Cräutlein et al. 2011).

The following subsections describe the taxa and accessions of vireyas that have been selected for conservation based on the methods outlined in this study. The taxa are arranged in the decreasing order of conservation priority.

7.4.1 *R. acrophilum* (Critically Endangered)

Authority:	Merr. & Quisumb., *Phil. J. Sc*. 82: 333, 1953.
IUCN Code:	**CR B1a+2ab(iii)**
Distribution:	Mt Mantalingahan (Philippines) (Argent 2006).
Status:	Known from the single type location. Nearby forest destruction and degradation; no evidence of direct population decline. Distribution <100 per km^2 (Gibbs et al. 2011).
Acc. Worldwide:	**RBGE**: 19922768, 19922773, 19922784, 19922785 and 19922786 (all wild collected in Philippines) (RBGE 2012). **RSBG**: 2002/018 (wild collected) (RSBG 2012).
Accessions in NZ:	**Pukeiti Gardens**: EK669 (Currie; wild collected); S2008115 (source unknown); S2003187 (4 plants – Currie; wild collected); S2007117 (Mitch; wild collected); S2007126 (5 plants – Mitch/Currie; wild collected).
MPI Listed:	No
Prioritization:	Due to the threatened Red List status, this species need to be conserved.
Action:	Only a single known accession of *R. acrophilum* was available for study from New Zealand, since then 11 plants representing four accessions have been found at Pukeiti. The additional accessions found need to undergo genetic diversity and

phylogenetic analyses. Propagation and distribution to other gardens of these accessions need to be carried out. DNA is available for the RSBG accession 2002/018 and need to be included in future studies.

7.4.2 *R. taxifolium* (Critically Endangered)

Authority: Merr., *Phil. J. Sc.* 30: 419, 1926.

IUCN Code: **CR B1ab(iii)**

Distribution: Mt Pulag, Luzon (Philippines) (Argent 2006).

Status: Threatened by agricultural encroachment and habitat loss, which has led to >30% reduction in habitat over its <100 km^2 range. All nearby mountains are already devoid of montane forest and therefore not suitable to support additional populations (Gibbs et al. 2011).

Acc. Worldwide: **RBGE**: 19922826 (wild collected in Philippines) (RBGE 2012).

Accessions in NZ: **Pukeiti Gardens**: EK578**,** EK580 and EK605 (wild collected).

Victoria Esplanade Gardens: single mature accession (origin unknown; PFR accession number absent as this accession was acquired after the conclusion of the molecular work for this study).

Bovees Nursery (USA): V675 (source unknown) (Bovees Nursery 2012).

MPI Listed: No

Prioritization: Due to the threatened Red List status, this species needs to be conserved.

Action: There are four known accessions of *R. taxifolium* in New Zealand and due to its conservation priority these accessions must be conserved. The genetic diversity analysis using microsatellites revealed that the genetic distance ($\delta\mu^2$) between the accessions EK578 and EK580 is 98.5. This result shows that these two accessions are genetically significantly different, and thus both require conservation. Further genetic diversity studies need to be carried out on the remaining two accessions, and if they are

significantly genetically different, they also need to be conserved. Material from genetically different accessions also needs to be exchanged between the gardens holding this species.

The genetic diversity between the New Zealand accessions and the overseas accessions also need to be evaluated. If the New Zealand accessions are genetically (significantly) different to than those in the overseas collections, these should be sent to the overseas collections to increase their genetic diversity of this species.

7.4.3 *R. mendumiae* (Critically Endangered)

Authority: Argent, *Gardens Bull. Singapore* 56(1 & 2): 82, 2004.

IUCN Code: **CR B2ab(i)**

Distribution: Philippines, Palawan, Cleopatra Needle. Known only from the type locality (Argent 2006; Gibbs et al. 2011).

Status: Only known from a very small population at the type locality in mossy submontane forest on Palawan, Philippines. Due to habitat type and population size, this species is at risk from habitat disturbances such as those caused by *El Niño* events (Gibbs et al. 2011).

Acc. Worldwide: **RBGE**: 19981798, 19981800, 19981815 and 20031269 (wild collected in Philippines) (RBGE 2012).

Bovees Nursery (USA): V909 (source unknown) (Bovees Nursery 2012).

Accessions in NZ: **Pukeiti Gardens**: 2008118 (source unknown).

MPI Listed: No

Prioritization: Due to the threatened Red List status, this species must be conserved.

Action: There is only a single known accession of *R. mendumiae* in New Zealand, and due to its conservation priority this accession must be conserved. Propagation and distribution to other gardens of this accession need to be carried out, and further genetically

different accessions need to be sought from overseas to increase the genetic makeup of the existing single accession. The New Zealand accession was not found during the specimen collection excursions for this study, and thus require further analysis to determine its placement in the vireya phylogeny.

7.4.4 *R. santapaui* (Endangered)

Authority: Sastry, Kataki, P. A. Cox, E. P. Cox & Hutchinson, *J. Bombay Nat. Hist. Soc.* 65: 744, 1969.

IUCN Code: **EN B2ab(ii,iii,v)**

Distribution: Endemic and known from two localities, at a narrow altitudinal range in montane forest of Arunachal Pradesh (NE India) (Argent 2006; Mao 2011).

Status: Under threat due to habitat fragmentation, dam construction and agricultural practices. Known to be in cultivation but no additional conservation measures are known (Gibbs et al. 2011).

Acc. Worldwide: **RBGE**: 19830996 (wild collected in Arunachal Pradesh) and 19830536 (cultivated material in India) (RBGE 2012).

RSBG: 1998/020 (wild collected by Peter Cox in NE India in 1965) (RSBG 2012).

Accessions in NZ: **Pukeiti Gardens**: EK581 (source unknown).

MPI Listed: No

Prioritization: Due to the threatened Red List status of *R. santapaui* and as it represents a phylogenetically distinct group within *Pseudovireya* this species need to be conserved. Genetic diversity analysis using nucleotide sequence data (*rpb*2i) showed that the genetic distance (Tamura-Nei) between the New Zealand accession and the accession in published data (AY765625) is 0.005694. The branch containing these two accessions has 94% bootstrap support and 100% Bayesian posterior probability; i.e. even though these two accessions are genetically very close, they are not identical.

Action: There is only a single known accession of *R. santapaui* in New Zealand, and due to its conservation priority, this accession must

be conserved. Propagation and distribution to other gardens of this accession need to be carried out, and further genetically different accessions need to be sought from overseas to increase the genetic makeup of the existing single accession.

7.4.5 *R. alborugosum* (Endangered)

Authority: Argent & J. Dransfield, *Notes RBG Edinburgh* 46(1): 27, 1989.

IUCN Code: **EN D**

Distribution: S Kalimantan (Borneo) (Argent 2006; Gibbs et al. 2011).

Status: Known only from a single population in upper montane, mossy forest on a single peak; although not currently under direct decline or fluctuations there are fewer than 250 individuals which makes the species at risk from stochastic events (Gibbs et al. 2011).

Acc. Worldwide: **RBGE**: 19962356, 19962364, 19962365, 19962366, 19962368, 19962369, 19962370, 19962371, 19962372 and 19970113 (wild collected in Indonesia) (RBGE 2012).

Bovees Nursery (USA): V717 (source unknown).

Accessions in NZ: **Pukeiti Gardens**: EK536 (S1998106; wild collected in Borneo).

MPI Listed: No

Prioritization: Due to the threatened Red List status, this species must be conserved.

Action: There is only a single known accession of *R. alborugosum* in New Zealand, and due to its conservation priority this accession must be conserved. Propagation and distribution to other gardens of this accession need to be carried out, and further genetically different accessions need to be sought from overseas to increase the genetic makeup of the existing single accession. Since RBGE has ten accessions, DNA from these accessions needs to be procured to determine whether the New Zealand accession is different from the RBGE's. If the RBGE accessions are different from the local accession, these need to be acquired to increase the genetic diversity of the local accessions of *R. alborugosum*.

7.4.6 *R. baconii* (Endangered)

Authority: Argent, A. Lamb & Phillipps, *Notes RBG Edinburgh* 42(1): 115, 1984.

IUCN Code: **EN D**

Distribution: Yunnan (China), Vietnam. Known from a very restricted area on the China-Vietnam border region (Argent 2006; Gibbs et al. 2011).

Status: Known from one small site on Mt Tambuyukon, <1km^2, with a very small but stable population of fewer than 100 mature individuals (Gibbs et al. 2011).

Acc. Worldwide: **RBGE**: 19952766, 19952769, 19952770 (wild collected in Sabah) (RBGE 2012). Two further accessions representing the hybrid *R. baconii* × *meijeri* Argent, A. Lamb & Phillips are also cultivated at RBGE.

Accessions in NZ: **Pukeiti Gardens**: 2007115 (wild source; PFR number not assigned as the accession was found in the collection after the completion of the molecular studies).

MPI Listed: No

Prioritization: Due to the threatened Red List status, this species must be conserved.

Action: There is only a single known accession of *R. baconii* in New Zealand, and due to its conservation priority this accession must be conserved. Propagation and distribution to other gardens of this accession need to be carried out, and further genetically different accessions need to be sought from overseas to increase the genetic makeup of the existing single accession. The New Zealand accession was not found during the specimen collection excursions for this study, and thus require further analysis to determine its placement in the vireya phylogeny.

7.4.7 *R. lochiae* (Vulnerable)

At present the major characteristic between these two taxa are the shape of the corolla and the arrangement of the stamens around the corolla mouth (Section 6.2.1) (Argent 2006; Craven 2003; Craven & Withers 1996a; Withers 1992). However, flowers with varying degrees of curvature have been observed on single plants in different populations (Andrew Small *pers. comm.*). Further studies need to be carried out with true wild origin specimens to determine the status of these taxa. Since the IUCN Red List has categorized *R. lochiae* as VU D2 and *R. viriosum* as LC, *R. lochiae* has been chosen as a candidate for conservation in the interim.

Authority:	F. Muell., *Vict. Nat.* 3: 157, 1887.
IUCN Code:	**VU D2**
Distribution:	Queensland (Australia) (Argent 2006; Gibbs et al. 2011).
Status:	Only known from two localities (Bellenden Ker Range and on Bell Peak in Malbon Thompson Range (Gibbs et al. 2011).
Acc. Worldwide:	**RBGE**: 19961298, 19961303 and 20021043 (wild collected in Australia); there are additional six accessions not cited to be of wild origin. *R. viriosum* has five known accessions in collections worldwide, while RBGE has three accessions (two of which are of wild origin) (RBGE 2012).
Accessions in NZ:	**Pukeiti Gardens**: EK606 (source unknown).
MPI Listed:	Basic (importation of cuttings and whole plants allowed).
Prioritization:	Due to the threatened Red List status, this species must be conserved. Since the demarcation between *R. lochiae* and *R. viriosum* are not well-established, the latter also should be included in the conservation programme.
Action:	The large number of accessions allied to *R. lochiae* and *R. viriosum* show significant genetic variation. The presence of possible hybrid taxa may also increase the genetic diversity values. For the interim, all the accessions assigned to these two taxa should be conserved until further studies are carried out to determine their taxonomic status.

7.4.8 *R. pudorinum* (Vulnerable)

Authority: Sleumer, *Reinwardtia* 5: 112, 1960.

IUCN Code: **VU D2**

Distribution: Latimodjong Range (Mt Pokapindjang and its spur to Tinabang) of Sulawesi (Argent 2006; Gibbs et al. 2011).

Status: Found in two locations (Gibbs et al. 2011).

Acc. Worldwide: **RBGE**: 19981659 and 20082118 (wild collected in Sulawesi) (RBGE 2012).

Accessions in NZ: **Pukeiti Gardens**: EK653 (source unknown).

MPI Listed: No

Prioritization: Due to the threatened Red List status, this species must be conserved.

Action: There is only a single known accession of *R. pudorinum* in New Zealand, and due to its conservation priority this accession must be conserved. Propagation and distribution to other gardens of this accession need to be carried out, and further genetically different accessions need to be sought from overseas to increase the genetic makeup of the existing single accession.

7.4.9 *R. lamrialianum ssp. lamrialianum* (Vulnerable)

Authority: Argent & Barkman, *The New Plantsman* 7(4): 209, 2000.

IUCN Code: **VU D2**

Distribution: Sabah (Borneo) (Argent 2006; Gibbs et al. 2011).

Status: Known only from a single population on one mountain (Mt Trus Madi) (Gibbs et al. 2011).

Acc. Worldwide: **RBGE**: 19841164 (wild collected in Sabah, Borneo) (RBGE 2012).

Accessions in NZ: This taxon is known to be in cultivation in New Zealand (MacKay et al. 2012), however no accessions were available for the current study.

MPI Listed: **No**

Prioritization: Due to the threatened Red List status, this species must be conserved.

Action: Accessions of this taxon need to be found in New Zealand collections. If an accession is found, need to obtain DNA samples from worldwide collections to determine the identity of the local accession and the placement of this taxon within the wider vireya phylogeny.

7.4.10 *R. renschianum* (Vulnerable)

Authority: Sleumer, *Bot. Jahr.* 71: 146, 1940.

IUCN Code: **VU D2**

Distribution: Lesser Sunda Islands, Flores: Mts Geli Mutu (Kelimutu), Mandaswai and Mt Desu (Argent 2006; Gibbs et al. 2011).

Status: Known from two locations on Flores (Mt Geli Mutu and Mt Desu) (Gibbs et al. 2011).

Acc. Worldwide: **RBGE**: 19942176 (collected in Lesser Sunda Islands) and 20070780 (wild collected in Nusa Tenggara Timur) (RBGE 2012).

Accessions in NZ: **Pukeiti Gardens**: 2007107 (wild collected).

MPI Listed: No

Prioritization: Due to the threatened Red List status, this species must be conserved.

Action: There is only a single known accession of *R. renschianum* in New Zealand, and due to its conservation priority this accession must be conserved. Propagation and distribution to other gardens of this accession need to be carried out, and further genetically different accessions need to be sought from overseas to increase the genetic makeup of the existing single accession. The New Zealand accession was not found during the specimen collection excursions for this study, and thus require further analysis to determine its placement in the vireya phylogeny.

7.4.11 *R. luraluense* ssp. *luraluense* (Vulnerable)

Authority: Argent & D. F. Chamberlain, *The New Plantsman* 3(4): 195, 1996.

IUCN Code: **VU D2**

Distribution: Yunnan (China), Vietnam. Known from a very restricted area on the China-Vietnam border region (Argent 2006; Gibbs et al. 2011).

Status: The forests in the species locality have been significantly damaged and threatened by road construction, thus may be more threatened than the assessment suggests (Gibbs et al. 2011).

Acc. Worldwide: **RBGE**: 19830534 and 19870129 (wild collected in Papua New Guinea); there is an additional accession not cited as of wild origin (RBGE 2012).

Accessions in NZ: **Pukeiti Gardens**: EK564 (source unknown); HF137 and HF138 (wild collected in Solomon Islands; S1984236).

Victoria Esplanade Gardens: EK141 and EI192 (all accessions of unknown source).

Pukekura Park: HF094 (source unknown).

MPI Listed: **No**

Prioritization: Due to the threatened Red List status, this taxon must be conserved. The genetic diversity analysis of the accessions EK564, HF094, HF137 and HF138 have shown that there is significant genetic diversity in New Zealand accessions. The accessions EK507, EI192, and EK141 are yet to be studied using microsatellites. The genetic diversity analysis using nucleotide sequence data have shown that EI192, HF094 and GU445776 (from published data) are genetically identical, and HF137 is genetically different from the other three.

Action: Due to incomplete data analysis of all the accessions of *R. luraluense* ssp. *luraluense* all the accessions need to be conserved, until they are suitably studied for genetic diversity.

7.4.12 *R. rushforthii* (Vulnerable)

Authority: Argent & D. F. Chamberlain, *The New Plantsman* 3(4): 195, 1996.

IUCN Code: **VU D2**

Distribution: Yunnan (China), Vietnam. Known from a very restricted area on the China-Vietnam border region (Argent 2006; Gibbs et al. 2011).

Status: The forests in the species locality have been significantly damaged and threatened by road construction, thus may be more threatened than the assessment suggests (Gibbs et al. 2011).

Acc. Worldwide: **RBGE**: 19933195, 0020502 (wild collected in Vietnam) (RBGE 2012).

RSBG: 1997/087 (wild collected) (RSBG 2012).

Accessions in NZ: **Pukeiti Gardens**: HF147 (source unknown).

MPI Listed: No

Prioritization: Due to the threatened Red List status, this species must be conserved.

Action: There is only a single known accession of *R. rushforthii* in New Zealand, and due to its conservation priority this accession must be conserved. Propagation and distribution to other gardens of this accession need to be carried out, and further genetically different accessions need to be sought from overseas to increase the genetic makeup of the existing single accession. DNA of the RSBG accession 1997/087 is available and should be included in future studies.

7.4.13 *R. ericoides* (Vulnerable)

Authority: Low ex Hook. *f., Hook. Icon. Pl.* t.887, 1852.

IUCN Code: **VU D1**

Distribution: Mt Kinabalu (Sabah, Borneo); in primary mossy forest, abundant terrestrially on open granite dome in exposed sunny places (Argent 2006; Gibbs et al. 2011).

Status: Point endemic; abundant terrestrially but with a population of <1,000 mature individuals (Gibbs et al. 2011).

Acc. Worldwide: **RBGE**: 19871806 and 200313313 (wild collected in Malaysia); there is an additional accession not of wild origin (RBGE 2012).

Accessions in NZ: **Pukeiti Gardens**: EK537 (wild collected; S97138).

MPI Listed: No

Prioritization: Due to the threatened Red List status, this species must be conserved.

Action: Due to the conservation priority of *R. ericoides* the single accession must be conserved. Further accessions of this species need to be acquired to determine the genetic uniqueness of the New Zealand accessions and further genetically diverse accessions need to be acquired outside New Zealand to complement the existing single accession.

7.4.14 *R. abietifolium* (Vulnerable)

Authority: Sleumer, *Blumea* 11: 122, 1961.

IUCN Code: **VU D1**

Distribution: Mt Kinabalu (Sabah, Borneo) (Argent 2006; Gibbs et al. 2011).

Status: Restricted endemic of Mt Kinabalu with a small population of <1,000 individuals (Argent 2006; Gibbs et al. 2011).

Acc. Worldwide: **RBGE**: 19801209 and 19801268 (wild collected in Sabah, Borneo) (RBGE 2012).

Accessions in NZ: This taxon is known to be in cultivation in New Zealand (MacKay et al. 2012), however no accessions were available for the current study.

MPI Listed: **No**

Prioritization: Due to the threatened Red List status, this species must be conserved.

Action: Accessions of this taxon need to be found in New Zealand collections. If an accession is found, need to obtain DNA samples from worldwide collections to determine the identity of the local accession and the placement of this taxon within the wider vireya phylogeny.

7.4.15 *R. nervulosum* (Vulnerable)

Authority: Sleumer, *Bot. Jahrb.* 71: 146, 1940.

IUCN Code: **VU D1**

Distribution: Sabah (Borneo) (Argent 2006; Gibbs et al. 2011).

Status: Terrestrial (rarely epiphytic) shrub known from two locations with <1,000 mature individuals (Argent 2006; Gibbs et al. 2011).

Acc. Worldwide: **RBGE**: 19801157, 19801179 and 19801218 (all three wild collected in Sabah, Borneo) (RBGE 2012).

Accessions in NZ: This taxon is known to be in cultivation in New Zealand (MacKay et al. 2012), however no accessions were available for the current study.

MPI Listed: **No**

Prioritization: Due to the threatened Red List status, this species must be conserved.

Action: Plants referred to as *R. nervulosum* are thought to be a hybrid between *R. crassifolium* and *R. stenophyllum* (e.g. HF027 at Pukeiti Gardens) (Argent 2006) that displays linear foliage which is typical of *R. stenophyllum* (Photo 24d). Accessions of this taxon need to be found in New Zealand collections. If an accession is found, need to obtain DNA samples from worldwide collections to determine the identity of the local accession and the placement of this taxon within the wider vireya phylogeny.

7.4.16 *R. intranervatum* (Vulnerable)

Authority: Sleumer, *Blumea* 11: 129, 1961.

IUCN Code: **VU D1**

Distribution: Borneo (Kalimantan, Mt Palimasan, near Tabang on Belajan River in W Kutei; Sarawak, Mt Penrissen and Mt Berumput) (Argent 2006; Gibbs et al. 2011).

Status: Known from three locations (Mt Palimasan, Mt Penrissen and Mt Berumput) in *Agathis* forest on waterlogged, white, acid sands or on granite rock faces. <1,000 adults make the species vulnerable to threats and climatic events (Gibbs et al. 2011).

Acc. Worldwide: **RBGE**: 19622876 (wild collected in Sarawak, Borneo) (RBGE 2012).

Bovees Nursery (USA): V734 (source unknown) (Bovees Nursery 2012).

Accessions in NZ: **Pukeiti Gardens**: 2007101 (wild collected).

MPI Listed: **No**

Prioritization: Due to the threatened Red List status, this species must be conserved.

Action: There is only a single known accession of *R. intranervatum* in New Zealand, and due to its conservation priority this accession must be conserved. Propagation and distribution to other gardens of this accession need to be carried out, and further genetically different accessions need to be sought from overseas to increase the genetic makeup of the existing single accession. The New Zealand accession was not found during the specimen collection excursions for this study, and thus require further analysis to determine its placement in the vireya phylogeny.

7.4.17 *R. album* (Vulnerable)

Authority: Blume, *Cat. Hort. Buitenz* 72, 1989.

IUCN Code: **VU B2ab(v)**

Distribution: Java (Argent 2006; Gibbs et al. 2011).

Status:	Restricted to montane forests. Not thought to be currently at risk from habitat decline; vulnerable due to low number of mature individuals and locations (Gibbs et al. 2011).
Acc. Worldwide:	**RBGE**: 19882540 and 198825443 (wild collected in Indonesia); there is an additional accession not of wild origin (RBGE 2012).
Accessions in NZ:	**Pukeiti Gardens**: EK570 (S2008122; wild collected).
MPI Listed:	No
Prioritization:	Due to the threatened Red List status, this species must be conserved.
Action:	There is only a single known accession of *R. album* in New Zealand, and due to its conservation priority this accession must be conserved. Propagation and distribution to other gardens of this accession need to be carried out, and further genetically different accessions need to be sought from overseas to increase the genetic makeup of the existing single accession. Since RBGE has ten accessions, DNA from these accessions needs to be procured to determine if the New Zealand accession is different from the RBGE's. If the RBGE accessions are different to the local accession, these need to be acquired to increase the genetic diversity of the local accessions of *R. album*.

The taxa discussed above are prioritized according to their IUCN Red List categories. However, there are a large number of taxa that have been categorized as Data Deficient (DD), Least Concern (LC) and Not Evaluated (NE), which are of conservation interest, based on the taxonomic and/or phylogenetic relationship with Red-listed taxa.

According to the phylogenetic analyses outlined in the previous chapters, the core vireyas consisted of a large cluster of taxa of which the majority belongs to the Section *Euvireya sensu* Argent (2006) (for example the MP tree in Figure 41). The analyses showed that these taxa are very closely related to each other compared to the taxa found in the basal clades. In terms of germplasm conservation, a wider net has to be spread to capture more genetic diversity. That is, if a threatened taxon clusters with other taxa in a tight cluster, they all need to be considered for conservation. For example, *R. yelliotii* categorized as LC clusters with *R. luraluense* which is categorized as VU D2 (Figure 41). In this case

R. yelliotii appears to be closely related to *R. luraluense* and thus need to be conserved alongside, as they share genetic traits between them.

The following is a selection of additional taxa that need to be considered for conservation as part of the overall conservation of vireyas. The majority of the taxa discussed below are not listed on the MPI list of allowed species and measures need to be taken to add them to the list in the near future. However, accessions in the form of DNA samples could still be imported by research institutes and molecular analyses could be carried out.

7.4.18 *R. archboldianum* (Data Deficient)

Authority: Sleumer, *Reinwardtia* 5: 121, 1960.

IUCN Code: DD

Distribution: Papua New Guinea; known only from two mountains (Argent 2006; Gibbs et al. 2011).

Status: Rare in cultivation.

Acc. Worldwide: **RBGE**: 20021042A (not known to be of wild origin and held in the research collection only) (RBGE 2012).

Accessions in NZ: **Pukeiti Gardens**: HF002 (H1986506; wild collected) and HF003 (wild collected). The plant was originally collected by Norman Cruttwell before 1986 (prior to Graham Smith's collection) on Mt Suckling, Papua New Guinea and was grown at the Lipizauga Botanical Sanctuary on Mt Gahavisukar (from where it had been obtained by Graham Smith). It was referred to as a selection of *R. archboldianum* and named *R.* 'Starburst' and *R.* 'Pukeiti Skyrocket' (Leslie 2012). According to Argent (2006), *R.* 'Starburst' is thought to be a hybrid between *R. herzogii* and *R. culminicola* (Argent 2006).

MPI Listed: No

Prioritization: Due to data deficiency, rarity in cultivation and limited distribution, this species must be conserved.

Action: *R. archboldianum* is a highly ornamental epiphytic species growing to about 3 m high. Physical examination of herbarium samples suggests a relationship between *R. archboldianum*

accessions and the *R. herzogii* accession (EK639). However, a relationship between *R. archboldianum* and the taxa *R. culminicola* and *R. inundatum*, as suggested by Argent (2006) could not be established. The DNA samples of *R. archboldianum* accessions did not produce good quality DNA for sequencing purposes, and was not included in the phylogenetic analyses. Further investigation is needed to establish the placement of *R. archboldianum* within the vireya phylogeny and its relationship to other presumed relatives.

The genetic diversity analyses using eight microsatellite markers showed that the two accessions HF002 and HF003 have a genetic distance $(\delta\mu)^2 = 20.20$, which suggest that these two accessions might have originated from two different populations. HF002 and HF003 are thus suitable candidates for conservation. Accessions of taxa related to *R. archboldianum* should also be maintained to enable further study. Further studies need to be expanded to include accessions of *R. archboldianum* and its relatives held in worldwide collections. RBGE has a single accession of *R. archboldianum* (20021042A), but is not of wild origin. However, this accession should be including future studies to establish the genetic diversity among the accessions and the placement of this species within the vireya phylogeny.

7.4.19 *R. arenicola* (Data Deficient)

Authority: Sleumer, *Reinwardtia* 5: 113, 1960.

IUCN Code: **DD**

Distribution: SW Central Sulawesi (Latimodjong Range: 2,600–3,000 m) (Argent 2006; Gibbs et al. 2011).

Status: Common in the wild but rare in cultivation (Gibbs et al. 2011). *R. arenicola* was recently introduced into cultivation by Galloway and Smith from Mt Rantemario, where it was common at 2,700 m (Argent 2006).

Acc. Worldwide: **RBGE**: 20000587 and 20031268 (both wild collected in Sulawesi) (RBGE 2012).

Accessions in NZ: Two accessions labelled as *R. arenicola* (EK596 and EK660) were found in New Zealand (Pukeiti Gardens); however they do not match the physical description of the species and were eventually excluded in the phylogenetic analyses. A third accession (EK573) previously labelled as *R. lagunculicarpum* was identified as *R. arenicola* but was not sequenced for phylogenetic analyses.

MPI Listed: **No**

Prioritization: Due to data deficiency, rarity in cultivation and limited distribution, this species must be conserved.

Action: *R. arenicola* is very similar to *R. lagunculicarpum* and the molecular phylogenetic analyses of this study (using published data) show a close relationship between these two species. *R. arenicola* clusters together with the core vireyas at Node 13 (Group G, Figure 41) and not allied to the traditional classification under *Albovireya*. Phylogenetic analyses show a relationship between *R. arenicola* and *R. zollingeri*, but require further investigation. Accessions at RBGE would be useful in future phylogenetic analyses to establish the identity of New Zealand accessions (EK573, EK596 and EK660), and study the genetic diversity among worldwide accessions. As an immediate action EK573 need to be sequenced and included in the vireya phylogeny to establish its identity (comparing with published data) and its exact placement within vireya phylogeny.

7.4.20 *R. arfakianum* (Data Deficient)

Authority: Becc., *Malesia* 1: 201, 1878.

IUCN Code: DD

Distribution: New Guinea (Arfak and Nettoti Mts) (Argent 2006; Gibbs et al. 2011).

Status: Data deficient and accessions in cultivation are doubtful (Argent 2006).

Acc. Worldwide: **RBGE**: 20090797 and 20090736, both wild collected in Papua (Irian Jaya) (RBGE 2012).

Accessions in NZ: The identity of the accession (EK608) is questionable and appears to be related to *R. culminicola*. This accession clusters with the accession of *R. villosulum* in the majority of the phylogenetic analyses of this study.

MPI Listed: **No**

Prioritization: Due to data deficiency, rarity in cultivation and limited distribution, this species must be conserved.

Action: DNA from RBGE accessions need to be obtained for phylogenetic and genetic diversity analyses, and also to establish identity and to study the genetic differentiation among the worldwide accessions.

7.4.21 *R. baenitzianum* (Data Deficient)

Authority: Lauterb., *Nachtr.* 337, 1905.

IUCN Code: DD

Distribution: Papua New Guinea (Argent 2006; Gibbs et al. 2011).

Status: A lowland species and therefore likely to be at risk from habitat loss, but considered by taxonomists not to be distinct from *R. englerianum* Koord. (Argent 2006; Gibbs et al. 2011).

Acc. Worldwide: **RBGE**: 19901315 (wild collected in Papua New Guinea) and 19973613 (cultivated material in the research collection) (RBGE 2012).

Accessions in NZ: **Pukeiti Gardens**: EK658 and another accession with no label,

MPI Listed: No

Prioritization: Due to data deficiency, rarity in cultivation and limited distribution, this species must be conserved.

Action: DNA from RBGE accessions need to be obtained for phylogenetic analyses (to determine true identity of the New Zealand accessions) and genetic diversity analyses (to study the genetic differentiation among the worldwide accessions).

7.4.22 *R. bryophilum* (Data Deficient)

Authority: Sleumer, *Reinwardtia* 5: 79, 1960.

IUCN Code: **DD**

Distribution: New Guinea (Argent 2006; Gibbs et al. 2011).

Status: Data deficient. Found only on the Cycloop Mts (W New Guinea) (Argent 2006).

Acc. Worldwide: **RSBG**: 1980/141 (wild collected from Cycloop Mts, W New Guinea) (RSBG 2012).

Accessions in NZ: Three accessions (EK502, EK649 and HF023) labelled as this species were collected; however none of these match the physical identities, and all keys to *R. dielsianum*. According to Argent (2006), the distinction between these two species is not clearly established and 'the best difference appears to be that *R. dielsianum* has a glabrous style except for a few hairs at the base whereas in *R. bryophilum* the style is covered simple hairs for most of its length'.

MPI Listed: No

Prioritization: Due to data deficiency, rarity in cultivation and limited distribution, this species must be conserved.

Action: The extent of the misidentification between *R. bryophilum* and *R. dielsianum* need to be investigated and the accessions labelled according in the collections. Genuine material representing *R. bryophilum* also need to be sought from worldwide collections for further analysis and comparison. *R. dielsianum* categorized by

IUCN as LC should be included in the conservation programme due to the affinity of this species to the DD categorized *R. bryophilum*.

7.4.23 *R. dianthosmum* (Data Deficient)

Authority: Sleumer, *Blumea* 12: 100, 1963.

IUCN Code: **DD**

Distribution: Known from a single location on Mt Dafonsero () (Argent 2006; Gibbs et al. 2011).

Status: Data deficient (Argent 2006; Gibbs et al. 2011).

Acc. Worldwide: **RSBG**: 1983/063 (wild collected); DNA from this accession is presently available in New Zealand (stored at PFR) (RSBG 2012).

Bovees Nursery (USA): accession number and origin unknown (Bovees Nursery 2012).

Accessions in NZ: The accession labelled as *R. dianthosmum* did not match the physical description and keys out to *R. superbum*. An accession typical of *R. dianthosmum* has yet to be found in New Zealand collections.

MPI Listed: No

Prioritization: Due to data deficiency, rarity in cultivation and limited distribution, this species must be conserved.

Action: The RSBG accession (1983/063) need to be sequenced and subjected to phylogenetic analyses with closely related taxa (and accessions with affinities to *R. dianthosmum*). Further surveying is required to seek any accessions typical of *R. dianthosmum* to determine a true sample of this species.

7.4.24 *R. goodenoughii* (Data Deficient)

Authority: Sleumer, *Reinwardtia* 5: 131, 1960.

IUCN Code: **DD**

Distribution: Goodenough Island (Papua New Guinea) (Argent 2006; Gibbs et al. 2011).

Status: Known only from a single mountain on Goodenough Island. Needs further research to establish the conservation status.(Argent 2006; Gibbs et al. 2011).

Acc. Worldwide: **RBGE**: 19670828 (cultivated from material wild collected in Papua New Guinea) and 19772400 (wild collected in Papua New Guinea) (RBGE 2012).

RSBG: 1983/053 (wild collected) (RSBG 2012).

Bovees Nursery (USA): V53 (source unknown) (Bovees Nursery 2012).

Accessions in NZ: **Victoria Esplanade Gardens**: EI146, EI170, EI171 and EI172 (source unknown).

Pukeiti Gardens: EK611 (source unknown).

MPI Listed: Basic (importation of cuttings and whole plants allowed).

Prioritization: Due to data deficiency, rarity in cultivation and limited distribution, this species must be conserved.

Action: The presence of five accessions of this species means that there is a useful opportunity for propagation and distribution of the New Zealand material to other international collections. Identity of EK611 has yet to be physically verified.

7.4.25 *R. bloembergenii* (Data Deficient)

Authority: Sleumer, *Reinwardtia* 5: 204, 1960.

IUCN Code: **DD**

Distribution: Sulawesi (Argent 2006; Gibbs et al. 2011).

Status: Needs further research to establish the conservation status (Argent 2006; Gibbs et al. 2011).

Acc. Worldwide: Not known to be in cultivation. According to Argent (2006), this species is not known to have been in cultivation.

Accessions in NZ: Exact whereabouts of this species in New Zealand is yet unknown; however preliminary review of literature indicates

	collections by David Binney from Sulawesi may be present in collections in Taranaki and Auckland regions.
MPI Listed:	No
Prioritization:	Due to data deficiency, rarity in cultivation and limited distribution, this species must be conserved.
Action:	Need to locate the New Zealand accession of this species. Once found molecular phylogenetic analysis should be carried out possibly including the herbarium samples of this species held at RBGE and Kew. The lack of cultivated material of this species worldwide strongly indicates the revision of the conservation status, and perhaps raising the category to VU.

7.4.26 *R. leucogigas* (Data Deficient)

Authority:	Sleumer, *Blumea* 12: 102, 1963.
IUCN Code:	**DD**
Distribution:	New Guinea (Argent 2006; Gibbs et al. 2011).
Status:	Known from a single location and needs further research to establish conservation status (Argent 2006; Gibbs et al. 2011).
Acc. Worldwide:	**RBGE**: (RBGE 2012).
Accessions in NZ:	Accession of typical form not found.
MPI Listed:	**No**
Prioritization:	Due to data deficiency, rarity in cultivation and limited distribution, this species must be conserved.
Action:	Known from a single location and needs further research to establish its conservation status (Gibbs et al. 2011). The single accession belonging to this species is *R. leucogigas* 'Hunstein's Surprise' (HF051) at Pukeiti Gardens. Further research is needed to locate additional accessions of this species in New Zealand collections.

7.4.27 *R. maxwellii* (Data Deficient)

Authority:	Gibbs, *J. Linn. Soc. Bot.* 42: 103, 1914.

IUCN Code: DD

Distribution: Mt Kinabalu, Sabah, Borneo (Argent 2006; Gibbs et al. 2011).

Status: Known from a single location, but thought to be widespread in its locality and further research is needed to establish its conservation status (Argent 2006).

Acc. Worldwide: **RBGE**: 19801235, 19801241, 19801374 (all wild collected from Mt Kinabalu, Sabah, Borneo) (RBGE 2012).

Accessions in NZ: **Victoria Esplanade Gardens**: EI138 & EI156 (source unknown).

Pukeiti Gardens: EK523 (wild collected); HF033 (source unknown).

MPI Listed: **No**

Prioritization: Due to data deficiency, rarity in cultivation and limited distribution, this species must be conserved.

Action: Further research is needed to determine the distribution of this taxon in New Zealand collections and future molecular studies need to include additional accessions and comparison of these to accessions in worldwide collections.

7.4.28 *R. rhodopus* (Data Deficient)

Authority: Sleumer, *Reinwardtia* 5: 199, 1960.

IUCN Code: DD

Distribution: Sulawesi (Argent 2006; Gibbs et al. 2011).

Status: First collected as living material by Keith Adams in 1997; also collected by L A Craven and G K Brown in 2002 and grown in Australia (Argent 2006). Brown (2002) reported that *R. rhodopus* Sleumer was found on Gunung[52] Sesean, north of Rantepao. Further research needed to establish conservation status (Argent 2006; Gibbs et al. 2011).

Acc. Worldwide: **RBGE**: 19973620 (wild collected in Celebes) (RBGE 2012).

Accessions in NZ: **Pukeiti Gardens**: EK597 (source unknown).

[52] Malay name for mountain (also Mt, Mount).

MPI Listed: **No**

Prioritization: Due to data deficiency, rarity in cultivation and limited distribution, this species must be conserved.

Action: The accession EK597 need to be sequenced and used in the phylogenetic analyses to determine the placement of this species in the vireya phylogeny. The accession should also be used to propagate and distribution of material to other New Zealand and worldwide collections for *ex situ* conservation.

7.4.29 *R. leptanthum* (Least Concern)

Authority: F. Muell., *Trans. R. Soc. Vict. n.s.* 1(2): 24, 1889. (Syn: *R. warianum* Schltr., *Bot. Jahrb.* 55: 151, 1918). Argent (2006) reduced *R. warianum* to a synonym of *R. leptanthum* based on morphological similarities, and differing only in the extreme leaf shape and general vigour. Craven (2009) used bark morphology to support the recognition, at species rank, of *R. warianum* Schltr., which was earlier reduced to varietal rank within *R. leptanthum* F. Muell. by Argent (1995) and later reduced to a synonym of *R. leptanthum* by Argent (2006) without taxonomic recognition.

IUCN Code: **LC**

Distribution: Madang to Milne Bay Districts (E Papua New Guinea) (Argent 2006; Gibbs et al. 2011).

Status: Sometimes common (Argent 2006).

Acc. Worldwide: **RBGE**: 19614123, 19671322, 19681089, 19681327, 19681436, 19681505, 19861562, 19861575, 19861579, 20061808, 20061809 and 20061810 (wild collected in Papua New Guinea). (RBGE 2012). 19681517, 19682248 and 19630476 (cultivated from material wild collected in Papua New Guinea). 19861635 collected in the wild as *R. leptanthum* var. *warianum* (Schltr.) Argent.

RSBG: 1985/043 (wild collected by Michael Black (#75); flowers rose-coloured with golden brown scales), 1987/041 (wild

collected) and 1987/042 (wild collected by Michael Black (#75); flowers rose-coloured with golden brown scales) (RSBG 2012).

Accessions in NZ: **Pukeiti Gardens**: EK670 (source unknown).

MPI Listed: Basic (importation of cuttings and whole plants allowed).

Prioritization: *R. leptanthum* is designated LC but related to *R. warianum* designated DD.

Action: Molecular analyses need to be carried out using the accession EK670 to confirm the identity against published data and to determine the genetic distance among the accessions of this species worldwide. Multiple accessions representing *R. leptanthum* and *R. warianum* from worldwide collections need to be studied to ascertain the species boundary of these two species.

7.4.30 *R. jasminiflorum* (Least Concern)

Authority: Hook, *Bot. Mag.* t4525, 1850.

IUCN Code: **LC**

Distribution: Peninsular Malaysia to E Malesia (Argent 2006; Gibbs et al. 2011).

Status: Most subspecies are common, however the subspecies in the Philippines are vulnerable (VU) due to habitat loss (Argent 2006; Gibbs et al. 2011).

Acc. Worldwide: **RBGE**: *R. jasminiflorum* ssp. *jasminiflorum* – 19672707 (collected from cultivated material) (RBGE 2012); 19680606 (wild collected at Selangor/Pahang border); 19820742, 19871301, 19943011, 19943018, 19960914 and 19960915 (wild collected in Sarawak); 19680638 (wild collected in Malaysia; exact locality not specified); 20110248 (wild collected in Indonesia; exact locality not specified). *R. jasminiflorum* ssp. *oblongifolium* – 19943003 and 19943012 (wild collected in Sarawak). *R. jasminiflorum* ssp. *copelandii* – 19922739 and 19922827 (wild collected in Mindanao). *R. jasminiflorum* ssp.

heusseri – 20010247 and 20010351 (wild collected in Aceh, Sumatra).

RSBG: 78/102 and 82/209 (both wild collected from Malay Peninsula) (RSBG 2012).

Bovees Nursery (USA): *R. jasminiflorum* ssp. *oblongifolium* – V610 (oblong). *R. jasminiflorum* ssp. *jasminiflorum* – V580 and V302 (source unknown) (Bovees Nursery 2012).

Accessions in NZ: **Pukeiti Gardens**: *R. jasminiflorum* ssp. *jasminiflorum* – EK548 (wild collected); EK612 and EK656 (source unknown). *R. jasminiflorum* ssp. *oblongifolium* – EK645 (wild collected) and EK590 (source unknown).

Victoria Esplanade Gardens: *R. jasminiflorum* ssp. *jasminiflorum* – EI135, EI136, EI137, EI153, EI154 and EI155 (all accessions of unknown origin).

MPI Listed: Basic (importation of cuttings and whole plants allowed).

Prioritization: *R. jasminiflorum* consists of six intraspecific taxa and the subspecies *copelandii* is Red-Listed as VU D2. To conserve genetic diversity of *R. jasminiflorum* all the subspecific taxa need to be included in the conservation programme.

Action: *R. jasminiflorum* is a highly variable and widely distributed species in W Malesia. Further assessment of accessions need to be carried out to determine the intraspecific variation of this species. DNA of other subspecies needs to be obtained from international collections to determine the intraspecific boundaries and genetic diversity among the accessions of each subspecies.

7.5 Conservation Actions and Strategies

The conservation plan highlights the modes and management of germplasm applicable to vireyas cultivated in New Zealand and worldwide. These actions and strategies can also be adapted to apply for *in situ* conservation programmes.

7.5.1 Immediate Conservation Actions for Vireyas

Due to the availability of facilities to store and maintain germplasm in New Zealand, there are several conservation actions that can be carried out at present. They include:

1. Contribute information regarding taxa and accessions of vireyas in New Zealand plant collections to global registers such as the BGCI database and other similar resources.

2. Expand the conservation of vireyas beyond the subgeneric level and include other non-vireya rhododendron taxa in New Zealand collections.

3. Conduct a detailed survey of rhododendron collections within New Zealand to ascertain and update the total number of taxa and accessions. A centralized register of these accessions hosted on an accessible resource would allow growers and researchers to exchange information and develop a comprehensive database of rhododendron in New Zealand.

4. Exchange of germplasm between other international rhododendron repositories such as the Rhododendron Species Botanical Garden (USA) and Royal Botanic Garden Edinburgh (UK) for those taxa with accessions in cultivation in New Zealand.

5. Initiate registration of existing *Rhododendron* taxa (threatened or not) in New Zealand with MPI. Those few taxa that are presently listed on the MPI website can be imported without any restrictions. Those taxa in cultivation since 1998 that are not on the MPI register need to be assessed by them to be added to the register. As these processes take time, measures to register them should be initiated as soon as possible. Those taxa brought into the country after 1998 could also be registered with MPI, but may take longer and should be initiated if possible (Ministry for Primary Industries 2012).

6. Initiate collection of seeds of *Rhododendron* taxa in New Zealand and organise the storage of these at the Kew Millennium Seed Bank. Storage of duplicate specimens at the German Rhododendron Gene Bank should also be reviewed (Spethmann et al. 2010).

7. Seek funding for further study on conservation management of *Rhododendron* germplasm.

Urgent action is required to conserve the most threatened vireya taxa, in particular those assessed as Critically Endangered, which have now been reduced to only a few individuals (Gibbs et al. 2011). The BGCI's plant database shows that worldwide botanic gardens currently hold over 65% of all threatened *Rhododendron* taxa compared to over 75% of LC category taxa (BGCI 2012). The collection of the remaining 35% of threatened taxa for *ex situ* conservation should therefore be prioritized to insure against extinction of these.

Vireyas being taxonomically complex need to be studied further to understand the wide variation among the taxa and to ascertain the taxonomic boundaries of taxonomic groups and individual taxa. To achieve this, a complete phylogeny of vireyas is required that includes all the representatives of vireyas (extinct and extant). Currently, over 100 vireya taxa have their nucleotide sequences published using either plastid or nuclear genomes (NCBI 2012), and the current study adds a further 75 taxa. At present, not all of the taxa have been sequenced for a universal nucleotide region. For future studies the utilization of the *rpb*2i nuclear region would be ideal as this represents the largest number of nucleotide sequences of vireya.

7.5.2 Long-Term Collaborative Action Plan

To achieve the goals of the Conservation Plan of vireyas a collaborative Action Plan is needed. This collaboration needs to be between all the stakeholders related to the global conservation of vireyas. Most threatened species are found in developing countries where funds for conservation programmes are limited. *Ex situ* conservation of forest genetic resources may be the only option in some instances, but this also is a long-term commitment with a large initial investment and recurrent costs. Funding organizations have increasingly incorporated environmental considerations into their international development activities, but support is generally provided for protection of plants *in situ* because of the urgent need to protect ecosystems from imminent change (FAO et al. 2004).

To ease the financial burden on a single institution to manage conservation, *ex situ* germplasm conservation programmes may be successfully carried out by multiple organizations. An example is the Central America and Mexico Coniferous Resources Cooperative (CAMCORE), formed in 1980 by cooperation between North Carolina State University, private forest industry, and government agencies around the world. *Ex situ* conservation is also the concern of international agencies such as IUFRO, IPGRI and FAO that have been instrumental in drawing global attention to the need for collection and conservation of genetic resources. *Ex situ* conservation programmes coordinated through multilateral organizations usually have a reasonable time horizon for funding because of the commitment from member governments (Dvorak 2000; FAO et al. 2004).

To achieve global conservation of vireyas, several stakeholders need to be brought on board that should include the representative nations which hold the in situ populations of the threatened taxa, existing *ex situ* collections worldwide, possible future germplasm collections and funding agencies (FAO et al. 2004). Many botanic gardens have for example established conservation programs into their missions, and support from international organizations has also increased in tandem, such as the Centre for Plant Conservation (CPC), the International Union for the Conservation of Nature (IUCN), Species Survival Commission (SSC), the International Plant Genetic Resources Institute (IPGRI), and Botanic Gardens Conservation International (BGCI). These organizations collaborate by helping many botanic gardens and other *ex situ* conservation bodies improve their conservation programs, such as BGCI's global agenda for botanic gardens (Botanic Gardens Conservation International 2001).

7.6 Collection Management Aspects

This section describes in detail the conservation action that needs to be carried out on the selected threatened vireya accessions presently held in New Zealand collections. *Ex situ* conservation of plants is often held back by the very large number of taxa that require protection such as in the vireyas. Larger number of taxa demands larger areas for their cultivation, and adequate methods for long-term storage of germplasm (Bawa & Ashton 1991).

The decision to implement an *ex situ* conservation programme as part of a conservation management or recovery plan will depend largely on the taxon's circumstances and conservation needs. A taxon-specific conservation plan therefore should include a range of *ex situ* objectives, including short-, medium- and long-term maintenance of accessions. The maintenance can be achieved by employing a variety of techniques including propagation, germplasm storage, and where possible re-introduction into the wild (Maunder & Byers 2005). Taxa-specific actions are outlined under each taxa described. In addition, the following general conservation measures for vireya accessions in New Zealand should be considered:

- Propagation of threatened taxa – Propagation from accessions is essential to increase the survivability of threatened taxa, and since threatened taxa has limited number of accessions often restricted to only a single collection, material need to be distributed to other gardens (Fay 1992; Maunder et al. 2001b). For example, Pukeiti Gardens has the largest known collection of threatened vireyas in New Zealand, and accessions of these need to be propagated and sent to other gardens such as the Victoria Esplanade Gardens, which already has a significant number of vireya accessions.

- Where accessions of threatened taxa are available in collections outside New Zealand, DNA samples from these need to be obtained for genetic diversity analysis in the first instance. If any of these accessions are genetically different from those cultivated in New Zealand, these need to be imported under existing regulations and added to the existing collections to improve the genetic diversity of the taxon in New Zealand.

- When collecting species, intraspecific taxa should also be considered as they could possess higher levels of genetic characteristics, or features that will help to protect them from future climate change (Brown & Hardner 2000; Muller-Starck & Schubert 2001; Stern & Roche 1974)

- Since vireyas are perennials, rapid production of new variations among populations is not possible, thus existing genetic diversity collected from wild

populations is important and fundamental to the *ex situ* conservation programme (Brown & Hardner 2000; Muller-Starck & Schubert 2001; Stern & Roche 1974).

- A comprehensive survey of vireyas in cultivation in New Zealand needs to be urgently carried out to determine the existence of threatened taxa in other collections than the Pukeiti Gardens and the Victoria Esplanade Gardens.

- Role of a garden with climatic conditions more suitable to large-scale growing of vireyas (e.g. in Singapore or Hawai'i) also need to be considered. Growing vireyas in a tropical climate can reduce costs, and incorporating threatened taxa into existing collections will be very cost effective. The importation of plant material into some countries is also problematic such as in New Zealand, thus countries with more relaxed importation policies are more desirable.

The primary selection criteria of threatened vireya taxa at present are based on the IUCN Red List categories assigned to them. The following subsections outline those taxa that have been categorized as threatened (IUCN categories CR, EN and VU).

Wild source material is always preferable for *ex situ* conservation, however some key taxa lack collection records and thus cannot be confirmed as of wild origin (see Appendices). This raises the question, how would one determine what wild source material is. The accessions at Pukeiti for instance, have been collected both as live plants, cuttings and as seeds. These are all wild source material, and will remain as such even after propagation. Unless these accessions are crossed with other accessions, they should be treated as of wild source origin (Adams 1996; Allen 1971; Binney 2003; Black 1965, 1969; Smith 2003).

Molecular methods can play a role in determining whether some of the questionable accessions are of wild origin or not. Although a simple phylogenetic analysis may not be able to identify hybrids or garden varieties, it can identify to a certain extent which taxa the questionable accessions are related to. Genetic diversity and parentage analysis using marker systems such as microsatellites and RAPDs can indicate the origins of these accessions. It is also important to note that selections (for horticultural purposes) still are of wild origin, just selected for specific traits from the gene pool. In the absence of wild

collection notes, accessions of conservation interest should be included in the *ex situ* conservation programmes, at least until they can be replaced with bona fide wild source material (Rieseberg 1997; Rieseberg et al. 1993).

The Pukeiti Gardens and Victoria Esplanade Gardens should therefore be designated as the primary sites for *ex situ* conservation of vireyas in New Zealand, as they hold the largest documented collections of vireyas in the country. Pukeiti Gardens is already involved in the *ex situ* conservation of vireyas in New Zealand, presently managing threatened taxa and supporting research activities such as this study. The Victoria Esplanade Gardens has yet to formally come to an understanding to partake in the *ex situ* conservation of vireyas in New Zealand.

7.6.1 Collection of germplasm

Collecting involves gathering samples of a species from populations in the field or natural habitats for conservation and subsequent use. The unit of collection may be botanic seeds or vegetative propagules, depending on the breeding system of the species. Collecting may be easy in species producing small seeds in abundance. However, it becomes problematic when seeds are unavailable or non-viable due to: damage of plants by grazing or diseases; large and fleshy seeds that are difficult to transport; or where samples are not likely to remain viable during transportation due to remoteness of the collecting site from the genebank. Advances in biotechnology provide useful solutions for collecting such problem species (Withers 1995; Engelmann 2011). *In vitro* collecting methods were also developed for a range of other species including oil palm, forage grasses, banana, coffee, grape, *Prunus* and *Citrus* spp. (Withers & Engelmann 1997).

7.6.2 Maintenance of Germplasm

The nature of the germplasm (whole plants, plant tissues, seeds, etc.) that need to be conserved must to be determined prior to implementation of any conservation strategy. Conservation of wild species globally has increased, reflecting a growing interest in securing such material before it is lost, as well as for their potential use in genetic improvement programmes (FAO 2010a). The most common mode of conservation of plant material is via whole plants, seeds and tissue samples. Additionally, isolated DNA

can be maintained at low temperatures (frozen at -80°C) or electronically as sequence data *in silico* (FAO 2010a, 2010b).

Germplasm maintenance methods are vital in any conservation programme, and a suitable maintenance regime that guarantees the survival of the accessions of conservation interest is of utmost importance. The maintenance methodology however will depend largely on the mode of storage of germplasm. In case of maintaining live plant specimens for instance, this can be achieved by a combination of methods involving proper collection management practices and record keeping (Maunder & Byers 2005). During the course of the current study, a handful of accessions have perished, such as the mature single accession of the Subsection *Saxifragoidea*, *R. saxifragoides* grown at the Pukeiti Gardens. Fortunately, germplasm in the form of clonal accessions (raised at Pukeiti Gardens), leaf samples and DNA (held at the Plant and Food Research, Palmerston North) have been secured for further study and re-plantation.

The selection of site for carrying out the *ex situ* conservation need to be strategically chosen, so as to reduce costs, easy access, possibility for expansion, etc. The role of botanic gardens in the conservation of threated plant species has strengthened over the years. They also play now play an important role in implementing the Convention on Biological Diversity (CBD) and other international treaties such as IUCN and CITES. Additionally, the majority of international gardens have committed to the conservation of these species through the Botanic Gardens Conservation International. The added advantage of botanic gardens is the exposure it creates to the general public in raising public awareness on the importance of preserving biodiversity and threatened species. The collections in these botanic gardens have been accumulated over centuries and represent a huge investment in human resources and infrastructure. Also, botanic gardens that do not emphasize plant conservation in their mission program, whether in education or in the *ex situ* conservation of species or habitats, are not adequately responding to the challenges of today's world (Wyse Jackson & Sutherland 2000).

There are several sites in New Zealand where the germplasm could be housed. Ideally, the sites which already have resources and infrastructure in place are more suitable, such as the Pukeiti Gardens. This would reduce the overall costs involved in setting up and long-term maintenance of the germplasm collections. Site selection is also highly

dependent on the mode of germplasm storage. In the case of planting live specimens, the two main factors determining the suitability of a site are: (1) suitable climate and soil conditions, and (2) proximity to resources needed for the maintenance of the germplasm collection. However, it would be difficult to predetermine how a plant would perform in a new site, thus the host site should match the original provenance as closely as possible (Engels & Visser 2003).

In New Zealand several modes for the storage of germplasm are available including botanic gardens, seed banks and gene banks. One of the largest germplasm repositories in New Zealand is the Margot Forde Germplasm Centre[53] (MFGC) based in Palmerston North and managed under AgResearch Ltd. The centre is caters for the New Zealand's national gene bank of grassland plants, the New Zealand Endangered Species Seed Bank and also Australia's gene bank for perennial grasses and legumes. The germplasm held at this centre is publicly available to all breeders and makes the site more desirable (AgResearch 2010). This facility is highly suitable for the storage of vireya (and perhaps other rhododendron) seeds, and is more feasible than setting up a brand new site to cater for vireyas. A duplicate collection should also be held at another international germplasm repository, such as the Kew Millennium Seed Bank (UK).

In terms of botanical gardens in New Zealand, the ideal sites would be Pukeiti Gardens (Taranaki) and the Victoria Esplanade Gardens (Palmerston North), at least for initiating the conservation programme, and also since these two sites have the largest number of recorded vireya accessions. Additional sites discussed in Section 2.2.5 such as the Eden Garden (Auckland) could be included in subsequent stages of the conservation programme, as this garden presently has a basic collection of vireyas in cultivation.

Measures and guidelines that need to be considered for maintenance of germplasm have been suggested for various plant types (medicinal, crop, forage, etc.) for various modes of storage (FAO 2010b; Guerrant et al. 2004; Maunder & Byers 2005; WHO et al. 1993). A consensus of these measures and guidelines that could be applicable for the maintenance of threatened germplasm:

[53] www.agresearch.co.nz/business/services/germplasm-centre/Pages/default.aspx

(i) Propagation.
(ii) Routine maintenance.
(iii) Extensive record keeping.
(iv) Partnership with other collections.
(v) Acquiring additional accessions.
(vi) Assessment of environmental impact.
(vii) Monitoring and evaluation.
(viii) Funding and personnel.

7.6.3 Propagation

Advances in biotechnology techniques have generated new opportunities for genetic resources conservation and utilization. Techniques such as *in vitro* culture and cryopreservation have made it easy to collect and conserve genetic resources, especially of species that are difficult to conserve as seeds (Rao 2004). Cryopreservation of azalea accessions have been recently shown successful and has the potential to be applied to vireyas (Kholina & Voronkova 2008; Van Huylenbroeck & Calsyn 2009).

Propagation of all accessions of conservation interest is very important, as it allows replacement of any perished ones, and to provide material for herbarium collections and scientific studies. Production of sufficient numbers of vegetatively propagated material also would allow the general public to endeavour in growing these threatened taxa. Vegetative propagation is a preferred method, as the material often includes a genotype that has been subjected to a selection process during different stages of its life cycle and thus will be acclimatized to the extant conditions of the facility (usually the botanical garden housing the accessions). When introducing a species to a new environment from seeds it is a good idea to sow them directly to an environment most similar to the wild habitat in order to let selective forces eliminate non-viable seeds and test the acclimatization of the species (Engels & Visser 2003; Govil 1999; Maunder & Byers 2005; Rao 2004).

Where only a few accessions of a selected taxon exist, it is important to make genetically appropriate crossings in order to obtain healthy progeny and to capture any remaining genetic diversity. Molecular methods can be used to monitor the purity of a species and

ensure that hybridization has not taken place within an *ex situ* collection. Hybridization is a risk when plants are grown in botanic gardens often in proximity, and not enough care is taken to avoid it. Tissue culture thus is an invaluable tool for propagating threatened and rare taxa and obtaining disease-free lines (Guerrant et al. 2004).

The two main methods of propagating vireyas are by vegetative methods and seeds. *Rhododendron* propagation via conventional methods has been carried out by utilizing seeds and vegetative methods (Singh et al. 2003). The most common vegetative method is by cuttings, which is a rapid way of increasing plants and retaining the true identity of the taxon. Other vegetative methods include grafting, layering and micropropagation (e.g. tissue culture) (Argent 2006).

7.6.3.1 Vegetative Methods

The study Singh et al. (2003) included the vireya taxon *R. vaccinioides* (the northernmost representative of the vireyas), and showed all the studied taxa could be successfully propagated using the conventional methods suitable for *ex situ* conservation programmes. In the same study, cutting, grafting and layering were examined, and with the improvements in rooting methods, propagation through direct rooting of the cuttings were found effective in several temperate *Rhododendron* species. The tender branchlet cuttings were shown to be more vigorous in root production in comparison to the mature branches. Roots have also been induced in *R. grande* and *R. dalhousiae* using the air-wet technique (Singh et al. 2008).

Layering and air-layering are often the best way for the propagation because this is more rapid and more successful in wet condition and where there is plenty of organic matter that does not dry out readily. The advantage of this method is that the root system of the parent plant is not damaged or disturbed (Singh et al. 2003).

According to Argent (2006), vireya cuttings are very easy to root using semi-ripe nodal cuttings, when the stem is beginning to firm yet still remains flexible, and can be propagated using the following method:

1. **Prepare the cutting** – The size of the cutting is dependent on the size of the taxon, and may be in the range of 1–10 cm long and should include at least two nodes. On larger woodier taxa wounding can be done by slicing away either one or two very thin slivers ~1–2 cm long from the base of the cutting. This provides a larger surfaced area for root initiation by exposing more cambium (Argent & Galloway 2003). Since a large number of vireya taxa exhibit scales on most of the plant parts, a brush could be used to clear the scales from the base of the cutting. Remove all the leaves in the basal half of the cutting and cut the top half off the upper leaves, as this will reduce water loss, make the cuttings less top-heavy and enable more cuttings to be packed into the propagation area. A fungicide could be applied to prevent rotting but not compulsory, however application of a proprietary rooting hormone is advantageous. The cuttings should carry a label at this stage.
2. **Preparation of propagation media** – A very light open acid compost or a mix of 50:50 propagation bark and vermiculite or perlite is required. Bark separates readily when potting on which keeps damage to the delicate roots to a minimum. After insertion of the cuttings into the compost, they are watered in thoroughly with tepid water and ideally placed in a closed case or a mist bench with a basal temperature of approximately 21–25°C together with supplementary lighting. Rooting usually takes place within 8–12 weeks, and larger specimens with thicker stems take longer. An alternative method is placing the cuttings in a pot and enclosing the pot and cuttings in a polythene bag. Place the pot in a partially-sunny area. This method however takes longer to root.
3. **Hardening** – Once rooted, the cuttings should be hardened off for at least two weeks in an open case with reduced basal heat which still gently encourages root formation with supplementary lighting.
4. **Planting** – The hardened rooted cuttings can then be potted into regular vireya mix, taking great care not to damage the fine, delicate root system. After potting, the young plants should be returned to an open case where they should remain on basal heat and supplementary lighting until they show signs of new growth.

7.6.3.2 Seeds

Seed storage methods have been greatly improved in the last few decades, and there has been much research on dormancy breaking and recalcitrance (Guerrant et al. 2004). Growing vireyas from seed, although not difficult, can take up to six years from sowing to flowering, depending on the species. Many growers and collectors have successfully grown vireyas from seed, utilising several methods (Moyles 2001; Rouse 1985).

The following are the steps of a generalized method for raising vireyas from seeds:

1. **Collection of seeds** – The lifespan of vireya seeds is very short, and therefore it is preferable to collect them as soon as they are ripe. Ripening occurs as the capsule starts to split open. The capsules need to be collected and dried for 24 hours at room temperature and the fresh seeds extracted free of chaff (Rouse 1985).
2. **Storage of seeds** – The fresh seeds can be stored at –20°C, and then can be thawed and kept at the +4°C store for a few days before being sown (Rouse 1985).
3. **Preparation of sowing media** – Sterilize the containers and substrate and use boiled or distilled water. Substrates can be any compost such as a fine bark mix or mixed with sphagnum moss and perlite (Rouse 1985).
4. **Sowing of seeds** – Sow the seeds evenly and settle them on the surface by spraying with a fine mist. Place a glass lid on top, which creates a microclimate in which the seeds will not dry out (Argent 2006).
5. **Germination of seeds** – The temperature range for germinating seeds should be ideally 15–30°C. The relative humidity should be above 90% and there must be adequate water for imbibition. Although air is needed for germination, vireya seeds germinated after three weeks without air and the seedlings develop satisfactorily under water for a further three weeks. Lighting should be kept at a moderate level as very low and very high lighting conditions have adverse effects on germination. Vireya seeds take about three weeks after sowing for the cotyledons to appear, and occasionally can take as long as 5–6 weeks (Moyles 2001; Rouse 1985).

6. **Caring for the seedlings** – Once the cotyledons appear, the seedlings should be ventilated to reduce the relative humidity (Rouse 1985). Once the first leaves have developed remove the glass lids to avoid damping off. Once seedlings begin to grow lift them out into seeds trays or individual pots containing a vireya potting mix, and place them under the lights to avoid long etiolated seedlings (Argent 2006).
7. **Planting** – Once the seedlings have reached approximately 2–3 cm they can be potted in individual pots. Vireya seedlings can be quite slow to develop initially and can benefit from regular weak feeds (Argent 2006).

Presently, vireya seeds can be easily obtained from various collections worldwide. For example the Rhododendron Species Foundation (USA) conducts a very successful seed exchange program, through which additional accessions of existing taxa or accessions of new taxa can be added to existing collections (Rhododendron Species Foundation (RSF) 2012).

7.6.3.3 Pollen

Propagation from pollen is common in vireyas, especially in the production of hybrids for the horticultural trade. Pollen normally survives only a few days at ambient temperatures, but when dehydrated and frozen can be stored for pollination at any season of the year (Rouse 1984; Williams & Rouse 1987).

7.6.3.4 Micro-propagation

Micro-propagation is the practice of rapidly multiplying stock plant material to produce a large number of progeny plants, using modern plant tissue culture methods. Tissue culture has now become routine in commercial and research applications, and is an invaluable tool for propagating rare species and obtaining disease-free lines (Debergh & Zimmerman 1990; Fay 1992; Guerrant et al. 2004; Rao 2004; Sarasan et al. 2006). The *in vitro* micro-propagation through meristem culture has become one of the most economical and viable method for clonal propagation of rhododendrons and have been shown to be successful in several studies on temperate *Rhododendron* taxa (Gurung & Singh 2010; Mao et al. 2011; Singh 2008).

Very few studies using micro-propagation on vireyas have been carried out (Iapichino et al. 1991). Iapichino et al. (1991) demonstrated that that adventitious shoot regeneration from callus induced at wound sites of shoot tip explants is the most efficient plant propagation method for the vireya hybrid *R. laetum* × *aurigeranum*. In this study, plant regeneration was also undertaken using leaf strips of commercial *Rhododendron* cultivars.

7.6.3.5 Routine Maintenance

Routine maintenance includes the feeding, watering, dead-heading (unless seeds are collected), environmental control and monitoring, maintenance of proper labelling and pest control. Maintenance also should extend to collections of herbarium samples, seeds, pollen and DNA. These methods will require additional maintenance regimes suitable for them; however the physical space required is much lower than for the living collections. Cryogenic storage of seeds and DNA is becoming more popular and the costs of maintaining have become significantly low to become an alternative choice (Maunder & Byers 2005; Van Huylenbroeck & Calsyn 2009).

7.6.3.6 Extensive Record Keeping

Extensive record keeping needs to take higher priority as they will facilitate the formation of collections suitable for *ex situ* conservation. The retention of previous nomenclature changes for example need to be established in these collections (Ashton 1988; Furman et al. 2009; Maunder & Byers 2005). The best method of achieving this is by maintaining reliable passport information which is essential for record keeping, along with other information such as the conditions in which the accessions would likely adapt to and discrete morphological data of the accessions (Furman et al. 2009).

Another neglected area is the maintenance of herbarium samples of vireyas in New Zealand collections. Herbarium records will serve as a reference for future studies and to ascertain the identity of the accessions held. An extensive set of herbarium samples were collected for this study, and some of these have been already deposited at the national Te Papa Museum. More herbarium samples will be deposited at the conclusion of this study.

A duplicate set of herbarium samples of vireyas is also in progress to be held at Massey University herbarium.

7.6.3.7 Partnership with Other Collections

Collections must maintain strong partnerships between other similar collections, as this will have mutual benefits in the cultivation and exchange of plant material. The establishment of a network of all the involved collections will enable the rapid communication between them and exchange of relevant knowledge. Good record keeping and collaboration between the collections is thus essential to achieve this (Maunder & Byers 2005).

The loss of valuable genetic resources is a global concern and requires rapid international action. To combat loss of plant genetic resources many countries have initiated programmes and established genebanks for food and agriculture (Jaramillo & Baena 2007). The concept of germplasm conservation requires that the collection strategies used are successful in capturing maximum variation and subsequently, conservation and regeneration techniques employed to minimize losses through time (Astley 1992). To accomplish this, conservation activities for plant genetic resources comprise of collecting, conservation and management, identification of potentially valuable material by characterization, and evaluation for subsequent use. The recent advances in biotechnology such as *in vitro* culture techniques and molecular biology offer some valuable tools for improved conservation and management of plant genetic resources more effectively (Ramanatha Rao & Riley 1994).

7.6.3.8 Acquiring Additional Accessions

Further collection in the wild, though important, should be included in any conservation plan. This will increase the genetic diversity of the collections and thus expands the germplasm extensively for conservation exercises such as re-introduction into the wild. The importation of new plant material to New Zealand is highly restricted and costly, but importation of taxa for which accessions are already in New Zealand is relatively easy. This also highlights the importance of establishing a comprehensive database of vireya taxa currently cultivated in New Zealand (Maunder & Byers 2005).

Private and public collections will therefore be the source of additional germplasm for any vireya collection. In New Zealand, vireyas are commonly grown both in the North and the South island, and are becoming popular garden (or conservatory) plants. The exchange of accessions between the major collections is also important, as it will increase the genetic diversity of all the collections involved (Maunder & Byers 2005).

The number of accessions to be held at any location is debatable, but has several constraints such as the availability of physical space, other related resources and funding. However, the larger the number of genetically diverse accessions the more complete and useful the collections become. In case of crops, the suitable number of genetic accessions that need to be collected per population usually varies between 50 and 200 (Marshall & Brown 1975). If collecting seeds this usually means a total of about 5,000 accessions (ENSCONET 2009a). For threatened species with limited distribution, the numbers could be significantly lower, and numbers in the range of 2–10 are reasonable (Maunder & Byers 2005).

Since New Zealand allows germplasm of taxa already in the country to be re-imported, future collection expeditions to vireya provenances need to be investigated. Also, germplasm held at other repositories outside New Zealand could be imported if the taxa are already in cultivation in New Zealand (Ministry for Primary Industries 2012).

7.6.3.9 Assessment of Environmental Impact

Vireyas being exotic plants to New Zealand needs to be evaluated for the potential of becoming pests, which could harm the local flora and fauna. At present there are no records of vireyas having escaped from cultivation, and the plants being of tropical origin the risk of establishing them in the cooler New Zealand climate is limited. Garden hybrids are often grown outside, especially in the North Island and to-date there are no reported cases of them having escaped from cultivation.

Some taxa of vireyas are known to be poisonous to livestock, but this has no impact on New Zealand livestock as the vireyas at presently are mainly grown in enclosed spaces. There are however several benefits of vireyas in cultivation as they have been seen to attract several species of local birds and insects, attracted by the nectar (Argent 2006).

Diseases of vireyas are known but presently there is limited amount of literature on diseases and pathogens of vireyas in New Zealand. The restricted importation of new plant material also has reduced the risk of importation of new pathogens to the cultivated vireyas in New Zealand (Ministry for Primary Industries 2012).

7.6.3.10 Monitoring and Evaluation

Once the taxa of conservation interest have been established in a collection, comprehensive monitoring and evaluation of the accessions need to be carried out. This will allow maintaining the numbers and the health of accessions at an acceptable standard. The germplasm need to be routinely evaluated to maintain viability and utilization in future conservation programmes (Maunder & Byers 2005).

7.6.3.11 Funding and Personnel

The major limitation of maintenance of a plant collection is often funding, and this ultimately leads to limitations on personnel available for the maintenance of the collections. Established collections have the advantage of attracting funds compared to less established ones. Funds can originate from public and private sectors, in addition to fund-raising activities carried out by the collections themselves. Popular fund-raising activities include entry fees, sale of plants and guided tours (Maunder & Byers 2005).

Where limited funds are available to maintain the facilities and personnel at collections, volunteers can also be sought. Volunteers play a huge role in large collections worldwide and have mutual benefits to both the parties involved (Maunder & Byers 2005). Funding also need to be sought for the costs involved in maintenance of germplasm using modes other than botanic gardens, such as seed stores and gene banks. By using existing infrastructure, the costs can be significantly reduced in the long-term.

7.7 Recommendations for Future Work

The conservation plan outlined in this chapter highlights the opportunities available and challenges faced with the conservation of vireyas in *ex situ* collections in New Zealand. The strict adherence to the conservation plan will reduce numerous pitfalls associated

with taxon selection and resource allocation. The conservation plan also highlights the need to carry out further research on establishing additional collections in more central locations in New Zealand such as Auckland and Wellington. The plan also stresses the need to establish robust assessment methodologies such as molecular analyses leading to the proper selection of taxa and accessions contributing to the *ex situ* conservation.

Suitable germplasm repositories for vireyas have been identified and include Pukeiti Gardens and the Victoria Esplanade Gardens (for live plants). The availability of these existing facilities makes the New Zealand and the vireya collections in New Zealand ideal for contributing towards the global *ex situ* conservation.

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Chapter 8

Conclusions

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8 Conclusions

The research outlined in this study aim to explore and understand the taxonomic and conservation issues related to vireyas. One of the main outcomes expected of this study is a plan for the *ex situ* conservation of vireyas in New Zealand collections. The first chapter provides an overview of the study, by laying out the aims and objectives of the study. The second chapter is a comprehensive review of the systematics of the genus *Rhododendron* and the vireyas leading up to the most recent systematics studies on this plant group. The chapter discussed both the classical and the modern molecular methods used in vireya systematics and their impact on the current understanding of this diverse plant group. Also discussed in the chapter are conservation of biodiversity and methodology used in the measurement of biodiversity. The third chapter describes in detail the materials and various research methods employed this study. The fourth chapter describes the results and analysis of the work carried out in the third chapter. The fifth chapter discusses in detail the findings of the results and analysis in the fourth chapter. The sixth chapter combines the findings of this research to build a plan for the *ex situ* conservation of vireyas in New Zealand collections.

This study also proposed sectional arrangement and an insight into the evolutionary history of several species complexes of Subgenus *Vireya* Argent. The study also provided systematic investigations using models of evolution leading to more probable representation of the evolutionary history of the sequences which generated well-supported phylogenetic hypotheses. The new inference of phylogenetic relationships based on molecular data from the nuclear genome, specifically the *rpb*2i gene is more robust. A five subsectional arrangement has been proposed to replace the previous seven subsectional arrangements of Sleumer (1966a) and seven sectional arrangements of Argent (2006) that were based on morphological characters (see Table 1 for a comparison of these two classical taxonomic systems). However, further studies are still required to determine the relationships within the Section *Euvireya*.

The proposed phylogeny is broadly consistent with the recent classifications presented by Goetsch et al. (2011) and Craven et al. (2011), which differs in having *Discovireya* sister

Pseudovireya. The current study however proposes that *Pseudovireya* is sister to *Discovireya*, which appears to be more evolved and thus more probable.

Section *Pseudovireya* is shown to be paraphyletic consisting of a few subclusters that correspond to separate geographic regions. One lineage of the vireyas 'expanded' eastwards to Taiwan, while the other expanded southwards through the Malay Peninsula to New Guinea becoming the bulk of the vireyas and the Section *Euvireya*. Despite the taxa of Section *Discovireya* having a wide distribution in Malesia, they are genetically very closely related to each other forming a well-supported clade. Section *Discovireya* can be treated as monophyletic if the taxon *R. perakense* is excluded.

The sections *Siphonovireya*, *Phaeovireya*, *Malayovireya*, *Albovireya* and *Euvireya* (including the subsections) were not recovered in this analysis as monophyletic groups. However, it is interesting to note that numerous taxa belonging to the Subsection *Solenovireya* appear in the basal clades of the core vireyas, suggesting a probable group representing the traditional Subsection *Solenovireya* proposed by Sleumer (1966a). Overall the core vireyas seem to cluster corresponding to geographic origin and supports the out of Asia hypothesis for the evolution of the vireyas.

The traditional arrangement of the subsections within *Euvireya was* not fully resolved in this study, and these subsections appear to be paraphyletic and polyphyletic. Lack of support for the monophyly of Section *Euvireya* leaves the important question regarding its taxonomic status unanswered. Lack of resolution in part may be due to incomplete lineage sorting[54] of ancestral polymorphisms among recently diverged species. However the *rpb*2i intron region examined did distinguish at sectional level the sections *Pseudovireya* and *Discovireya*.

The phylogenetic analyses in this study resolved several taxonomic issues related to the vireyas, in particular those related to species of conservation interest. Higher level relationships are more apparent than lower ones, and more data may be necessary to resolve finer and intricate taxonomic issues of closely related taxa, such as the taxa within Section *Euvireya*. This study confirmed the monophyly of several species of conservation

[54] Due to populations going their separate ways carrying genetic diversity with them.

interest with taxonomic issues, thus supporting their eligibility for receiving conservation attention. The single *rpb*2i intron region used for this study was also able to distinguish between subspecies especially that of *R. jasminiflorum*, for which the two subspecies *jasminiflorum* and *oblongifolium* was segregated.

The phylogenetic analyses using the *rpb*2i intron 23F–24R sequence data resolved numerous taxonomic issues, some of which were applicable to conservation. Some taxonomic issues related to *R. jasminiflorum*, *R. superbum* and *R. konori* for example have been greatly resolved.

There are still pending taxonomic issues which need resolution and need to be further studied, possibly with additional data. This study focussed on a single intron of the *rpb*2i gene, instead of all the introns of the gene. Sequence data from the *rpb*2i combined with other nuclear regions such as ITS and *mat*K will further resolve many of the outstanding taxonomic issues. Comparison of this study with those of Goetsch et al. (2011) and Craven et al. (2011) for example demonstrates that different parts of the genome reveal different aspects of species relationships and stress the importance of using multiple genes in future for the reconstruction of phylogenies and taxonomic relationships.

The application of microsatellites in determining the genetic diversity of selected taxa and taxa complexes showed that several of them have relatively high genetic diversity suitable for conservation planning. The major limitation during the genetic diversity analyses was the low number of accessions available for the taxa studied. Further study on the phylogenetics of the vireyas will contribute towards future genetic diversity studies.

Overall the study showed that genetic differentiation between accessions of the taxa can be determined using microsatellites and the methods outlined. The genetic differentiation shown is not always very large, and this is due to the limited number of accessions available for the study. A larger selection of accessions and more specific microsatellite markers could be used to improve these results. The microsatellite markers used in this study were originally designed for temperate rhododendrons.

The combination of phylogenetic and genetic diversity analyses in this study shows that the outlined methods can be used in taxonomically complex plant groups such as vireyas. The phylogenetic analyses resolved several taxonomic issues among vireya taxa and these results combined with the results of genetic diversity analyses provided selection and prioritization of taxa and their constituent accessions for conservation. A total of 30 taxa have been identified as suitable candidates for *ex situ* conservation in New Zealand of which 17 are threatened (with IUCN categories VU, EN and CR). The remaining 13 taxa consist of 11 Data Deficient (DD) and two of Least Concern (LC). There are further taxa suitable with conservation potential cultivated in New Zealand and further research is needed to determine the extent of their distribution in New Zealand. These taxa and their representative accessions need to be studied using molecular methods outlined in this research to determine their taxonomic status and conservation potential.

8.1 Future Research

Robust phylogenies are crucial in answering many of the taxonomic and conservation issues outlined in the previous chapters. These need to incorporate more extensive sampling than was possible here, however, practical and financial limitations are likely to prohibit the sampling necessary to achieve this. However, the sectional and subsectional groupings outlined in this study provide an opportunity to target likely clades of vireyas. The drawback of utilizing limited number of accessions is that sufficient genetic variability within the taxon is not revealed. Another limitation is the limitation on the molecular markers used, for both phylogenetic and genetic diversity analyses. This study utilized an approximately 1 kb long nucleotide sequence obtained from a single intron region which was insufficient for finer resolution within the sectional groupings. Sequences from multiple intron regions and multiple genes may provide better resolution of the taxa within the groupings. Using DNA of higher quality and cleaning of the PCR products prior to sequencing would also increase the accuracy in base-calling.

The results of this study also highlighted the need for an integrated approach to further studies on vireyas and also in the management of their collections. It is clear that some of the hypotheses regarding sectional and subsectional relationships of vireyas based on morphology are not supported and taxonomic revisions are warranted. One particular area of research should be focussed at the basal vireyas (*Pseudovireya*) in which a larger

representation of this group is needed. Such a study should include a substantial representation of the Subgenus *Rhododendron*, and especially Subsection *Cinnabarina* on the basis of morphological similarities.

8.2 Summary

This study used a relatively novel approach for alignment, analysis and presentation of sequence data. The monophyly of Subgenus *Vireya* is strongly supported based on the molecular sequence data. The taxa sampled formed well-supported, monophyletic groups within the Subgenus *Vireya* relative to the outgroup. Some of the hypotheses regarding sectional and subsectional level relationships based on morphology were not supported and taxonomic revisions are warranted.

Overall, the phylogenetic study presented here has contributed to our understanding of vireya systematics. Also, this study is presently one of the largest molecular analyses of the vireyas to date and thus creates a baseline for future genetic work on vireyas.

Genetic diversity study showed that several taxa or taxa complexes showed significant genetic diversity sufficient for them to be conserved. The method employed in the genetic diversity study can be further extended to other conservation taxa and perhaps other plant groups of conservation interest.

This research highlighted the importance of employing more than one molecular marker system in making taxonomic or conservation decisions. The research also highlighted that results of molecular analyses should integrate other sources of information such as morphological characteristics in making taxonomic deductions leading to conservation decisions.

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Glossary

clade – a group consisting of a taxon and all its descendants.

Consistency Index (CI) – this is a measure of the relative amount of homoplasy in a phylogenetic tree, and assesses the level of difficulty in fitting a given data set to a given tree; Consistency Index is defined as, $CI = \frac{m}{s}$, where m is the minimum possible parsimony steps on a phylogenetic tree (number of character states – 1) and s is the actual number of reconstructed steps.

choripetalous *adj*. – having separate petals.

homology *noun* (**homologous** *adj*.) – similarity due to common evolutionary origin, i.e. derived from the same ancestral character.

homoplasy *noun* (**homoplasious** *adj*.) – describes characteristics of an organ that are shared by different species because of shared evolution.

log likelihood – a test based on the likelihood ratio, which expresses how many times more likely the data are under one model than the other (i.e. comparing the fit of two models).

***mat*K** – a chloroplast gene coding for the protein maturase k, ~1,500 base pairs in length, and located within the intron of the chloroplast gene *trn*K, on the large single-copy section adjacent to the inverted repeat.

monophyletic *adj*. **(monophyly** *noun*) – a group of organisms which forms a clade that includes all of the descendants of a single common ancestor.

Nested clade analysis (NCA) – a flexible and powerful method to study the phylogeography of species and populations, implemented in the software GEODIS.

outgroup – a taxon (or group of taxa) used to help resolve the polarity of characters (by assigning as the root, in rooted trees), and which is hypothesised to be less closely related to each of the taxa studied than any are to each other.

paraphyletic *adj*. (**paraphyly** *noun*) – a group in phylogenetics that do not include all of the descendants of a single common ancestor.

phylogeny *noun* (**phylogenetic** *adj*.) – the unique historical relationship as a result of evolution, represented as a phylogenetic tree (or cladogram).

phyllotaxy *noun* –the arrangement of leaves on a plant stem.

plesiomorphic *adj*. – in cladistics, an ancestral or primitive character, or generalized characteristics that arose early in the evolutionary history of a taxonomic group.

polyphyletic *adj*. (**polyphyly** *noun*)

Retention Index (RI) – measures the proportion of synapomorphy expected from a data set that is retained as synapomorphy on a phylogenetic tree (i.e. a measure of the proportion of similarities on a phylogenetic tree).

sympetalous *adj*. (**sympetaly** *noun*) – the condition of some flowers in which the petals are fused together (or **connate**).

synapomorphy *noun* (synapomorphic *adj*.) –a trait that is shared by two or more taxa and their most recent common ancestor, whose own ancestor in turn does not possess the trait.

tree length – the total number of character state changes necessary to support the relationship of the configurations in a phylogenetic tree.

***trn*L–*trn*F** – a region located in the large single-copy region of the chloroplast genome and consists of the *trn*L gene, a group I intron, and the *trn*L–F intergenic spacer.

Appendices

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A1 Accessions of taxa selected for the study

Table 30 Accessions of taxa collected for the study. (Key: DNA – DNA isolated; SEQ – DNA Sequenced; SSR – Analysed with microsatellites; RAPD – Analysed with RAPDs).

OUTGROUP TAXA

Subgenus *Azaleastrum* Section *Azaleastrum* Subsection *Tsutsusi*

#	Taxon	Location	PFR #	Accession #	Origin (Collector)	DNA	Seq	SSR	RAPD
1	*R. leptothrium*	Esplanade	EI151			✓			

Subgenus *Rhododendron* Section *Rhododendron* Subsection *Maddenia*

#	Taxon	Location	PFR #	Accession #	Origin (Collector)	DNA	Seq	SSR	RAPD
2	*R. lindleyi*	Pukeiti	EK631			✓			
3	*R. maddeni* ssp. *maddeni* (*crassum*)	Pukeiti	EK672			✓			
4	*R. maddeni* ssp. *maddeni*	Pukeiti	EK673			✓			
5	*R. maddeni* ssp. *maddeni* (*odoriferum*)	Pukeiti	EK674			✓			

INGROUP TAXA

Subgenus *Vireya*

Section *Pseudovireya*

#	Taxon	Location	PFR #	Accession #	Origin (Collector)	DNA	Seq	SSR	RAPD
6	*R. emarginatum*	Pukeiti	HF050	S1998111	N Vietnam (Jury AC446)	✓	✓		
7	*R. kawakamii*	Pukeiti	HF059	S1973392	(RSF USA 73152)	✓		✓	

8	*R. kawakamii*	Pukeiti	HF072			✓	✓	✓	
9	*R. rushforthii*	Pukeiti	HF147	S2001118	(Binney; RHS seed)	✓	✓	✓	
10	*R. santapaui*	Pukeiti	EK581	S2001136	(Binney)	✓	✓	✓	

Section *Discovireya*

#	Taxon	Location	PFR #	Accession #	Origin (Collector)	DNA	Seq	SSR	RAPD
11	*R. adinophyllum*	Pukeiti	EK602	S2001110	Gunung Keniri, Sumatra (Binney)	✓	✓		
12	*R. borneense* ssp. *borneense*	Pukeiti	EK554	S1989387A	Bukit Lumut, Sarawak, Borneo (K Adams A1489)	✓			
13	*R. borneense* ssp. *villosum*	Pukeiti	EK521	S1990387B	Mt Kinabalu, Borneo (Blumhardt; cuttings)	✓			
14	*R. borneense* ssp. *villosum*	Pukeiti	EK532	S1990387B	Mt Kinabalu, Borneo (Binney)	✓			
15	*R. ericoides*	Pukeiti	EK537	S1997138	Borneo	✓		✓	
16	*R. perakense*	Pukeiti	EK553	S1990273	Gunung Brinchang, Cameron Highlands, Malaysia (K Adams A7909; cuttings)	✓		✓	
17	*R. perakense*	Pukeiti	HF026	S1990273	Borneo	✓		✓	
18	*R. quadrasianum*	Esplanade	EI143			✓			
19	*R. quadrasianum*	Esplanade	EI144			✓			
20	*R. quadrasianum*	Esplanade	EI145			✓			
21	*R. quadrasianum*	Esplanade	EK516			✓			
22	*R. quadrasianum*	Esplanade	EK517			✓		✓	
23	*R. quadrasianum*	Esplanade	EK518			✓			
24	*R. quadrasianum* var. *malindangense*	Pukeiti	EK663	S2008107	(Currie 2000)	✓		✓	
25	*R. quadrasianum* var. *rosmarinifolium*	Pukeiti	EK662	S2008131	(Currie 2002)	✓		✓	
26	*R. retusum* var. *retusum*	Pukeiti	EK571			✓		✓	
27	*R. retusum* (?)	Pukeiti	EK675			✓		✓	

Section *Malayovireya*

#	Taxon	Location	PFR #	Accession #	Origin (Collector)	DNA	Seq	SSR	RAPD
28	*R. fallacinum* (Mt Trus Madi form)	Pukeiti	EK527	S1998100	Trus Madi, Sabah, Borneo (Binney)	✓		✓	
29	*R. fallacinum*	Pukeiti	EK531	S1989178B	Borneo (Blumhardt)	✓		✓	
30	*R. fallacinum*	Pukeiti	EK582	S2001137		✓		✓	
31	*R. himantodes* var. *himantodes*	Pukeiti	EK535	S1998107	Borneo	✓			
32	*R. malayanum* var. *malayanum* *f. malayanum*	Pukeiti	EK555	S1990241A	Gunung Bunga Buah, Genting Highlands, Malaysia (K Adams A79013; cuttings)	✓		✓	
33	*R. micromalayanum*	Pukeiti	EK542	S1992397	Borneo (K Adams A02109; cuttings)	✓		✓	

Section *Siphonovireya*

#	Taxon	Location	PFR #	Accession #	Origin (Collector)	DNA	Seq	SSR	RAPD
34	*R. herzogii*	Pukeiti	EK622	S1986197	Mt Gahavsukaar PNG. Smith GFS10713. Cuttings	✓			
35	*R. herzogii* (Mt Yakananda form)	Pukeiti	EK639	S1983197	Mt Yakananda, PNG (G Smith GFS111/10)	✓	✓	✓	
36	*R. inundatum*	Pukeiti	EK654	S2008161	(051200)	✓		✓	
37	*R. inundatum*	Pukeiti	HF042	S2005192	(Currie JA9B)	✓		✓	
38	*R. inundatum*	Pukeiti	HF102		(K Adams)	✓			
39	*R. searleanum*	Pukeiti	HF038	S2008170	(Currie ex Binney 2000 BI0100)	✓		✓	

Section *Phaeovireya*

#	Taxon	Location	PFR #	Accession #	Origin (Collector)	DNA	Seq	SSR	RAPD
40	*R. asperum*	Pukeiti	EK666	S2008133	(Currie 1999)	✓	✓	✓	
41	*R. dielsianum* (labelled *R. bryophilum*)	Esplanade	EK502			✓			
42	*R. dielsianum* (labelled *R. bryophilum*)	Pukeiti	EK649	S2008164	(Currie ex Jury JU0599)	✓	✓	✓	

43	*R. caliginis*	Pukeiti	EK638			✓	✓		
44	*R. caliginis* (Mt Miap form)	Pukeiti	HF020	S1986391	Mt Miap, PNG, 2,800 m, 21/08/1986(G Smith GFS04621; cuttings)	✓			
45	*R. superbum* (labelled *R. dianthosmum*)	Pukeiti	EK565	S1989169	PNG (Blumhardt)	✓	✓	✓	
46	*R. dielsianum*	Pukeiti	HF023			✓	✓	✓	
47	*R. gardenia* 'Odyssey'	Esplanade	EI148			✓			
48	*R. gardenia* 'Odyssey'	Esplanade	EI169			✓	✓		
49	*R. gardenia* 'Odyssey'	Pukeiti	HF012	S1990184	(Blumhardt; via Australia)	✓	✓	✓	
50	*R. hellwigii*	Pukeiti	HF004	S1976195	Mts Finisterre, PNG (P Kores; seed; collected as *R. superbum*)	✓	✓	✓	
51	*R. konori* (white form)	Pukeiti	EK619	S1979215C	W Irian (J Rouse)	✓			✓
52	*R. konori* (Kasenombi form)	Pukeiti	HF001	S2005110	Kasenombi (Currie 2001)	✓	✓		
53	*R. superbum*	Pukeiti	HF010			✓		✓	
54	*R. konori*	Esplanade	EI187			✓		✓	
55	*R. konori*	Esplanade	EI188			✓			
56	*R. leptanthum* (Syn: *R. warianum*)	Pukeiti	EK670	S2008121	(Currie 2005)	✓		✓	
57	*R. phaeochitum*	Pukeiti	HF019	S1979275A	PNG	✓		✓	
58	*R. phaeochitum* (*aff. R. scabridibracteum*)	Pukeiti	HF022	S1979376	(J Rouse; collected as *R. scabridibracteum*)	✓		✓	
59	*R. rarum*	Pukeiti	EK618	S1976305	(E Boswell; cutting)	✓	✓		✓
60	*R. rarum* (hybrid?)	Pukeiti	EK655	S2008130	(Currie 120999)	✓			✓
61	*R. solitarium*	Pukeiti	HF089			✓		✓	
62	*R. solitarium*	Pukeiti	EK614	S1983338	Mt Kaindi, PNG (G Smith GS6983; cutting)	✓	✓	✓	✓
63	*R. solitarium* (Bulldog Rd form)	Pukeiti	EK617	S1976338	Bulldog Rd, PNG (P Kores K10376; cuttings)	✓	✓	✓	✓
64	*R. superbum* ssp. *superbum*	Pukeiti	EK616	S1983352	Mt Miap, PNG, 2,800 m (G Smith GS2983; seedling)	✓	✓	✓	✓

65	*R. konori* (labelled *R. superbum*)	Pukeiti	EK651	S2008162	(Currie 1989)	✓	✓	✓	✓
66	*R. superbum* ssp. *superbum*	Pukeiti	HF006			✓		✓	
67	*R. superbum* ssp. *superbum*	Pukeiti	HF046			✓		✓	
68	*R. superbum* (labelled *R. hyacinthosmum*)	Pukeiti	EK588	S2005188	(Currie 2000)	✓	✓	✓	
69	*aff. R. superbum* (Edie Creek form) (labelled *R. konori*)	Pukeiti	EK613	S1976215	Edie Creek, PNG (P Kores K11576; cuttings)	✓		✓	✓
70	*R. truncicola*	Pukeiti	HF028	S2008173	(Currie 2001 ex Blumhardt)	✓		✓	

Section *Albovireya*

#	Taxon	Location	PFR #	Accession #	Origin (Collector)	DNA	Seq	SSR	RAPD
71	*R. album*	Pukeiti	EK570	S2008122	Indonesia (Currie)	✓		✓	
72	*R. lagunculicarpum* (labelled *R. arenicola*)	Pukeiti	EK573	S2001131	(Binney)	✓		✓	
73	*R. yelliotii*	Esplanade	EI164			✓		✓	
74	*R. zollingeri*	Pukeiti	EK530	S1998103	Sulawesi (Binney)	✓			
75	*R. zollingeri*	Pukeiti	EK601	S2001111	(Binney)	✓		✓	
76	*R. zollingeri*	Keith Adams[55]	HF097		(K Adams)	✓	✓	✓	

Section *Euvireya* Subsection *Linnaeopsis*

#	Taxon	Location	PFR #	Accession #	Origin (Collector)	DNA	Seq	SSR	RAPD
77	*R. gracilentum*	Pukeiti	EK576	S1986190C		✓		✓	
78	*R. gracilentum*	Pukeiti	EK603	S1986190C		✓		✓	✓
79	*R. gracilentum*	Pukeiti	EK635	S1976190	(E Boswell; cutting)	✓	✓	✓	
80	*R. gracilentum*	Pukeiti	HF076	S2008138	(Currie 260400)	✓		✓	
81	*R. gracilentum* (Mt Miap form)	Pukeiti	EK621	1986190C	Mt Miap, PNG (G Smith GS05121)	✓		✓	✓

[55] A private collection of vireyas in New Plymouth.

82	*R. rubineiflorum*	Pukeiti	EK524	S1986389	Kaiap Orchid Lodge Wabag, Wabag, PNG, 2,300 m (G Smith GS05722)	✓			
83	*R. womersleyi*	Pukeiti	HF052	S1983378	Mt Giluwe, PNG (G Smith GS33083)	✓			
Section *Euvireya* Subsection *Saxifragoidea*									
#	**Taxon**	**Location**	**PFR #**	**Accession #**	**Origin (Collector)**	**DNA**	**Seq**	**SSR**	**RAPD**
84	*R. saxifragoides*	Pukeiti	EK541	S19862325	PNG	✓			
Section *Euvireya* Subsection *Solenovireya*									
#	**Taxon**	**Location**	**PFR #**	**Accession #**	**Origin (Collector)**	**DNA**	**Seq**	**SSR**	**RAPD**
85	*R. alborugosum*	Pukeiti	EK536	S1998106	Borneo (Binney)	✓	✓		
86	*R. archboldianum* (labelled *R.* 'Starburst')	Pukeiti	HF002	H1986506	Mt Gahavisukar, PNG, 2,800 m (G Smith 1986)	✓		✓	
87	*R. archboldianum* (labelled *R.* 'Starburst')	Pukeiti	HF003			✓		✓	
88	*R. armitii*	Pukeiti	HF032	S2008156	(Currie 1999 ex D Brown)	✓	✓	✓	
89	*R. carringtoniae*	Pukeiti	EK626	S2008166	(Currie)	✓	✓		
90	*R. cruttwellii*	Esplanade	EK504	(EI193)		✓			
91	*R. cruttwellii*	Esplanade	EK505	(EI194)		✓			
92	*R. cruttwellii*	Esplanade	EK506			✓			
93	*R. cruttwellii*	Pukeiti	HF016	S2008103	(possibly Currie)	✓	✓	✓	
94	*R. cruttwellii*	Pukekura	HF084			✓		✓	
95	*R. cruttwellii*	Pukekura	HF095			✓		✓	
96	*R. edanoi* ssp. *pneumonanthum*	Pukeiti	EK549	S1987281	Gunung Murud, Borneo, 7,000 ft (K Adams1982)	✓			
97	*R. goodenoughii*	Esplanade	EI146			✓			
98	*R. goodenoughii*	Esplanade	EI170			✓			
99	*R. goodenoughii*	Esplanade	EI171			✓			

100	*R. goodenoughii*	Esplanade	EI172			✓			
101	*R. goodenoughii*	Pukeiti	EK611	H1987189	(Blumhardt)	✓		✓	
102	*R. jasminiflorum* ssp. *jasminiflorum*	Pukeiti	EK548	S1986209	Gunung Bunga Buah, Genting Highlands, Peninsular Malaysia (K Adams 1986; cuttings)	✓	✓	✓	✓
103	*R. jasminiflorum* ssp. *oblongifolium*	Pukeiti	EK645	S2008125	Gunong Berumput, Borneo [border Sarawak/Kalimantan] (Currie ex Binney)	✓	✓	✓	✓
104	*R. jasminiflorum* ssp. *jasminiflorum*	Esplanade	EI135			✓			
105	*R. jasminiflorum* ssp. *jasminiflorum*	Esplanade	EI136			✓			
106	*R. jasminiflorum* ssp. *jasminiflorum*	Esplanade	EI137			✓			
107	*R. jasminiflorum* ssp. *jasminiflorum*	Esplanade	EI153			✓	✓	✓	✓
108	*R. jasminiflorum* ssp. *jasminiflorum*	Esplanade	EI154			✓		✓	✓
109	*R. jasminiflorum* ssp. *jasminiflorum*	Esplanade	EI155			✓			
110	*R. jasminiflorum* ssp. *jasminiflorum*	Pukeiti	EK612			✓		✓	✓
111	*R. jasminiflorum* ssp. *jasminiflorum* (labelled *R. jasminiflorum* ssp. *punctatum*)	Pukeiti	EK656	S2008167	(Currie1995; KI 1295)	✓		✓	✓
112	*R. jasminiflorum* ssp. *oblongifolium*	Pukeiti	EK590	S2001105	Gunung Penrissen, Sarawak, Borneo (Binney)	✓	✓	✓	✓
113	*R. jasminiflorum* 'X' (hybrid)	Pukeiti	HF139			✓	✓	✓	
114	*R. loranthiflorum*	Esplanade	EI140			✓			
115	*R. loranthiflorum*	Esplanade	EK501			✓			
116	*R. loranthiflorum* (labelled as *R. loranthiflorum* Sri Chinmoy 140306)	Pukeiti	HF044	S2008120	(Currie; seed raised)	✓			
117	*R. loranthiflorum*	Pukeiti	HF090			✓	✓	✓	
118	*R. loranthiflorum*	Pukeiti	EK515		Solomon Is	✓			
119	*R. loranthiflorum*	Pukeiti	HF058	S1985234	(H Greer, USA)	✓			
120	*R. majus*	Esplanade	EI150			✓			

121	*R. majus*	Esplanade	EI157			✓			✓
122	*R. majus*	Esplanade	EI158			✓	✓		✓
123	*R. majus*	Esplanade	EI159			✓			
124	*R. majus*	Pukeiti	EK657	S2008140	(Currie ex Binney 2003; B10103)	✓	✓		✓
125	*R. baenitzianum* (labelled as *R. majus*)	Pukeiti	EK658	S2008127	(Currie)	✓	✓		✓
126	*R. majus*	Ex Jury	HF087			✓		✓	
127	*R. majus*	Ex Jury	HF088			✓		✓	
128	*R. multinervium* (ID by Andrew, Pukeiti)	Pukeiti	HF015			✓		✓	
129	*R. pleianthum*	Pukeiti	EK566	S1983251	PNG	✓			
130	*R. pleianthum*	Pukeiti	HF036	S2008148	(Currie ex Jury 1998 JU1298)	✓	✓	✓	
131	*R. radians*	Pukeiti	EK667	S2008104	(Currie 2004)	✓	✓		
132	*R. rhodoleucum*	Pukeiti	HF034	S2008123	(Currie ex Jury)	✓		✓	
133	*R. rutenii*	Pukeiti	EK647	S2008157	(Currie ex Binney 2003)	✓	✓		
134	*R. stapfianum*	Pukeiti	EK583	S2001101	Mt Kinabalu, Sabah, Borneo (Binney ex Argent; seed)	✓	✓		
135	*R. suaveolens*	Pukeiti	EK544	S1989393A	Mt Kinabalu, Sabah, Borneo (Blumhardt)	✓	✓	✓	
136	*R. suaveolens*	Pukekura	HF081		(ex Jury)	✓		✓	
137	*R. tuba*	Pukeiti	HF007	S1990360	(Blumhardt)	✓		✓	
138	*R. tuba*	Pukeiti	HF100			✓	✓		
139	*R. viriosum* (labelled *lochiae*) × *javanicum* (ID Andrew; EK636 was previously recorded as *R. tuba*; require ID verification, re-collection and tagging)	Pukeiti	EK636			✓			

Section *Euvireya* Subsection *Malesia*

#	Taxon	Location	PFR #	Accession #	Origin (Collector)	DNA	Seq	SSR	RAPD
140	*R. acrophilum*	Pukeiti	EK669	S2008115	(Currie)	✓		✓	
141	*R. bagobonum*	Pukeiti	EK525	S1990124A	Gunung Batu Lawi, Sarawak, Borneo (Adams 1990 A6908; cuttings)	✓	✓	✓	
142	*R. burttii*	Pukeiti	EK543	S1990395	Borneo (M Cullinane)	✓		✓	
143	*R. burttii*	Pukeiti	HF043	S2008152	(Currie 2003)	✓		✓	
144	*R. citrinum*	Pukeiti	EK579	S2008168	(Currie 2002)	✓	✓		
145	*R. commonae*	Pukeiti	EK632	S1986155G	Kain Swamp, PNG 2,700 m (G Smith 03719; cuttings)	✓	✓		
146	*R. commonae* (coral-pink form)	Pukeiti	EK640	S1986155G		✓			
147	*R. commonae* (coral-pink form)	Pukeiti	HF062	S1986155G		✓	✓	✓	
148	*R. commonae* (cream form)	Pukeiti	EK633	S1983155	Highland Orchid Collection, Laiagam, PNG (G Smith1983; seed)	✓	✓		
149	*R. commonae* (red form)	Pukeiti	EK637			✓	✓		
150	*R. inconspicuum*	Pukeiti	HF055	S1975205	(I Gordon ex NZRA; cutting)	✓		✓	
151	*R. pauciflorum*	Pukeiti	EK559	S1984272	Malaysia	✓			
152	*R. pauciflorum*	Pukeiti	HF061	S1984272	Gunung Batu Brinchang, Cameron Highlands, Malay Peninsula (K Adams)	✓			
153	*R. pubigermen*	Pukeiti	HF053	S2008111	(Currie ex Binney 2001 BI0901)	✓	✓	✓	
154	*R. rousei*	Pukeiti	HF014	S2008160	(Currie 1999 ex D Brown)	✓	✓	✓	
155	*R. stevensianum*	Esplanade	EI175			✓			
156	*R. stevensianum*	Pukeiti	EK607	H1986502		✓			
157	*R. stevensianum* (Mt Gahavisuka form) (considered natural hybrid, originally collected by N Crutwell; later collected by G Smith)	Pukeiti	EK623	H1986502? (HF108)	PNG	✓			

158	*R. stevensianum* (Mt Gahavisuka form) (considered natural hybrid, originally collected by N Crutwell; later collected by G Smith)	Pukeiti	EK624	H1986502? (HF108)	PNG	✓			
159	*R. taxifolium*	Pukeiti	EK578	S2001130		✓		✓	
160	*R. taxifolium*	Pukeiti	EK580	S2001130		✓		✓	
161	*R. taxifolium*	Pukeiti	EK605	2001130	Mt Pulag (also Pulog), Philippines (Binney ex Argent)	✓		✓	
162	*R. vitis-idaea*	Pukeiti	EK574	S1986373	Mt Yakananda, PNG 2,800 m (G Smith GS04120)	✓	✓		✓
163	*R. vitis-idaea*	Pukeiti	EK575	S1986373	Mt Yakananda, PNG 2,800 m (G Smith GS04120)	✓			✓
164	*R. wilkiei*	Pukeiti	EK671	S2007123	(Currie)	✓	✓	✓	
165	*R. wilkiei*	Pukeiti	HF013	S2008174	(Currie 2004 171204)	✓		✓	
166	*R. wrightianum* (white form)	Pukeiti	HF018	S2008128	(Currie ex Binney BI0103)	✓			

Section *Euvireya* Subsection *Euvireya*

#	Taxon	Location	PFR #	Accession #	Origin (Collector)	DNA	Seq	SSR	RAPD
167	*R. arenicola* (*aff. R. lagunculicarpum*)	Pukeiti	EK596	S2001140		✓		✓	
168	*R. arenicola*	Pukeiti	EK660	S2008110	Sulawesi (Currie ex Binney Bl1000)	✓		✓	
169	*R. arfakianum*	Pukeiti	EK608	S1984116	(Strybing Arboretum, USA via Graham Snell. Australia)	✓	✓	✓	
170	*R. aurigeranum* ssp. *aurigeranum*	Pukeiti	HF068	S2008129	(Currie ex B Clancy, Australia; seed)	✓	✓		
171	*R. blackii*	Pukeiti	EK591			✓	✓	✓	✓
172	*R. blackii*	Pukeiti	EK592			✓	✓		✓
173	*R. blackii*	Pukeiti	EK593			✓			✓
174	*R. blackii*	Pukeiti	EK594			✓			

175	*R. blackii*	Pukeiti	EK625	S1983386	Kandep-Laiagam divide, Lagaip District, Enga Province (G Smith 1983 GFS3883; seedling)	✓			
176	*R. blackii*	Pukeiti	HF056			✓		✓	
177	*R. celebicum*	Pukeiti	HF070	S2007127	(Currie)	✓	✓	✓	
178	*R. celebicum* (?)	Pukeiti	HF071	S2008135	(Currie 2003)	✓			
179	*R. christi*	Esplanade	EI147			✓			
180	*R. christi*	Esplanade	EI179			✓			
181	*R. christi*	Pukeiti	EK610	1983148?	Mt Miap, PNG 2,800 m (G Smith GFS2383; seedling)	✓		✓	✓
182	*R. christi* (Mt Miap form)	Pukeiti	EK609			✓		✓	✓
183	*R. christi* (red form)	Pukeiti	HF048	S2208117	(Currie 2005)	✓		✓	
184	*R. christi* (small form)	Pukeiti	HF017	S2008113	(Currie 1999)	✓		✓	
185	*R. christianae*	Esplanade	EI152			✓			
186	*R. christianae*	Esplanade	EI182			✓			
187	*R. christianae*	Esplanade	EI185			✓			
188	*R. christianae*	Pukeiti	HF060	S1975149	(E B Perrott)	✓		✓	
189	*R. christianae* (ID by Andrew, Pukeiti)	Pukeiti	HF075			✓			
190	*R. crassifolium*	Pukeiti	EK522	S1989161C	Gunung Alab, Sabah, Borneo (Blumhardt; from seed collected by Argent)	✓		✓	
191	*R. crassifolium*	Pukeiti	EK560	S1980161	Mt Mulu, Sabah, Borneo (K Adams AD1280)	✓		✓	
192	*R. crassifolium*× *stenophyllum*	Pukeiti	HF027		Mt Kinabalu, Sabah, Borneo (Blumhardt; cutting)	✓		✓	
193	*R. culminicola* var. *culminicola* (Bulldog Rd form)	Pukeiti	EK629	S1976163	Bulldog Rd, PNG (P Kores K10576)	✓	✓	✓	
194	*R. curviflorum*	Pukeiti	HF031	S2008151	(Currie 2003 ex Binney)	✓	✓	✓	
195	*R. impositum*	Pukeiti	HF135	S2008158	(Currie ex Binney)	✓	✓	✓	

196	*R. javanicum* ssp. *brookeanum*	Pukeiti	EK547	S1990210D	Borneo (Blumhardt; grafted)	✓		✓	
197	*R. javanicum* ssp. *brookeanum*	Pukeiti	EK652	S2008142	(Currie ?)	✓		✓	
198	*R. javanicum* ssp. *moultonii*	Pukeiti	EK661	S2008109	(Currie 2002)	✓		✓	
199	*R. robinsonii*	Pukeiti	HF021	S1979210	(J Rouse; cuttings)	✓	✓	✓	
200	*R. kochii*	Pukeiti	EK600			✓	✓		
201	*R. kochii*	Pukeiti	HF035	S2008149	(Currie ex John Kenyon)	✓		✓	
202	*R. laetum*	Pukeiti	EK643	S2008145	(Currie ex John Kenyon 1998 KY0298)	✓		✓	
203	*R. laetum*	Pukeiti	EK644	S2008126	(Currie 2000)	✓	✓	✓	
204	*R. laetum*	Pukeiti	EK648	S2008143	(Currie ex Kings 2001 K10901)	✓	✓	✓	
205	*R. laetum* × *hellwigii*	Pukeiti	EK567	H1989143		✓			
206	*R. laetum* × *hellwigii*	Pukeiti	HF066	H1989143		✓	✓		
207	*R. lanceolatum*	Pukeiti	HF037	S2008154	(Currie 2000)	✓		✓	
208	*R. leptobrachion*	Pukeiti	HF039	S2008165	(Currie 2002 ex Binney)	✓			
209	*R. leucogigas* 'Hunstein's Surprise'	Pukeiti	HF051			✓	✓	✓	
210	*R. viriosum* (Mt Finnigan form) (labelled *R. lochiae*)	Esplanade	EK507			✓			
211	*R. viriosum* (labelled *R. lochiae*)	Esplanade	EK508			✓			
212	*R. viriosum*	Pukeiti	EK630			✓		✓	
213	*R. viriosum*	Pukeiti	HF054			✓		✓	
214	*R. viriosum* (Mt Finnigan[56] form)	Pukeiti	EK569	(EK696)		✓		✓	✓
215	Hybrid; labelled as *R. bryophilum*	Esplanade	EI139			✓			✓
216	*R. viriosum* (Mt Lewis form)	Pukeiti	HF045	S2005203	(Currie 2003)	✓		✓	
217	*R. viriosum* (Devil's Thumb form)	Pukeiti	HF049	S2008163	(Currie ex Binney 2001)	✓		✓	
218	*R. viriosum*	Pukeiti	EK572	S2002151A		✓		✓	✓

[56] Also known as Mt Finnegan.

219	*R. viriosum*	Pukeiti	EK604	S2002151A		✓		✓	✓
220	*R. viriosum* (Mt Finnigan form)	Pukeiti	EK589	S1984230	Mt Finnigan, N Queensland, Australia (G Snell; from wild collected seed)	✓		✓	✓
221	*R. viriosum* (Mt Finnigan form)	Pukeiti	HF077			✓	✓	✓	
222	*R. viriosum* (Mt Finnigan form)	Pukeiti	EK620	S2002151B		✓		✓	✓
223	*R. lochiae* 'Baby Bells'	Pukeiti	HF030	S2008112	(Currie ex John Kenyon 2002)	✓	✓	✓	✓
224	*R. lochiae* 'Highlander'	Pukeiti	HF029	S2008175	(Currie 2001)	✓		✓	
225	*R. lochiae* (labelled *R. notiale*)	Pukeiti	EK606	S2005186	(Currie 2000 BR0600)	✓		✓	
226	*R. longiflorum* var. *longiflorum*	Pukeiti	EK668	S2008139	(Currie ex Binney 2000 BI0100)	✓		✓	
227	*R. longiflorum* var. *longiflorum*	Pukeiti	HF047	S2005199	(Currie)	✓		✓	
228	*R. lowii*	Pukeiti	EK539	S1980235	Mt Kinabalu, Sabah, Borneo 10,000 ft (K Adams 1980 AD0180)	✓			
229	*R. lowii* (seedling)	Mark Jury	HF101			✓	✓	✓	
230	*R. luraluense* ssp. *luraluense*	Pukekura	HF094			✓	✓	✓	
231	*R. luraluense* ssp. *luraluense*	Esplanade	EI141			✓			
232	*R. luraluense* ssp. *luraluense*	Esplanade	EI192			✓			
233	*R. luraluense* ssp. *luraluense*	Pukeiti	HF137	S1984236	Solomon Is	✓		✓	
234	*R. luraluense* ssp. *luraluense*	Pukeiti	HF138	S1984236	Solomon Is	✓		✓	
235	*R. luraluense* ssp. *luraluense*	Pukeiti	EK564			✓		✓	
236	*R. macgregoriae*	Pukeiti	EK615	S1983238	Laiagam, PNG (G Smith GS11512)	✓		✓	
237	*R. macgregoriae* (? *R. christianae* hybrid)	Pukeiti	HF079			✓		✓	
238	*R. macgregoriae* (large form)	Pukeiti	EK585	S1977238F	(seed ?)	✓		✓	
239	*R. macgregoriae* (orange form)	Esplanade	EK513			✓			
240	*R. macgregoriae* (orange form)	Esplanade	EK514			✓		✓	
241	*R. macgregoriae* (orange form)	Pukeiti	HF057	S1976238?		✓		✓	

242	*R. macgregoriae* (red form)	Pukeiti	EK634	S1983239	Laiagam, PNG (G Smith GS4983)	✓		✓	
243	*R. maxwellii*	Esplanade	EI138			✓			
244	*R. maxwellii*	Esplanade	EI156			✓			
245	*R. maxwellii*	Pukeiti	EK523	S1995169	(Binney; grafted)	✓		✓	
246	*R. maxwellii*	Pukeiti	HF033			✓		✓	
247	*R. mindanaense*	Pukeiti	EK586	S2008150	(Currie ex Binney)	✓			
248	*R. multicolor*	Pukeiti	HF040	S2008169	(Currie ex John Kenyon 1997)	✓		✓	
249	*R. orbiculatum*	Esplanade	EI173			✓			
250	*R. orbiculatum*	Pukeiti	EK650	S2008144	(Currie ex John Kenyon 1999)	✓		✓	
251	*R. orbiculatum* (?)	Pukeiti	HF011			✓		✓	
252	*R. orbiculatum*	Jury	HF096			✓		✓	
253	*R. orbiculatum* (?)	Jury	HF092			✓	✓	✓	
254	*R.* × *planecostatum*	Pukeiti	HF009	S2002120		✓			
255	*R.* × *planecostatum*	Pukeiti	HF145			✓	✓	✓	
256	*R. polyanthemum*	Pukeiti	EK538	S1984282	Mt Kinabalu, Sabah, Borneo (Blumhardt; seed)	✓			
257	*R. praetervisum*	Pukeiti	EK558	S1984390	Mt Kinabalu, Sabah, Borneo (Blumhardt)	✓	✓		
258	*R. pudorinum*	Pukeiti	EK653	S2008153	(Currie ex Binney 2002 BI0902)	✓		✓	
259	*R. rarilepidotum*	Pukeiti	EK584			✓		✓	
260	*R. rarilepidotum*	Pukeiti	EK665	S2003199	(Currie ex B Clancy)	✓		✓	
261	*R. rarilepidotum* (yellow form)	Pukeiti	EK646	S2008146	(Currie ex Binney 2003)	✓		✓	
262	*R. retivenium*	Pukeiti	EK533	S1981307	Mt Kinabalu, Sabah, Borneo (Blumhardt 21981)	✓			
263	*R. retivenium*	Pukeiti	EK552	S1981307	Mt Kinabalu, Sabah, Borneo (same as S1981307, but different seedling)	✓			

264	*R. retivenium*	Esplanade	EI149			✓			
265	*R. retivenium*	Esplanade	EI176			✓			
266	*R. retivenium*	Esplanade	EI177			✓			
267	*R. impositum* (labelled as *R. rhodopus*)	Pukeiti	EK577	S2001138	(Binney)	✓		✓	
268	*R. rhodopus*	Pukeiti	EK597	S2005189	(Currie 2002 050202)	✓		✓	
269	*R. robinsonii*	Esplanade	EI168			✓			
270	*R. robinsonii*	Pukeiti	EK562	S1986317	Gunung Brinchang, Cameron Highlands, Malay Peninsula 4,000 ft (K Adams; cuttings)	✓			
271	*R. robinsonii*	Pukeiti	EK642	S2008188	(Currie B10600)	✓		✓	
272	*R. rugosum*	Pukeiti	EK540	S1980322	Mt Kinabalu, Sabah, Borneo (K Adams A18680)	✓		✓	
273	*R. rugosum*	Pukeiti	EK550		Mt Kinabalu, Sabah, Borneo (K Adams)	✓			
274	*R. rugosum*	Pukeiti	HF005	S1980321	Mt Kinabalu, Sabah, Borneo, 7,500 ft (K Adams A0280)	✓		✓	
275	*R. scabridibracteum*	Pukeiti	HF024	S1990327	Mt Gahavisukar, PNG (Blumhardt / G Smith 00513)	✓		✓	
276	*R. sessilifolium*	Pukeiti	EK546	S1997162	Sumatra	✓			
277	*R. stenophyllum* ssp. *angustifolium*	Pukeiti	EK526	S1992108	Borneo	✓		✓	
278	*R. stenophyllum* ssp. *stenophyllum*	Pukeiti	HF082			✓	✓	✓	
279	*R. stenophyllum* ssp. *stenophyllum*	Pukeiti	EK561			✓			
280	*R. sumatranum*	Pukeiti	EK659	S2008101	(Currie 1997)	✓		✓	
281	*R. sumatranum*	Pukekura	HF086			✓			
282	*R. sumatranum* (parent of HF086)	Pukekura	HF093			✓	✓	✓	
283	*R. sumatranum* × *retusum*	Pukeiti	EK528	S1984351	Sumatra (G Snell, Australia)	✓			
284	*R. vanvuurenii*	Pukeiti	EK641	S2008159	(Currie 2002; seed)	✓		✓	
285	*R. verticillatum*	Esplanade	EI160			✓			

286	*R. verticillatum*	Esplanade	EI161			✓			
287	*R. verticillatum*	Esplanade	EI162			✓			
288	*R. verticillatum*	Pukekura	HF091			✓	✓	✓	
289	*R. villosulum* (?)	Pukeiti	HF067	S2007108	(Currie)	✓	✓		
290	*R. yongii*	Pukeiti	EK545	S1980388	Gunung Mulu, Sarawak, Borneo (K Adams No7 A1580; cuttings)	✓			
291	*R. yongii*	Pukeiti	EK664	S2008106	(Currie 2003)	✓	✓		
292	*R. zoelleri*	K Adams	HF098			✓			
293	*R. zoelleri*	Pukeiti	EK628	S1976383	(E B Perrott)	✓	✓	✓	

Miscellaneous accessions collected

Natural Hybrids

#	Taxon	Location	PFR #	Accession #	Origin	DNA	Seq	SSR	RAPD
294	*R. sumatranum* × *retusum*	Pukeiti	EK528	S1984351	(G Snell, Australia)	✓			
295	*R. crassifolium* × *stenophyllum*	Pukeiti	HF027		Mt Kinabalu, Sabah, Borneo (Blumhardt; cutting)	✓		✓	

Garden Hybrids

#	Taxon	Location	PFR #	Accession #	Origin	DNA	Seq	SSR	RAPD
296	*R. beyerinckianum* × *culminicola*	Esplanade	EI142			✓			
297	*R.* 'Felicitas'	Esplanade	EI166			✓			
298	*R.* 'Satan's Gift'	Esplanade	EI174			✓			
299	*R.* 'Pink Ray'	Esplanade	EI178			✓			
300	*R.* 'Vladimir Bukousky'	Esplanade	EI183			✓			
301	*R.* 'Little Angel'	Esplanade	EK509			✓			
302	*R.* 'Pendance'	Esplanade	EK511			✓			

303	*R. beyerinckianum* × *culminicola*	Esplanade	EK512			✓			
304	*R.* 'Pacific Shower'	Esplanade	EK519			✓			
305	*R.* 'Just Peachy'	Esplanade	EK520			✓			
306	*R.* 'Pendance'	Pukeiti	EK551			✓			
307	*R. laetum* × *hellwigii*	Pukeiti	EK567	H1989143		✓			
308	*R.* 'Mossman'	Pukeiti	EK627		PNG	✓			

Unidentified *Rhododendron* accessions

#	Taxon	Location	PFR #	Accession #	Origin	DNA	Seq	SSR	RAPD
309	*Rhododendron sp.*	Esplanade	EI163			✓			
310	*Rhododendron sp.*	Esplanade	EI165			✓			
311	*Rhododendron sp.*	Esplanade	EI167			✓			
312	*Rhododendron sp.*	Esplanade	EI184			✓			
313	*Rhododendron sp.* (labelled *R. bryophilum*)	Esplanade	EK510			✓			
314	*Rhododendron sp.*	Pukeiti	EK529			✓			
315	*Rhododendron sp.*	Pukeiti	EK534			✓			
316	*Rhododendron sp.*	Pukeiti	EK556	S1983393	(J Rouse)	✓			
317	*Rhododendron sp.*	Pukeiti	EK557			✓			
318	*Rhododendron sp.*	Pukeiti	EK563			✓			
319	*Rhododendron sp.*	Pukeiti	EK568	S1990360 (HF007)	(Blumhardt)	✓		✓	
320	*Rhododendron sp.*	Pukeiti	EK587	S1986396	(RSBG, USA 80/148; from Boskoop, Holland)	✓			
321	*Rhododendron sp.*	Pukeiti	EK595			✓			
322	*Rhododendron sp.* (pink-flowered seedling)	Pukeiti	EK676			✓			
323	*Rhododendron sp.*	Pukeiti	EK677			✓		✓	
324	*Rhododendron sp.*	Pukeiti	HF008			✓			

325	*Rhododendron sp.*	Pukeiti	HF099			✓		✓	
326	*Rhododendron sp.*	Pukeiti	HF136			✓			
327	*Rhododendron* ‘Little Kisses’	Pukeiti	HF140			✓			
328	*Rhododendron* ‘Felicitas’	Pukeiti	HF141			✓			
329	*Rhododendron* ‘Minnie Mouse’	Pukeiti	HF142			✓			
330	*Rhododendron* ‘Popcorn’	Pukeiti	HF143			✓			
331	*Rhododendron* ‘Brightly’	Pukeiti	HF144			✓			

Table 31 Accessions of *Rhododendron* obtained from the US as DNA. All specimens collected from the Rhododendron Species Foundation Garden. (Key: DNA – DNA isolated; SEQ – DNA Sequenced; SSR – Analysed with microsatellites; RAPD – Analysed with RAPDs).

#	Taxon	Location	PFR #	Accession #	Origin	DNA	Seq	SSR	RAPD
1	*R. jasminiflorum* (ssp. not specified)	RSF, USA	n/a	1978/102		✓		✓	
2	*R. culminicola* var. *angiense*	RSF, USA	n/a	1983/059		✓		✓	
3	*R. dianthosmum*	RSF, USA	n/a	1983/063		✓		✓	
4	*R. dielsianum*	RSF, USA	n/a	1983/60		✓		✓	
5	*R.* × *sheilae*	RSF, USA	n/a	1987/048		✓		✓	
6	*R. javanicum* ssp. *gracile*	RSF, USA	n/a	1994/373		✓		✓	
7	*R. rushforthii*	RSF, USA	n/a	1997/087		✓		✓	
8	*R. culminicola* var. *culminicola*	RSF, USA	n/a	1999/286		✓		✓	
9	*R. javanicum* ssp. *teysmannii*	RSF, USA	n/a	1999/307		✓		✓	
10	*R. vaccinioides*	RSF, USA	n/a	1999/308		✓		✓	
11	*R. javanicum* ssp. *brookeanum*	RSF, USA	n/a	1999/318		✓		✓	
12	*R. emarginatum*	RSF, USA	n/a	1999/382		✓		✓	
13	*R. acrophilum*	RSF, USA	n/a	2002/018		✓	✓	✓	
14	*R. leptanthum*	RSF, USA	n/a	87/041		✓		✓	
15	*R. polyanthemum*	RSF, USA	n/a	94/333		✓		✓	
16	*R. dielsianum*	RSF, USA	n/a	99/330		✓	✓	✓	
17	*R. polyanthemum*	RSF, USA	n/a	994/336		✓		✓	
18	*R. solitarium*	RSF, USA	n/a	V112		✓		✓	

A2 Taxonomic & Conservation Issues of Vireyas

Table 32 Summary of IUCN categories assigned to vireyas.

Taxonomic Group	NE	LC	DD	NT	VU	EN	CR	EX	TOTAL
Section *Pseudovireya*	1	4	5	0	2	0	0	0	**12**
Section *Discovireya*	8	20	7	0	5	1	0	0	**41**
Section *Siphonovireya*	0	6	4	0	0	0	1	0	**11**
Section *Phaeovireya*	3	22	16	0	8	1	0	0	**50**
Section *Malayovireya*	6	9	3	2	1	2	0	0	**23**
Section *Albovireya*	0	7	5	0	2	1	0	0	**15**
Section *Euvireya* Subsection *Linnaeopsis*	2	6	6	0	2	0	0	0	**16**
Section *Euvireya* Subsection *Saxifragoidea*	0	1	0	0	0	0	0	0	**1**
Section *Euvireya* Subsection *Solenovireya*	1+3	20	14	0	6	0	0	1	**45**
Section *Euvireya* Subsection *Malesia*	2+4	27	16	0	11	0	3	0	**63**
Section *Euvireya* Subsection *Euvireya*	16	54	25	1	9	3	5	0	**113**
TOTAL	**46**	**176**	**101**	**3**	**46**	**8**	**9**	**1**	**390**

A2.1 Section *Pseudovireya* (Clarke) Sleumer

Table 33 Summary of the taxonomic and conservation issues of Section *Pseudovireya*. The taxa in boldface denote those analysed in this study. The taxa are arranged according to the classification of Argent (2006), and the taxon numbers in the leftmost column correspond to the taxon number used in Argent's (2006) classification. The column 'Range' shows the geographic region the taxa belong (Figure 21).

#	Taxon	Range	Taxonomic Issues	Questions Raised	IUCN Code
1	***R. vaccinioides*** Hook. f., *Rhod. Sikkim. Himal.* ii: 3, 1851.	IS	*R. vaccinioides* is a widespread species in the wild. This species is very similar to *R. asperulum* (categorized VU D2), and one form in cultivation has	(i) What is the genetic relationship between this species and *R. asperulum*?	LC

#	Taxon	Range	Taxonomic Issues	Questions Raised	IUCN Code
			distinctive pink undersides to the leaves (Argent 2006). The accession of *R. vaccinioides* at the Pukeiti collection is originated from the RGBE (Edinburgh, UK) collection. Tiwari & Chauhan (2006) reports that *R. vaccinioides* is 'out of danger' at present, but reports numbers as 'few'.	(ii) Where is this species placed within *Pseudovireya*?	
2	***R. santapaui*** Sastry, Kataki, P A Cox, E P Cox & Hutchison, *J. Bombay Nat. Hist. Soc*. 65: 744, 1969.	IS	The temperate species *R. campylogynum* seems to be very closely related to this species and often forming a separate clade together (Brown et al. 2005; Kurashige 2001). The authors of this species suggested that this species is allied to *R. kawakamii* (LC) but it is very distinct and crosses that species only with great difficulty. In New Zealand a hybrid in David Binney's collection was said to be of *R. santapaui* × *R. lochiae*. Certainly looks like *R. santapaui* but with red flowers. Origin unknown (Argent 2006).	(i) What is the genetic relationship between this species and *R. kawakamii*? (ii) Where is this species placed within *Pseudovireya*? (iii) Is there significant genetic distance between multiple accessions of this species?	DD
3	***R. asperulum*** Hutch. & Kingdon-Ward, *Notes RBG Edinb*. 16: 182, 1931.	EA	Very similar to *R. vaccinioides*. A very imperfectly known species. The isotype in Edinburgh is mixed with *R. insculptum*, and the paratype 7163 (also in Edinburgh) shows some variation in that it has faint pinnate venation not evident in the type, but this specimen is without flowers. A collection distributed under this name has yellow flowers and is not this species (Argent 2006).	(i) What is the genetic relationship between this species, *R. vaccinioides*, and *R. insculptum*? (ii) Where is this species placed within *Pseudovireya*?	VU D2
4	*R. insculptum* Hutch. & Kingdon-Ward, *Notes RBG Edinb*. 16: 182, 1931.	EA	Rarely collected and not yet known to be cultivated. This species is very similar to *R. emarginatum* (Argent 2006; The Herbarium Catalogue 2006). Known from two locations, rarely collected, and therefore additional field work is required before an assessment can be carried out (Gibbs et al. 2011).	(i) What is the genetic relationship between this species and *R. emarginatum*? (ii) Where is this species placed within *Pseudovireya*?	DD

#	Taxon	Range	Taxonomic Issues	Questions Raised	IUCN Code
5	*R. rupivalleculatum* P C Tam, *Guihaia* 2(2): 69, 1982.	EA	A very imperfectly understood species. The red spots on the flowers may be distinctive or could be an imperfect rendering of the orange spots which are common on *R. emarginatum* (Argent 2006).	(i) What is the genetic relationship between this species and *R. emarginatum*? (ii) Where is this species placed within *Pseudovireya*?	DD
6a	***R. emarginatum* var. *emarginatum*** Hemsl. & E H Wilson, *Kew Bull*. 118, 1910.	EA	*R. insculptum* (DD) is of conservation interest and related to *R. emarginatum*, but the former is not available in New Zealand. *R. emarginatum* is also closely related to *R. rushforthii* (Argent 2006). Sleumer compared the types of *R. euonymifolium* and *R. poilanei* and reduced these species to synonyms o f *R. emarginatum* (Sleumer 1958). *R. leiboense* was differentiated in having more slender branches, triangular calyx lobes and flowers in twos and does not appear to warrant even varietal on these characters (Argent 2006). Argent (2006) also reduced *R. maguanense* as a synonym after examining the type material. Feng (1983) said this species was close to *R. emarginatum* (Argent 2006). According to the molecular study by Brown et al. (2006b), *R. emarginatum* (Syn: *R. euonymifolium*) is shown to be related to *R. kawakamii*.	(i) What is the genetic relationship between this taxon and *R. kawakamii*? (ii) What is the placement of this species with respect to the other taxa of *Pseudovireya*? (iii) Is there significant genetic distance between multiple accessions of this species? (iv) What is the genetic differentiation between this taxon and *R. euonymifolium*?	LC
6b	*R. emarginatum* var. *eriocarpum* K M Feng, *Acta Bot. Yunnan*., 5(3) 268, 1983.	EA	This taxon is not yet in cultivation (Argent 2006), and need to be analysed to determine the genetic distance between the two varieties of *R. emarginatum*, and whether this species need to be promoted to a species.	(i) What is the genetic relationship between this variety and *R. emarginatum* var. *emarginatum*? (ii) What is the genetic relationship between this variety and *R. insculptum*? (iii) Where is this variety placed within *Pseudovireya*?	NE

#	Taxon	Range	Taxonomic Issues	Questions Raised	IUCN Code
7	***R. sororium*** Sleumer, *Blumea Suppl*. IV(2): 47, 1958.	EA	Differing from *R. emarginatum* in the smooth not rough twigs, a slightly longer (Argent 2006).	(i) What is the genetic relationship between this species and *R. emarginatum*? (ii) Where is this species placed within *Pseudovireya*?	LC
8	*R. densifolium* K M Feng, *Acta Bot. Yunnan*., 5(3) 266, 1983.	EA	Very similar to *R. emarginatum* and recently introduced into cultivation (Argent 2006). Eric Annal's specimen of *R. densifolium* is the first to flower in cultivation, on 6 October 2006[57].	(i) What is the genetic relationship between this species and *R. emarginatum*? (ii) Where is this species placed within *Pseudovireya*?	VU D2
9	***R. rushforthii*** Argent & D F Chamberlain, *The New Plantsman* 3(4): 195, 1996.	EA	This species of conservation interest as it has been assigned the DD category. This species is known at present from a very limited area in N Vietnam and from across the border in China. Apparently most closely related to *R. kawakamii* (LC) from Taiwan (Argent 2006). *R. densifolium* and *R. sororium* are also related to *R. rushforthii*, but they are not cultivated in New Zealand.	(i) How closely related is this species to *R. kawakamii*? (ii) What is the genetic distance between accessions of this species? (iii) Which other species are this species genetically related to?	DD
10	*R. datiandingense* Z J Feng, *J. South China Agr. Univ*., 17(1): 59, 1996.	EA	This species is said to be similar to *R. rupivalleculatum*. Also, very reminiscent of *R. rushforthii*. *R. datiandingense* is the older name by a few months and would take precedence (Argent 2006). Known only from the type specimen; needs further research to establish the conservation status (Argent 2006; Gibbs et al. 2011).	(i) What is the genetic relationship between this species and *R. rupivalleculatum*? (ii) Where is this species placed within *Pseudovireya*?	DD
11	***R. kawakamii*** Hayata, *J. Coll. Sci. Univ. Tokyo* 30(1): 171, 1911.	TW	The original description of this taxon does not include flower colour nor is it recorded on the type specimen, but it was reported as a 'red or white' by Liu and Chuang (Liu & Chuang 1960) when they described	(i) What is the genetic relationship between this species, *R. rushforthii* and *R. emarginatum*?	LC

[57] http://www.rhodogroup-rhs.org/Services/News/newsrhoNov2006.htm

#	Taxon	Range	Taxonomic Issues	Questions Raised	IUCN Code
			R. kawakamii var. *flaviflorum*. This was subsequently reported by Withers & Womersley (Withers & Womersley 1986) and has led to considerable confusion as to the status of the yellow-flowered plant in cultivation (Argent 2006). Shen-You Lu & Yuen-Po Yang (Lu & Yang 1989) firmly reduced *R. kawakamii* var. *flaviflorum* to *R. kawakamii* and there appears to be no evidence that pink- or white-flowered forms of this species ever existed (Argent 2006).	(ii) Where is this species placed within *Pseudovireya*? (iii) What is the genetic distance between accessions of this species?	

A2.2 Section *Discovireya* (Sleumer) Argent

Table 34 Summary of the taxonomic and conservation issues of Section *Discovireya*. The taxa in boldface denote those analysed in this study. The taxa are arranged according to the classification of Argent (2006), and the taxon numbers in the leftmost column correspond to the taxon number used in Argent's (2006) classification. The column 'Range' shows the geographic region the taxa belong (Figure 21).

#	Taxon	Range	Taxonomic Issues	Questions Raised	IUCN Code
12	***R. perakense*** King & Gamble, *J. As. Soc. Beng.* 74(2): 76, 1905.	MP	Locally common, and in several locations (Argent 2006; Gibbs et al. 2011). First introduced into cultivation by an unknown Japanese collector who supplied material to John Rouse. It was later collected by Keith Adams and grown at Pukeiti in New Zealand. Both these introductions have been widely distributed (Argent 2006). Recent molecular studies have shown that this species stands on its own outside the other taxa of *Discovireya* and sister to the core vireyas (Goetsch et al. 2011).	(i) Where is this species placed within *Discovireya*? (ii) Are there any close genetic affinities of this species to other taxa in this group? (iii) What is the genetic distance between accessions of this species? (iv) How closely related is this species to the other Malay Peninsula taxa of *Pseudovireya* (*R. scortechinii*, *R. seimundii* and *R. spathulatum*)?	LC

#	Taxon	Range	Taxonomic Issues	Questions Raised	IUCN Code
13	*R. scortechinii* King & Gamble, *J. As. Soc. Beng.* 74(2): 76, 1905.	MP	Apparently only recently introduced into cultivation and not yet reported to have flowered. A record from Borneo was a misidentification of *R. buxoides* (Argent 2006).	(i) Is there a genetic relationship between this species and *R. buxoides*?	LC
14	*R. seimundii* J. J. Sm., *Gard. Bull. S. S.* 8(3): 262, 1935.	MP	Not known in cultivation (Argent 2006).	No known taxonomic issues.	DD
15	*R. spathulatum* Ridl., *J. Str. Br. R. As. Soc.* 61: 25, 1912.	MP	Not recorded as ever cultivated. Said to be locally plentiful (Argent 2006).	No known taxonomic issues.	LC
16	***R. adinophyllum*** Merr., *Notes Natl. Acad. Nat. Sci. Philad.*, 47: 3, 1940.	SM	A wild hybrid of this species with *R. sumatranum* was collected by David Binney on Mt Kemiri, Sumatra (19982482) in 1998 (Argent 2006). Population on several mountains in Sumatra - one population in the National Park (but these are incursions into this park) (Gibbs et al. 2011).	(i) Is there a genetic relationship between this species and the out of section *R. sumatranum*? (ii) Are there any other out of section relationships with this taxon? (iii) Are there any close genetic affinities of this species to other taxa in this group? (iv) What is the genetic distance between accessions of this species?	LC
17a	***R. retusum* var. *retusum*** (Blume) Benn., *Pl. Jav. Rar.*, 86-88 t20, 1838.	SM JV	One of the earliest vireyas to be introduced into cultivation (Argent 2006). Since *R. retusum* var. *trichostylum* is listed as DD, this variety must be analysed to see the genetic difference between the two varieties.	(i) Where is this species placed within *Discovireya*? (ii) Are there any close genetic affinities of this species to other taxa in this group? (iii) What is the genetic distance between accessions of this species?	LC
17b	*R. retusum* var. *trichostylum*	SM	In three locations and no recent collections (Gibbs et al. 2011).	(i) What is the genetic relationship between this variety and var. *retusum*?	DD

#	Taxon	Range	Taxonomic Issues	Questions Raised	IUCN Code
	Sleumer, *Reinwardtia* 5: 55, 1960.			(ii) Where is this species placed within *Discovireya*?	
17c	*R.* × *epilosum* (J. J. Sm.) Argent,	SM	Unresolved name. Considered to be a hybrid with *R. sumatranum* (Argent 2006).	(i) Is this taxon related to the suspected parents (*R. retusum* and *R. sumatranum*)?	NE
18a	*R. borneense* ssp. *borneense* (J. J. Sm.) Argent, A. Lamb & Phillipps, *Rhododendrons of Sabah* 8: 74, 1988.	BN	Widespread (Gibbs et al. 2011). Not known to be in cultivation (Argent 2006).	(i) Where is this species placed within *Discovireya*? (ii) Are there any close genetic affinities of this species to other taxa in this group? (iii) What is the genetic relationship between other subspecies of this species?	LC
18b	***R. borneense* ssp. *villosum*** (J. J. Sm.) Argent, A. Lamb & Phillipps, *Rhododendrons of Sabah* 8: 75, 1988.	BN	Widespread (Gibbs et al. 2011). Cultivated in Edinburgh since 1982 (Argent 2006).	(i) Where is this species placed within *Discovireya*? (ii) Are there any close genetic affinities of this species to other taxa in this group? (iii) What is the genetic relationship between other subspecies of this species?	LC
18c	*R. borneense* ssp. *angustissimum* (J. J. Sm.) Argent, A. Lamb & Phillipps, *Edinb. Bot.* 8: 74, 1988.	BN	Only known from Mt Mulu (Sarawak, Borneo, Malaysia). Probably more than 1,000 plants, and no factors of decline (Gibbs et al. 2011). This is an extreme form of *R. borneense* which approaches *R. ericoides* in the size of its leaves. Locally abundant; not known to have been cultivated (Argent 2006). Sleumer (1963b) considered it as	(i) Where is this species placed within *Discovireya*? (ii) Are there any close genetic affinities of this species to other taxa in this group, especially *R. quadrasianum* and *R. ericoides*?	LC

#	Taxon	Range	Taxonomic Issues	Questions Raised	IUCN Code
			R. quadrasianum var. *angustissimum*, but this name is now reduced to a synonym.	(iii) What is the genetic relationship between other subspecies of this species?	
19	*R. buxoides* Sleumer, *Blumea* 21: 359, 1973.	BN	Sleumer (1973) commented that this species was 'close to' *R. perakense*. Not known in cultivation. *R. buxoides* very much parallels *R. inconspicuum* and *R. yelliotii* (Argent 2006). Small populations on 3 mountains, probably <1,000 individuals (Gibbs et al. 2011).	(i) Where is this species placed within *Discovireya*? (ii) What is the genetic relationship between this species and *R. perakense*? (iii) What is the genetic relationship between this species, *R. inconspicuum* and *R. yelliotii*?	VU
20a	*R. cuneifolium* var. *cuneifolium* Stapf, *Trans. Linn. Soc. London, II, Bot*. 4: 198 (t. 15, f.B,3), 1894.	BN SW	Hybridising in the wild with *R. ericoides* to give *R.* × *silvicola* (Argent 2006). Since *R. cuneifolium* var. *microcarpum* is Red-listed as VU D2, this taxon must be analysed to determine their genetic differentiation.	(i) Where is this species placed within *Discovireya*? (ii) What is the relationship between this variety and other varieties of this species?	LC
20b	*R. cuneifolium* var. *microcarpum* Argent, A. Lamb & Phillipps, *Notes RBG Edinb*. 42(1): 118, 1984.	BN	Found in a single location and vulnerable (Gibbs et al. 2011). An extreme form of *R. cuneifolium* known only from Mt Trus Madi (Sabah, Borneo, Malaysia). Brought into cultivation in 1984 (Argent 2006).	(i) Where is this species placed within *Discovireya*? (ii) What is the relationship between this variety and other varieties of this species?	VU D2
21a	***R. ericoides*** Low *ex* Hook. f., *Hook. Icon. Pl*. t. 887, 1852.	BN	Point endemic on Mt Kinabalu, with >1,000 individuals (Gibbs et al. 2011). Records of this species from other mountains are all referable to *R. borneense* (Argent 2006). Thus raises the issue of confusion between *R. borneense* and *R. ericoides* (Argent 2006). *R. ericoides* forms the hybrid *R.* × *silvicola* with *R. cuneifolium* var. *cuneifolium* (LC).	(i) Where is this species placed within *Discovireya*? (ii) What is the relationship between this species, *R. borneense* and *R. cuneifolium*? (iii) Are there any close genetic affinities of this species to other taxa in this group?	VU

#	Taxon	Range	Taxonomic Issues	Questions Raised	IUCN Code
				(iv) What is the genetic distance between accessions of this species?	
21b	*R.* × *silvicola* Sleumer, *Rhododendrons of Sabah* 8: 102, 1988.	BN	A hybrid formed from *R. cuneifolium* and *R. ericoides* (Argent 2006). Previously known as *R. ericoides* var. *silvicolum* Sleumer.	(i) Where is this species placed within *Discovireya*? (ii) Is there a genetic relationship between this taxon and its presumed parents (*R. ericoides* and *R. cuneifolium*)?	NE
22a	***R. nanophyton* var. *nanophyton*** Sleumer, *Reinwardtia* 5: 62, 1962.	SW	Critically endangered point endemic, known only from the type specimen and needs further research to establish its status (Gibbs et al. 2011). Cultivated since 2000 (Argent 2006).	(i) Where is this species placed within *Discovireya*? (ii) What is the genetic relationship between this variety and var. *petrophilum*?	EN D
22b	*R. nanophyton* var. *petrophilum* Sleumer, *Reinwardtia* 5: 63, 1960.	SW	Known only from type location (Argent 2006; Gibbs et al. 2011). Known only from the type collection (Argent 2006).	(i) Where is this species placed within *Discovireya*? (ii) What is the genetic relationship between this variety and var. *nanophyton*?	DD
23	*R. monodii* (H. J. Lam) Argent, *Rhododendrons of subgenus Vireya* 50, 2006.	SW	This name is a new combination of *R. quadrasianum* f. *monodii*, and is unresolved (The Plant List 2010). Another synonym of this taxon is *R. quadrasianum* f. *selebicum*. Superficially similar to the New Guinean *R. pulleanum* (Argent 2006). It would be useful to determine the genetic differentiation between this taxon and its related taxa, especially the varieties of *R. quadrasianum*.	(i) Where is this species placed within *Discovireya*? (ii) What is the genetic relationship between this species, *R. quadrasianum* and *R. pulleanum*?	DD
24	***R. meliphagidum*** J. J. Sm., *Fedde Rep*. 30: 162, 1932.	SW ML	Locally common to abundant in Maluku and other locations (Argent 2006; Gibbs et al. 2011).	No known taxonomic issues.	LC

#	Taxon	Range	Taxonomic Issues	Questions Raised	IUCN Code
			Introduced by David Binney from Sulawesi to New Zealand (Argent 2006).		
25a	***R. quadrasianum* var. *quadrasianum*** S. Vidal, *Rev. Pl. Vasc. Filip*. 170, 1886.	PH	Plants previously referred to *R. quadrasianum* from outside the Philippines are all referable to other species (*R. borneense*, *R. cuneifolium* and *R. monodii*) (Argent 2006). Sleumer (1966a) considered this as the 'typical' form, i.e. the first one described of this variable species, and reduced the infraspecific taxa that Copeland (1929) recognised to a more reasonable number in order to avoid overlapping (Argent 2006). This variety is collected repeatedly on Mayon Volcano, but not found elsewhere (Argent 2006). Plants abundant on rocky open slopes on Mt Isarog and Mt Mayon (Luzon, Philippines) (Nickrent & Barcelona 2011).	(i) Where is this variety placed within *Discovireya*? (ii) Are there any close genetic affinities of this species to other taxa in this group? (iii) What is the genetic distance between accessions of this species? (iv) How closely related are the different varieties of this species? (v) Are any *R. quadrasianum* varieties related to the non-Philippines species *R. borneense*, *R. cuneifolium* and *R. monodii*?	LC
25b	*R. quadrasianum* var. *davaoense* (H F Copeland) Sleumer, *Reinwardtia* 5: 65, 1960.	PH	Found in Mindanao, Leyte, Negros and S Luzon (Philippines), 1,600–2,440 m (Argent 2006; Nickrent & Barcelona 2011).	(i) Where is this variety placed within *Discovireya*? (ii) What is the genetic relationship between this variety and other varieties of this species?	NE
25c	***R. quadrasianum* var. *rosmarinifolium*** (S. Vidal) H F Copeland, *Phil. J. Sc.* 40: 144, 1929.	PH	This variety has been in cultivation since at least 1980 from an unknown source (Argent 2006). Found in Mindoro, Biliran, Luzon and Negros, Philippines (Argent 2006; Nickrent & Barcelona 2011).	(i) Where is this variety placed within *Discovireya*? (ii) Are there any close genetic affinities of this species to other taxa in this group? (iii) What is the genetic distance between accessions of this species?	NE

#	Taxon	Range	Taxonomic Issues	Questions Raised	IUCN Code
				(iv) How closely related are the different varieties of this species?	
25d	***R. quadrasianum* var. *malindangense*** (Merr.) H F Copeland, *Phil. J. Sc*. 40: 142, 1929.	PH	Found in Mindanao and Camiguin, Philippines (Argent 2006; Nickrent & Barcelona 2011). In cultivation from Mt Apo since 1993 (Argent 2006).	(i) Where is this variety placed within *Discovireya*? (ii) Are there any close genetic affinities of this species to other taxa in this group? (iii) What is the genetic distance between accessions of this species? (iv) How closely related are the different varieties of this species?	NE
25e	*R. quadrasianum* var. *marivelesense* (H F Copeland) Sleumer, *Reinwardtia* 5: 66, 1960.		Found in Luzon, Mindoro and Leyte, Philippines (Argent 2006; Nickrent & Barcelona 2011).	(i) Where is this variety placed within *Discovireya*? (ii) What is the genetic relationship between this variety and other varieties of this species?	NE
25f	*R. quadrasianum* var. *intermedium* Merr., *Phil. J. Sc. (Bot.)* 3: 382, 1908.		Found in Luzon, Philippines (Argent 2006).	(i) Where is this variety placed within *Discovireya*? (ii) What is the genetic relationship between this variety and other varieties of this species?	NE
26	*R. taxoides* J. J. Sm., *Nova Guinea* 18: 92, 1936.	NG	Known only from the two original collections from the same locality; never cultivated (Argent 2006).	No known taxonomic issues.	VU
27a	*R. pulleanum* var. *pulleanum* Koord., *Nova Guinea* 8: 879, 1912.	NG	Not known to have been cultivated (Argent 2006).	(i) Where is this variety placed within *Discovireya*?	LC

#	Taxon	Range	Taxonomic Issues	Questions Raised	IUCN Code
				(ii) What is the genetic relationship between this variety and other varieties of this species?	
27b	*R. pulleanum* var. *maiusculum* Sleumer, *Reinwardtia* 5: 56, 1960.	NG	This variety was said by Sleumer (1966a) to approach *R. hameliiflorum* in many respects. Common in certain places (Argent 2006).	(i) Where is this variety placed within *Discovireya*? (ii) What is the genetic relationship between this variety and other varieties of this species?	NE
28	*R. nummatum* J. J. Sm., *Nova Guinea* 18: 91, 1936.	NG	Has almost circular leaves but not as distinct as in *R. pulleanum*. Not known in cultivation (Argent 2006). Found in several locations across New Guinea (Gibbs et al. 2011).	(i) Where is this variety placed within *Discovireya*? (ii) What is the genetic relationship between species and *R. pulleanum*?	LC
29a	***R. gaultheriifolium var. gaultheriifolium*** J. J. Sm., *Nova Guinea* 18: 90, 1936.	NG	This species is widespread, and locally common (Argent 2006; Gibbs et al. 2011).	(i) Where is this variety placed within *Discovireya*? (ii) What is the genetic relationship between this variety and other varieties of this species?	LC
29b	*R. gaultheriifolium* var. *expositum* Sleumer, *Reinwardtia* 5: 56, 1960.	NG	This is a high altitude (alpine) form of *R. gaultheriifolium* (Gibbs et al. 2011). Locally abundant (Argent 2006).	(i) Where is this variety placed within *Discovireya*? (ii) What is the genetic relationship between this variety and other varieties of this species?	LC
30a	*R. oreites* var. *oreites* Sleumer, *Reinwardtia* 5: 57, 1960.	NG	Not known to have been cultivated (Argent 2006).	(i) Where is this variety placed within *Discovireya*? (ii) What is the genetic relationship between this variety and other varieties of this species?	LC

#	Taxon	Range	Taxonomic Issues	Questions Raised	IUCN Code
30b	*R. oreites* var. *chlorops* Sleumer, *Reinwardtia* 5: 58, 1960.	NG	Differing from the type variety in the colour of the flowers (Argent 2006).	(i) Where is this variety placed within *Discovireya*? (ii) What is the genetic relationship between this variety and other varieties of this species?	LC
31	*R. erosipetalum* J. J. Sm., *Nova Guinea* 18: 91, 1936.	NG	A widespread species; not known to have been cultivated. Said to be closely related to *R. detznerianum* (Argent 2006).	(i) Where is this variety placed within *Discovireya*? (ii) What is the genetic relationship between this species and *R. detznerianum*?	LC
32	*R. detznerianum* Sleumer, *Blumea* 21(2): 359, 1973.	NG	Taxonomic debate exists around the status of this species: poorly known species from just one location and is not known to be in cultivation (Gibbs et al. 2011). Said to be closely related to *R. erosipetalum* from the Vogelkop Peninsula (Argent 2006).	(i) Where is this variety placed within *Discovireya*? (ii) What is the genetic relationship between this species and *R. erosipetalum*?	DD
33	*R. hameliiflorum* Wernham, *Trans. Linn. Soc. London*, II, Bot. 9: 98, 1916.	NG	Found on Mt Jaya (W New Guinea, Indonesia) (Gibbs et al. 2011). Collected only once. A very imperfectly known species which has not been recollected from the type locality despite considerable recent botanical activity there (Argent 2006).	No known taxonomic issues.	DD
34a	*R. lindaueanum* var. *lindaueanum* Koord., *Nova Guinea* 8(4): 878, 1912.	NG	Large healthy populations; widespread and variable species (Argent 2006; Gibbs et al. 2011). The differences between *R. lindaueanum* and *R. erosipetalum* do not appear to be significant but there are considerable differences between the West New Guinea and East New Guinea specimens of this species (Argent 2006).	(i) Where is this variety placed within *Discovireya*? (ii) What is the genetic relationship between this variety and other varieties of this species?	LC

#	Taxon	Range	Taxonomic Issues	Questions Raised	IUCN Code
34b	*R. lindaueanum* var. *bantaengense* J. J. Sm., *Fedde Rep*. 30: 163, 1932.	SW	Found only in a single alpine location (Argent 2006).	(i) Where is this variety placed within *Discovireya*? (ii) What is the genetic relationship between this variety and other varieties of this species? (iii) Is this variety related to other taxa of *Discovireya* (or other sections) found on Sulawesi?	VU D2
35	*R. cyrtophyllum* Wernham, *Trans. Linn. Soc. London*, II, Bot. 9: 97, 1916.	NG	Found only in a single location. Not yet recollected and remaining poorly known. Never cultivated. Needs further research to establish the conservation status (Argent 2006; Gibbs et al. 2011).	No known taxonomic issues.	DD
36	*R. ciliilobum* Sleumer, *Reinwardtia* 5: 64, 1960.	NG	Not known in cultivation (Argent 2006). Widespread, but an imperfectly known species (Gibbs et al. 2011).	No known taxonomic issues.	LC

A2.3 Section *Siphonovireya* (Sleumer) Argent

Table 35 Summary of the taxonomic and conservation issues of Section *Siphonovireya*. The taxa in boldface denote those analysed in this study. The taxa are arranged according to the classification of Argent (2006), and the taxon numbers in the leftmost column correspond to the taxon number used in Argent's (2006) classification. The column 'Range' shows the geographic region the taxa belong (Figure 21).

#	Taxon	Range	Taxonomic Issues	Questions Raised	IUCN Code
37	*R. agathodaemonis* J. J. Sm., *Fedde Rep*. 1913	NG	Not known in cultivation. The differences between *R. agathodaemonis* and *R. herzogii* are not clearly established. Sleumer (1973) modified his view of the difference from his *Flora Malesiana* (Sleumer 1966a) in the light of observations on flower length made by Peter Stevens and then made the chief difference fruit size. On the basis of limited herbarium specimens	(i) What is the genetic relationship between this species and *R. herzogii*?	DD

#	Taxon	Range	Taxonomic Issues	Questions Raised	IUCN Code
			especially of *R. agathodaemonis* there does seem to be a difference in the corolla tube shape (Argent 2006).		
38	*R. incommodum* Sleumer, *Reinwardtia* 5: 70, 1960.	NG	Not known in cultivation. Not recollected recently. It is the only really red-flowered species in *Siphonovireya*. Tempting to regard it as a hybrid between a *Siphonovireya* and a red-flowered species from another section if it was not reported as locally common (in three locations; quite widespread) (Argent 2006).	(i) Is this species related to any taxa outside Section *Siphonovireya*?	LC
39	***R. inundatum*** Sleumer, *Blumea* 12: 92-93, 1963.	NG	Common in a large population. A natural hybrid between this species and a red-flowered *Rhododendron* is in cultivation with deep pink flowers. Another wild collected hybrid (probably with *R. konori*) is in cultivation (Argent 2006).	(i) What is the genetic relationship between this species and *R. konori*? (ii) Which other taxa does this species cluster with on the phylogenetic tree?	LC
40	*R. protandrum* Sleumer, *Blumea* 11: 114, 1961.	NG	Found in a single location; common. Not known in cultivation (Argent 2006).	No known taxonomic issues.	DD
41	*R. habbemae* Koord, *Nova Guinea* 8(4): 877, 1912.	NG	Not known in cultivation (Argent 2006).	No known taxonomic issues.	LC
42	*R. cinchoniflorum* Sleumer, *Reinwardtia* 5: 68, 1960.	NG	Widespread species. Introduced into cultivation in 2001 but not yet established. The leaves of this species are not aromatic as they are in *R. herzogii*. A single hybrid has been recorded, probably with *R. schlechteri* that was growing with this species in the Mt Jaya region (Argent 2006).	(i) Is there a genetic relationship between this species, *R. herzogii* and *R. schlechteri*? (ii) Is this species genetically related to any other taxa outside Section *Siphonovireya*?	LC
43	***R. herzogii*** Warb., *Bot. Jahr*. 16: 52, 1892.	NG	Introduced repeatedly into cultivation from Papua New Guinea in the early 1960s and also subsequently. Resinously aromatic foliage and scented flowers. This species is closely related to *R. agathodaemonis* and	(i) What is the genetic relationship between this species, *R. inundatum*, *R. culminicola*, R. *archboldianum*	LC

#	Taxon	Range	Taxonomic Issues	Questions Raised	IUCN Code
			the differences between these species are not clearly established (Argent 2006). *R. herzogii* crosses with *R. macgregoriae* (Section *Euvireya* Subsection *Euvireya*) which is also categorized as LC. According to molecular studies by Brown et al. (2006a), *R. herzogii* is very closely related to *R. inundatum* (LC).	'Starburst', *R. archboldianum* and *R. macgregoriae*?	
44	*R. gideonii* Argent, *Folia Malaysiana* 4(2): 104, 2003.	NG	Not yet introduced into cultivation and at present known only from two herbarium collections. This species is in some respects similar to *R. protandrum* (Argent 2006). Overall the impression of this species is of a smaller and more delicate plant than *R. herzogii* (Argent 2006).	(i) What is the genetic relationship between this species and *R. protandrum*? (ii) Is there a genetic relationship between this species and *R. herzogii*?	DD
45	*R. searleanum* Sleumer, *Blumea* 21(2) 367, 1973.	NG	Cultivated since 1974 from type material collected by Lou Searle. Sleumer (1973) placed this species in 'Solenovireya', commenting that 'the scales are almost entire in the dry specimens'. In fact the scales are entire with large centres in the live specimens, especially so on the pedicels and corollas, as is quite typical of 'Siphonovireya' and hence its placement in Section *Siphonovireya* (Argent 2006).	(i) Is this species related to any taxa from Section *Solenovireya*? (ii) Is this species closely related to other taxa of Section *Siphonovireya*?	LC
NEW	*R. dutartrei* F. Danet, *Adansonia*, sér 3 29(1): 106–108, 2007.	NG	Discovered in 2007, and known only from type collection and the single population, <250 individuals , is restricted to the edge of secondary forest which is often damaged by fire and erosion. This species is close to *R. incommodum* (Danet 2007).	(i) What is the placement of this species within Subgenus *Vireya* relative to Section *Siphonovireya*? (ii) What is the genetic relationship between this species and *R. incommodum*?	CR C2a(ii)
NEW	*R. kogo* F. Danet, *Adansonia*, sér 3 29(1): 108–110, 2007.	NG	Discovered in 2007, and known only from type collection. This species is close to *R. agathodaemonis* J. J. Sm. (Danet 2007).	(i) What is the placement of this species within Subgenus *Vireya* relative to Section *Siphonovireya*?	DD

#	Taxon	Range	Taxonomic Issues	Questions Raised	IUCN Code
				(ii) What is the genetic relationship between this species and *R. agathodaemonis*?	

A2.4 Section *Phaeovireya* (Sleumer) Argent

Table 36 Summary of the taxonomic and conservation issues of Section *Phaeovireya*. The taxa in boldface denote those analysed in this study. The taxa are arranged according to the classification of Argent (2006), and the taxon numbers in the leftmost column correspond to the taxon number used in Argent's (2006) classification. The column 'Range' shows the geographic region the taxa belong (Figure 21).

#	Taxon	Range	Taxonomic Issues	Questions Raised	IUCN Code
46	***R. eymae*** Sleumer, *Reinwardtia* 5: 74, 1960.	SW	Point endemic from the summit of Mt Rantemario, very small but healthy population (<100 mature individuals) and range (<1 km^2). No known current threats (Gibbs et al. 2011). Common in the open summit area of Mt Rantemario (Sulawesi, Indonesia) (Argent 2006).	No known taxonomic issues.	EN D
47	*R. psilanthum* Sleumer, *Reinwardtia* 5: 81, 1960.	SW	Known only from the type collection and needs further research to establish its status (Gibbs et al. 2011). Not yet recollected since the original find, and never cultivated (Argent 2006).	No known taxonomic issues.	DD
48	*R. asperrimum* Sleumer, *Blumea* 12: 97, 1963.	NG	Not known in cultivation (Argent 2006).	No known taxonomic issues.	DD
49	***R. asperum*** J. J. Sm., *Nova Guinea* 12: 137, 1914.	NG	Sleumer (1966a) described wild hybrids of this species with *R. laetum* (Argent 2006). Healthy; several populations (Gibbs et al. 2011).	(i) What is the genetic relationship between this species and *R. laetum*? (ii) Is this species related to taxa outside Section *Phaeovireya*?	LC

#	Taxon	Range	Taxonomic Issues	Questions Raised	IUCN Code
50	***R. beyerinckianum*** Koord., *Nova Guinea* 8(4): 876, 1912.	NG	A plant corresponding to one of van Royen & Kores (1982) lower altitude forms is in cultivation at Pukeiti in New Zealand, and has larger flatter leaves and pink flowers (Argent 2006). Sleumer (1966a) conceived this species in a broad sense and acknowledged that it might ultimately be united with *R. phaeochitum* (Argent 2006). Van Royen & Kores (1982) reported that this species had been found on all major mountain ranges from the Nassau Mts to Mt Daymana and that 'it is an extremely polymorphic species. Plants from different geographic locations vary considerably in stature, flower colour, leaf size, leaf shape and texture (Argent 2006).	(i) What is the genetic relationship between this species and *R. phaeochitum*?	LC
51	***R. bryophilum*** Sleumer, *Reinwardtia* 5: 79, 1960.	NG	The distinction between this species and *R. dielsianum* is not clearly established; the best difference appears to be that *R. dielsianum* has a glabrous style except for a few hairs at the base whereas in *R. bryophilum* the style is covered with simple hairs for most of its length (Argent 2006).	(i) What is the relationship between this species and *R. dielsianum*?	DD
52	*R. bullifolium* Sleumer, *Blumea* 12: 93, 1963.	NG	Known only from the type specimen which is of poor quality and therefore taxonomic debate exists with the name remaining unresolved (Gibbs et al. 2011). Once collected and still apparently known only from the very imperfect type specimen and the collector's field notes (Argent 2006).	(i) Where is this species placed within the Subgenus *Vireya*? (ii) What taxa are closely related to this species?	DD
53	***R. caliginis*** Kores, *Blumea* 30(1): 45, 1984.	NG	This species is similar to *R. hooglandii*. Widely cultivated; a pink form in cultivation under the name has broader narrowly elliptic leaves and is probably a hybrid (Argent 2006).	(i) What is the genetic relationship between this species and *R. hooglandii*?	LC
54a	*R. delicatulum* var. *delicatulum*	NG	Only known from type specimen; additional field work required prior to conservation assessment (Gibbs et al. 2011).	(i) What is the genetic differentiation between the two varieties of this species?	DD

#	Taxon	Range	Taxonomic Issues	Questions Raised	IUCN Code
	Sleumer, *Reinwardtia* 5: 78, 1960.				
54b	*R. delicatulum* var. *lanceolatoides* Sleumer, *Blumea* 11: 116, 1961.	NG	Only known from type specimen; additional field work required prior to conservation assessment (Gibbs et al. 2011).	(i) What is the genetic differentiation between the two varieties of this species?	DD
55	*R. dianthosmum* Sleumer, *Blumea* 12: 100, 1963.	NG	An epiphytic species known from a single location on Mt Dafonsero (Gibbs et al. 2011). In cultivation since 1961 when Professor Sleumer sent seed from New Guinea to the USA from where it has been distributed. Probably all genuine materials of this species are from this introduction (Argent 2006).	(i) Where is this species placed within Subgenus *Vireya*? (ii) Are there genetic relationships between this species and other similar-flowered taxa?	VU D2
56a	***R. dielsianum* var. *dielsianum*** Schltr., *Bot. Jahr*. 55: 150, 1918.	NG	A common species (Argent 2006).	(i) What is the genetic differentiation between the two varieties of this species?	LC
56b	*R. dielsianum* var. *stylotrichum* Sleumer, *Reinwardtia* 5: 80, 1960.	NG	Not known to be in cultivation (Argent 2006). This name is not yet resolved. The type specimen (Hoogland & Pullen 5307) notes that the flowers were pinkish red (The Herbarium Catalogue 2006).	(i) What is the genetic differentiation between the two varieties of this species?	LC
57	*R. extrorsum* J. J. Sm., *Nova Guinea* 18: 95, 1936.	NG	Only known from one collection at one location and has not been recollected. Needs further research to establish the conservation status (Gibbs et al. 2011).	No known taxonomic issues.	DD
58	***R. gardenia*** Schltr., *Bot. Jahr*. 55: 158, 1918.	NG	Cultivated locally in New Guinea around Telefomin. Two forms now in cultivation, both with creamy very strongly perfumed flowers, one with flat overlapping lobes, and the other with lobes having strongly revolute lateral margins. Supposedly grown in Australia but most if not all early plants were identified as a hybrid: *R.* 'Gardenia Odyssey' (Argent 2006; Clancy 2005; Craven 1993). Recent visits to the Bele River Valley have failed to re-find this species	(i) What is the genetic differentiation between this species and *R.* 'Gardenia Odyssey'? (ii) What is the genetic relationship between *R. gardenia* and *R. superbum*?	LC

#	Taxon	Range	Taxonomic Issues	Questions Raised	IUCN Code
			in the type locality although a very similar plant which keys out to *R. superbum* is common there (Argent 2006).	(iii) Are there genetic relationships between this species and other similar-flowered taxa?	
59	*R. haematophthalmum* Sleumer, *Reinwardtia* 5: 89, 1960.	NG	Introduced into cultivation in 1992 but failed to establish (Argent 2006).	No known taxonomic issues.	LC
60	***R. hellwigii*** Warb, *Bot. Jahr*. 16: 26, 1892.	NG	Commonly forms hybrids at the western end of its range with *R. superbum*. Paul Kores collected the first living plants that flowered in cultivation from the Finistere Mts in 1976. These were distributed as seed of *R. superbum* but they were confirmed as *R. hellwigii* by Withers & Rouse (1988) when they flowered for the first time in March 1988 in Melbourne (Australia) and simultaneously at Pukeiti in New Zealand. Subsequent collections of this species were made by Sandham in 1986 from above Iloko village (near Konge) on the slopes of Mt Bangeta; these have also flowered and been distributed. The poor pollen reported by Withers & Rouse (1988) was probably due not to hybridity but to the fact that the plants were insufficiently vigorous to produce fully fertile anthers (Argent 2006). Healthy populations present in montane forest, thus minimal threat (Gibbs et al. 2011).	(i) What is the genetic relationship between this species and *R. superbum*? (ii) Are there genetic relationships between this species and other similar-flowered taxa?	LC
61	*R. hooglandii* Sleumer, *Reinwardtia* 5: 75, 1960.	NG	There is still some confusion between this species and *R. caliginis*. Kores (1984) distinguished *R. caliginis* from *R. hooglandii* 'on the basis of its patent, slightly or non-revolute leaves. Not in cultivation at present (Argent 2006).	(i) What is the genetic relationship between this species and *R. caliginis*?	DD
62	***R. hyacinthosmum*** Sleumer, *Blumea* 21(2): 363, 1973.	NG	Seedlings were reported growing in Melbourne in 1971 from material sent by Canon Cruttwell at an unconfirmed date. It was material from these plants from which the species was later described. It has	(i) Is this species genetically related to similar-flowered taxa?	LC

#	Taxon	Range	Taxonomic Issues	Questions Raised	IUCN Code
			been widely distributed but also confused, so that not all plants grown under this name are correctly attributable to this species (Argent 2006).		
63	*R. kerowagiense* Argent, *Folia Malaysiana* 4(2): 108, 2003.	NG	It is closely related to *R. rarum* (Argent 2006). This name is not yet resolved (The Plant List 2010).	(i) What is the genetic relationship between this species and *R. rarum*?	VU
64a	***R. konori* var. *konori*** Becc., *Malesia* I: 200, 1878.	NG	Introduced into cultivation the 1960s, and subsequently from various places in New Guinea, especially in the Arfak Mts, where it grows in abundance. It apparently hybridises with *R. asperum*, which is often found in the same places. In one place with abundant *R. konori* and *R. laetum*, growing together. Sleumer (1966a) recorded a fruiting specimen which was apparently intermediate in character between these species (Argent 2006).	(i) What is the genetic relationship between this species, *R. laetum* and *R. asperum*? (ii) What is the genetic differentiation between the two varieties of this species?	LC
64b	*R. konori* var. *phaeopeplum* (Sleumer) Argent, *Edinb. J. Bot*. 52(3): 364, 1995.	NG	A small form of *R. konori*, and possibly in the Wissel Lakes region a hybrid of *R. konori* with a related species of the same subsection with smaller flowers, possibly *R. rappardii*. A number of natural intermediate hybrids have been observed with the 5-lobed, orange-red-flowered *R. zoelleri*, which is abundant in the same locality. A few specimens of *R. zoelleri*, typical except for the white corollas with a yellowish colour, at least at the tube, were found in the same place; these are probably due to a slight introgression with *R. phaeopeplum* (Argent 2006; Sleumer 1966a).	(i) What is the genetic relationship between this species, *R. rappardii*, *R. zoelleri* and *R. asperum*? (ii) What is the genetic differentiation between the two varieties of this species?	NE
65	***R. leptanthum*** F. Muell., *Trans. R. Soc. Vict*. 1(2): 24, 1889.	NG	The first recorded introduction was from seed collected at Edie Creek above Wau which was sent by John Womersley to Edinburgh in 1961. Other introductions were made in the late 1960s by Paddy Woods and Michael Black and this species was introduced to Australia by Lyn Craven in 1966	(i) Is there a genetic relationship between this species and *R. gracilentum*?	LC

#	Taxon	Range	Taxonomic Issues	Questions Raised	IUCN Code
			(Argent 2006). Widespread species; previously known as *R. warianum* Schltr. Forms agreeing with the description of *R. warianum* were collected by John Sandham (Argent 2006). According to molecular studies *R. leptanthum* is closely related to *R. gracilentum* (Section *Euvireya* Subsection *Linnaeopsis*) with strong bootstrap support (Brown et al. 2006b).	(ii) What physical characters unite the species *R. leptanthum* and *R. gracilentum*? (iii) Does *R. leptanthum* ally with any other taxa from Section *Euvireya*?	
66	*R. melantherum* Schltr., *Bot Jahr*. 55: 152, 1918.	NG	Found once in a single location in mountain forest at 2,070 m. No type material is known to be preserved and remains an imperfectly known species (Argent 2006).	(i) Where is this species placed within Subgenus *Vireya*? (ii) What are its closest relatives?	DD
67	*R. neobritannicum* Sleumer, *Blumea* 21: 361, 1973.	NB	Vulnerable. Found in four mountains, at low altitudes (800–1,585 m). Sleumer (1973) notes the similarity between this species and *R. rarum*. Not known in cultivation (Argent 2006).	(i) What is the genetic relationship between this species and *R. rarum*?	VU C1
68	*R. neriifolium* Schltr., *Bot. Jahr*. 55: 149, 1918.	NG	Neither type material preserved, nor recollected. The description of this species was based on the original diagnosis. Known from the type locality and no recent collections. The type material was destroyed in Berlin and this species has still to be recollected (Argent 2006).	No known taxonomic issues.	DD
69	*R. opulentum* Sleumer, *Reinwardtia*, 5: 85, 1960.	NG	Said to be related to *R. kawir*. Known from two locations (Argent 2006).	(i) What is the genetic relationship between this species and *R. kawir*?	LC
70	*R. phaeochitum* F. Muell., *Trans. R. Soc. Vict*. 1(2): 23, 1889.	NG	Widespread species; still not clearly distinguishable from *R. beyerinckianum* (Argent 2006).	(i) What is the genetic relationship between this species, *R. beyerinckianum* and *R. phaeochitum*?	LC

#	Taxon	Range	Taxonomic Issues	Questions Raised	IUCN Code
71	*R. phaeochristum* Sleumer, *Blumea* 12: 95, 1963.	NG	Common on Arfak Mountains. A natural hybrid of *R. phaeochristum* apparently with *R. culminicola* var. *angiense* was found once in the Arfak Mts (Argent 2006).	(i) What is the genetic relationship between this species and *R. culminicola* var. *angiense*?	LC
72	*R. phaeops* Sleumer, *Reinwardtia* 5: 90, 1960.	NG	Known from a single location, and a single collection. Not re-collected or reported since the original collection and never cultivated (Argent 2006).	No known taxonomic issues.	DD
73	*R. prainianum* Koord., *Nova Guinea* 8: 187, 1909.	NG	Widespread species. Introduced into cultivation in 2000 from Mt Jaya (Argent 2006).	(i) The identity of this species needs to be confirmed to match the original type description.	LC
74	*R. rappardii* Sleumer, *Reinwardtia* 5: 93, 1960.	NG	Said to be related to *R. kawir*. A tentative hybrid with *R. rosendahlii* is recorded by Sleumer in the Leiden herbarium. Not known to be in cultivation (Argent 2006).	(i) What is the genetic relationship between this species and *R. kawir*? (ii) What is the genetic relationship between this species and *R. rosendahlii*?	LC
75	***R. rarum*** Schltr., *Bot. Jahr*. 55: 150, 1918.	NG	A widespread species. Introduced into the RBGE in 1961 from material sent by the Dept. of Forests in Lae. It was grown in Strybing from material collected by Prof Sleumer in 1965 and this was distributed to Kew. Very similar *R. stelligerum* (Argent 2006).	(i) What is the genetic relationship between this species and *R. stelligerum*?	LC
76	*R. revolutum* Sleumer, *Reinwardtia* 5: 74, 1960.	NG	Said to be close to *R. tintinnabellum*. Known only from the type collection and never cultivated. Restricted to crevices and sandy niches on sterile limestone slopes, at 3,225 m (Argent 2006).	(i) What is the genetic relationship between this species and *R. tintinnabellum*?	DD
77	*R. rhodochroum* Sleumer, *Reinwardtia* 5: 87, 1969.	NG	Common; apparently not recollected since the original single collection, and never cultivated (Argent 2006).	No known taxonomic issues.	DD
78	*R. rubellum* Sleumer, *Reinwardtia* 5: 94, 1960.	NG	Collections from widespread localities have shown this to be a variable species (van Royen & Kores	No known taxonomic issues.	LC

#	Taxon	Range	Taxonomic Issues	Questions Raised	IUCN Code
			1982). Introduced into cultivation in 1976 (Argent 2006).		
79	***R. solitarium*** Sleumer, *Blumea* 12: 94, 1963.	NG	Introduced into cultivation in Edinburgh by Paddy Woods from Mt Kaindi in 1968, there have doubtless been other introductions and it is now widely grown although not all plants grown under this name are this species (Argent 2006).	(i) Do all the accessions of this species cluster together on the phylogenetic tree? (ii) If not clustering together, which taxa do the accessions ally with? (iii) Does the physical examination of the accessions match with the type description and the holotype?	VU
80	*R. spondylophyllum* F. Muell., *Trans. R. Soc. Vict*. n.s. 1(2): 23, 1889.	NG	Van Royen & Kores (1982) reduced *R. cyatheicolum* as a synonym under this name. Sleumer (1966a) separated the species from *R. cyatheicolum* having longer flowers and leaf size. Seems safe to include *R. cyatheicolum* under *R. spondylophyllum*; also closely related to *R. evelyneae* (Argent 2006).	(i) What is the genetic relationship between this species and *R. evelyneae*? (ii) What is the genetic relationship between this species and *R. cyatheicolum*?	LC
81	*R. stelligerum* Sleumer, *Blumea* 11: 115, 1961.	NG	Not known in cultivation. Wild hybrids between *R. stelligerum* and *R. delicatulum* were reported by Sleumer from the Star Mts. This species is very similar to *R. rarum* and replaces it further to the west (Argent 2006).	(i) What is the genetic relationship between this species and *R. delicatulum*?	LC
82	*R. stolleanum* Schltr., *Bot. Jahr*. 55: 143, 1917.	NG	The holotype was destroyed in Berlin and no isotype has yet been found; the description is derived from Prof Sleumer's translation of the original which was incomplete. Sleumer (1966a) noted: 'The position of *R. stolleanum* both in and within the Subsection *Phaeovireya* thus remains somewhat doubtful; yet most of the characters given are those of *R. dielsianum*' (Argent 2006).	(i) What is the genetic relationship between this species and *R. dielsianum*?	DD

#	Taxon	Range	Taxonomic Issues	Questions Raised	IUCN Code
83a	***R. superbum* ssp. *superbum*** Sleumer, *Reinwardtia* 5: 76, 1960.	NG	Hybridising in the wild with *R. hellwigii* where these species overlap to give deep pink intermediate forms. It is possible that all the really pink forms of R. superbum are of hybrid origin. First introduced into cultivation by Prof Sleumer (Argent 2006). Stonor (1952) described this subspecies as '*R. devriesianum vel*. *aff*. Flower scent similar to that of *R. agathodaemonis* (Argent 2006).	(i) What is the genetic relationship between this subspecies and *R. hellwigii*? (ii) What is the genetic relationship between this subspecies, *R. gardenia* and *R. inundatum*? (iii) What is the genetic relationship between this subspecies and other similar-flowered taxa? (iv) How much are the two subspecies of this species genetically differentiated? (v) Does the physical characteristics of the accessions of this subspecies match the type description, or perhaps the other subspecies *ibele*?	LC
83b	*R. superbum* ssp. *ibele* Argent, *Rhododendrons of subgenus Vireya*, 103-104, 2006.	NG	This collection keys out to *R. superbum* but differs in the very short corolla tube. It was found while searching for *R. gardenia* in the type locality of this species and was at first thought to be *R. gardenia* but that species has the ovary covered by long simple hairs as well as the scales. This species was growing with *R. inundatum* and a hybrid between these species was collected. It is tempting to think that the distinctive rounded scales of this subspecies might be due to introgression with *R. inundatum* (Argent 2006).	(i) What is the genetic relationship between this subspecies, *R. gardenia* and *R. inundatum*? (ii) What is the genetic relationship between this subspecies and other similar-flowered taxa? (iii) How much are the two subspecies of this species genetically differentiated?	VU
84	*R. thaumasianthum* Sleumer, *Blumea*, 12: 98, 1963.	NG	Sleumer (1966a) commented that this species was 'similar to *R. konori*, but [the] style [was] completely glabrous'. Awaiting further collections but it seems very likely that this will turn out to be just a variant of	(i) What is the genetic relationship between this species and *R. konori*?	DD

#	Taxon	Range	Taxonomic Issues	Questions Raised	IUCN Code
			R. konori. Known only from the type collection (Argent 2006).		
85	***R. truncicola*** Sleumer, *Reinwardtia* 5: 91, 1960.	NG	Introduced into cultivation in New Zealand by Michael Cullinane in 1988, from Mt. Simpson where it was growing at 2,025 m (Argent 2006).	No known taxonomic issues.	LC
86	*R. tuberculiferum* J. J. Sm., *Med. Rijksherb*. 25: 4, 1915.	NG	Only known from two old collections. Never cultivated (Argent 2006).	No known taxonomic issues.	DD
87	*R. evelyneae* Danet, *Adansonia* 27(2): 270, 2005.	NG	Closely related to *R. spondylophyllum*. Discovered in 2005 and known at present only from the type location. Not known in cultivation (Argent 2006).	(i) What is the genetic relationship between this species and *R. spondylophyllum*?	VU
88	*R. kawir* Danet, *Adansonia* 27(2): 273, 2005.	NG	Said to be similar to *R. rappardii* and *R. opulentum*. Discovered in 2005 and known only from the type collection (Argent 2006).	(i) What is the genetic relationship between this species, *R. rappardii* and *R. opulentum*?	VU
89	*R. tintinnabellum* Danet, *Adansonia* 27(2): 268, 2005.	NG	Said to be close to *R. revolutum*. Discovered in 2005 and known only from the original type collection. Not yet cultivated (Argent 2006).	(i) What is the genetic relationship between this species and *R. revolutum*?	VU
89a	*R.* × *gilliardii* Sleumer, *Reinwardtia* 5: 88, 1960.	NG	Natural hybrid; once found near timber line at 2,285–3,655m. Considered to be a hybrid between *R. macgregoriae* and an unknown species. This plant has not been recollected despite the site being well visited. Described as similar to *R. macgregoriae*, but differing in having stellate scales on distinct tubercles, typical of Section *Phaeovireya*. Van Royen & Kores (1982) suggested a strong resemblance to material tentatively identified as *R. macgregoriae* × *R. dielsianum*; Sleumer (1973) had suggested the possibility of it being *R. beyerinckianum* × *R. macgregoriae* (Argent 2006).	(i) What is the genetic relationship between this taxon and *R. macgregoriae*? (ii) What is the genetic relationship between this taxon and *R. beyerinckianum*? (ii) What is the genetic relationship between this taxon and *R. dielsianum*?	NE

#	Taxon	Range	Taxonomic Issues	Questions Raised	IUCN Code
89b	*R.* × *schoddei* Sleumer, *Blumea* 12: 95, 1963.	NG	Natural hybrid; originally placed in Subsection *Phaeovireya* (Sleumer 1963a). *R.* × *schoddei* shows a general resemblance to *R. christi*, as noted by van Royen & Kores (1982). They also stated that *R. christi* is often found growing sympatrically with *R. beyerinckianum* (Argent 2006).	(i) Does this taxon cluster together with other taxa of Section *Phaeovireya*? (ii) What is the genetic relationship between this taxon, *R. christi* and *R. beyerinckianum*? (iii) Does this taxon cluster with other taxa of Section *Euvireya* on the phylogenetic tree?	NE

A2.5 Section *Malayovireya* (Sleumer) Argent

Table 37 Summary of the taxonomic and conservation issues of Section *Malayovireya*. The taxa in boldface denote those analysed in this study. The taxa are arranged according to the classification of Argent (2006), and the taxon numbers in the leftmost column correspond to the taxon number used in Argent's (2006) classification. The column 'Range' shows the geographic region the taxa belong (Figure 21).

#	Taxon	Range	Taxonomic Issues	Questions Raised	IUCN Code
90	*R. acuminatum* Hook. *f.*, *Ic. Pl.* t. 886, 1852.	BN	Restricted range and population diminished to about 50% by drought. Dramatic change in population caused by El Nino drought. One natural hybrid recorded with *R. fallacinum*. Thought to hybridize with *R. lamrialianum*. Most records of it growing successfully when checked have proved to be *R. rugosum* (Argent 2006). Vegetatively different from *R. fallacinum* which grows together with *R. acuminatum* in part of its range on Mt Kinabalu (Argent 2006).	(i) Where is this species placed within the Subgenus *Vireya*, given the peculiar physical characters? (ii) Does this species cluster with the rest or majority of the taxa of Section *Malayovireya*? (iii) What is the genetic relationship between this species, *R. fallacinum*, *R. lamrialianum* and *R. rugosum*?	EN
91	***R. apoanum*** Stein, *Gartenflora* 34: 194, t. 1196, 1885.	PH	Copeland (1929) discussed the existence of 2 forms which had also been suggested by Elmer (Elmer 1911). This species might be regarded as an extreme	(i) Is this species related to the *R. malayanum* complex?	LC

#	Taxon	Range	Taxonomic Issues	Questions Raised	IUCN Code
			variant of the *R. malayanum* complex. It is distinct from the only other *Malayovireya* so far known from the Philippines – *R. nortoniae*. Found in two mountains, but populations healthy. Common on Mt Apo (Argent 2006).		
92a	*R. durionifolium* ssp. *durionifolium* Becc., *Malesia* 1: 202, 1878.	BN	Common and widespread (Argent 2006).	No known taxonomic issues.	LC
92b	*R. durionifolium* ssp. *sabahense* Argent, A. Lamb & Phillipps, *Notes RBGE* 42(1), 119: 1984.	BN	More restricted than the other subspecies but healthy populations present. Occurs in fairly uniform populations (Argent 2006).	No known taxonomic issues.	LC
93	***R. fallacinum*** Sleumer, *Reinwardtia* 5: 99, 1960.	BN	It was noted by Sleumer (1966a) that 'sterile specimens [were] hardly distinguishable from those of *R. durionifolium*'. In fact this species is not clearly distinguished from *R. durionifolium* (Argent 2006). Common species. Earliest cultivation started from 1980 from a series of introductions to Australia, New Zealand and the UK. Plants are difficult to grow satisfactorily and to propagate (Argent 2006).	(i) What is the relationship between *R. fallacinum* and *R. durionifolium*?	LC
94	*R. fortunans* J. J. Sm., *Bull. Jard. Bot. Buit. III*, 1: 401 t49, 1920.	BN	Found in a single location. Relatively common on that mountain. Location is in a National Park. The flowers are, as can be compared from dry samples, identical with those of *R. himantodes* but the leaves are markedly different (Argent 2006).	No known taxonomic issues.	NT
95a	***R. himantodes* var. *himantodes*** Sleumer, *Bot. Jahr*. 71: 145, 1940.	BN	First introduced by Bill Burtt and Paddy Woods in 1962. Reintroduced in 1978 and subsequently to both UK and NZ. Found in several locations. *R. himantodes* var. *himantodes* has morphological	(i) Is *R. himantodes* var. *himantodes* genetically related to *R. stenophyllum*, *R. vinicolor*, *R. lineare* and *R. fortunans*?	LC

#	Taxon	Range	Taxonomic Issues	Questions Raised	IUCN Code
			similarities with *R. stenophyllum*, *R. vinicolor*, *R. lineare* and *R. fortunans* (Argent 2006).		
95b	*R. himantodes* var. *lavandulifolium* Sleumer, *Reinwardtia* 5: 97, 1960.	BN	Further collection needed to rule out the flower colour difference. Known only from the type locality (Argent 2006).	No known taxonomic issues.	NE
96a	*R. lamrialianum* ssp. *lamrialianum* Argent & Barkman, *The New Plantsman* 7(4): 209, 2000.	BN	Found only on a single mountain, but population healthy (Argent 2006).	No known taxonomic issues.	VU
96b	*R. lamrialianum* ssp. *gunsalamianum* Argent & Barkman, *The New Plantsman* 7(4): 214, 2000.	BN	Found only on a single mountain. Protected, but population small. Probably <250 individuals (Gibbs et al. 2011).	No known taxonomic issues.	EN
97	*R. lineare* Merr., *J. Str. Br. As. Soc.* 76: 108, 1917.	BN	In low altitudes, epiphytic in dipterocarp forest. Forest under threat. Widespread though in that forest (Argent 2006).	No known taxonomic issues.	NT
98a	***R. malayanum* var. *malayanum f. malayanum*** Jack, *Mal. Misc*. 2: 17, 1822.	MP, BN, SM, JV, SW, ML	Widespread and subtly variable species. Thought to be related to *R. apoanum*. *R. vinicolor* might be considered an extreme form. *R. micromalayanum* has identical flowers although smaller leaves. Similar to *R. nortoniae* from Philippines, but with larger flowers. Possibly *R.* × *variolosum* in southern Sarawak is the hybrid between *R. malayanum* and *R. javanicum*. *R. malayanum* also hybridizes with *R. vinicolor* and *R. jasminiflorum* (Argent 2006).	(i) What is the genetic relationship between *R. malayanum* and *R. apoanum*? (ii) Is *R. malayanum* related to *R. micromalayanum*? (iii) Is *R. malayanum* related to *R. jasminiflorum* and/or *R. javanicum*?	LC
98b	*R. malayanum* var. *malayanum f. latifolium* Sleumer, *Reinwardtia* 5: 103, 1960.	BN	No known issues.	No known taxonomic issues.	NE

#	Taxon	Range	Taxonomic Issues	Questions Raised	IUCN Code
98c	*R. malayanum* var. *pubens* Sleumer, *Reinwardtia* 5: 102, 1960.	ML	One record at 1,000 m, on a steep limestone ridge (Argent 2006).	No known taxonomic issues.	DD
98d	*R. malayanum* var. *pilosifilum* Sleumer, *Reinwardtia* 5: 102, 1960.	BN, ML	Widespread species (Argent 2006).	No known taxonomic issues.	LC
99	*R. micromalayanum* Sleumer, *Blumea* 21: 364, 1973.	BN	In several locations with healthy populations (Gibbs et al. 2011).	No known taxonomic issues.	LC
100	*R. nortoniae* Merr., *Phil. J. Sc.* 1: Supp. 220, 1906.	PH	Found in 3 locations (Gibbs et al. 2011).	No known taxonomic issues.	DD
101	*R. obscurum* Sleumer, *Reinwardtia* 5: 104, 1960.	MP	Found in a single location (Gibbs et al. 2011). Sleumer (1966a) suggested that this could be a natural hybrid with a species of *Pseudovireya* (*R. perakense*, *R. scortechinii* or *R. spathulatum*).	No known taxonomic issues.	DD
102	*R. vinicolor* Sleumer, *Reinwardtia* 5: 98, 1960.	SM	No known issues.	No known taxonomic issues.	LC
102a	*R.* × *andersonii* (Ridl.) Argent, *Rhod. Subg. Vireya* 126, 2006.	BN	No known issues.	No known taxonomic issues.	NE
102b	*R.* × *hybridogenum* Sleumer, *Reinwardtia* 5: 106, 1960.	MP	Once found. Thought to be a natural hybrid between *R. malayanum* and *R. jasminiflorum* var. *punctatum* (Argent 2006).	See *R. malayanum*.	NE
102c	*R.* × *variolosum* (Becc.) Argent, *Rhod. Subg. Vireya* 126, 2006.	BN	Hybrid between *R. malayanum* and *R. jasminiflorum* (Argent 2006).	See *R. malayanum*.	NE
102d	*R.* × *wilhelminae* Hochr., *Candollea* 2:493, 1925.	JV	Only a single collection known, found in a shrubbery near the crater at 1,350m. Thought to be a hybrid of *R. javanicum* and *R. malayanum* (Argent 2006).	See *R. malayanum*.	NE

A2.6 Section *Albovireya* Sleumer

Table 38 Summary of the taxonomic and conservation issues of Section *Albovireya*. The taxa in boldface denote those analysed in this study. The taxa are arranged according to the classification of Argent (2006), and the taxon numbers in the leftmost column correspond to the taxon number used in Argent's (2006) classification. The column 'Range' shows the geographic region the taxa belong (Figure 21).

#	Taxon	Range	Taxonomic Issues	Questions Raised	IUCN Code
103	***R. aequabile*** J. J. Sm., *Bull. Jard. Bot. Buit.* III, 13: 451, 1953.	SM	First introduced into cultivation by Dr Willem Meijr who collected seed from the type locality which was distributed by the Rijksherbarium in 1957 (Argent 2006).	No known taxonomic issues.	LC
104	*R. lampongum* Miq., *Fl. Ind. Bat.* 251 (581) Suppl., 1860.	SM	Not recently recollected or cultivated (Argent 2006).	No known taxonomic issues.	DD
105	*R. cernuum* Sleumer, *Reinwardtia* 5: 111, 1960.	SM	Known only from two specimens, from two mountains; not collected since. Not known to be in cultivation. Forest on these mountains vulnerable to destruction by human (Argent 2006)	No known taxonomic issues.	EN
106	***R. album*** Blume, *Cat. Hort. Buitenz.* 72, 1823.	JV	Restricted to two mountains, and habitat protected. Problem is with the number of mature individuals. This species is locally common but not recollected recently (Argent 2006). Hooker (1855) reported the species as free flowering with *R. rubriflorum* (scarlet-flowered) and *R. javanicum* (forming a thicket). According to molecular studies, *R. album* is very closely related to *R. sumatranum* (Section *Euvireya* Subsection *Euvireya*), *R. culminicola* (Section *Euvireya* Subsection *Euvireya*) and *R. aequabile* with very good bootstrap support (Brown et al. 2006b).	(i) Is this species related to *R. rubriflorum* and/or *R. javanicum*? (ii) What is the genetic relationship between this species, *R. sumatranum*, *R. culminicola* and *R. aequabile*? (iii) Does this species cluster together with the rest of the taxa of Section *Albovireya*?	VU
107	***R. zollingeri*** J. J. Sm., *Ic. Bog.* 4: 73 t322, 1910.	JV, LS, SW, PH	There is some confusion between this species and *R. lagunculicarpum* (Argent 2006).	(i) What is the genetic relationship between this species and *R. lagunculicarpum*?	LC

#	Taxon	Range	Taxonomic Issues	Questions Raised	IUCN Code
108	***R. arenicola*** Sleumer, *Reinwardtia* 5: 113, 1960.	SW	Plants less scaly in cultivation. Introduced into cultivation by Galloway and Smith from Mt Rantemario, where it was common at 2,700 m (Argent 2006)	(i) Where is this species placed within the *Vireya* phylogeny?	DD
109	*R. pudorinum* Sleumer, *Reinwardtia* 5: 112, 1960.	SW	The plants are less scaly in cultivation than in the wild. Vegetatively this species approaches *R. impositum*. Found in two locations. Introduced into cultivation in 1998 by David Binney (Argent 2006).	(i) What is the relationship between this species and *R. impositum*?	VU D2
110	***R. lagunculicarpum*** J. J Sm., *Bot. Jahr.* 68: 200 1937.	SW	Introduced into cultivation by David Binney in 1998. Cultivated plants are less scaly than they are in the wild. Have taxonomic issues with *R. correoides* and *R. zollingeri* (Argent 2006).	(i) What is the genetic relationship between this species and *R. correoides* and *R. zollingeri*?	LC
111	***R. correoides*** J. J. Sm. *Med. Rijksherb.* 25: 2, 1915.	NG	There is some confusion with this species and *R. lagunculicarpum*. Very common at high altitudes (Argent 2006).	(i) What is the relationship between this species and *R. lagunculicarpum*?	LC
112	*R. proliferum* Sleumer, *Blumea* 12: 101, 1963.	NG	Known only from type collection. Apparently rare. Apparently rare. Known only from the imperfect type collection (Argent 2006).	Species not available for study.	DD
113	*R. giulianettii* Lauterb., *Nachtr.* 338, 1905.	NG	This species has been confused with *R. comptum*. Found in three locations. Not known to have been in cultivation (Argent 2006).	(i) What is the relationship between *R. giulianettii* and *R. comptum*?	DD
114a	*R. comptum* var. *comptum* C. H. Wright, *Kew Bull.* 103, 1899.	NG	This species have been confused with *R. giulianettii*. Found in three locations. Not known to be cultivated (Argent 2006).	(i) What is the relationship between *R. giulianettii* and *R. comptum*?	DD
114b	*R. comptum* var. *trichodes* Sleumer, *Reinwardtia* 5: 111, 1960.	NG	Found on a single mountain (Argent 2006).	No known taxonomic issues.	DD
115	***R. yelliotii*** Warb. , *Bot. Jahrb. Syst.* xvi.: 25, 1893.	NG	Often confused with *R. inconspicuum*. *R. saruwagedicum* is reduced to a synonym. Great variation in many specimens due to broad altitudinal range (Argent 2006). The scales are at least in part	(i) What is the relationship of this species with *R. inconspicuum*?	LC

#	Taxon	Range	Taxonomic Issues	Questions Raised	IUCN Code
	(Syn: ***R. saruwagedicum*** F.Först., *Repert. Spec. Nov. Regni* Veg. 13: 222. 1914)		rather intermediate between those found in Subsect. *Pseudovireya*, Subsect. *Euvireya*, and Subsect. *Albovireya* (Sleumer 1966a). Apparently, growing with *R. papuanum* on Arfak Mts and with *R. yellioti* on the Finisterre Mts (Saruwaged region) (Förster 1915).	(ii) Is the reduction of *R. saruwagedicum* to a synonym of this species supported by molecular data? (iii) Is there a relationship between this species and the sections *Pseudovireya* and *Euvireya*?	
116	*R. versteegii* J. J. Sm. *Med. Rijksherb.* 25: 2, 1915.	NG	This species is being used extensively around Mt Jaya (Carstensz) mine in New Guinea in the rehabilitation of disturbed land, where it is one of the most favoured species, growing well at high altitude and flowering continuously (Argent 2006).	No known taxonomic issues.	LC

A2.7 Section *Euvireya* (H F Copeland) Argent

A2.7.1 Subsection *Linnaeopsis* (Schlechter) Sleumer

Table 39 Summary of the taxonomic and conservation issues of Subsection *Linnaeopsis*. The taxa in boldface denote those analysed in this study. The taxa are arranged according to the classification of Argent (2006), and the taxon numbers in the leftmost column correspond to the taxon number used in Argent's (2006) classification. The column 'Range' shows the geographic region the taxa belong (Figure 21). There are 16 taxa in this subsection, 10 of which are of conservation interest, none of which are in New Zealand. *R. gracilentum* (LC) has taxonomic issues with Section *Phaeovireya* and thus are included in the study.

#	Taxon	Range	Taxonomic Issues	Questions Raised	IUCN Code
117	*R. caespitosum* Sleumer, *Reinwardtia* 5: 63, 1960.	NG	Only known from a small area at Mt Wilhelmina. Not much known about the species (Argent 2006).	No known taxonomic issues.	VU
118	*R. schizostigma* Sleumer	NG	Not known in cultivation (Argent 2006).	No known taxonomic issues.	LC
119	*R. pusillum*	NG	Not known in cultivation (Argent 2006).	No known taxonomic issues.	DD

#	Taxon	Range	Taxonomic Issues	Questions Raised	IUCN Code
	J. J. Sm., *Med. Rijksherb*. 25: 1, 1915.				
120	*R. microphyllum* J. J. Sm., *Bull. Jard. Bot. Buit. III*, 8:51, 1920.	NG	Found in three locations, and poorly known (Argent 2006).	No known taxonomic issues.	DD
121	*R. coelorum* Wernham, *Trans. Linn. Soc. London, II Bot*. 9: 96, 1916.	NG	Found in two locations, wide apart. Poorly known species and not known to have been cultivated. Sleumer (1973) commented that it might have to be united with *R. schizostigma* and *R. disterigmoides* but both of these species have a much more vigorous erect habit (Argent 2006).	No known taxonomic issues.	DD
122	*R. muscicola* J. J. Sm., *Nova Guinea* 18: 93 t19 1, 1936.	NG	Found in two locations (Argent 2006).	No known taxonomic issues.	DD
123	*R. xenium* Gillian Brown & Craven, *Novon* 13: 26, 2003.	NG	Long pedicels reminiscent of *R. saxifragoides*. Not yet in cultivation (Argent 2006).	No known taxonomic issues.	NE
124	*R. parvulum* Sleumer, *Reinwardtia* 5: 139, 1960.	NG	Known only from type collection, never cultivated (Argent 2006).	No known taxonomic issues.	DD
125	*R. oxycoccoides* Sleumer, *Reinwardtia* 5: 139, 1960.	NG	Known only from type collection and never cultivated (Argent 2006).	No known taxonomic issues.	DD
126a	*R. disterigmoides* ssp. *disterigmoides* Sleumer, *Reinwardtia* 5: 140, 1960.	NG	Found in a single location and little known (Argent 2006).	No known taxonomic issues.	LC
126b	*R. disterigmoides* ssp. *astromontium* Argent, *Folia Malaysiana* 4(2): 101–128, 2003.	NG	Found in two locations (Argent 2006).	No known taxonomic issues.	NE

#	Taxon	Range	Taxonomic Issues	Questions Raised	IUCN Code
127	*R. anagalliflorum* Wernham, *Trans. Linn. Soc. London, II Bot*. 9: 94, 1916.	NG, NB	This species was for some time confused with *R. rubineiflorum*. Reasonably widespread (Argent 2006; Craven 1980).	(i) What is the relationship between this species and *R. rubineiflorum*?	LC
128	***R. rubineiflorum*** Craven, *Notes RBGE* 38(1):141 f1, 1984.	NG	This species was for some time confused with *R. anagalliflorum* (Argent 2006).	(i) What is the relationship between this species and *R. anagalliflorum*?	LC
129	*R. capellae* Kores, *Blumea*, 24: 181 f1, 1978.	NG	Found on a single mountain. Known only from the type collection and not in cultivation (Argent 2006). Closely related to *R. vinkii* and *R. pulleanum* (Kores 1978).	(i) Where is this species placed within the *Vireya* phylogeny? (ii) Is this species related to *R. pulleanum* or *R. vinkii*?	VU D2
130	*R. womersleyi* Sleumer, *Reinwardtia* 5:136, 1960.	NG	Known to hybridise with *R. atropurpureum* and *R. commonae* on Mt. Wilhelm (Argent 2006).	(i) What is the relationship between this species, *R. atropurpureum* and *R. commonae*?	LC
131	***R. gracilentum*** F. Muell., *Trans. R. Soc. Vict*. n.s. 1(2): 22, 1889.	NG	Widely cultivated species (Argent 2006).	No known taxonomic issues.	LC

A2.7.2 Subsection *Saxifragoidea* (Sleumer) Argent

Table 40 Summary of the taxonomic and conservation issues of Subsection *Saxifragoidea*. The taxa in boldface denote those analysed in this study.

#	Taxon	Range	Taxonomic Issues	Questions Raised	IUCN Code
132	***R. saxifragoides*** J. J. Sm., *Med. Rijksherb.* 25 : 3, 1915.	NG	No known issues.	No known issues.	LC

A2.7.3 Subsection *Solenovireya* H F Copeland

Table 41 Summary of the taxonomic and conservation issues of Subsection *Solenovireya*. The taxa in boldface denote those analysed in this study. The taxa are arranged according to the classification of Argent (2006), and the taxon numbers in the leftmost column correspond to the taxon number used in Argent's (2006) classification. The column 'Range' shows the geographic region the taxa belong (Figure 21).

#	Taxon	Range	Taxonomic Issues	Questions Raised	IUCN Code
133a	***R. jasminiflorum* ssp. *jasminiflorum*** Hook., *Bot. Mag.* t4525, 1850.	MP	The material figured for *Curtis's Botanical Magazine* (t. 4524) in the Kew Herbarium is hairy to above halfway up the outside of the tube so the distinctness of var. *punctatum* is not valid and this variety is included within the type subspecies which probably always has at least a few hairs on the outside of the corolla tube. *R. jasminiflorum* is known to hybridize with *R. malayanum* (Section *Malayovireya*) (Argent 2006).	(i) What is the distinction between the taxon *R. jasminiflorum* ssp. *jasminiflorum* and its synonym *R. jasminiflorum* ssp. *punctatum*? (ii) Is there a close genetic relationship between this subspecies and *R. malayanum*? (iii) Are the subspecies status supported by molecular data?	LC
133b	*R. jasminiflorum* ssp. *chaemaepitys* (Sleumer) Argent, *Rhod. Subg. Vireya*. 158, 2006.	BN	Known only from a single location. Introduced into cultivation by John Dransfield in 1981 but no longer growing. Although very distinctive with its narrow leaves, this appears to be no more than an extreme form of *R. jasminiflorum* at the edge of its range in Borneo (Argent 2006).	(i) Does molecular data support this subspecific status?	NE
133c	*R. jasminiflorum* ssp. *copelandii* (Merr.) Argent, *Rhod. Subg. Vireya*. 158, 2006.	PH	The original description describes the twigs as glabrous; the living material in Edinburgh which is from the type locality has minutely hairy stems, which are also brown-scaly. It is very difficult to determine whether the type material has any simple hairs since it is covered with crystals of mercuric chloride. Found in a single location, at high altitude (Argent 2006).	(i) Does molecular data support this subspecific status?	VU D2

#	Taxon	Range	Taxonomic Issues	Questions Raised	IUCN Code
133d	*R. jasminiflorum* ssp. *heusseri* (J. J. Sm.) Argent, *Rhod. Subg. Vireya*. 158, 2006.	SM	Widespread in Sumatra. A recent search of the Samosir Peninsula failed to find the multi-coloured forms reported by Sleumer (1966a); these would appear to be hybrids with *R. longiflorum* as suggested in that account. Populations elsewhere have uniformly white flowers. Only recently been brought into cultivation (Argent 2006).	(i) What is the relationship between this species and *R. longiflorum*?	LC
133e	***R. jasminiflorum* ssp. *oblongifolium*** (Sleumer) Argent, *Rhod. Subg. Vireya*. 158, 2006.	MP	Widespread subspecies (Argent 2006).	(i) What is the distinction between this subspecies and ssp. *jasminiflorum*? (ii) Is there a close genetic relationship between this subspecies and *R. malayanum*? (iii) Is the subspecies status supported by molecular data?	LC
134a	***R. edanoi* ssp. *edanoi*** Merr. & Quisumb., *Phil. J. Sc.* 83: 333, 1953.	PH	Restricted to two peaks. Introduced into cultivation in 1998 (Argent 2006).	(i) Is there a genetic relationship between this species and *R. jasminiflorum*?	VU
134b	*R. edanoi* ssp. *pneumonanthum* (Sleumer) Argent, *Gardens Bull. Sing.* 56: 79, 2004.	BN	In many locations. Introduced into cultivation in 1984 by Keith Adams to New Zealand; it is now widely cultivated. This subspecies is superficially similar to *R. jasminiflorum* (Argent 2006).	(i) Can this subspecies status be supported by molecular data?	LC
135	***R. stapfianum*** Hemsl. *ex.* Prain, *Bot. Mag.* t8372, 1911.	BN	Not reported as hybridising with any others. It replaces *R. jasminiflorum* in the northern part of Borneo (Argent 2006).	(i) Is there any relationship between this species and *R. jasminiflorum*?	LC
136	***R. alborugosum*** Argent & J. Dransfield, *Notes RBGE* 46(1): 27 1984.	BN	Originally confused with *R. rugosum* because of superficial resemblance in the rugose leaves. Also confused with *R. suaveolens*. Known only from the mountain in which it was originally collected. In cultivation since 1996 (Argent 2006). *R. suaveolens* was previously confused with *R. orbiculatum* (LC)	(i) What is the genetic relationship between *R. alborugosum*, *R. rugosum*, *R. suaveolens* and *R. orbiculatum*?	VU

#	Taxon	Range	Taxonomic Issues	Questions Raised	IUCN Code
			(Sect. *Euvireya* Subsect. *Euvireya*) but was later shown by Hunt (1972) to be different.		
137a	***R. suaveolens* f. *suaveolens*** Sleumer, *Bot. Jahr*. 71: 147, 1940.	BN	This species is confused with *R. orbiculatum*, and very similar to *R. lambianum* (Argent 2006).	(i) What is the genetic relationship between this form, *R. orbiculatum* and *R. lambianum*? (ii) Is there a genetic relationship between *R. suaveolens* and *R. niveoflorum*?	LC
137b	*R. suaveolens* f. *roseum* Argent, A. Lamb & Phillipps, *Notes RBGE* 42(1): 117, 1984.	BN	This form differs from the type in the uniformly pink colour of the flowers (Argent 2006).	(i) Is the status of form supported by molecular data?	NE
138	*R. lambianum* Argent, *Folia Malaysiana* 4(2): 109 pl2, 2003.	BN	This species is still known from two localities. It has been in cultivation since 1980 (Argent 2006).	(i) What is the genetic relationship between this species, *R. orbiculatum* and *R. suaveolens*?	VU
139	*R. niveoflorum* Argent, *Folia Malaysiana* 4(2): 115 pl4–5, 2003.	BN	Very similar to *R. suaveolens* (Argent 2006). Further field observations needed to determine further taxonomic issues.	(ii) Is there a genetic relationship between this species and *R. suaveolens*?	LC
140	*R. pseudotrichanthum* Sleumer, *Blumea* 12: 340, 1964.	BN	Found in a single location, where the type was collected, and never cultivated (Argent 2006).	No known taxonomic issues.	DD
141	*R. mogeanum* Argent, *Folia Malaysiana* 4(2): 111 pl3, 2003.	BN	This species is similar to *R. suaveolens* and *R. niveoflorum* but the single herbarium collection is incomplete. Found only in a single location, and known only from the type sample (Argent 2006).	(i) What is the relationship between this species, *R. suaveolens* and *R. niveoflorum*?	VU
142	*R. amabile* Sleumer, *Reinwardtia* 5: 127, 1960.	SW	Said to be common but not recently seen. Known only from the type collection, and not cultivated (Argent 2006).	No known taxonomic issues.	DD
143a	***R. radians* var. *radians*** J. J. Sm., *Bull. Jard. Bot. Buit. III*, 1: 403 t51, 1920.	SW	No known issues.	No known taxonomic issues.	LC

#	Taxon	Range	Taxonomic Issues	Questions Raised	IUCN Code
143b	*R. radians* var. *minahasae* Sleumer, *Reinwardtia* 5: 130, 1960.	SW	In several volcanoes. Sleumer (1966a) notes that the corolla of the type specimen form Mt Soputan is given as white and very fragrant.	No known taxonomic issues.	LC
143c	*R. radians* var. *pubitubum* (Sleumer) Argent, *Rhod. Subg. Vireya.* 170, 2006.	SW	The distinction between *R. radians* and *R. pubitubum* is not satisfactory and is reduced to varietal status. Not known to be in cultivation (Argent 2006).	(i) Is this varietal status supported by molecular data?	DD
144	***R. rutenii*** J. J. Sm., *Fedde Rep.* 30: 170, 1932.	ML	Very similar to *R. jasminiflorum*. Likely to hybridize with *R. malayanum* which grows commonly with *R. rutenii* (Argent 2006).	(i) What is the genetic relationship between *R. rutenii*, *R. jasminiflorum* and *R. malayanum*?	LC
145	*R. brachypodarium* Sleumer, *Blumea*, 12: 103, 1963.	NG	Found in several locations; not known in cultivation. This species is reminiscent of *R. jasminiflorum* (Argent 2006).	(i) What is the genetic relationship between this species and *R. jasminiflorum*?	LC
146	*R. carstensense* Wernham, *Trans. Linn. Soc. London II Bot*, 9: 96, 1916.	NG	Once collected, and so far not recollected and never cultivated. Very similar to *R. syringoideum* (Argent 2006).	(i) What is the genetic relationship between this species and *R. syringoideum*?	DD
147	*R. cinerascens* Sleumer, *Reinwardtia* 5: 130, 1960.	NG	Known from two collections. This species has never been cultivated (Argent 2006).	No known taxonomic issues.	DD
148	*R. macrosiphon* Sleumer, Blumea, 11: 118, 1963.	NG	This species is said to be locally common. Leaves are similar to those of *R. scabridibracteum*. Said to be similar to *R. carringtoniae* and *R. carstensense* (Argent 2006).	(i) Is there any genetic relationships between this species, *R. scabridibracteum*, *R. carringtoniae* and *R. carstensense*?	LC
149	*R. oreadum* Wernham, *Trans. Linn. Soc. London, II Bot*. 9: 98, 1916.	NG	Known from a single location, and not known to have been cultivated (Argent 2006).	No known taxonomic issues.	DD
150	*R. rhodosalpinx* Sleumer, *Blumea*, 11: 121, 1961.	NG	Known only from the single type location and collection. This species should possibly not be included in *Solenovireya* on account of flower colour and the large corolla lobes in relation to the tube.	(i) Where is this species placed within the Section *Solenovireya* and the overall *Vireya* phylogeny?	DD

#	Taxon	Range	Taxonomic Issues	Questions Raised	IUCN Code
			Further specimens are badly needed to establish its proper identity (Argent 2006).		
151	*R. roseiflorum* P. F. Stevens, *Adansonia* Ser 2 18(1): 55, 1978.	NG	Not yet known in cultivation (Argent 2006). No known issues.	No known taxonomic issues.	DD
152	*R. syringoideum* Sleumer, *Blumea*, 12: 104, 1963.	NG	*R. syringoideum* is very similar to *R. carstensense* (Argent 2006).	(i) What is the genetic relationship between *R. syringoideum* and *R. carstensense*?	DD
153	***R. majus*** (J. J. Sm.) Sleumer, *Reinwardtia* 5: 120 '*maius*', 1960.	NG	Wide distribution, but at high altitude (Argent 2006). No known taxonomic issues.	No known taxonomic issues.	LC
154	*R. archboldianum* Sleumer, *Reinwardtia* 5: 97, 1960.	NG	*R. archboldianum* is known only from two mountains. (Argent 2006). There is an accession at Pukeiti collected by Graham Smith, which was initially named as *R. archboldianum* 'Starburst', collected in the same locality as the type specimen of *R. archboldianum*. *R. archboldianum* 'Starburst' is thought to be a hybrid between *R. herzogii* and *R. culminicola* (Argent 2006).	(i) What is the relationship between this species, *R. herzogii* and *R. culminicola*?	DD
155	***R. armitii*** F. M. Bailey, *Bot. Bull. Queensl. Dep. Agr.* 10: 39, 1895.	NG	Found in at least four locations. First introduced to Edinburgh by Paddy Woods in 1968 (Argent 2006). No known issues.	No known taxonomic issues.	LC
156	*R. carrii* Sleumer, *Reinwardtia* 5: 124, 1960.	NG	Vegetatively similar to *R. blackii*. Found in a single location. Introduced into cultivation by Paul Kores in 1976 (Argent 2006).	(i) What is the genetic relationship between this species and *R. blackii*?	VU
157	***R. carringtoniae*** F. Muell., *Vict. Nat.* 4: 110, 1887.	NG	Found in several locations. Introduced into cultivation by Canon Cruttwell in Australia in 1972 (Cruttwell 1972). Cruttwell regarded this species as 'in the top rank of the tubular (*Solenovireya*) rhododendrons' (Argent 2006).	(i) Where is this species placed within the Section *Solenovireya* and in the wider Subgenus *Vireya* phylogeny?	LC

#	Taxon	Range	Taxonomic Issues	Questions Raised	IUCN Code
158	***R. cruttwellii*** Sleumer, *Reinwardtia* 5: 120, 1960.	NG	'Close to *R. hartleyi*' according to Sleumer (1973). Found in several locations. An earlier introduction under this name by G Herklots in 1965, possibly from the Telefomin area, is now considered to be *R. multinervium* (Argent 2006).	(i) Is there a genetic relationship between this species, *R. hartleyi* and *R. multinervium*?	LC
159	*R. hartleyi* Sleumer, *Blumea*, 21: 366, 1973.	NG	Said to be close to *R. cruttwellii* (Sleumer 1973). Known only from the type locality (Argent 2006).	(i) Is there a genetic relationship between this species and *R. cruttwellii*?	DD
160	*R. goodenoughii* Sleumer, *Reinwardtia* 5: 131, 1960.	GI	Found in one mountain on Goodenough Island. Cultivated widely, and introduced multiple times (Argent 2006). No known taxonomic issues.	No known taxonomic issues.	DD
161	*R. multinervium* Sleumer, *Reinwardtia* 5: 117, 1960.	NG	Found in many locations. Similar to *R. cruttwellii* (Argent 2006).	(i) What is the relationship between this species and *R. cruttwellii*?	LC
162	*R. natalicium* Sleumer, *Reinwardtia* 5: 118, 1960.	NG	The status of this species is not clear; it would appear to be relatively rare. Never been cultivated (Argent 2006).	No known taxonomic issues.	DD
163	*R. retrorsipilum* Sleumer, *Blumea*, 11: 120, 1961.	NG	Extinct species. Very restricted distribution. Never cultivated. Michael Black commented that 'its small white tubular campanulate flowers were of little decorative value' (Black 1965). Now almost certainly extinct in the type locality, which has totally lost its forest to native gardens (Argent 2006).	No known taxonomic issues.	EX
164	*R. oliganthum* Sleumer, *Reinwardtia* 5: 123, 1960.	NG	Known only from the single type collection and never cultivated (Argent 2006). No known taxonomic issues.	No known taxonomic issues.	DD
165	***R. pleianthum*** Sleumer, *Reinwardtia* 5: 122, 1960.	NG	Found in many locations (Argent 2006).	No known taxonomic issues.	LC

#	Taxon	Range	Taxonomic Issues	Questions Raised	IUCN Code
166	***R. tuba*** Sleumer, *Reinwardtia* 5: 125, 1960.	NG	*R. tuba* is in many respects intermediate between *R. carringtoniae* and *R. rhodoleucum*. *R. carringtoniae* and *R. rhodoleucum* are also known from the Maneau Range, thus Sleumer (1966a) suggesting that *R. tuba* could be a natural hybrid of these two (Argent 2006). Molecular studies by Brown et al. (2006a, 2006b) suggest that *R. tuba* is closely related to *R. culminicola* (Section *Euvireya* Subsection *Euvireya*).	(i) What is the genetic relationship between *R. tuba*, *R. carringtoniae*, *R. rhodoleucum* and *R. culminicola*?	LC
167	*R. rhodoleucum* Sleumer, *Blumea*, 11: 119, 1961.	NG	Found in several locations (Argent 2006).	No known taxonomic issues.	LC
168a	***R. loranthiflorum* ssp. *loranthiflorum*** Sleumer, *Notizbl. Berl.-Dahl.* 12: 485, 1953.	NG, SI	This species appears to be vegetatively almost identical to *R. luraluense* (Argent 2006).	(i) What is the genetic differentiation between *R. loranthiflorum* and *R. luraluense*?	LC
168b	*R. loranthiflorum* ssp. *lakekamuensis* W. N. Takeuchi, *Edinb. J. Bot.* 57(3): 333, 2000.	NG	Not known to be in cultivation (Argent 2006).	No known taxonomic issues.	DD

A2.7.4 Subsection *Malesia* H F Copeland

Table 42 Summary of the taxonomic and conservation issues of Subsection *Malesia*. The taxa in boldface denote those analysed in this study. The taxa are arranged according to the classification of Argent (2006), and the taxon numbers in the leftmost column correspond to the taxon number used in Argent's (2006) classification. The column 'Range' shows the geographic region the taxa belong (Figure 21).

#	Taxon	Range	Taxonomic Issues	Questions Raised	IUCN Code
169	*R. chevalieri* Dop, *Rev. de Bot. Appl. et d'Agric. Trop.* 9(92): 256 t10, 1929.	EA	No known issues.	No known taxonomic issues.	LC

#	Taxon	Range	Taxonomic Issues	Questions Raised	IUCN Code
170	***R. pauciflorum*** King & Gamble, *J. As. Soc. Beng.* 74(2): 75, 1905.	MP	The taxon '*calocodon*' does not have any significance and has been reduced to synonymy (Argent 2006).	No known taxonomic issues.	LC
171	*R. pubigermen* J. J. Sm., *Contr. Arn. Arb.* 8: 122, 1934.	SM	Resembling *R. frey-wysslingii* in the foliage. Superficially similar to *R. banghamiorum* (Argent 2006).	(i) What is the genetic relationship between this species, *R. frey-wysslingii* and *R. banghamiorum*?	LC
172	*R. frey-wysslingii* J. J. Sm., *Contr. Arn. Arb.* 8: 123, 1934.	SM	Found in a single location, and a single collection. Not known in cultivation (Argent 2006).	No known taxonomic issues.	DD
173	***R. multicolor*** Miq., *Fl. Ind. Bat.* Suppl. 1: 251 & 586, 1860.	SM	Locally common and widespread. Very similar to *R. salicifolium*, The flower shape very reminiscent of *R. ripleyi* (Argent 2006).	(i) What is the relationship between this species, *R. salicifolium* and *R. ripleyi*?	LC
174	*R. pyrrhophorum* Sleumer, *Reinwardtia* 5: 165, 1960.	SM	Known only from the type collection and a single location. Never cultivated. (Argent 2006).	No known taxonomic issues.	DD
175	*R. banghamiorum* (J. J. Sm.) Sleumer, *Reinwardtia* 5: 163, 1960.	SM	This species is similar to *R. ultimum* and *R. brassii* (Sleumer 1973). Previously known only from the type collection, it was recently re-found and introduced into cultivation in 2001. Superficially similar to *R. pubigermen* (Argent 2006).	(i) What is the genetic relationship between this species, *R. ultimum*, *R. brassii* and *R. pubigermen*?	VU
176a	*R. ripleyi* var. *ripleyi* Merr., *Notes Natl. Acad. Nat. Sci. Philad.* 47: 4, 1940.	SM	Not recently recollected and never cultivated (Argent 2006).	(i) What is the genetic relationship between this variety and *R. pubigermen*? (ii) Is the varietal status supported by molecular data?	DD
176b	*R. ripleyi* var. *basitrichum* Sleumer, *Reinwardtia* 5: 164, 1960.	SM	Sleumer in the original publication noted that this was possibly a hybrid of *R. ripleyi* with *R. pubigermen*. However, this matter has not been resolved (Argent 2006).	(i) What is the genetic relationship between this variety, *R. pubigermen* and *R. malayanum*? (ii) Is the varietal status supported by molecular data?	LC

#	Taxon	Range	Taxonomic Issues	Questions Raised	IUCN Code
176c	*R. ripleyi* var. *cryptogonium* Sleumer, *Reinwardtia* 5: 164, 1960.	SM	Sleumer suggested this to be a hybrid of *R. ripleyi* with *R. malayanum* (Argent 2006).	(i) What is the genetic relationship between this variety, *R. pubigermen* and *R. malayanum*? (ii) Is the varietal status supported by molecular data?	LC
177a	***R. citrinum* var. *citrinum*** (Hassk.) Hassk., *Cat. Hort. Bog.* 161, 1844.	JV	This species sometimes produces petaloid calyces, giving the flowers a semi-double appearance (Argent 2006).	No known taxonomic issues.	LC
177b	*R. citrinum* var. *discoloratum* Sleumer, *Reinwardtia* 5: 145, 1960.	SM	The fact that pale yellow flowers from Sumatra makes this variety of doubtful significance as some Javan specimens have the ovaries entirely covered with scales (Argent 2006).	(i) Is the varietal status supported by molecular data?	LC
178	*R. meijeri* Argent, A. Lamb & Phillipps, *Notes RBGE* 42(1): 116, 1984.	BN	Occasionally hybridises in the wild with *R. baconii*; the hybrid has been cultivated. Found in a single location, which is a very small area, with a very small population (Argent 2006; Gibbs et al. 2011).	(i) What is the relationship between this species and *R. baconii*?	CR
179a	*R. abietifolium* Sleumer, *Blumea*, 11: 122, 1961.	BN	This species has an extraordinarily restricted distribution. Found only in the vicinity of the type locality. Introduced into cultivation in 1980. Commonly hybridises with *R. buxifolium* to give *R.* × *sheilae* (Argent 2006). Found in a small population with fewer than 1,000 individuals (Gibbs et al. 2011).	(i) Is there a close genetic relationship between *R. abietifolium*, *R. buxifolium* and *R.* × *sheilae*?	VU D1
179b	*R.* × *sheilae*	BN	Hybrid between *R. abietifolium* and *R. buxifolium* (Argent 2006).	(i) Is there a close genetic relationship between *R. abietifolium*, *R. buxifolium* and *R.* × *sheilae*?	NE
180	***R. burttii*** P. Woods, *Notes RBGE* 37(1): 57, 1978.	BN	This species superficially most closely resembles *R. borneense* ssp. *villosum*. Also similar to *R.sugaui* (Argent 2006).	(i) What is the relationship between this species, *R. borneense* ssp. *villosum* and *R.sugaui*?	LC

#	Taxon	Range	Taxonomic Issues	Questions Raised	IUCN Code
181	*R. sugaui* Argent, *Rhod. Subg. Vireya*. 158, 2006.	BN	Very similar to *R. burtii*. Very likely to be endemic. Known only from the type collection (Argent 2006).	(i) What is the relationship between this species, *R. borneense* ssp. *villosum* and *R.sugaui*?	DD
182	*R. buxifolium* Low ex Hook. *f.*, In Hook. *Ic. Pl.* t890, 1852.	BN	The variety *robustum* recognised by Sleumer (1960) and thus reduced to a synonym. The taxonomic position of this species is anomalous. It has the scale type of *Discovireya* and has just a few hairs on the margins of the bracts, but the flower shape is much more typical of *Euvireya* where it was placed by Sleumer (1960). This species is similar to *R. tuhanensis* (Argent 2006).	(i) Where is this species placed within the *Vireya* phylogeny? (ii) Is there a relationship between this species and the taxa of Section *Discovireya*? (iii) What is the relationship between this species and *R. tuhanensis*?	VU
183	*R. tuhanensis* Argent & Barkman, *The New Plantsman* 7(4): 214–219, 2000.	BN	Similar to *R. buxifolium*. Its true relationships may be with *R. baconii* or *R. rugosum* (Argent 2006).	(i) Where is this species placed within the *Vireya* phylogeny? (ii) What is the relationship between this species, *R. buxifolium*, *R. baconii* or *R. rugosum*?	CR
184	*R. nieuwenhuisii* J. J. Sm., *Ic. Bog.* 4: 75 t323, 1910.	BN	This very distinctive species is unlikely to be confused with any other. With its rugose leaves and broad 'saucer-shaped', mostly solitary yellow flowers it is unlike any other (Argent 2006).	No known taxonomic issues.	LC
185	***R. taxifolium*** Merr., *Phil. J. Sc.* 30: 419, 1926.	PH	A threatened species in the wild with restricted range and subjected to habitat loss (Argent 2006). Sleumer (1966a) placed this species in his series Stenophylla on account of its narrow linear leaves and suggested to be related to *R. stenophyllum* and similar taxa. A relationship with *R. vidalii* was suggested by Copeland (1929).	(i) Where is this species placed within the *Vireya* phylogeny? (ii) Is this species related to *R. stenophyllum* and/or *R. vidalii*? (iii) What is the genetic diversity among the accessions of *R. taxifolium.*	CR B1ab(iii)
186	***R. acrophilum*** Merr. & Quisumb., *Phil. J. Sc.* 82: 333, 1953.	PH	Usually produces bicoloured, orange and yellow flowers. The original collection was erroneously described with white flowers. This species is similar to *R. wilkiei* (Argent 2006; Argent & Madulid 1995).	(i) What is the relationship between *R. acrophilum* and *R. wilkiei*?	CR

#	Taxon	Range	Taxonomic Issues	Questions Raised	IUCN Code
187	***R. wilkiei*** Argent, *Gardens Bull. Sing.* 56(1&2): 88 f4, 2004.	PH	This species is similar to *R. acrophilum* (Argent 2006).	(i) What is the relationship between *R. acrophilum* and *R. wilkiei*?	VU
188	***R. rousei*** Argent & Madulid, The New Plantsman 5(1): 25, 1998.	PH	This species is related to *R. vidalii* (Argent 2006).	(i) What is the relationship between this species and *R. vidalii*?	DD
189	*R. whiteheadii* Rendle, *J. Bot.* 34: 356, 1896.	PH	Considered to be rare, and not known in cultivation. Sleumer (1966a) sank this species into *R. vidalii* on the grounds that only flower colour separated the two species but later (Sleumer 1973) asserted the distinctness due to the large fruit of a specimen collected on Mt Pulag which meant fruit size could be used as well. Hybrids between these two species with pink flowers appear to exist (Argent 2006).	(i) What is the relationship between this species and *R. vidalii*?	DD
190a	*R. vidalii* ssp. *vidalii* Rolfe, *J. Bot.* 24: 348, 1886.	PH	Related to *R. rousei* and *R. whiteheadii*. An earlier collection from Sibuyan Island which was distributed under the name *R. vidalii* is now considered *R. rousei* (Argent 2006).	(i) What is the relationship between *R. vidalii*, *R. rousei* and *R. whiteheadii*? (ii) Is this subspecific status supported by molecular data?	
190b	*R. vidalii* ssp. *brachystemon* Argent, *Folia Malaysiana* 4(2): 119 pl7b, 2003.	PH	Related to *R. rousei* and *R. whiteheadii*. Presently known only from the type locality (Argent 2006).	(i) What is the relationship between *R. vidalii*, *R. rousei* and *R. whiteheadii*? (ii) Is this subspecific status supported by molecular data?	VU
191	*R. scarlatinum* Sleumer, *Reinwardtia* 5: 168, 1960.	SW	Known only from the type collection, and never been cultivated (Argent 2006).	No known taxonomic issues.	VU
192	*R. leptomorphum* Sleumer, *Reinwardtia* 5: 160, 1960.	SW	Known only from the type collection. Never cultivated (Argent 2006).	No known taxonomic issues.	DD

#	Taxon	Range	Taxonomic Issues	Questions Raised	IUCN Code
193	***R. alternans*** Sleumer, *Reinwardtia* 5: 159, 1960.	SW	Not recollected and never cultivated (Argent 2006).	No known taxonomic issues.	DD
194	***R. bagobonum*** H. F. Copel., *Phil. J. Sc.* 40: 151 t4 f1–2, 1929.	PH, BN, SW, ML	Closely related to *R. exuberans* and *R. nervulosum*. It hybridises in the wild with *R. crassifolium* to give *R.* × *planecostatum*. *S*uperficially resembles, and has been confused with, species in Section *Discovireya* (Argent 2006). *R.* × *sarcodes* is a hybrid between *R. bagobonum* and *R. javanicum* ssp. *schadenbergii* (Argent 2006).	(i) What is the relationship between this species and the taxa of Section *Discovireya*, specifically *R. borneense* and *R. cuneifolium*? (ii) What is the genetic differentiation between accessions of this species collected from the different geographic localities? (iii) What is the relationship between this species, *R. exuberans*, *R. nervulosum*, *R. crassifolium* and *R.* × *planecostatum*? (iv) What is the relationship between *R.* × *sarcodes*, *R. bagobonum* and *R. javanicum* ssp. *schadenbergii*?	LC
195	***R. pseudobuxifolium*** Sleumer, *Reinwardtia* 5: 154, 1960.	SW	A point endemic from Mt. Rantemario in C Sulawesi. Possibly hybridising with *R. celebicum* (Gibbs et al. 2011)	(i) What is the relationship between this species and *R. celebicum*?	VU D2
196	*R. nubicola* Wernham, *Trans. Linn. Soc. London, II Bot*. 9: 98, 1916.	NG	Changed from *R. culminicola* var. *nubicola* to *R. nubicola*. Not known to have been cultivated (Argent 2006).	(i) Is the specific status of this taxon supported by molecular data? (ii) Is there a genetic relationship between this species and *R. culminicola*?	LC
197	*R. vinkii* Sleumer, *Blumea*, 12: 91, 1963.	NG	Once found, and not yet recollected and never cultivated. This species was anomalous amongst the New Guinea discovireyas. It is tempting to regard it	(i) What is the relationship between this species and the taxa of Section *Discovireya*?	DD

#	Taxon	Range	Taxonomic Issues	Questions Raised	IUCN Code
			as a hybrid but only observations in the field will provide the answer to this (Argent 2006).	(ii) Does this species cluster together with other taxa of Subsection *Malesia* on the *Vireya* phylogeny?	
198	*R. flavoviride* J. J. Sm., *Med. Rijksherb.* 25: 4, 1915.	NG	Similar to *R. milleri* (Argent 2006). The flower colour was described by the author as yellowish-green (Smith 1915).	(i) What is the relationship between this species and the newly described *R. milleri* by Argent?	LC
199	***R. vitis-idaea*** Sleumer, *Reinwardtia* 5: 156, 1960.	NG	Van Royen & Kores (1982) synonymised *R. vandeursenii* with *R. vitis-idaea* as a result of field work.	No known taxonomic issues.	LC
200	*R. stevensianum* Sleumer, *Blumea*, 21(2): 371, 1963.	NG	No known issues.	No known taxonomic issues.	LC
201	*R. hatamense* Becc., *Malesia* 1: 202, 1878.	NG	Found at low altitudes on three mountains, with a scattered distribution in western New Guinea (Gibbs et al. 2011).	No known taxonomic issues.	VU D2
202	*R. cornu-bovis* Sleumer, *Reinwardtia* 5: 152, 1960.	NG	Only known from type, and never cultivated. Said to be common in that location (Argent 2006; Gibbs et al. 2011).	No known taxonomic issues.	DD
203	***R. commonae*** Foerster, *Fedde Rep.* 13: 223, 1914.	NG	Van Royen & Kores (1982) reduced *R. pseudonitens* to this species on the basis of field work. They also recorded wild hybrids with *R. culminicola* and *R. womersleyi* in the Finisterre Mts and with *R. macgregoriae* in the Tari Gap. At least three colour forms are in cultivation: bright red, pink and a very pale yellow (Argent 2006).	(i) Is there a genetic relationship between this species, *R. culminicola*, *R. womersleyi* and *R. macgregoriae*?	LC
204	*R. rhodostomum* Sleumer, *Reinwardtia* 5: 157, 1960.	NG	Found in several locations; never cultivated (Argent 2006).	No known taxonomic issues.	LC
205	*R. takeuchii* Argent, *Folia Malaysiana* 4(2): 117 pl6a–b, 2003.	NG	Very similar to small-leafed forms of *R. culminicola* from the New Guinea. *R. takeuchii* superficially	(i) What is the relationship between this species, *R. culminicola*, *R. neobritannicum*?	VU

#	Taxon	Range	Taxonomic Issues	Questions Raised	IUCN Code
			resembles *R. neobritannicum*. Not known in cultivation (Argent 2006).	(ii) Does this species cluster with taxa of Section *Phaeovireya*?	
206	*R. helodes* Sleumer, *Reinwardtia* 5: 161, 1960.	NG	A poorly known species which has not been recollected recently, and never cultivated (Argent 2006).	No known taxonomic issues.	DD
207	*R. psammogenes* Sleumer, *Reinwardtia* 5: 150, 1960.	NG	Known only from the type collection. Never cultivated (Argent 2006).	No known taxonomic issues.	DD
208a	*R. brassii* Sleumer, *Reinwardtia* 5: 170, 1960.	NG	Sleumer (1973) notes under *R. ultimum* that it may not be specifically different from the species. A hybrid with *R. versteegii* (*R.* × *nebulicola*) has been described from the wild by Danet (2005).	(i) Is there a relationship between this species and *R. ultimum*? (ii) Is this species related to *R. versteegii* and/or *R.* × *nebulicola*?	LC
208b	*R.* × *nebulicola* Danet	NG	Thought to be a hybrid between *R. brassi* and *R. versteegii* (Argent 2006).	(i) Is this species related to *R. brassii* and/or *R. versteegii*?	
209	*R. porphyranthes* Sleumer, *Blumea*, 12: 108, 1963.	NG	Not cultivated (Argent 2006).	No known taxonomic issues.	DD
210	*R. rubrobracteatum* Sleumer, *Reinwardtia* 5: 175, 1960.	NG	Similar to *R. calosanthes*. Also very similar to and possibly to be united with *R. subcrenulatum*. Never cultivated (Argent 2006).	(i) Is this species related to *R. calosanthes* and *R. subcrenulatum*?	LC
211	*R. myrsinites* Sleumer, *Reinwardtia* 5: 142, 1960.	NG	Known only from the type collection, and never cultivated (Argent 2006).	No known taxonomic issues.	DD
212	*R. purpureiflorum* J. J. Sm., *Med. Rijksherb*. 25: 3, 1915.	NG	No recent collections and never cultivated (Argent 2006).	No known taxonomic issues.	DD
213	*R. ultimum* Wernham, *Trans. Linn. Soc. London, II Bot*. 9: 99, 1916.	NG	Related to *R. brassii* and *R. banghamiorum* (Argent 2006). This species is endemic to Mt Jaya, Papua (Gibbs et al. 2011).	(i) What is the relationship between this species, *R. brassii* and *R. banghamiorum*?	VU B1ab(ii,iv); D2

#	Taxon	Range	Taxonomic Issues	Questions Raised	IUCN Code
214	*R. atropurpureum* Sleumer, Reinwardtia 5: 172, 1960.	NG	A hybrid with *R. commonae* has been recorded from Mt Wilhelm and hybrids with *R. womersleyi* are also found (Argent 2006).	(i) What is the relationship between this species, *R. commonae* and *R. womersleyi*?	LC
215	*R. subuliferum* Sleumer, *Reinwardtia* 5: 171, 1960.	NG	Not known to have been cultivated (Argent 2006).	No known taxonomic issues.	LC
216	***R. inconspicuum*** J. J. Sm., *Med. Rijksherb*. 25: 1, 1915.	NG	This species is easily confused with *R. yelliotii* (Section *Albovireya*) and superficially looks very similar (Argent 2006).	(i) Is there a genetic relationship between this species and *R. yelliotii*? (ii) Is this species clustering with taxa from Section *Albovireya* within the *Vireya* phylogeny?	LC
217	*R. lamii* J. J. Sm., *Nova Guinea* 18: 96 t20 1, 1936.	NG	Not recently recollected and never cultivated (Argent 2006). Known from one location, not recently recollected; additional field work required (Gibbs et al. 2011).	No known taxonomic issues.	DD
218	*R. simulans* Sleumer, *Reinwardtia* 5: 168, 1960.	NG	Not recently collected and never cultivated (Argent 2006).	No known taxonomic issues.	LC
219	*R. papuanum* Becc., *Malesia* 1: 201, 1878.	NG	Found in several locations. Not known in cultivation (Argent 2006).	No known taxonomic issues.	LC
220a	*R. wrightianum* var. *wrightianum* Koord., *Nova Guinea* 8: 880, 1912.	NG	No known issues.	No known taxonomic issues.	LC
220b	*R. wrightianum* var. *cyclopense* J. J. Sm., *Nova Guinea* 12: 130, 1914.	NG	No known issues.	No known taxonomic issues.	
220c	*R. wrightianum* var. *insulare*	NG	Not known to have been cultivated (Argent 2006).	No known taxonomic issues.	LC

#	Taxon	Range	Taxonomic Issues	Questions Raised	IUCN Code
	Sleumer, *Reinwardtia* 5: 174, 1960.				
221	*R. subcrenulatum* Sleumer, *Reinwardtia* 5: 174, 1960.	NG	Never cultivated. Similar to *R. rubrobracteatum* (Argent 2006).	(i) What is the relationship between this species and *R. rubrobracteatum*?	LC
222	*R. calosanthes* Sleumer, *Blumea*, 11: 125, 1961.	NG	Never cultivated. Sleumer (1961) commented that this species was related to *R. rubrobracteatum*.	(i) What is the relationship between this species and *R. rubrobracteatum*?	VU D2
222a	***R.* × *sarcodes*** Argent & Madulid, *The New Plantsman* 2(3): 156, 1995.	PH	A hybrid between *R. bagobonum* and *R. javanicum* ssp. *schadenbergii*. Very similar to *R.* × *planecostatum* but lacking the hairs on the ovary of that hybrid (Argent 2006).	(i) What is the relationship between this taxon, between *R. bagobonum* and *R. javanicum* ssp. *schadenbergii*, *R.* × *planecostatum*?	NE

A2.7.5 Subsection *Euvireya* H F Copeland

Table 43 Summary of the taxonomic and conservation issues of Subsection *Euvireya*. The taxa in boldface denote those analysed in this study. The taxa are arranged according to the classification of Argent (2006), and the taxon numbers in the leftmost column correspond to the taxon number used in Argent's (2006) classification. The column 'Range' shows the geographic region the taxa belong (Figure 21).

#	Taxon	Range	Taxonomic Issues	Questions Raised	IUCN Code
223	*R. triumphans* Yersin & A. Chev., *Rev. de Bot. Appl. et d'Agric. Trop.* 9(92): 256 t11, 1929.	EA	Under threat and from a very restricted area (Gibbs et al. 2011). Commonly confused with the superficially similar hybrid *R.* 'Triumphans'. Sleumer (1958) considered it 'practically identical to *R. brookeanum* [*R. javanicum* ssp. *brookeanum*]' but found 'a striking difference however, between these species in the petiole'. This species will most likely be included in the *R. javanicum* complex (Argent 2006).	(i) What is the relationship between this species and the *R. javanicum* complex, especially *R. javanicum* ssp. *brookeanum*? (iii) What is the genetic differentiation between this species and the hybrid *R.* 'Triumphans'?	EN B1ab(ii,iii,v)
224a	*R. longiflorum* var. *longiflorum* Lindl., *J. Hort. Soc. Lond.* 3: f89, 1848.	MP, BN, SM	Known to hybridise with *R. jasminiflorum* ssp. *heusseri* (Argent 2006).	(i) What is the relationship between this species and *R. jasminiflorum*	LC

#	Taxon	Range	Taxonomic Issues	Questions Raised	IUCN Code
				complex, especially *R. jasminiflorum* ssp. *heusseri*? (ii) Is the varietal status of this taxon supported by molecular data and what is the genetic difference between the four varieties of this species?	
224b	*R. longiflorum* var. *longipetalum* Argent, A. Lamb & Phillipps, *Notes RBGE* 42(1): 114, 1984.	BN	Low altitude epiphytic forest species under threat from deforestation and degradation. Originally known from two locations, one of which has been completely lost due to deforestation (Gibbs et al. 2011). It is found in an area where *R. javanicum* ssp. *brookeanum* is common and it is possible that this variety is a hybrid between this species and *R. longiflorum*; however, the leaves are typical of *R. longiflorum* var. *longiflorum* (Argent 2006).	(i) What is the relationship between this species and the *R. javanicum* complex, especially *R. javanicum* ssp. *brookeanum*? (ii) Is the varietal status of this taxon supported by molecular data?	CR B2ab(iii)
224c	*R. longiflorum* var. *bancanum* Sleumer, *Reinwardtia* 5: 210, 1960.	SM	Severely fragmented lowland species from Bangka Islands (top of Mt Maras, Menumbing, R. Liat) and under threat from mining operations (Gibbs et al. 2011).	(i) Is the varietal status of this taxon supported by molecular data?	CR B2ab(iii)
224d	*R. longiflorum* var. *subcordatum* (Becc.) Argent, *Rhod. of Sabah, Sabah Parks Publ.* 8: 32, 1988.	BN	Differs from the typical variety mainly by smaller flowers and longer petioles (Argent 2006).	(i) Is the varietal status of this taxon supported by molecular data?	LC
225	***R. robinsonii*** Ridl., *J. Fed. Mal. St. Mus.* 4: 44, 1909.	MP	Superficially similar to *R. javanicum*. Sleumer comments on its similarity to *R. rarilepidotum* (Argent 2006).	(i) Is there a relationship between this species and the *R. javanicum* complex? (ii) Is this species related to *R. rarilepidotum*?	LC
226	***R. rarilepidotum*** J. J. Sm., *Contr. Arn. Arb*. 8: 126, 1934.	SM	Sleumer (1966a) noted that '*R. rarilepidotum* is closely related to *R. robinsonii*'. A collection by David Binney is yellow-flowered (Argent 2006).	(i) Is this species related to *R. sumatranum*?	LC

#	Taxon	Range	Taxonomic Issues	Questions Raised	IUCN Code
226i	*R.* × *ootrichum* Sleumer, *Reinwardtia* 5: 97 (*R. rarilepidotum* × *R. sumatranum*), 1960.	SM	Hybrid between *R. rarilepidotum* and *R. sumatranum* (Argent 2006).	(i) Is there a genetic relationship between *R.* × *ootrichum* and its parents *R. rarilepidotum* × *R. sumatranum*?	NE
227a	*R. javanicum* ssp. *javanicum* (Blume) Benn., *Pl. Jav. Rar*. 85 excl. t19, 1838.	SM	Introduced into cultivation around 1845 (Argent 2006).	(i) What is the genetic differentiation between the various subspecies of *R. javanicum*? (ii) Is the subspecific status of this taxon supported by molecular data?	LC
227b	***R. javanicum* ssp. *brookeanum*** (Low ex Lindl.) Argent, A. Lamb & Phillipps, *Notes RBGE* 42(1): 113, 1984.	BN	Previously known as *R. brookeanum* (now reduced to a synonym). Unlike the typical subspecies (from Sumatra), this subspecies is widely distributed throughout Borneo (Argent 2006).	(i) What is the relationship between this species and other taxa of this Subsection *Euvireya*? (ii) Is the subspecific status of this taxon supported by molecular data?	LC
227c	*R. javanicum* ssp. *gracile* (Lindl.) Argent, A. Lamb & Phillipps, *Notes RBGE* 42(1): 114, 1984.	BN	This subspecies includes all the slender-leafed forms of *R. javanicum*, some of which may merely be impoverished forms of ssp. *brookeanum*. Shows strong similarities to *R. salicifolium* from Sarawak. It is possible that this plant and many similar forms from Sarawak are the result of hybridisation with *R. longiflorum* (Argent 2006).	(i) What is the genetic differentiation between this taxon and other subspecies? (ii) Is there a genetic relationship between this subspecies, *R. salicifolium* and *R. longiflorum*? (iii) Is the subspecific status of this taxon supported by molecular data?	LC
227d	*R. javanicum* ssp. *cladotrichum* (Sleumer) Argent, *Rhod. Subg. Vireya.* 158, 2006.	BN	Similar to Javan forms of *R. javanicum* (Argent 2006).	(i) Is the subspecific status of this taxon supported by molecular data?	NE
227e	*R. javanicum* ssp. *cockburnii* Argent, A. Lamb & Phillipps, *Notes RBGE* 42(1): 113, 1984.	BN	Known from two locations with a total population size of fewer than 1,000 mature individuals (Gibbs et al. 2011). Most similar to ssp. *schadenbergii*.	(i) Is the subspecific status of this taxon supported by molecular data? (ii) What is the relationship of this taxon with ssp. *schadenbergii*?	VU D1

#	Taxon	Range	Taxonomic Issues	Questions Raised	IUCN Code
			Approaches *R. triumphans* from Vietnam (Argent 2006).	(iii) Is there a relationship with *R. triumphans*?	
227f	*R. javanicum* ssp. *schadenbergii* (Warb.) Argent, *Rhod. Subg. Vireya*. 247, 2006.	PH, SW	The status of *R. clementis* (a synonym of this subspecies) is not clear, and the variations in the Philippines are still far from well understood (Argent 2006).	(i) Is the subspecific status of this taxon supported by molecular data? (ii) What is the relationship of this taxon with ssp. *cockburnii*?	LC
227g	*R. javanicum* ssp. *palawanense* Argent, *Rhod. Subg. Vireya*. 248, 2006.	PH	This subspecies is very similar to ssp. *kinabaluense* from Mt Kinabalu (Argent 2006)..	(i) Is the subspecific status of this taxon supported by molecular data? (ii) What is the relationship of this taxon with ssp. *kinabaluense*?	NE
227h	*R. javanicum* ssp. *kinabaluense* (Argent, A. Lamb & Phillipps) Argent, *Rhod. Subg. Vireya*. 248, 2006.	BN	Similar to ssp. *palawanense*. One of the clones collected was named 'Mandarin' (Argent 2006).	(i) Is the subspecific status of this taxon supported by molecular data? (ii) What is the relationship of this taxon with ssp. *palawanense*?	NE
227i	*R. javanicum* ssp. *moultonii* (Ridl.) Argent, *Rhod. Subg. Vireya*. 249, 2006.	BN	Similar to ssp. *brookeanum* but with the ovary completely glabrous (Argent 2006).	(i) Is the subspecific status of this taxon supported by molecular data? (ii) What is the relationship of this taxon with ssp. *brookeanum*?	LC
227j	***R. javanicum* ssp. *teysmannii*** (Miq.) Argent, *Rhod. Subg. Vireya*. 249, 2006.	MP, SM, JV	Sleumer (1966a) noted relationships with *R. beccarii* and *R. basirotundatum*. Introduced into cultivation before 1860 if the Fitch painting preserved at Kew is to be believed, but this may be ssp. *brookeanum*. It is hardly distinct from ssp. *brookeanum* (Argent 2006; Sleumer 1966a). *R. teysmannii* was figured by Blume and Miquel as having yellow flowers. Presumably an error by an omission of the draughtsman, similarly as was made for *R. album*; there is no difference in colour with *R. javanicum* (Sleumer 1966a). There is no reason to doubt the flower colour as both colour forms occur (Argent 2006). *R.* × *wilhelminae* is	(i) Is the subspecific status of this taxon supported by molecular data? (ii) What is the relationship of this taxon with ssp. *brookeanum*? (iii) Is this taxon related to *R. beccarii*, *R. basirotundatum*, and *R. album*? (iv) Is there any molecular evidence to suggest that *R.* × *wilhelminae* is a hybrid between *R. javanicum* var. *teysmannii* and *R. malayanum*?	NE

#	Taxon	Range	Taxonomic Issues	Questions Raised	IUCN Code
			thought to be a natural hybrid between *R. javanicum* var. *teysmannii* and *R. malayanum* (Sleumer 1966a).		
228	***R. sumatranum*** Merr., *Pap. Mich. Ac. Sc.* 19: 182, 1933.	SM	Commonly hybridising with *R. retusum*. Also hybridising with *R. rarilepidotum* to give a range of forms. A probable hybrid with *R. adinophyllum* was collected on Mt Kemiri by David Binney (982482). This species is confused with *R. ripleyi* (Argent 2006).	(i) Is this species related to *R. rarilepidotum*, *R. adinophyllum* or *R. ripleyi*?	LC
229	*R. perplexum* Sleumer, *Reinwardtia* 5: 197, 1960.	SM	Only known from the type specimen (Gibbs et al. 2011). Similar to the Bornean *R. crassifolium* Further collections needed to clarify the position of this species which could possibly be a hybrid with *R. sessilifolium* as one of the parents. Never cultivated (Argent 2006).	(i) Is this species related to *R. crassifolium* or *R. sessilifolium*?	DD
230	*R. sessilifolium* J. J. Sm., *Contr. Arn. Arb*. 8: 125, 1934.	SM	David Binney in New Zealand grows two distinct forms: a large-flowered and a small-flowered. They differ mainly in the size of the corolla lobes (Argent 2006).	(i) Is this species related to *R. perplexum* or *R. beccarii*?	LC
231	*R. beccarii* Sleumer, *Reinwardtia* 5: 192, 1960.	SM	Apparently rare, not recently recollected and never cultivated, known from two locations; status of remains uncertain. The difference between this species and *R. sessilifolium* remain matters of degree. The orange or red flower colour suggests that this might be a hybrid between *R. sessilifolium* and perhaps *R. rarilepidotum* (Argent 2006; Gibbs et al. 2011).	(i) Is this species related to *R. sessilifolium* or *R. rarilepidotum*?	DD
232	*R. loerzingii* J. J. Sm., *Bijdr.* 13: 105 & 107, 1914.	JV	Apparently rare and known from just two locations; not recently recollected (Gibbs et al. 2011). Never cultivated (Argent 2006).	No known taxonomic issues.	VU D2
233	***R. renschianum***	LS	Epiphytic in *Casuarina* forest and summit vegetation, terrestrial on stony ground on slopes and crater edge.	No known taxonomic issues.	VU D2

#	Taxon	Range	Taxonomic Issues	Questions Raised	IUCN Code
	Sleumer, *Bot. Jahr.* 71: 146, 1940.		Known from two locations on Flores (Mt Geli Mutu and Mt Desu) (Gibbs et al. 2011; Sleumer 1966a).		
234a	*R. stenophyllum* ssp. *stenophyllum* Hook. *f.* ex Stapf, *Trans. Linn. Soc. London, II Bot*. 4(2): 196, 1894.	BN	Sleumer (1966a) placed this species in a separate series 'Stenophylla' with three other species, based on the linear or narrowly-lanceolate leaves (and other characters). Hybrids with this subspecies have not been recorded (Argent 2006).	(i) Is the subspecific status of this taxon supported by molecular data?	LC
234b	*R. stenophyllum* ssp. *angustifolium* (J. J. Sm.) Argent, A. Lamb & Phillipps, *Notes RBGE* 42(1): 115, 1984.	BN	Hybrids with this species appear to be common and fairly easily identified as the narrow leaves are characteristic in the progeny. *R.* × *liewianum* has been described from Mt Kinabalu (Argent 1988b) as it is quite prominent with its pink flowers and narrow leaves, *R. fallacinum* × *R. stenophyllum* has been reported from Mt Alab, and there is some evidence that at least some plants referred to *R. nervulosum* may be *R. crassifolium* × *R. stenophyllum* in origin (Argent 2006).	(i) Is the subspecific status of this taxon supported by molecular data? (ii) Is this taxon related to *R.* × *liewianum*, *R. nervulosum*, *R. crassifolium* or *R. fallacinum*?	NE
235	***R. verticillatum*** Low ex Lindl., *J. Hort. Soc. Lond.* 3: 86–87, 1848.	BN	*R. verticillatum* is similar to *R. polyanthemum* (Argent 2006).	(i) Is this species related to *R. polyanthemum*?	LC
236a	***R. crassifolium* var. *crassifolium*** Stapf, *Trans. Linn. Soc. London, II Bot*. 4: 195, 1894.	BN	Hybrids with *R. stenophyllum* have been recorded and are very similar to *R. nervulosum*. *R.* × *planecostatum* is the hybrid with *R. bagobonum* (Argent 2006). *R. brevitubum* was reduced to synonymy by Argent (1988b).	(i) Is the varietal status of this taxon supported by molecular data? (ii) Is this taxon related to *R. stenophyllum*, *R. nervulosum*, *R. crassifolium*, *R. brevitubum*, *R. bagobonum* or *R.* × *planecostatum*?	LC
236b	*R. crassifolium* var. *pseudomurudense* (Sleumer) Argent, *Rhod. Subg. Vireya*. 258, 2006.	BN	This variety differs only in the glabrous filament (Argent 2006).	(i) Is the varietal status of this taxon supported by molecular data?	DD

#	Taxon	Range	Taxonomic Issues	Questions Raised	IUCN Code
237	*R. jiewhoei* Argent, *Rhod. Subg. Vireya*. 259, 2006.	BN	This species was described in 2005, and looks in many respects like a miniature version of *R. crassifolium* (Argent 2006).	(i) Is this species related to *R. crassifolium*?	NE
238	*R. kemulense* J. J. Sm., *Bull. Jard. Bot. Buit. III*, 13:448, 1935.	BN	Known from a single location; additional field work required before any conservation assessment (Gibbs et al. 2011). This species is somewhat intermediate between *R. polyanthemum* and *R. verticillatum*. A plant from Mt Mulu is in cultivation but has yet to be evaluated (Argent 2006).	(i) Is this species related to *R. polyanthemum* or *R. verticillatum*?	DD
239	*R. monkoboense* Argent, *Folia Malaysiana* 4(2): 113, 2003.	BN	A very rare point endemic. No other obvious potential locations and therefore at risk from stochastic events (Gibbs et al. 2011). Most similar to *R. lowii*. The ovary indumentum is very similar to that of *R. retivenium*. Never cultivated (Argent 2006).	(i) Is this species related to *R. lowii* or *R. retivenium*?	CR B1ab(i)
240	*R. apiense* Argent, *Folia Malaysiana* 4(2): 102, 2003.	BN	This species is reminiscent of *R. intranervatum*. Probably closely related to *R. javanicum* ssp. *brookeanum* (Argent 2006).	(i) Is this species related to *R. intranervatum*, *R. javanicum* ssp. *brookeanum* or the *R. javanicum* complex in general?	NE
241a	*R. rugosum* var. *rugosum* Low ex Hook. *f.*, *Ic. Pl.* t885, 1852.	BN	At least some of the records of *R. rugosum* from Mt Murud (Sarawak) are referable to *R. yongii*. Often confused with *R. acuminatum*. Natural hybrids with various species have been recorded (*R.* × *coriifolium*, *R.* × *keditii* and *R.* × *liewianum*). Hybrids with *R. maxwellii* and *R. fallacinum* have also been recorded (Argent 2006).	(i) Is the varietal status of this taxon supported by molecular data? (ii) Is this taxon related to *R. acuminatum*, *R. yongii*, *R. maxwellii* or *R. fallacinum*? (iii) Is this taxon parent to the hybrids: *R.* × *coriifolium*, *R.* × *keditii* and *R.* × *liewianum*?	LC
241b	*R. rugosum* var. *kinabaluense* (Merr.) Argent, *Rhod. Subg. Vireya*. 263, 2006.	BN	Differs from the typical variety in the larger, smooth leaves (Argent 2006).	(i) Is the varietal status of this taxon supported by molecular data?	NE

#	Taxon	Range	Taxonomic Issues	Questions Raised	IUCN Code
241x	*R.* × *coriifolium* (Sleumer) Sleumer, *Blumea*, 12: 340 (*R. buxifolium* × *R. rugosum*), 1963.	BN	A hybrid between *R. buxifolium* and *R. rugosum*; common in the zone of overlap between the two species. It virtually replaces pure *R. rugosum* above 3,000 m and forms hybrid swarms with great variability and back crossing to at least *R. rugosum* (Argent 2006).	(i) Are *R. buxifolium* and *R. rugosum* the parents of this taxon?	NE
242	*R. nervulosum* Sleumer, *Bot. Jahr*. 71: 146, 1940.	BN	Known from two locations with fewer than 1,000 mature individuals (Gibbs et al. 2011). Very similar to hybrids that occur on Mt Kinabalu between *R. crassifolium* and *R. stenophyllum*. It is of lanky growth and intermediate in appearance between *R. stenophyllum* and *R. exuberans* (might be a hybrid between these two species) (Argent 2006).	(i) Is this species related to *R. exuberans*, *R. crassifolium* or *R. stenophyllum*?	VU D1
243	***R. salicifolium*** Becc., *Malesia* 1: 202, 1878.	BN	This species superficially resembles narrow-leafed forms of *R. javanicum*. It also resembles some forms or *R. multicolor* (Argent 2006).	(i) Is this species related to *R. multicolor* or the *R. javanicum* complex?	LC
244	***R. yongii*** Argent, *Bot. J. Linn. Soc*. 85: 12, 1982.	BN	Vegetatively this species is very similar to *R. praetervisum*. Some earlier collections of this species were ascribed to *R.* × *keditii* (Argent 2006).	(i) Is this species related to *R. praetervisum* or *R.* × *keditii*?	LC
245	*R. baconii* Argent, A. Lamb & Phillipps, *Notes RBGE* 42(1): 115, 1984.	BN	Known from one small site on Mt Tambuyukon, <1km^2, with a very small but stable population of fewer than 100 mature individuals (Gibbs et al. 2011). *R. baconii* hybridises in the wild with *R. meijeri* and *R. rugosum*. Wild collected seed produced some attractive vigorous plants with delicate pink flowers which are probably hybrids with *R. meijeri* (Argent 2006).	(i) Is this species related to *R. meijeri* or *R. rugosum*?	EN D
246	***R. praetervisum*** Sleumer, *Blumea*, 21(2): 376, 1963.	BN	It was grown as *R. longiflorum* before *R. praetervisum* was described. For some years specimens of this species accumulated in herbaria under *R. longiflorum* (Argent 2006).	(i) Is this species related to *R. longiflorum*?	LC

#	Taxon	Range	Taxonomic Issues	Questions Raised	IUCN Code
247	***R. orbiculatum*** Ridl., *J. Str. Br. As. Soc.* 63: 60, 1912.	BN, SW	Similar to *R. edanoi*, *R. suaveolens* and *R. lambianum*. The species has been moved from Section *Solenovireya* (Sleumer 1966a) as the corolla lobes are much longer than is usual in this group, often being almost as long as the tube. Specimens collected on Mt Penrissen (Sarawak) by David Binney and now in cultivation have much smaller lobes. This is one of the most strongly perfumed forms of this species (Argent 2006).	(i) Is this species related to *R. edanoi* or *R. suaveolens*? (ii) Is the placement of this species within Subsection *Euvireya* supported by molecular data? (iii) Are there any genetic relationships of this species with taxa of Subsection *Solenovireya*?	LC
248	*R. lanceolatum* Ridl., *J. Str. Br. As. Soc*. 63: 60, 1912.	BN	*R. partitum* was reduced to synonymy by Sleumer (1966a). It was however described (possibly in error) as having orange-yellow flowers, which *R. lanceolatum* apparently never does. Keith Adams reintroduced a clone in 1990 from Batu Lawei (Sarawak) to Pukeiti in New Zealand. Here it thrives in their covered area and has now been distributed to the USA and the UK and is in many collections (Argent 2006).	(i) Is there significant genetic differentiation between the accessions of this species?	LC
249	*R. exuberans* (Sleumer) Argent, *Bot. J. Linn. Soc*. 85: 12, 1982.	BN	No known issues.	No known issues.	LC
250	*R. commutatum* Sleumer, *Reinwardtia* 5: 201, 1960.	BN	Not yet known to have been cultivated (Argent 2006). No known taxonomic issues.	No known issues.	LC
251	*R. intranervatum* Sleumer, *Blumea*, 11: 129, 1961.	BN	Known from three locations. Fewer than 1,000 adults make the species vulnerable to threats and climatic events (Gibbs et al. 2011). Related to the *R. javanicum* complex. Introduced into cultivation in 1962 by Bill Burtt and Paddy Woods from seedlings collected on Mt Berumput (Burt & Woods 2829). A slightly narrower leaf form was introduced by David Binney from Mt Penrissen (Argent 2006).	(i) Is this species related to the *R. javanicum* complex?	VU D1

#	Taxon	Range	Taxonomic Issues	Questions Raised	IUCN Code
252	***R. maxwellii*** Gibbs, *J. Linn. Soc. Bot*. 42: 103, 1914.	BN	Only known from one location on Mt Kinabalu, but thought to be widespread in that location; needs further research to establish the conservation status (Gibbs et al. 2011). This species appears to hybridise with *R. rugosum* giving rise to forms very similar to those of pure *R. maxwellii*. A beautifully scented plant with significantly larger flowers and intermediate leaves found on Kinabalu's Eastern Ridge recently is thought to be a hybrid with *R. lowii* (Argent 2006).	(i) Is this species related to *R. rugosum* or *R. lowii*?	DD
253	*R. retivenium* Sleumer, *Reinwardtia* 5: 222, 1960.	BN	This species hybridises with *R. lowii*. A hybrid with *R. crassifolium* has also been recorded. This species is closely related to the *R. javanicum* complex. It is similar in some respects to *R. monkoboense*. Introduced by Os Blumhardt to New Zealand (Argent 2006).	(i) Is this species related to *R. crassifolium*, *R. monkoboense*, *R. lowii* or the *R. javanicum* complex?	LC
254	*R. polyanthemum* Sleumer, *Blumea*, 12: 111, 1963.	BN	This species is probably most closely related to *R. verticillatum*. There are no records of wild hybrids (Argent 2006).	(i) Is this species related to *R. verticillatum*?	LC
255	***R. lowii*** Hook. *f.*, *Ic. Pl.* t883 '*lowei'*, 1852.	BN	No known issues.	No known issues.	LC
256	*R. mendumiae* Argent, *Gardens Bull. Sing*. 56(1&2): 82 f2, 2004.	PH	Only known from a very small population at the type locality in mossy submontane forest on Palawan, Philippines. Due to habitat type and population size, this species is at risk from habitat disturbances such as those caused by *El Niño* events (Gibbs et al. 2011). *R. mendumiae* is similar to *R. madulidii*. Vegetatively, it looks very similar to *R. jasminiflorum* ssp. *copelandii*. Associated with Section *Euvireya* on morphological basis. The seeds have unusually short tails (characteristic of vireyas in open situations on mountain peaks such as	(i) Is this species related to *R. madulidii* or *R. jasminiflorum* ssp. *copelandii*? (ii) Is the placement of this species within Subsection *Euvireya* supported by molecular data? (iii) Does this species show close relationships with other taxa from the Philippines?	CR B2ab(i)

#	Taxon	Range	Taxonomic Issues	Questions Raised	IUCN Code
			R. retusum, *R. adinophyllum* and *R. abietifolium*) (Argent 2006).		
257	***R. kochii*** Stein, *Gartenflora* 34: 193 t1195, 1885.	PH	All material examined with the name *R. williamsii* has turned out to be *R. kochii* (Argent 2006).	(i) Is this species related to *R. williamsii*?	LC
258	***R. williamsii*** Merr. ex H. F. Copel., *Phil. J. Sc*. 40: 163 t9 & t11 f2, 1926.	PH	Often reported in cultivation but all material examined with this name has turned out to be *R. kochii* (Argent 2006).	(i) Is this species related to *R. kochii*?	LC
259	*R. mindanaense* Merr., *Publ. Gov. Lab. Philipp*. 29: 41, 1905.	PH	No known issues.	No known issues.	LC
260	*R. reynosoi* Argent, *Gardens Bull. Sing*. 56(1&2): 84 f3, 2004.	PH	Only known from a very small population at one site (Gibbs et al. 2011). Similar in some respects to *R. leytense*. A unique feature, at least amongst the Philippine rhododendrons, is the gradual transition from foliage leaves to bracts. It is much smaller leaved than *R. javanicum* ssp. *schadenbergii* as conceived by Sleumer (1966a). Cultivated since 1998 (Argent 2006).	(i) Is this species related to *R. leytense*, *R. reynosoi*, *R. javanicum* ssp. *schadenbergii* or any taxa from the *R. javanicum* complex?	CR B2ab(i)
261	*R. brachygynum* H. F. Copel., *Phil. J. Sc*. 40(2): 165 pl11 f4–6, 1929.	PH	Based on a single specimen which has since been destroyed (Sleumer saw no material); additional collections needed to determine the conservation status (Argent 2006; Gibbs et al. 2011). Copeland (1929) regarded this species as related to *R. teysmannii* (*R. javanicum* ssp. *teysmannii*) and *R. kochii*. It would appear to belong to *R. javanicum* complex but it is clearly distinct from *R. kochii* morphologically. Copeland also reported relationships with *R. leytense* and *R. loheri* (Argent 2006).	(i) Is this species related to *R. brachygynum*, *R. kochii*, *R. williamsii*, the *R. javanicum* complex, *R. leytense* or *R. loheri*?	DD

#	Taxon	Range	Taxonomic Issues	Questions Raised	IUCN Code
262a	*R. leytense* var. *leytense* Merr., *Phil. J. Sc. Bot.* 10: 55, 1915.	PH	Taxonomic issues with *R. brachygynum* and *R. reynosoi* (see under these species for more details).	(i) Is the varietal status of this taxon supported by molecular data? (ii) Is this taxon related to *R. brachygynum* or *R. reynosoi*?	LC
262b	*R. leytense* var. *loheri* (H. F. Copel.) Sleumer, *Reinwardtia* 5: 218, 1960.	PH	Only known from the type specimen and needs further research to establish its status (Gibbs et al. 2011).	(i) Is the varietal status of this taxon supported by molecular data?	DD
263	*R. loboense* H. F. Copel., *Phil. J. Sc.* 40: 172 t15 f3 t16 f5–6, 1929.	PH	The differences between the three known locations do not appear to warrant any subspecific recognition. It is very similar to *R. leytense*, and hardly distinguishable from the *R. javanicum* complex (Argent 2006).	(i) Is this species related to *R. leytense* or the *R. javanicum* complex?	LC
264	*R. xanthopetalum* Merr., *Publ. Gov. Lab. Philipp*. 29: 41, 1905.	PH	Apparently rare and an imperfectly known. A recent expedition to Mt Mariveles failed to find this species in the type locality, but material closely matching the description was collected in 1999. Further field research required (Argent 2006; Gibbs et al. 2011). This accession at RBGE appear to be very similar to *R. javanicum* ssp. *schadenbergii* (Argent 2006).	(i) Is this species related to taxa of the *R. javanicum* complex or in particular to *R. javanicum* ssp. *schadenbergii*?	DD
265	***R. madulidii*** Argent & Madulid, *The New Plantsman* 5(4): 204, 1998.	PH	Point endemic from Mt Mantalingahan, at a higher altitude than *R. acrophilum*. Grows in sub-montane shrubbery on ultramafic rocks; there is deforestation occurring in the area (Gibbs et al. 2011). This species is thought to be similar to *R. mendumiae* (Argent 2006).	(i) Is this species related to *R. mendumiae* or *R. acrophilum*?	EN B2ab(i)
266	*R. impressopunctatum* J. J. Sm., *Fedde Rep*. 30: 164, 1932.	ML	Only known from one location and the type specimen; therefore needs research to establish its status (Gibbs et al. 2011). Size and colour of the scales suggest, that *R. impressopunctatum* is a hybrid of *R. malayanum* with another species of the *R. javanicum* complex, presumably *R. seranicum*;	(i) Is this species related to *R. seranicum*, *R. malayanum* or the *R. javanicum* complex?	DD

#	Taxon	Range	Taxonomic Issues	Questions Raised	IUCN Code
			these three species grow together at Kunturun (Sleumer 1966a). There are no recent observations to confirm or refute this (Argent 2006).		
267	*R. seranicum* J. J. Sm., *Fedde Rep*. 30: 165, 1932.	ML, SW	Possibly related to *R. impressopunctatum* (Sleumer 1966a).	(i) Is this species related to *R. impressopunctatum*?	LC
268	***R. celebicum*** (Blume) DC., *Bijdr*. 855, 1826.	SW	Two distinct colour forms are in cultivation, the commoner pink and a deep red (Argent 2006).	(i) Is there molecular evidence that this species have two genetically distinct forms?	LC
269	***R. rhodopus*** Sleumer, *Reinwardtia* 5: 199, 1960.	SW	First collected as living material by Keith Adams in 1997; also collected by L A Craven and G K Brown in 2002 and now grown in Australia (Argent 2006). Brown (2002) reported that *R. rhodopus* was found on Gunung Sesean, north of Rantepao. *R. rhodopus* was also found growing with another species (probably *R. seranicum*) in Rantepao. Also growing nearby was a bush ~2 m high and covered with small red blossoms, '*R. quadrasianum* var. *celebicum*'. Another species found in this area was *R. vanvuurenii* (Brentel 2001).	(i) Is this species related to *R. seranicum*, *R. quadrasianum* or *R. vanvuurenii*?	DD
270	*R. bloembergenii* Sleumer, *Reinwardtia* 5: 204, 1960.	SW	Needs further research to establish the conservation status of this species (Gibbs et al. 2011). The collection notes on the herbarium sample at Kew (Paratype K000769919) by Hugo Cool says '*aff. R. schadenbergii*' (now *R. javanicum* ssp. *schadenbergii*).	(i) Is this species related to taxa of the *R. javanicum* complex?	DD
271	*R. poromense* J. J. Sm., *Bot. Jahr*. 68: 203, 1937.	SW	Known only from the type specimen and needs further research to establish its status. Never cultivated (Argent 2006; Gibbs et al. 2011).	No known taxonomic issues.	DD
272	*R. leptobrachion* Sleumer, *Reinwardtia* 5: 203, 1960.	SW	No known issues.	No known issues.	LC

#	Taxon	Range	Taxonomic Issues	Questions Raised	IUCN Code
273	***R. vanvuurenii*** J. J. Sm., *Bull. Jard. Bot. Buit. III*, 1: 399 t48, 1920.	SW	Thought to be related *R. rhodopus. R. vanvuurenii*; locally common, but known to be poisonous to livestock and actively removed. Collected as living material by Craven and Brown in 2002 and being grown in Canberra. A white form flowered in NZ (Argent 2006; Brown 2002).	(i) Is this species related to *R. rhodopus*?	LC
274	*R. stresemannii* J. J. Sm., *Fedde Rep*. 30: 166, 1932.	ML	Only known from the type collection and not known to be in cultivation. Further field research required (Argent 2006; Gibbs et al. 2011).	No known taxonomic issues.	DD
275	***R. impositum*** J. J. Sm., *Fedde Rep*. 68: 201, 1937.	SW	Reintroduced in 1998 to New Zealand by David Binney and in 2000 to Edinburgh by Smith and Galloway (Argent 2006). No known issues.	No known issues.	LC
276	*R. buruense* J. J. Sm., *Fedde Rep*. 30: 168, 1932.	ML	Not known to have been recollected recently and taxonomic uncertainty remains over the status of the species. Not known to have ever been cultivated (Argent 2006; Gibbs et al. 2011). Sleumer (1966a) listed *R. lompohense* var. *grandifolium* as a synonym of *R. buruense*.	No known taxonomic issues.	DD
277	*R. toxopei* J. J. Sm., *Fedde Rep*. 30: 168, 1932.	ML	Not recently recollected, not known to be in cultivation, therefore additional field work is required before any conservation assessment (Gibbs et al. 2011).	No known taxonomic issues.	DD
278	*R. lompohense* J. J. Sm., *Bull. Jard. Bot. Buit. III*, 1: 402 t50, 1920.	SW	Known only from one location and needs further research to establish its status (Gibbs et al. 2011). An intermediate specimen between this species and *R. bloembergenii* at Kew casts doubt on the distinctness of this species. Never cultivated (Argent 2006). Perhaps related to *R. buruense* (see under this species).	(i) Is this species related to *R. bloembergenii* or *buruense*?	DD

#	Taxon	Range	Taxonomic Issues	Questions Raised	IUCN Code
279	*R. subulosum* Sleumer, *Reinwardtia* 5: 143, 1960.	NG	Only known from the type collections and not known to be in cultivation. Further field research required (Argent 2006; Gibbs et al. 2011).	No known taxonomic issues.	DD
280	*R. glabriflorum* J. J. Sm., *Med. Rijksherb*. 25: 6, 1915.	NG	The flowers were originally described as red (Smith 1915) but all the plants recently collected have been yellow. It is possible that the colour recorded on the type collection is an error and that this species always has yellow flowers (Argent 2006).	(i) What colour forms of this species are in cultivation?	LC
281	*R. pachycarpon* Sleumer, *Reinwardtia* 5: 186, 1960.	NG	Van Royen & Kores (1982) noted the similarity of this species to *R. brassii* and suggested that they may have to be united if intermediates are collected.	(i) Is this species related to *R. brassii*?	LC
282	*R. pachystigma* Sleumer, *Blumea*, 12: 110, 1963.	NG	Known only from the type collection and never cultivated (Argent 2006).	No known taxonomic issues.	LC
283	*R. angulatum* J. J. Sm., *Bull. Jard. Bot. Buit. II*, 8: 50, 1912.	NG	Only known from the type collection on Mt Goliath in W New Guinea. Never cultivated (Argent 2006; Gibbs et al. 2011).	No known taxonomic issues.	DD
284	*R. alticola* Sleumer, *Reinwardtia* 5: 164, 1960.	NG	Van Royen & Kores (1982) regarded *R. alticola* as a widespread and polymorphic species, transferring it to series *Javanica* (*sensu* Sleumer 1966). Very similar to *R. culminicola*. The hybrid *R. alticola* × *R. spondylophyllum* was collected by Paul Kores from Mt Victoria and it would be surprising if hybrids with *R. culminicola* did not occur (Argent 2006).	(i) Is this species related to *R. spondylophyllum*, *R. alticola* or *culminicola*?	LC
285	*R. sayeri* Sleumer, *Reinwardtia* 5: 188, 1960.	NG	Known only from the type collection and needs further research to establish its status. Never cultivated (Argent 2006; Gibbs et al. 2011).	No known taxonomic issues.	DD
286a	***R. aurigeranum* ssp. *aurigeranum*** Sleumer, *Reinwardtia* 5: 214, 1960.	NG	The flower buds are very distinctive in this taxon, with short reflexed tips which are scaly both inside and out (Argent 2006).	(i) Is the subspecific status of this taxon supported by molecular data?	LC

#	Taxon	Range	Taxonomic Issues	Questions Raised	IUCN Code
286b	*R. aurigeranum* ssp. *hirsutum* Argent, *Folia Malaysiana* 4(2): 120, 2003.	NG	The flowers are described as yellow in one collection but with a yellow tube and salmon pink lobes in the other. Remaining colour in the herbarium sheets clearly indicates a yellow tube and pink or orange lobes. Not known to have been cultivated (Argent 2006).	(i) Is the subspecific status of this taxon supported by molecular data? (ii) What colour forms of this taxon are in cultivation?	NE
287	***R. laetum*** J. J. Sm., *Nova Guinea* 12: 139 t35, 1914.	NG	In one place with abundant *R. konori* and *R. laetum*, a fruiting specimen was found with apparently intermediate in leaves. This species is also much related to *R. zoelleri* (Sleumer 1966a). It differs mainly in the pure yellow colour of the flowers, at least when they first open (Argent 2006).	(i) Is this species related to *R. konori* or *R. zoelleri*?	LC
288a	***R. christi*** Foerster, *Fedde Rep*. 13: 222, 1914.	NG	Some specimens could be hybrids with *R. curviflorum* (van Royen & Kores 1982), these hybrids having uniformly pink flowers (see under *R.* × *schoddei*). Several different forms are in cultivation for many years. In Edinburgh (UK) there are a large-leafed form and small-leafed form. These forms remain true to type growing side by side and Graham Snell reports growing at least two different forms in Queensland. In cultivation it has a sprawling habit (Argent 2006). *R. christi* is thought to be much related to *R. villosulum* (Sleumer 1966a).	(i) Is this species related to *R. curviflorum*, *R. villosulum* or *R.* × *schoddei*?	NE
288b	***R. christi* (Mt Miap form)**	NG	This form has a sprawling habit.	(i) Is there a genetic differentiation between this form and the typical form?	NE
289	***R. villosulum*** J. J. Sm., *Med. Rijksherb*. 25: 5, 1915.	NG	Sleumer (1966a) noted that this species is 'much related to *R. christi*. In cultivation it has a much more erect habit than *R. christi* and has uniformly red, not bicoloured flowers, but it is certainly very similar (Argent 2006).	(i) Is this species related to *R. christi*?	LC

#	Taxon	Range	Taxonomic Issues	Questions Raised	IUCN Code
290	***R. curviflorum*** J. J. Sm., *Bull. Jard. Bot. Buit. II*, 8: 50, 1912.	NG	Common. It would be odd for this species to occur in the lilac mentioned in the original type description. This may have been an error in the field descriptions or we may still be dealing with two different species. Further collections in the Keyts Mts needed to resolve this issue (Argent 2006).	(i) Is this species related to *R. christi*?	LC
291	*R. milleri* Argent, *Rhod. Subg. Vireya*. 304, 2006.	NG	Known only from an open sub-alpine shrubbery by a roadside, at its type locality in the Darnell Ridge in W New Guinea (Gibbs et al. 2011). This species is superficially similar to *R. flavoviride* (Argent 2006).	(i) What is the relationship between this species and *R. flavoviride*?	VU D1
292	***R. macgregoriae*** F. Muell., *J. Bot.* 29: 177, 1891.	NG	This species is well known to the local people in many places as poisonous to grazing animals and therefore is often removed; human deaths are also recorded (Henty 1981). Natural hybrids are common, especially with *R. zoelleri*, the two species sometimes forming hybrid swarms in disturbed areas where the forest has been cleared. Hybrids with longer tubes and scented flowers have been attributed to crossing with *R. herzogii*. Records of this species with pink flowers and corollas which are glabrous inside are now referable to *R. glabrifilum*; plants with pink flowers and hairy corollas may be hybrids with that species but more careful observations are needed (Argent 2006).	(i) What is the genetic differentiation between the various forms of this species, and does any of these forms warrant varietal or subspecific status? (ii) What is the relationship between this species, *R. zoelleri*, *R. glabrifilum* and *R. herzogii*?	LC
293	*R. christianae* Sleumer, *Reinwardtia* 5: 211, 1960.	NG	No known issues.	No known issues.	LC
294	*R. rosendahlii* Sleumer, *Reinwardtia* 5: 207, 1960.	NG	No known issues.	No known issues.	LC
295a	***R. culminicola* var. *culminicola***	NG	The variety *nubicola sensu* Sleumer (1966a) has been reinstated as a good species by Argent (2006).	(i) Is the varietal status of this taxon supported by molecular data?	LC

#	Taxon	Range	Taxonomic Issues	Questions Raised	IUCN Code
	F. Muell., *Trans. R. Soc. Vict.* n.s. 1(2): 23, 1889.			(ii) Is this taxon related to *R. nubicola*? (iii) Is this taxon related to *R. archboldianum* and/or *R. herzogii*?	
295b	*R. culminicola* var. *angiense* (J. J. Sm.) Sleumer, *Blumea*, 12: 114, 1963.	NG	Said to be closely related to *R. arfakianum* (Argent 2006). Previously known as *R. angiense* J. J. Sm. (now been reduced to a synonym).	(i) Is the varietal status of this taxon supported by molecular data? (ii) Is this taxon related *R. arfakianum*?	NE
296	***R. arfakianum*** Becc., *Malesia* 1: 201, 1878.	NG	Said to be close to *R. angiense* J. J. Sm. (*R. culminicola* var. *angiense*). It is probably a hybrid but it does have the minute hairs on the petiole and mid-vein described for this species (Argent 2006).	(i) Is this taxon related *R. culminicola* var. *angiense*?	DD
297	***R. blackii*** Sleumer, *Blumea*, 21: 375, 1973.	NG	Sleumer (1973) records this as being in cultivation at the time of publication of the species both at Michael Black's garden in Grasmere in the UK and in Australia from the seeds from a Vink collection (17041) of 1966 (Argent 2006).	No known issues.	LC
298	*R. hirtolepidotum* J. J. Sm., *Nova Guinea* 12: 135 t32, 1914.	NG	Known from just one location. Not known to be currently in cultivation (Argent 2006; Gibbs et al. 2011).	No known taxonomic issues.	VU D2
299	*R. comparabile* Sleumer, *Reinwardtia* 5: 208, 1960.	NG	Only known from one mountain; needs further research to establish the conservation status (Gibbs et al. 2011). Sleumer (1966a) noted that this species is much related to *R. lochiae*. Not known to be in cultivation (Argent 2006).	(i) Is this species related to *R. lochiae* or *R. viriosum*?	DD
300a	***R. luraluense* ssp. *luraluense*** Sleumer, *Notizbl. Berl. Dahl*. 12: 485, 1935.	NG	Thought to be locally common at the single known location on Bougainville Island (Gibbs et al. 2011). No known taxonomic issues.	(i) Is the subspecific status of this taxon supported by molecular data?	VU D2
300b	***R. luraluense* ssp. *whitmorei***	SI	Differing from the type subspecies of *R. luraluense* by style morphology (Argent 2006).	(i) Is the subspecific status of this taxon supported by molecular data?	NE

#	Taxon	Range	Taxonomic Issues	Questions Raised	IUCN Code
	Argent, *Rhod. Subg. Vireya*. 313, 2006.				
301	*R. wentianum* Koord., *Nova Guinea* 8: 188, 1909.	NG	Introduced into cultivation in 1974 as a low altitude form of *R. christi* (it had not been collected in flower in the wild). The foliage is very reminiscent of *R. christi* but the flowers are quite different in shape and indumentum, lacking the distinctive white hairs on the outside of the corolla tube that *R. christi* always has (Argent 2006).	(i) Is this species genetically related to *R. christi*?	LC
302	*R. glabrifilum* J. J. Sm., *Nova Guinea* 12: 134 t31, 1914.	NG	This species has been reinstated after having been reduced to a variety of *R. macgregoriae* by Sleumer. Not known to be cultivated although pink forms of *R. macgregoriae* are in cultivation which if examined carefully could be this species (Argent 2006).	(i) Is this species genetically related to any forms of *R. macgregoriae*?	NE
303	*R. schlechteri* Lauterb., *Nachtr*. 338, 1905.	NG	This species is very similar to *R. leucogigas*. There are also strong similarities to *R. konori* (Argent 2006).	(i) Is this species related to *R. leucogigas* or *R. konori*?	LC
304	***R. leucogigas*** Sleumer, *Blumea*, 12: 102, 1963.	NG	Only known from one location and needs further research to establish its status (Gibbs et al. 2011). A second collection made in the Hunstein Mts by Lyn Craven was initially known as *R. gardenia aff*. It was later christened *R. leucogigas* 'Hunstein's Secret'. The scales are also more typical of Section *Phaeovireya* (Argent 2006).	(i) Is this species related to *R. gardenia*, *R. schlechteri* or *R. konori*? (ii) Where is this species placed within the *Vireya* phylogeny and does this species cluster with other taxa of Section *Phaeovireya*?	DD
305	*R. brevipes* Sleumer, *Reinwardtia* 5: 213, 1960.	NG	Known from type collection only; additional fieldwork required before conservation assessment (Gibbs et al. 2011). A poorly known species very reminiscent of *R. aurigeranum*. Never cultivated (Argent 2006).	(i) Is this species related to *R. aurigeranum*?	DD
306	*R. englerianum*	NG	Widespread but a low altitude species and possibly under threat from deforestation. Nearly meets VU	(i) Is this species related to *R. baenitzianum* or *R. cuspidellum*?	NT

#	Taxon	Range	Taxonomic Issues	Questions Raised	IUCN Code
	Koord., *Nova Guinea* 8(1): 186, 1909.		B2ab(iii,v) (Gibbs et al. 2011). Very similar to *R. baenitzianum*. Baron von Mueller used the invalid name '*megalostigma*' for this species which has extremely divided stigmatic lobes. Not known to have been cultivated (Argent 2006).		
307	*R. mollianum* Koord., *Nova Guinea* 8(1): 187, 1909.	NG	Only known from the type specimen and needs further research to establish its status (Gibbs et al. 2011). Very reminiscent of *R. englerianum* (Argent 2006).	(i) Is this species related to *R. englerianum*?	DD
308	*R. cuspidellum* Sleumer, *Reinwardtia* 5: 200, 1960.	NG	Very similar to *R. baenitzianum*; more collections are badly needed to evaluate this. Not known to have been cultivated (Argent 2006).	(i) Is this species related to *R. englerianum* or *R. baenitzianum*?	LC
309	*R. baenitzianum* Lauterb., *Nachtr*. 337, 1905.	NG	A lowland species and therefore likely to be at risk from habitat loss, but considered by taxonomists not to be distinct from *R. englerianum* (Gibbs et al. 2011). This species has sometimes been confused with *R. zoelleri*. Sleumer (1973) commented on its relationship with *R. englerianum*. Further work needed to fully understand the variation in these two species (Argent 2006).	(i) Is this species related to *R. englerianum*, *R. cuspidellum* or *R. zoelleri*?	DD
310	*R. scabridibracteum* Sleumer, *Reinwardtia* 5: 215, 1960.	NG	No known issues.	No known issues.	LC
311	***R. zoelleri*** Warb., *Bot. Jahr*. 16: 24, 1892.	NG, ML	A form different only by smaller anthers (3–4 mm) apparently limited to SE New Guinea (Central and Milne Bay District) was observed (Sleumer 1966a). Forms natural hybrids with *R. konori* var. *phaeopeplum* (previously known as *R. phaeopeplum sensu* Sleumer 1966). Natural hybrids apparently formed with *R. macgregoriae* are not rare locally, often forming hybrid swarms. Such plants show the general habit and foliage of *R. macgregoriae*, but	(i) Do the accessions of this species form genetically differentiated clusters? (ii) Is this species related to *R. konori*, *R. macgregoriae* or *R. laetum*?	LC

#	Taxon	Range	Taxonomic Issues	Questions Raised	IUCN Code
			have larger flowers. This species is also much related to *R. laetum* (Sleumer 1966a). Flower colour is reported to be variable, usually with yellow at least at the base of the tube but with the upper tube and lobes orange and reddish, and reported (Sleumer 1966a) as very rarely white or greenish white and sweet-scented. This he regarded as a hybrid with *R. konori* var. *phaeopeplum* (Argent 2006). This species has been confused with *R. baenitzianum* which was unsatisfactorily keyed in Sleumer (1966a).		
312	***R. lochiae*** F. Muell., *Vict. Nat.* 3: 157, 1887.	AU	Known only from two locations (Gibbs et al. 2011). Although recorded as being in cultivation on numerous occasions, all early records of this species as live plants are referable to *R. viriosum* and similarly all records of hybrids formed from this species in fact used *R. viriosum* as parent, not *R. lochiae*. D L Jones collected material in 1975 which has grown and used to describe *R. notiale* (Craven & Withers 1996b). This name had to be abandoned when the International Nomenclatural Committee rejected the conservation of *R. lochiae* as the name which had been long misapplied to what is now known as *R. viriosum* (Argent 2006).	(i) What is the genetic relationship between *R. lochiae* and *R. viriosum*? (ii) What is the status of the taxon *R. notiale*?	VU D2
313	***R. viriosum*** Craven, *Edinb. J. Bot.* 59(3): 448, 2002.	AU	*R. lochiae* and *R. viriosum* were initially treated as a single taxon. Their separation was based on morphological differences mainly in stature, corolla shape and placement of stamens. The earliest formal report of *R. viriosum* growing in cultivation was from Kew (as *R. lochiae*) where it was recorded flowering in the temperate house in 1939. This species also has the distinction of being one of the only species of *Vireya* to have been reliably recorded as successfully	(i) What is the genetic relationship between *R. lochiae* and *R. viriosum*?	LC

#	Taxon	Range	Taxonomic Issues	Questions Raised	IUCN Code
			being crossed with a rhododendron outside *Vireya* (Argent 2006).		

A3 DNA Sequences of Vireyas for the *rpb*2i Intron 23

Table 44 DNA sequences of vireya accessions for the *rpb*2i intron 23 region.

Acc. Num.	Taxon	1 140
2002-018	R. acrophilum	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
99-0330	R. dielsianum	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
EI153	R. jasminiflorum	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
EI158	R. majus	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTGACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
EI169	R. gardenia 'Odyssey'	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
EK507	R. lochiae	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
EI192	R. luraluense	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
EK525	R. bagobonum	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
EK536	R. alborugosum	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
EK544	R. suaveolens	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCTTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACAA
EK548	R. jasminiflorum	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACAA
EK558	R. praetervisum	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACAA
EK565	R. superbum	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
EK574	R. vitis-idaea	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATTTCAAAGAGGGTTGCTTATGGGCACGA
EK579	R. citrinum	TTATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
EK581	R. santapaui	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
EK583	R. stapfianum	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACAA
EK588	R. superbum	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
EK590	R. jasminiflorum	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATAAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACAA
EK591	R. blackii	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
EK592	R. blackii	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGACGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
EK600	R. kochii	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
EK602	R. adinophyllum	TCATGGGGAAGGTTGCTGCCCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACAA
EK608	R. arfakianum	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
EK614	R. solitarium	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
EK616	R. superbum	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
EK617	R. solitarium	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTTACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACAA
EK618	R. rarum	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAACTCTCAAAGAGGGTTGCTTATGGGCACGA
EK626	R. carringtoniae	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
EK628	R. zoelleri	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
EK629	R. culminicola	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
EK632	R. commonae	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
EK633	R. commonae	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
EK635	R. gracilentum	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
EK637	R. commonae	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
EK638	R. caliginis	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAACTCTCAAAGAGGGTTGCTTATGGGCACGA
EK639	R. herzogii	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
EK644	R. laetum	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
EK645	R. jasminiflorum	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATAAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACAA
EK647	R. rutenii	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACAA
EK648	R. laetum	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
EK649	R. dielsianum	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
EK651	R. superbum	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTCGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
EK657	R. majus	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
EK658	R. majus	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
EK664	R. yongii	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACAA

EK666	R. asperum	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
EK667	R. radians	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACAA
EK671	R. wilkiei	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTTACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACAA
HF001	R. konori	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTCGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
HF004	R. superbum	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
HF012	R. gardenia	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
HF014	R. rousei	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACAA
HF016	R. cruttwellii	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
HF021	R. javanicum	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACAA
HF023	R. dielsianum	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
HF028	R. truncicola	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
HF030	R. lochiae	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
HF031	R. curviflorum	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
HF032	R. armitii	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
HF036	R. pleianthum	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
HF043	R. burttii	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTCTATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACAA
HF050	R. emarginatum	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAACAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACAA
HF051	R. leucogigas	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
HF053	R. pubigermen	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTTACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACAA
HF062	R. commonae	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
HF066	R. laetum	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
HF067	R. villosulum	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
HF068	R. aurigeranum	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
HF070	R. celebicum	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
HF072	R. kawakamii	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
HF077	R. viriosum	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
HF082	R. suaveolens	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
HF090	R. loranthiflorum	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCCAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
HF091	R. verticillatum	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
HF092	R. orbiculatum	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
HF093	R. sumatranum	TCATGGGGAAGGTTGCTGCCCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACAA
HF094	R. luraluense	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
HF097	R. zollingeri	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
HF100	R. tuba	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
HF101	R. lowii	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
HF135	R. impositum	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTTTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
HF137	R. luraluense	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
HF139	R. jasminiflorum	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
HF145	R. × planecostatum	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
HF147	R. rushforthii	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACAA

Acc. Num.	Taxon	141 280
2002-018	R. acrophilum	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
99-0330	R. dielsianum	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
EI153	R. jasminiflorum	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
EI158	R. majus	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTGACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
EI169	R. gardenia 'Odyssey'	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
EK507	R. lochiae	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
EI192	R. luraluense	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
EK525	R. bagobonum	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
EK536	R. alborugosum	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
EK544	R. suaveolens	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCTTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACAA
EK548	R. jasminiflorum	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACAA
EK558	R. praetervisum	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACAA
EK565	R. superbum	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
EK574	R. vitis-idaea	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATTTCAAAGAGGGTTGCTTATGGGCACGA
EK579	R. citrinum	TTATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
EK581	R. santapaui	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
EK583	R. stapfianum	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACAA
EK588	R. superbum	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
EK590	R. jasminiflorum	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATAAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACAA
EK591	R. blackii	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
EK592	R. blackii	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGACGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
EK600	R. kochii	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
EK602	R. adinophyllum	TCATGGGGAAGGTTGCTGCCCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACAA
EK608	R. arfakianum	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
EK614	R. solitarium	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
EK616	R. superbum	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
EK617	R. solitarium	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTTACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACAA
EK618	R. rarum	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAACTCTCAAAGAGGGTTGCTTATGGGCACGA
EK626	R. carringtoniae	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
EK628	R. zoelleri	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
EK629	R. culminicola	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
EK632	R. commonae	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
EK633	R. commonae	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
EK635	R. gracilentum	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
EK637	R. commonae	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
EK638	R. caliginis	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAACTCTCAAAGAGGGTTGCTTATGGGCACGA
EK639	R. herzogii	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
EK644	R. laetum	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
EK645	R. jasminiflorum	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATAAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACAA
EK647	R. rutenii	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACAA
EK648	R. laetum	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
EK649	R. dielsianum	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
EK651	R. superbum	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTCGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
EK657	R. majus	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
EK658	R. majus	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
EK664	R. yongii	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACAA
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EK667	R. radians	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACAA
EK671	R. wilkiei	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTTACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACAA
HF001	R. konori	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTCGAAATCTCAAAGAGGGTTGCTTATGGGCACGA

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HF012	R. gardenia	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
HF014	R. rousei	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACAA
HF016	R. cruttwellii	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
HF021	R. javanicum	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACAA
HF023	R. dielsianum	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
HF028	R. truncicola	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
HF030	R. lochiae	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
HF031	R. curviflorum	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
HF032	R. armitii	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
HF036	R. pleianthum	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
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HF050	R. emarginatum	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAACAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACAA
HF051	R. leucogigas	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
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HF062	R. commonae	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
HF066	R. laetum	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
HF067	R. villosulum	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
HF068	R. aurigeranum	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
HF070	R. celebicum	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
HF072	R. kawakamii	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
HF077	R. viriosum	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
HF082	R. suaveolens	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
HF090	R. loranthiflorum	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCCAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
HF091	R. verticillatum	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
HF092	R. orbiculatum	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
HF093	R. sumatranum	TCATGGGGAAGGTTGCTGCCCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACAA
HF094	R. luraluense	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
HF097	R. zollingeri	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
HF100	R. tuba	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
HF101	R. lowii	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
HF135	R. impositum	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTTTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
HF137	R. luraluense	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
HF139	R. jasminiflorum	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
HF145	R. x planecostatum	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACGA
HF147	R. rushforthii	TCATGGGGAAGGTTGCTGCTCACATGGGAAAGGAGGGAGATGCAACTCCTTTCACTGATGTCACTGTAAGTATATTTTGTTAACATGATATCAAAAGCATGCCTCTTTGAAATCTCAAAGAGGGTTGCTTATGGGCACAA

Acc. Num.	Taxon	281 420
2002-018	R. acrophilum	GCCCATTACTTTTTAGTAGTCTTCTTGGCTGGAATCAGAGTTAGCAGCATTGGTGTGAATACAATTCTATAACCTTAGAAGTCATACCTCTAGATTTGGTGCTAATTAATATCCCAATTCCCAAAGAGAGTTGACATGCC
99-0330	R. dielsianum	GCCCATTACTTTTTAGTAGTCTTCTTGGCTGGAATCAGAGTTAGCAGCATTGGTGTGAATACAATTCTATAACCTTAGAAGTCATACCTCTAGATTTGGTGCTAATTAATATCCCAATTCCCAAAGAGAGTTGACWTGCC
EI153	R. jasminiflorum	GCCCATTACTTTTTAGTAGTCTTCTTGGCTGGAATCAGAGTTAGCAGCATTGGTGTGAATACAATTCTATAACCTTAGAAGTCATACCTCTAGATTTGGTGCTAATTAATATCCCAATTCCCAAAGAGAGTTGACATGCC
EI158	R. majus	GCCCATTACTTTTTAGTAGTCTTCTTGGCTGGAATCAGAGTTAGCAGCATTGGTGTGAATACAATTCTATAACCTTAGAAGTCATACCTCTAGATTTGGTGCTAATTAATATCCCAATTCCCAAAGAGAGTTGACTTGCC
EI169	R. gardenia 'Odyssey'	GCCCATTACTTTTTAGTAGTCTTCTTGGCTGGAATCAGAGTTAGCAGCATTGGTGTGAATACAATTCTATAACCTTAGAAGTCATACCTCTAGATTTGGTGCTAATTAATATCCCAATTCCCAAAGAGAGTTGACATGCC
EK507	R. lochiae	GCCCATTACTTTTTAGTAGTCTTCTTGGCTGGAATCAGAGTTAGCAGCATTGGTGTGAATACAATTCTATAACCTTAGAAGTCATACCTCTAGATTTGGTGCTAATTAATATCCCAATTCCCAAAGAGAGTTGACATGCC
EI192	R. luraluense	GCCCATTACTTTTTAGTAGTCTTCTTGGCTGGAATCAGAGTTAGCAGCATTGGTGTGAATACAATTCTATAACCTTAGAAGTCATACCTCTAGATTTGGTGCTAATTAATATCCCAATTCCCAAAGAGAGTTGACATGCC
EK525	R. bagobonum	GCCCATTACTTTTTAGTAGTCTTCTTGGCTGGAATCAGAGTTAGCAGCATTGGTGTGAATACAATTCTATAACCTTAGAAGTCATACCTCTAGATTTGGTGCTAATTAATATCCCAATTCCCAAAGAGAGTTGACATGCC
EK536	R. alborugosum	GCCCATTACTTTTTAGTAGTCTTCTTGGCTGGAATCAGAGTTAGCAGCATTGGTGTGAATACAATTCTATAACCTTAGAAGTCATACCTCTAGATTTGGTGCTAATTAATATCCCAATTCCCAAAGAGAGTTGACTTGCC
EK544	R. suaveolens	GCCCATTACTTTTTAGTAGTCTTCTTGGCTGGAATCAGAGTTAGCAGCATTGGTGTGAATACAATTCTATAACCTTAGAAGTCATACCTCTAGATTTGGTGCTAATTAATATCCCAATTCCCAAAGAGAGTTGACATCCC
EK548	R. jasminiflorum	GCCCATTACTTTTTAGTAGTCTTCTTGGCTGGAATCAGAGTTAGCAGCATTGGTGTGAATACAATTCTATAACCTTAGAAGTCATACCTCTAGATTTGGTGCTAATTAATATCCCAATTCCCAAAGAGAGTTGACATGCC
EK558	R. praetervisum	GCCCATTACTTTTTAGTAGTCTTCTTGGCTGGAATCAGAGTGAGCAGCATTGGTGTGAATACGATTCTATCACCTTAGAAGTCATACCTCTAGATTTGGTGCTAATTAATATCCCAATTCCCAAAGAGAGGTGACTTGCC
EK565	R. superbum	GCCCATTACTTTTTAGTAGTCTTCTTGGCTGGAATCAGAGTTAGCAGCATTGGTGTGAATACAATTCTATAACCTTAGAAGTCATACCTCTAGATTTGATGCTAATTAATATCCCAATTCCCAAAGAGAGTTGACTTGCC
EK574	R. vitis-idaea	GCCCATTACTTTTTAGTAGTCTTCTTGGCTGGAATCAGAGTTAGCAGCATTGGTGTGAATACAATTCTATAACCTTAGAAGTCATACCTCTAGATTTGGTGCTAATTAATATCCCAATTCCCAAAGAGAGTTGACATGCC
EK579	R. citrinum	GCCCATTACTTTTTAGTAGTCTTCTTGGCTGGAATCAGAGTTAGCAGCATTGGTGTGAATACAATTCTATAACCTTAGAAGTCATACCTCTAGATTTGGTGCTAATTAATATCCCAATTCCCAAAGAGAGTTGACATGCC
EK581	R. santapaui	GCCCATTACTTTTTAGTAGTCTTCTTGGCTGGAATCAGAGTTAGCAGCATTGGTGTGAATACAATTCTATAACCTTAGAAGTCATACCTCTAGATTTGGTGCTAATTAATATCCCAATTCCCAAAGAGAGTTGACATGCC
EK583	R. stapfianum	GCCCATTACTTTTTAGTAGTCTTCTTGGCTGGAATCAGAGTTAGCAGCATTGGTGTGAATACAATTCTATAACCTTAGAAGTCATACCTCTAGATTTGGTGCTAATTAATATCCCAATTCCCAAAGAGAGTTGACTTGCC
EK588	R. superbum	GCCCATTACTTTTTAGTAGTCTTCTTGGCTGGAATCAGAGTTAGCAGCATTGGTGTGAATACAATTCTATAACCTTAGAAGTCATACCTCTAGATTTGGTGCTAATTAATATCCCAATTCCCAAAGAGAGTTGACTTGCC
EK590	R. jasminiflorum	GCCCATTACTTTTTAGTAGTCTTCTTGGCTGGAATCAGAGTTAGCAGCATTGGTGTGAATACAATTCTATAACCTTAGAAGTCATACCTCTAGATTTGGTGCTAATTAATATCCCAATTCCCAAAGAGAGTTGACATGCC
EK591	R. blackii	GCCCATTACTTTTTAGTAGTCTTCTTGGCTGGAATCAGAGTTAGCAGCATTGGTGTGAATACAATTCTATAACCTTAGAAGTCATACCTCTAGATTTGGTGCTAATTAATATCCCAATTCCCAAAGAGAGTTGACTTGCC
EK592	R. blackii	GCCCATTACTTTTTAGTAGTCTTCTTGGCTGGAATCAGAGTTAGCAGCATTGGTGTGAATACAATTCTATAACCTTAGAAGTCATACCTCTAGATTTGGTGCTAATTAATATCCCAATTCCCAAAGAGAGTTGACATGCC
EK600	R. kochii	GCCCATTACTTTTTAGTAGTCTTCTTGGCTGGAATCAGAGTTAGCAGCATTGGTGTGAATACGATTCTATAACCTTAGAAGTCATACCTCTAGATTTGGTGCTAATTAATATCCCAATTCCCAAAGAGAGTTGACATGCC
EK602	R. adinophyllum	GCCCATTACTTTTTAGTAGTCTTCTTGGCTGGAATCAGAGTTAGCAGCATTGGTGTGAATACAATTCTATAACCTTAGAAGTCATACCTCTAGATTTGGTGCTAATTAATATCCCAATTCCCAAAGAGAGTTGACATGCC
EK608	R. arfakianum	GCCCATTACTTTTTAGTAGTCTTCTTGGCTGGAATCAGAGTTAGCAGCATTGGTGTGAATACAATTCTATAACCTTAGAAGCCATAGCTCTAGATTTGGAGCTAATTAATATCCCAATTCCCAAAGAGAGTTGACTTGCC
EK614	R. solitarium	GCCCATTACTTTTTAGTAGTCTTCTTGGCTGGAATCAGAGTTAGCAGCATTGGTGTGAATACAATTCTATAACCTTAGAAGTCRTACCTCTAGATTTGGTGCTAATTGATATCCCAATTCCCAAAGAGAGTTGACWTGCC
EK616	R. superbum	GCCCATTACTTTTTAGTAGTCTTCTTGGCTGGAATCAGAGTTAGCAGCATTGGTGTGAATACAATTCTATAACCTTAGAAGTCATAGCTCTAGATTTGATGCTAATTAATATCCCAATTCCCAAAGAGAGTTGACATGCC
EK617	R. solitarium	GCCCATTACTTTTTAGTAGTCTTCTTGGCTGGAATCAGAGTTAGCAGCATTGGTGTGAATACAATTCTATAACCTTAGAAGTCATACCTCTAGATTTGGTGCTAATTAATATCCCAATTCCCAAAGAGAGTTGACATGCC
EK618	R. rarum	GCCCATTACTTTTTAGTAGTCTTCTTGGCTGGAATCAGAGTTAGCAGCATTGGTGTGAATACAATTCTATAACCTTAGAAGTCATACCTCTAGATTTGGTGCTAATTAATATCCCAATTCCCAAAGAGAGTTGACTTGCC
EK626	R. carringtoniae	GCCCATTACTTTTTAGTAGTCTTCTTGGCTGGAATCAGAGTTAGCAGCATTGGTGTGAATACAATTCTATAACCTTAGAAGTCATACCTCTAGATTTGGTGCTAATTAATATCCCAATTCCCAAAGAGAGTTGACATGCC
EK628	R. zoelleri	GCCCATTACTTTTTAGTAGTCTTCTTGGCTGGAATCAGAGTTAGCAGCATTGGTGTGAATACAATTCTATGACCTTAGAAGTCATACCTCTAGATTTGGTGCTAATTAATATCCCAATTCCCAAAGAGAGTTGACATGCC
EK629	R. culminicola	GCCCATTACTTTTTAGTAGTCTTCTTGGCTGGAATCAGAGTTAGCAGCATTGGTGTGAATACAATTCTATAACCTTAGAAGTCATACCTCTAGATTTGGTGCTAATTAATATCCCAATTCCCAAAGAGAGTTGACATGCC
EK632	R. commonae	GCCCATTACTTTTTAGTAGTCTTCTTGGCTGGAATCAGAGTTAGCAGCATTGGTGTGAATACAATTCTATAACCTTAGAAGTCATACCTCTAGATTTGGTGCTAATTAATATCCCAATTCCCAAAGAGAGTTGACATGCC
EK633	R. commonae	GCCCATTACTTTTTAGTAGTCTTCTTGGCTGGAATCAGAGTTAGCAGCATTGGTGTGAATACAATTCTATAACCTTAGAAGTCATACCTCTAGATTTGGTGCTAATTAATATCCCAATTCCCAAAGAGAGTTGACATGCC
EK635	R. gracilentum	GCCCATTACTTTTTAGTAGTCTTCTTGGCTGGAATCAGAGTTAGCAGCATTGGTGTGAATACGATTCTATAACCTTAGAAGTCATACCTCTAGATTTGGTGCTAATTAATATCCCAATTCCCAAAGAGAGTTGACATGCC
EK637	R. commonae	GCCCATTACTTTTTAGTAGTCTTCTTGGCTGGAATCAGAGTTAGCAGCATTGGTGTGAATACAATTCTATAACCTTAGAAGTCATACCTCTAGATTTGGTGCTAATTAATATCCCAATTCCCAAAGAGAGTTGACATGCC
EK638	R. caliginis	GCCCATTACTTTTTAGTAGTCTTCTTGGCTGGAATCAGAGTTAGCAGCATTGGTGTGAATACAATTCTATAACCTTAGAAGTCATACCTCTAGATTTGGTGCTAATTAATATCCCAATTCCCAAAGAGAGTTGACATGCC
EK639	R. herzogii	GCCCATTACTTTTTAGTAGTCTTCTTGGCTGGAATCAGAGTTAGCAGCATTGGTGTGAATACAATTCTATAACCTTAGAAGTCRTACCTCTAGATTTGGTGCTAATTAATATCCCAATTCCCAAAGAGAGTTGACWTGCC
EK644	R. laetum	GCCCATTACTTTTTAGTAGTCTTCTTGGCTGGAATCAGAGTTAGCAGCATTGGTGTGAATACAATTCTATAACCTTAGAAGTCATACCTCTAGATTTGGTGCTAATTAATATCCCAATTCCCAAAGAGAGTTGACATGCC
EK645	R. jasminiflorum	GCCCATTACTTTTTAGTAGTCTTCTTGGCTGGAATCAGAGTTAGCAGCATTGGTGTGAATACAATTCTATAACCTTAGAAGTCATACCTCTAGATTTGGTGCTAATTAATATCCCAATTCCCAAAGAGAGTTGACTTGCC
EK647	R. rutenii	GCCCATTACTTTTTAGTAGTCTTCTTGGCTGGAGTCAGAGTTAGCAGCATTGGTGTGAATACAATTCTATAACCTTAGAAGTCATACCTCTAGATTTGGTGCTAATTAATATCCCAATTCCCAAAGAGAGTTGACTTGCC
EK648	R. laetum	GCCCATTACTTTTTAGTAGTCTTCTTGGCTGGAATCAGAGTTAGCAGCATTGGTGTGAATACAATTCTATAACCTTAGAAGTCATACCTCTAGATTTGGTGCTAATTAATATCCCAATTCCCAAAGAGAGTTGACATGCC
EK649	R. dielsianum	GCCCATTACTTTTTAGTAGTCTTCTTGGCTGGAATCAGAGTTAGCAGCATTGGTGTGAATACAATTCTATAACCTTAGAAGTCRTACCTCTAGATTTGGTGCTAATTAATATCCCAATTCCCAAAGAGAGTTGACWTGCC
EK651	R. superbum	GCCCATTACTTTTTAGTAGTCTTCTTGGCTGGAATCAGAGTTAGCAGCATTGGTGTGAATACAATTCTATAACCTTAGAAGTCATACCTCTAGATTTGGTGCTAATTAATATCCCAATTCCCAAAGAGAGTTGACATGCC
EK657	R. majus	GCCCATTACTTTTTAGTAGTCTTCTTGGCTGGAATCAGAGTTAGCAGCATTGGTGTGAATACAATTCTATAACCTTAGAAGTCATACCTCTAGATTTGGTGCTAATTAATATCCCAATTCCCAAAGAGAGTTGACATGCC
EK658	R. majus	GCCCATTACTTTTTAGTAGTCTTCTTGGCTGGAATCAGAGTTAGCAGCATTGGTGTGAATACGATTCTATAACCTTAGAAGTCATACCTCTAGATTTGGTGCTAATTAATATCCCAATTCCCAAAGAGAGTTGACATGCC
EK664	R. yongii	GCCCATTACTTTTTAGTAGTCTTCTTGGCTGGAATCAGAGTTAGCAGCATTGGTGTGAATACAATTCTATAACCTTAGAAGTCATACCTCTAGATTTGGTGCTAATTAATATCCCAATTCCCAAAGAGAGTTGACTTGCC
EK666	R. asperum	GCCCATTACTTTTTAGTAGTCTTCTTGGCTGGAATCAGAGTTAGCAGCATTGGTGTGAATACAATTCTATAACCTTAGAAGTCATACCTCTAGATTTGGTGCTAATTAATATCCCAATTCCCAAAGAGAGTTGACATGCC
EK667	R. radians	GCCCATTACTTTTTAGTAGTCTTCTTGGCTGGAGTCAGAGTTAGCAGCATTGGTGTGAATACAATTCTATAACCTTAGAAGTCATACCTCTAGATTTGGTGCTAATTAATATCCCAATTCCAAAAGAGAGTTGACTTGCC
EK671	R. wilkiei	GCCCATTACTTTTTAGTAGTCTTCTTGGCTGGAATCAGAGTTAGCAGCATTGGTGTGAATACAATTCTATAACCTTAGAAGTCATACCTCTAGATTTGGTGCTAATTAATATCC-AATTCCCAAAGAGAGTTGACTTGCC
HF001	R. konori	GCCCATTACTTTTTAGTAGTCTTCTTGGCTGGAATCAGAGTTAGCAGCATTGGTGTGAATACAATTCTATAACCTTAGAAGTCATACCTCTAGATTTGGTGCTAATTAATATCCCAATTCCCAAAGAGAGTTGACATGCC

HF004	R. hellwigii	GCCCATTACTTTTTAGTAGTCTTCTTGGCTGGAATCAGAGTTAGCAGCATTGGTGTGAATACAATTCTATAACCTTAGAAGTCRTACCTCTAGATTTGGTGCTAATTAATATCCCAATTCCCAAAGAGAGTTGACATGCC
HF012	R. gardenia	GCCCATTACTTTTTAGTAGTCTTCTTGGCTGGAATCAGAGTTAGCAGCATTGGTGTGAATACAATTCTATAACCTTAGAAGTCATACCTCTAGATTTGGTGCTAATTAATATCCCAATTCCCAAAGAGAGTTGACATGCC
HF014	R. rousei	GCCCATTACTTTTTAGTAGTCTTCTTGGCTGGAGTCAGAGTTAGCAGCATTGGTGTGAATACAATTCTATAACCTTAGAAGTCATACCTCTAGATTTGGTGCTAATTAATATCCCAATTCCCAAAGAGAGTTGACTTGCC
HF016	R. cruttwellii	GCCCATTACTTTTTAGTAGTCTTCTTGGCTGGAATCAGAGTTAGCAGCATTGGTGTGAATACAATTCTATAACCTTAGAAGTCATACCTCTAGATTTGGTGCTAATTAATATCCCAATTCCCAAAGAGAGTTGACATGCC
HF021	R. javanicum	GCCCATTACTTTTTAGTAGTCTTCTTGGCTGGAATCAGAGTTAGCAGCATTGGTGTGAATACAATTCTATAACCTTAGAAGTCATACCTCTAGATTTGGTGCTAATTAATATCCCAATTCCCAAAGAGAGTTGACTTGCC
HF023	R. dielsianum	GCCCATTACTTTTTAGTAGTCTTCTTGGCTGGAATCAGAGTTAGCAGCATTGGTGTGAATACAATTCTATAACCTTAGAAGTCATACCTCTAGATTTGGTGCTAATTAATATCCCAATTCCCAAAGAGAGTTGACATGCC
HF028	R. truncicola	GCCCATTACTTTTTAGTAGTCTTCTTGGCTGGAATCAGAGTTAGCAGCATTGGTGTGAATACAATTCTATAACCTTAGAAGTCATACCTCTAGATTTGGTGCTAATTAATATCCCAATTCCCAAAGAGAGTTGACATGCC
HF030	R. lochiae	GCCCATTACTTTTTAGTAGTCTTCTTGGCTGGAATCAGAGTTAGCAGCATTGGTGTGAATACAATTCTATAACCTTAGAAGTCATACCTCTAGATTTGGTGCTAATTAATATCCCAATTCCCAAAGAGAGTTGACATGCC
HF031	R. curviflorum	GCCCATTACTTTTTAGTAGTCTTCTTGGCTGGAATCAGAGTTAGCAGCATTGGTGTGAATACAATTCTATAACCTTAGAAGTCATACCTCTAGATTTGGTGCTAATTAATATCCCAATTCCCAAAGAGAGTTGACATGCC
HF032	R. armitii	GCCCATTACTTTTTAGTAGTCTTCTTGGCTGGAATCAGAGTTAGCAGCATTGGTGTGAATACAATTCTATAACCTTAGAAGTCATASCTCTAGATTTGGTGCTAATTAATATCCCAATTCCCAAAGAGAGTTGACWTGCC
HF036	R. pleianthum	GCCCATTACTTTTTAGTAGTCTTCTTGGCTGGAATCAGAGTTAGCAGCATTGGTGTGAATACAATTCTATAACCTTAGAAGTCATACCTCTAGATTTGGTGCTAATTAATATCCCAATTCCCAAAGAGAGTTGACATGCC
HF043	R. burttii	GCCCATTACTTTTTAGTAGTCTTCTTGGCTGGAATCAGAGTTAGCAGCATTGGTGTGAATACAATTCTATAACCTTAGAAGTCATACCTCTAGATTTGGTGCTAATTAATATCCCAATTCCCAAAGAGAGTTGACTTGCC
HF050	R. emarginatum	GCCCATTACTTTTTAGTAGTCTTCTTGGCTGGAATCAGAGTTAGCAGAATTGGTGTGAATACAATTCTATAACCTTAGAAGTCATACCTCTAGATTTGGTGCTAATTAATATCCCAATTCCCAAAGAGAGTTGACTTGCC
HF051	R. leucogigas	GCCCATTACTTTTTAGTAGTCTTCTTGGCTGGAATCAGAGTTAGCAGCATTGGTGTGAATACAATTCTATACCCTTAGAAGTCATACCTCTAGATTTGGTGCTAATTAATATCCCAATTCCCAAAGAGAGTTGACATGCC
HF053	R. pubigermen	GCCCATTACTTTTTAGTAGTCTTCTTGGCTGGAATCAGAGTTAGCAGCATTGGTGTGAATACAATTCTATAACCTTAGAAGTCATACCTCTAGATTTGGTGCTAATTAATATCCCAATTCCCAAAGAGAGTTGACTTGCC
HF062	R. commonae	GCCCATTACTTTTTAGTAGTCTTCTTGGCTGGAATCAGAGTTAGCAGCATTGGTGTGAATACAATTCTATAACCTTAGAAGTCATACCTCTAGATTTGGTGCTAATTAATATCCCAATTCCCAAAGAGAGTTGACATGCC
HF066	R. laetum	GCCCATTACTTTTTAGTAGTCTTCTTGGCTGGAATCAGAGTTAGCAGCATTGGTGTGAATACAATTCTATAACCTTAGAAGTCATACCTCTAGATTTGGTGCTAATTAATATCCCAATTCCCAAAGAGAGTTGACATGCC
HF067	R. villosulum	GCCCATTACTTTTTAGTAGTCTTCTTGGCTGGAATCAGAGTTAGCAGCATTGGTGTGAATACAATTCTATAACCTTAGAAGTCATACCTCTAGATTTGGTGCTAATTAATATCCCAATTCCCAAAGAGAGTTGACATGCC
HF068	R. aurigeranum	GCCCATTACTTTTTAGTAGTCTTCTTGGCTGGAATCAGAGTTAGCAGCATTGGTGTGAATACAATTCTATAACCTTAGAAGTCATACCTCTAGATTTGGTGCTAATTAATATCCCAATTCCCAAAGAGAGTTGACATGCC
HF070	R. celebicum	GCCCATTACTTTTTAGTAGTCTTCTTGGCTGGAATCAGAGTTAGCAGCATTGGTGTGAATACAATTCTATAACCTTAGAAGTCRTACCTCTAGATTTGGTGCTAATTAATATCCCAATTCCCAAAGAGAGTTGACWTGCC
HF072	R. kawakamii	GCCCATTACTTTTTAGTAGTCTTCTTGGCTGGAATCAGAGTTAGCAGCATTGGTGTGAATACAATTCTATAACCTTAGAAGTCATACCTCTAGATTTGGTGCTAATTAATATCCCAATTCCCAAAGAGAGTTGACTTGCC
HF077	R. viriosum	GCCCATTACTTTTTAGTAGTCTTCTTGGCTGGAATCAGAGTTAGCAGCATTGGTGTGAATACAATTCTATAACCTTAGAAGTCATACCTCTAGATTTGGTGCTAATTAATATCCCAATTCCCAAAGAGAGTTGACATGCC
HF082	R. suaveolens	GCCCATTACTTTTTAGTAGTCTTCTTGGCTGGAATCAGAGTTAGCAGCATTGGTGTGAATACAATTCTATAACCTTAGAAGTCATACCTCTAGATTTGGTGCTAATTAATATCCCAATTCCCAAAGAGAGTTGACATGCC
HF090	R. loranthiflorum	GCCCATTACTTTTCAGTAGTCTTCTTGGCTGGAATCAGAGTTAGCAGCATTGGTGTGAATACAATTCTATAACCTTAGAAGTCATACCTCTAGATTTGGTGCTAATTAATATCCCAATTCCCAAAGAGAGTTGACATGCC
HF091	R. verticillatum	GCCCATTACTTTTTAGTAGTCTTCTTGGCTGGAATCAGAGTTAGCAGCATTGGTGTGAATACAATTCTATAACCTTAGAAGTCATACCTCTAGATTTGGTGCTAATTAATATCCCAATTCCCAAAGAGAGTTGACATGCC
HF092	R. orbiculatum	GCCCATTACTTTTTAGTAGTCTTCTTGGCTGGAATCAGAGTTAGCAGCATCGGTGTGAATACAATTCTATAACCTTAGAAGTCATACCTCTAGATTTGGTGCTAATTAATATCCCAATTCCCAAAGAGAGTTGACATGCC
HF093	R. sumatranum	GCCCATTACTTTTTAGTAGTCTTCTTGGCTGGAATCAGAGTTAGCAGCATTGGTGTGAATACAATTCTATAACCTTAGAAGTCATAGCTCTAGATTTGGTGCTAATTAATATCCCAATTCCCAAAGAGAGTTGACATGCC
HF094	R. luraluense	GCCCATTACTTTTTAGTAGTCTTCTTGGCTGGAATCAGAGTTAGCAGCATTGGTGTGAATACAATTCTATAACCTTAGAAGTCATACCTCTAGATTTGGTGCTAATTAATATCCCAATTCCCAAAGAGAGTTGACATGCC
HF097	R. zollingeri	GCCCATTACTTTTTAGTAGTCTTCTTGGCTGGAATCAGAGTTAGCAGCATTGGTGTGAATACAATTCTATAACCTTAGAAGTCATACCTCTAGATTTGGTGCTAATTAATATCCCAATTCCCAAAGAGAGTTGACATGCC
HF100	R. tuba	GCCCATTACTTTTTAGTAGTCTTCTTGGCTGGAATCAGAGTTAGCAGCATTGGTGTGAATACAATTCTATAACCTTAGAAGTCATACCTCTAGATTTGGTGCTAATTAATATCCCAATTCCCAAAGAGAGTTGACATGCC
HF101	R. lowii	GCCCATTACTTTTTAGTAGTCTTCTTGGCTGGAATCAGAGTTAGCAGCATTGGTGTGAATACAATTCTATAACCTTAGAAGTCATACCTCTAGATTTGGTGCTAATTAATATCCCAATTCCCAAAGAGAGTTGACATGCC
HF135	R. impositum	GCCCATTACTTTTTAGTAGTCTTCTTGGCTGGAATCAGAGTTAGCAGCATTGGTGTGAATACAATTCTATAACCTTAGAAGTCATACCTCTAGATTTGGTGCTAATTAATATCCCAATTCCCAAAGAGAGTTGACATGCC
HF137	R. luraluense	GCCCATTACTTTTTAGTAGTCTTCTTGGCTGGAATCAGAGTTAGCAGCATTGGTGTGAATACAATTCTATAACCTTAGAAGTCATACCTCTAGATTTGGTGCTAATTAATATCCCAATTCCCAAAGAGAGTTGACATGCC
HF139	R. jasminiflorum	GCCCATTACTTTTTAGTAGTCTTCTTGGCTGGAATCAGAGTTAGCAGCATCGGTGTGAATACAATTCTATACCCTTAGAAGTCATACCTCTAGATTTGGTGCTAATTAATATCCCAATTCCCAAAGAGAGTTGACATGCC
HF145	R. x planecostatum	GCCCATTACTTTTTAGTAGTCTTCTTGGCTGGAATCAGAGTTAGCAGCATTGGTGTGAATACAATTCTATAACCTTAGAAGTCATACCTCTAGATTTGGTGCTAATTAATATCCCAATTCCCAAAGAGAGTTGACATGCC
HF147	R. rushforthii	GCCCATTACTTTTTAGTAGTCTTCTTGGCTGGAATCAGAGTTAGCAGCATTGGTGTGAATACAATTCTATAACCTTAGAAGTCATACCTCTAGATTTGGTGCTAATTAATATCCCAATTCCCAAAGAGAGTTGACTTGCC

Acc. Num.	Taxon	421 560
2002-018	R. acrophilum	ACAATAAAATCACTATGCTCGCTTGGCATTTCTTTGACTTTGATAGGTTGGGTACGCAAAGTGTATTAGTGGTTTTAGAGCATCTCCTTGACAATTGACATCACCAAAATTTGTTAGATGAGAAAAATTGCAACTCCCCT
99-0330	R. dielsianum	ACAATAAAATCACTATGCTCGCTTGGCATTTCTTTGACTTTGATAGGTTGGGTACGCAAAGTGTATTAGTGGTTTTAGAGCATCTCCTTGACAATTGACATCACCAAAATTTGTTAGATGAGAAAAATTGCAACTCCACT
EI153	R. jasminiflorum	ACAATAAAATCACTATGCTCGCTTGGCATTTCTTTGACTTTGATAGGTTGGGTACGCAAAGTGTATTAGTGGTTTTAGAGCATCTCCTTGACAATTGACATCACCAAAATTTGTTAGATGAGAAAAATTGCAACTCCACT
EI158	R. majus	ACAATAAAATCACTATGCTCGCTTGGCATTTCTTTGACTTTGATAGGTTGGGTACGCAAAGTGTATTAGTGGTTTTAGAGCATCTCCTTGACAATTGACATCACCAAAATTTGTTAGATGAGAAAAATTGCAACTCCACT
EI169	R. gardenia 'Odyssey'	ACAATAAAATCACTATGCTCGCTTGGCATTTCTTTGACTTTGATAGGTTGGGTACGCAAAGTGTATTAGTGGTTTTAGAGCATCTCCTTGACAATTGACATCACCAAAATTTGTTAGATGAGAAAAATTGCAACTCCACT
EK507	R. lochiae	ACAATAAAATCACTATGCTCGCTTGGCATTTCTTTGACTTTGATAGGTTGGGTACGCAAAGTGTATTAGTGGTTTTAGAGCATCTCCTTGACAATTGACATCACCAAAATTTGTTAGATGAGAAAAATTGCAACTCCACT
EI192	R. luraluense	ACAATAAAATCACTATGCTCGCTTGGCATTTCTTTGACTTTGATAGGTTGGGTACGCAAAGTGTATTAGTGGTTTTAGAGCATCTCCTTGACAATTGACATCACCAAAATTTGTTAGATGAGAAAAATTGCAACTCCACT
EK525	R. bagobonum	ACAATAAAATCACTATGCTCGCTTGGCATTTCTTTGACTTTGATAGGTTGGGTACGCAAAGTGTATTAGTGGTTTTAGAGCATCTCCTTGACAATTGACATCACCAAAATTTGTTAGATGAGAAAAATTGCAACTCCACT
EK536	R. alborugosum	ACAATAAAATCACTATGCTCGCTTGGCATTTCTTTGACTTTGATAGGTTGGGTACGCAAAGTGTATTAGTGGTTTTAGAGCATCTCCTTGACAATTGACATCACCAAAATTTGTTAGATGAGAAAAATTGCAACTCCACT
EK544	R. suaveolens	ACAATAAAATCACTATGCTCGCTTGGCATTTCTTTGACTTTGATAGGTTGGGTACGCAAAGTGTATTAGTGGTTTTAGAGCATCTCCTTGACAATTGACATCACCAAAATTTGTTAGATGAGAAAAATTGCAACTCCACT
EK548	R. jasminiflorum	ACAATAAAATCACTATGCTCGCTTGGCATTTCTTTGACTTTGATAGGTTGGGTACGCAAAGTGTAGTAGTGGTTTTAGAGCATCTCCTTGACAATTGACATCACCAAAATTTGTTAGATGAGAAAAATTGCAACTCCACT
EK558	R. praetervisum	ACAATAAAATCACTATGCTCGCTTGGCATTTCTTTGACTTTGATAGGTTGGGTACGCAAAGTGTATTAGTGGTTTTAGAGCATCTCCTTGACAATTGACATCACCAAAATTTGTTAGATGAGAAAAATTGCAACTCCACT
EK565	R. superbum	ACAATAAAATCACTATGCTCGCTTGGCATTTCTTTGACTTTGATAGGTTGGGTACGCAAAGTGTATTAGTGGTTTTAGAGCATCTCCTTGACAATTGACATCACCAAAATTTGTTAGATGAGAAAAATTGCAACTCCACT
EK574	R. vitis-idaea	ACAATAAAATCACTATGCTCGCTTGGCATTTCTTTGACTTTGATAGGTTGGGTACGCAAAGTGTATTAGTGGTTTTAGAGCATCTCCTTGACAATTGACATCACCAAAATTTGTTAGATGAGAAAAATTGCAACTCCACT
EK579	R. citrinum	ACAATAAAATCACTATGCTCGCTTGGCATTTCTTTGACTTTGATAGGTTGGGTACGCAAAGTGTATTAGTGGTTTTAGAGCATCTCCTTGACAATTGACATCACCAAAATTTGTTAGATGAGAAAAATTGCAACTCCACT
EK581	R. santapaui	ACAATAAAATCACTATGCTCGCTTGGCATTTCTTAGACTTTGATAGGTTGGGTACGCAAAGTGTATTAGTGGTTTTAGAGCATCTCCTTGACAATTGACATCACCAAAATTTGTTAGATGAGAAAAATTGCAACTCCACT
EK583	R. stapfianum	ACAATAAAATCACTATGCTCGCTTGGCATTTCTTTGACTTTGATAGGTTGGGTACGCAAAGTGTATTAGTGGTTTTAGAGCATCTCCTTGACAATTGACATCACCAAAATTTGTTAGATGAGAAAAATTGCAACTCCACT
EK588	R. superbum	ACAATAAAATCACTATGCTCGCTTGGCATTTCTTTGACTTTGATAGGTTGGGTACGCAAAGTGTATTAGTGGTTTTAGAGCATCTCCTTGACAATTGACATCACCAAAATTTGTTAGATGAGAAAAATTGCAACTCCACT
EK590	R. jasminiflorum	ACAATAAAATCACTATGCTCGCTTGGCATTTCTTTGACTTTGATAGGTTGGGTACGCAAAGTGTATTAGTGGTTTTAGAGCATCTCCTTGACAATTGACATCACCAAAATTTGTTAGATGAGAAAAATTGCAACTCCACT
EK591	R. blackii	ACAATAAAATCACTATGCTCGCTTGGCATTTCTTTGACTTTGATAGGTTGGGTACGCAAAGTGTATTAGTGGTTTTAGAGCATCTCCTTGACAATTGACATCACCAAAATTTGTTAGATGAGAAAAATTGCAACTCCACT
EK592	R. blackii	ACAATAAAATCACTATGCTCGCTTGGCATTTCTTTGACTTTGATAGGTTGGGTACGCAAAGTGTATTAGTGGTTTTAGAGCATCTCCTTGACAATTGACATCACCAAAATTTGTTAGATGAGAAAAATTGCAACTCCACT
EK600	R. kochii	ACAATAAAATCACTATGCTCGCTTGGCATTTCTTTGACTTTGATAGGTTGGGTACGCAAAGTGTATTAGTGGTTTTAGAGCATCTCCTTGACAATTGACATCACCAAAATTTGTTAGATGAGAAAAATTGCAACTCCACT
EK602	R. adinophyllum	ACAATAAAATCACTATGCTCGCTTGGCATTTCTTTGACTTTGATAGGTTGGGTACGCAAAGTGTATTAGTGGTTTTAGAGCATCTCCTTGACAATTGACATCACCAAAATTTGTTAGATGAGAAAAATTGCAACTCCACT
EK608	R. arfakianum	ACAATAAAATCACTATGCTCGCTTGGCAATTCTTTGACTTTGATAGGTTGGGTACGCAAAGTGGATTAGTGGTTTTAGAGCATCTCCTTGACAATTGACATCACCAAAATTTGTTAGATGAGAAAAATTGCAACTCCACT
EK614	R. solitarium	ACAATAAAATCACTATGCTCGCTTGGCATTTCTTTGACTTTGATAGGTTGGGTACGCAAAGTGTATTAGTGGTTTTAGAGCATCTCCTTGACAATTGACATCACCAAAATTTGTTAGATGAGAAAAATTGCAACTCCACT
EK616	R. superbum	ACAATAAAATCACTATGCTCGCTTGGCATTTCTTTGACTTTGATAGGTTGGGTACGCAAAGTGTATTAGTGGTTTTAGAGCATCTCCTTGACAATTGACATCACCAAAATTTGTTAGATGAGAAAAATTGCAACTCCACT
EK617	R. solitarium	ACAATAAAATCACTATGCTCGCTTGGCATTTCTTTGACTTTGATAGGTTGGGTACGCAAAGTGTATTAGTGGTTTTAGAGCATCTCCTTGACAATTGACATCACCAAAATTTGTTAGATGAGAAAAATTGCAACTCCACT
EK618	R. rarum	ACAATAAAATCACTATGCTCGCTTGGCATTTCTTTGACTTTGATAGGTTGGGTACGCAAAGTGTATTAGTGGTTTTAGAGCATCTCCTTGACAATTGACATCACCAAAATTTGTTAGATGAGAAAAATTGCAACTCCACT
EK626	R. carringtoniae	ACAATAAAATCACTATGCTCGCTTGGCATTTCTTTGACTTTGATAGGTTGGGTACGCAAAGTGTATTAGTGGTTTTAGAGCATCTCCTTGACAATTGACATCACCAAAATTTGTTAGATGAGAAAAATTGCAACTCCACT
EK628	R. zoelleri	ACAATAAAATCACTATGCTCGCTTGGCATTTCTTTGACTTTGATAGGTTGGGTACGCAAAGTGTATTAGTGGTTTTAGAGCATCTCCTTGACAATTGACATCACCAAAATTTGTTAGATGAGAAAAATTGCAACTCCACT
EK629	R. culminicola	ACAATAAAATCACTATGCTCGCTTGGCATTTCTTTGACTTTGATAGGTTGGGTACGCAAAGTGTATTAGTGGTTTTAGAGCATCTCCTTGACAATTGACATCACCAAAATTTGTTAGATGAGAAAAATTGCAACTCCACT
EK632	R. commonae	ACAATAAAATCACTATGCTCGCTTGGCATTTCTTTGACTTTGATAGGTTGGGTACGCAAAGTGTATTAGTGGTTTTAGAGCATCTCCTTGACAATTGACATCACCAAAATTTGTTAGATGAGAAAAATTGCAACTCCACT
EK633	R. commonae	ACAATAAAATCACTATGCTCGCTTGGCATTTCTTTGACTTTGATAGGTTGGGTACGCAAAGTGTATTAGTGGTTTTAGAGCATCTCCTTGACAATTGACATCACCAAAATTTGTTAGATGAGAAAAATTGCAACTCCACT
EK635	R. gracilentum	ACAATAAAATCACTATGCTCGCTTGGCATTTCTTTGACTTTGATAGGTTGGGTACGCAAAGTGTATTAGTGGTTTTAGAGCATCTCCTTGACAATTGACATCACCAAAATTTGTTAGATGAGAAAAATTGCAACTCCACT
EK637	R. commonae	ACAATAAAATCACTATGCTCGCTTGGCATTTCTTTGACTTTGATAGGTTGGGTACGCAAAGTGTATTAGTGGTTTTAGAGCATCTCCTTGACAATTGACATCACCAAAATTTGTTAGATGAGAAAAATTGCAACTCCACT
EK638	R. caliginis	ACAATAAAATCACTATGCTCGCTTGGCATTTCTTTGACTTTGATAGGTTGGGTACGCAAAGTGTATTAGTGGTTTTAGAGCATCTCCTTGACAATTGACATCACCAAAATTTGTTAGATGAGAAAAATTGCAACTCGACT
EK639	R. herzogii	ACAATAAAATCACTATGCTCGCTTGGCATTTCTTTGACTTTGATAGGTTGGGTACGCAAAGTGTATTAGTGGTTTTAGAGCATCTCCTTGACAATTGACATCACCAAAATTTGTTAGATGAGAAAAATTGCAACTCCACT
EK644	R. laetum	ACAATAAAATCACTATGCTCGCTTGGCATTTCTTTGACTTTGATAGGTTGGGTACGCAAAGTGTATTAGTGGTTTTAGAGCATCTCCTTGACAATTGACATCACCAAAATTTGTTAGATGAGAAAAATTGCAACTCCACT
EK645	R. jasminiflorum	ACAATAAAATCACTATGCTCGCTTGGCATTTCTTTGACTTTGATAGGTTGGGTACGCAAAGTGTATTAGTGGTTTTAGAGCATCTCCTTGACAATTGACATCACCAAAATTTGTTAGATGAGAAAAATTGCAACTCCACT
EK647	R. rutenii	ACAATAAAATCACTATGCTCGCTTGGCATTTCTTTGACTTTGATAGGTTGGGTACGCAAAGTGTATTAGTGGTTTTAGAGCATCTCCTTGACAATTGACGTCACCAAAATTTGTTAGATGAGAAAAATTGCAACTCCACT
EK648	R. laetum	ACAATAAAATCACTATGCTCGCTTGGCATTTCTTTGACTTTGATAGGTTGGGTACGCAAAGTGTATTAGTGGTTTTAGAGCATCTCCTTGACAATTGACATCACCAAAATTTGTTAGATGAGAAAAATTGCAACTCCACT
EK649	R. dielsianum	ACAATAAAATCACTATGCTCGCTTGGCATTTCTTTGACTTTGATAGGTTGGGTACGCAAAGTGTATTAGTGGTTTTAGAGCATCTCCTTGACAATTGACATCACCAAAATTTGTTAGATGAGAAAAATTGCAACTCCACT
EK651	R. superbum	ACAATAAAATCACTATGCTCGCTTGGCATTTCTTTGACTTTGATAGGTTGGGTACGCAAAGTGTATTAGTGGTTTTAGAGCATCTCCTTGACAATTGACATCACCAAAATTTGTTAGATGAGAAAAATTGCAACTCCACT
EK657	R. majus	ACAATAAAATCACTATGCTCGCTTGGCATTTCTTTGACTTTGATAGGTTGGGTACGCAAAGTGTATTAGTGGTTTTAGAGCATCTCCTTGACAATTGACATCACCAAAATTTGTTAGATGAGAAAAATTGCAACTCCACT
EK658	R. majus	ACAATAAAATCACTATGCTCGCTTGGCATTTCTTTGACTTTGATAGGTTGGGTACGCAGAGTGTATTAGTGGTTGTAGAGCATCTCCTTGACAATTGACATCACCAAAATTTGTTAGATGAGAAAAATTGCAACTCCACT
EK664	R. yongii	ACAATAAAATCACTATGCTCGCTTGGCATTTCTTTGACTTTGATAGGTTGGGTACGCAAAGTGTATTAGTGGTTTTAGAGCATCTCCTTGACAATTGACATCACCAAAATTTGTTAGATGAGAAAAATTGCAACTCCACT
EK666	R. asperum	ACAATAAAATCACTATGCTCGCTTGGCATTTCTTTGACTTTGATAGGTTGGGTACGCAAAGTGTATTAGTGGTTTTAGAGCATCTCCTTGACAATTGACATCACCAAAATTTGTTAGATGAGAAAAATTGCAACTCCACT
EK667	R. radians	ACAATAAAATCACTATGCTCGCTTGGCATTTCTTTGACTTTGATAGGTTGGGTACGCAAAGTGTATTAGTGGTTTTAGAGCATCTCCTTGACAATTGACGTCACCAAAATTTGTTAGATGAGAAAAATTGCAACTCCACT
EK671	R. wilkiei	ACAATAAAATCACTATGCTCGCTTGGCATTTCTTTGACTTTGATAGGTTGGGTACGCAAAGTGTATTAGTGGTTTTAGAGCATCTCCTTGACAATTGACATCACCAAAATTTGTTAGATGAGAAAAATTGCAACTCCACT
HF001	R. konori	ACAATAAAATCACTATGCTCGCTTGGCATTTCTTTGACTTTGATAGGTTGGGTACGCAAAGTGTATTAGTGGTTTTAGAGCATCTCCTTGACAATTGACATCACCAAAATTTGTTAGATGAGAAAAATTGCAACTCCACT

HF004	R. hellwigii	ACAATAAAATCACTATGCTCGCTTGGCATTTCTTTGACTTTGATAGGTTGGGTACGCAAAGTGTATTAGTGGTTTTAGAGCATCTCCTTGACAATTGACATCACCAAAATTTGTTAGATGAGAAAAATTGCAACTCCACT
HF012	R. gardenia	ACAATAAAATCACTATGCTCGCTTGGCATTTCTTTGACTTTGATAGGTTGGGTACGCAAAGTGTATTAGTGGTTTTAGAGCATCTCCTTGACAATTGACATCACCAAAATTTGTTAGATGAGAAAAATTGCAACTCCACT
HF014	R. rousei	ACAATAAAATCACTATGCTTGCTTGGCATTTCTTTGACTTTGATAGGTTGGGTACGCAAAGTGTATTAGTGGTTTTAGAGCATCTCCTTGACAATTGACATCTCCAAAATTTGTTGGATGAGAAAAATTGCAACTCCACT
HF016	R. cruttwellii	ACAATAAAATCACTATGCTCGCTTGGCATTTCTTTGACTTTGATAGGTTGGGTACGCAAAGTGTATTAGTGGTTTTAGAGCATCTCCTTGACAATTGACATCACCAAAATTTGTTAGATGAGAAAAATTGCAACTCCACT
HF021	R. javanicum	ACAATAAAATCACTATGCTCGCTTGGCATTTCTTTGACTTTGATAGGTTGGGTACGCAGAGTGTATTAGTGGTTTTAGAGCATCTCCTTGACAATTGACATCACCAAAATTTGTTAGATGAGAAAAATTGCAACTCCACT
HF023	R. dielsianum	ACAATAAAATCACTATGCTCGCTTGGCATTTCTTTGACTTTGATAGGTTGGGTACGCAAAGTGTATTAGTGGTTTTAGAGCATCTCCTTGACAATTGACATCACCAAAATTTGTTAGATGAGAAAAATTGCAACTCCACT
HF028	R. truncicola	ACAATAAAATCACTATGCTCGCTTGGCATTTCTTTGACTTTGATAGGTTGGGTACGCAAAGTGTATTAGTGGTTTTAGAGCATCTCCTTGACAATTGACATCACCAAAATTTGTTAGATGAGAAAAATTGCAACTCCACT
HF030	R. lochiae	ACAATAAAATCACTATGCTCGCTTGGCATTTCTTTGACTTTGATAGGTTGGGTACGCAAAGTGTATTAGTGGTTTTAGAGCATCTCCTTGACAATTGACATCACCAAAATTTGTTAGATGAGAAAAATTGCAACTCCACT
HF031	R. curviflorum	ACAATAAAATCACTATGCTCGCTTGGCATTTCTTTGACTTTGATAGGTTGGGTACGCAAAGTGTATTAGTGGTTTTAGAGCATCTCCTTGACAATTGACATCACCAAAATTTGTTAGATGAGAAAAATTGCAACTCCACT
HF032	R. armitii	ACAATAAAATCACTATGCTCGCTTGGCATTTCTTTGACTTTGATAGGTTGGGTACGCAAAGTGTATTAGTGGTTTTAGAGCATCTCCTTGACAATTGACATCACCAAAATTTGTTAGATGAGAAAAATTGCAACTCCACT
HF036	R. pleianthum	ACAATAAAATCACTATGCTCGCTTGGCATTTCTTTGACTTTGATAGGTTGGGTACGCAAAGTGTATTAGTGGTTTTAGAGCATCTCCTTGACAATTGACATCACCAAAATTTGTTAGATGAGAAAAATTGCAACTCCACT
HF043	R. burttii	ACAATAAAATCACTATGCTCGCTTGGCATTTCTTTGACTTTGATAGGTTGGGTACGCAAAGTGTATTAGTGGTTTTAGAGCATCTCCTTGACAATTGACATCACC-AAATTTGTTAGATGAGAAAAACAACAAATCC---
HF050	R. emarginatum	ACAATAAAATCACTATGCTCGCTTGGCATTTCTTTGACTTTGATAGGTTGGGTACGCAAAGTGTATTAGTGGTTTTAGAGCATCTCCTTGACAATTGACATCACCAAAATTTGTTAGATGAGAAAAATTGCAACTCCACT
HF051	R. leucogigas	ACAATAAAATCACTATGCTCGCTTGGCATTTCTTTGACTTTGATAGGTTGGGTACGCAAAGTGTATTAGTGGTTTTAGAGCATCTCCTTGACAATTGACATCACCAAAATTTGTTAGATGAGAAAAATTGCAACTCCACT
HF053	R. pubigermen	ACAATAAAATCACTATGCTCGCTTGGCATTTCTTTGACTTTGATAGGTTGGGTACGCAAAGTGTATTAGTGGTTTTAGAGCATCTCCTTGACAATTGACATCACCAAAATTTGTTAGATGAGAAAAATTGCAACTCCACT
HF062	R. commonae	ACAATAAAATCACTATGCTCGCTTGGCATTTCTTTGACTTTGATAGGTTGGGTACGCAAAGTGTATTAGTGGTTTTAGAGCATCTCCTTGACAATTGACATCACCAAAATTTGTTAGATGAGAAAAATTGCAACTCCACT
HF066	R. laetum	ACAATAAAATCACTATGCTCGCTTGGCATTTCTTTGACTTTGATAGGTTGGGTACGCAAAGTGTATTAGTGGTTTTAGAGCATCTCCTTGACAATTGACATCACCAAAATTTGTTAGATGAGAAAAATTGCAACTCCACT
HF067	R. villosulum	ACAATAAAATCACTATGCTCGCTTGGCATTTCTTTGACTTTGATAGGTTGGGTACGCAAAGTGTATTAGTGGTTTTAGAGCATCTCCTTGACAATTGACATCACCAAAATTTGTTAGATGAGAAAAATTGCAACTCCACT
HF068	R. aurigeranum	ACAATAAAATCACTATGCTCGCTTGGCATTTCTTTGACTTTGATAGGTTGGGTACGCAAAGTGTATTAGTGGTTTTAGAGCATCTCCTTGACAATTGACATCACCAAAATTTGTTAGATGAGAAAAATTGCAACTCCACT
HF070	R. celebicum	ACAATAAAATCACTATGCTCGCTTGGCATTTCTTTGACTTTGATAGGTTGGGTACGCAAAGTGTATTAGTGGTTTTAGAGCATCTCCTTGACAATTGACATCACCAAAATTTGTTAGATGAGAAAAATTGCAACTCCACT
HF072	R. kawakamii	ACAATAAAATCACTATGCTCGCTTGGCATTTCTTTGACTTTGATAGGTTGGGTACGCAAAGTGTATTAGTGGTTTTAGAGCATCTCCTTGACAATTGACATCACCAAAATTTGTTAGATGAGAAAAATTGCAACTCCACT
HF077	R. viriosum	ACAATAAAATCACTATGCTCGCTTGGCATTTCTTTGACTTTGATAGGTTGGGTACGCAAAGTGTATTAGTGGTTTTAGAGCATCTCCTTGACAATTGACATCACCAAAATTTGTTAGATGAGAAAAATTGCAACTCCACT
HF082	R. suaveolens	ACAATAAAATCACTATGCTCGCTTGGCATTTCTTTGACTTTGATAGGTTGGGTACGCAAAGTGTATTAGTGGTTTTAGAGCATCTCCTTGACAATTGACATCACCAAAATTTGTTAGATGAGAAAAATTGCAACTCCACT
HF090	R. loranthiflorum	ACAATAAAATCACTATGCTCGCTTGGCATTTCTTTGACTTTGATAGGTTGGGTACGCAAAGTGTATTAGTGGTTTTAGAGCATCTCCTTGACAATTGACATCACCAAAATTTGTTAGATGAGAAAAATTGCAACTCCACT
HF091	R. verticillatum	ACAATAAAATCACTATGCTCGCTTGGCATTTCTTTGACTTTGATAGGTTGGGTACGCAAAGTGTATTAGTGGTTTTAGAATATCTCCTTGACAATTGACATCACCAAAATTTGTTAGATGAGAAAAATTGCAACTCCACT
HF092	R. orbiculatum	ACAATAAAATCACTATGCTCGCTTGGCATTTCTTTGACTTTGATAGGTTGGGTACGCAAAGTGTATTAGTGGTTTTAGAGCATCTCCTTGACAATTGACATCACCAAAATTTGTTAGATGAGAAAAATTGCAACTCCACT
HF093	R. sumatranum	ACAATAAAATCACTATGCTCGCTTGGCATTTCTTTGACTTTGATAGGTTGGGTACGCAAAGTGTATTAGTGGTTTTAGAGCATCTCCTTGACAATTGACATCACCAAAATTTGTTAGATGAGAAAAATTGCAACTCCACT
HF094	R. luraluense	ACAATAAAATCACTATGCTCGCTTGGCATTTCTTTGACTTTGATAGGTTGGGTACGCAAAGTGTATTAGTGGTTTTAGAGCATCTCCTTGACAATTGACATCACCAAAATTTGTTAGATGAGAAAAATTGCAACTCCACT
HF097	R. zollingeri	ACAATAAAATCACTATGCTCGCTTGGCATTTCTTTGACTTTGATAGGTTGGGTACGCAAAGTGTATTAGTGGTTTTAGAGCATCTCCTTGACAATTGACATCACCAAAATTTGTTAGATGAGAAAAATTGCAACTCCACT
HF100	R. tuba	ACAATAAAATCACTATGCTCGCTTGGCATTTCTTTGACTTTGATAGGTTGGGTACGCAAAGTGTATTAGTGGTTTTAGAGCATCTCCTTGACAATTGACATCACCAAAATTTGTTAGATGAGAAAAATTGCAACTCCACT
HF101	R. lowii	ACAATAAAATCACTATGCTCGCTTGGCATTTCTTTGACTTTGATAGGTTGGGTACGCAAAGTGTATTAGTGGTTTTAGAGCATCTCCTTGACAATTGACATCACCAAAATTTGTTAGATGAGAAAAATTGCAACTCCACT
HF135	R. impositum	ACAATAAAATCACTATGCTCGCTTGGCATTTCTTTGACTTTGATAGGTTGGGTACGCAAAGTGTATTAGTGGTTTTAGAGCATCTCCTTGACAATTGACATCACCAAAATTTGTTAGATGAGAAAAATTGCAACTCCACT
HF137	R. luraluense	ACAATAAAATCACTATGCTCGCTTGGCATTTCTTTGACTTTGATAGGTTGGGTACGCAAAGTGTATTAGTGGTTTTAGAGCATCTCCTTGACAATTGACATCACCAAAATTTGTTAGATGAGAAAAATTGCAACTCCACT
HF139	R. jasminiflorum	ACAATAAAATCACTATGCTCGCTTGGCATTTCTTTGACTTTGATAGGTTGGGTACGCAAAGTGTATTAGTGGTTTTAGAGCATCTCCTTGACAATTGACATCACCAAAATTTGTTAGATGAGAAAAATTGCAACTCCACT
HF145	R. x planecostatum	ACAATAAAATCACTATGCTCGCTTGGCATTTCTTTGACTTTGATAGGTTGGGTACGCAAAGTGTATTAGTGGTTTTAGAGCATCTCCTTGACAATTGACATCACCAAAATTTGTTAGATGAGAAAAATTGCAACTCCACT
HF147	R. rushforthii	ACAATAAAATCACTATGCTCGCTTGGCATTTCTTTGACTTTGATAGGTTGGGTACGCAAAGTGTATTAGTGGTTTTAGAGCATCTCCTTGACAATTGACATCACCAAAATTTGTTAGATGAGAAAAATTGCAACTCCACT

Acc. Num.	Taxon	561 700
2002-018	R. acrophilum	CATTG---GGCAAATCCCTTGC--CACAGCTAGCCAAAACCATTTCTTGTGCCAAATTTACCCCAAGTACCTTTGGCAAACTTGCCATATCAACATCAAATATCAATGCTTCTTGACCTCCGTCGCTTTACATCCAACTG
99-0330	R. dielsianum	CATTG---GGCAAATCCCTAGC--CACAGCTAGCCAAAACCATTTCTTGTGCCAAATTTACCCCAAGTACCTTTGGCAAACTTGCCATATCTACATCAAATATCAATGCTTCTTGACCTCCGTTGCTTTACATTCGACTG
EI153	R. jasminiflorum	CAGTG---GGCAAATCCCTTGC--CACAGTTAGCCAAAACCATTTCTTGTGCCAAATTTACCCCAAGTACCTTTGGCAAACTTGCCATATCTACATCAAATATCAATGCTTCTTGACCTCCGTCGCTTTACATCCAACTG
EI158	R. majus	CATTG---GGCAAATCCCTTGC--CACAGTTAGCCAAAACCATTTCTTGTGCCAAATTTAACCCAAGTACCTTTGGCAAGCTTGCCATATCAACATCAAATATCAATGCTTCTTGACCTCCGTTGCTTTACATCCAACTG
EI169	R. gardenia 'Odyssey'	CATTG---GGCAAATCCCTTGC--CACAGTTAGCCAAAACCATTTCTTGTGCCAAATTTACCCCAAGTACCTTTGGCAAACTTGCCATATCAACATCAAATATCAATGCTTCTTGACCTCCGTCGCTTTACATCCAACTG
EK507	R. lochiae	CATTG---GGCAAATCCCTTGC--CACAGTTAGCCAAAACCATTTCTTGTGCCAAATTTACCCCAAGTACCTTTGGCAAACTTGCCATATCAACATCAAATATCAATGCTTCTTGACCTCCGTCGCTTTACATCCAACTG
EI192	R. luraluense	CATTG---GGCAAATCCCTTGC--CACAGTTAGCCAAAACCATTTCTTGTGCCAAATTTACCCCAAGTACCTTTGGCAAACTTGCCATATCAACATCAAATATCAATGCTTCTTGACCTCCGTCGCTTTACATCCAACTG
EK525	R. bagobonum	CATTG---GGCAAATCCCTTGC--CACAGTTAGCCAAAACCATTTCTTGTGCCAAATTTACCCCAAGTACCTTTGGCAAACTTGCCATATCAACATCAAATATCAATGCTTCTTGACCTCCGTCGCTTTACATCCAACTG
EK536	R. alborugosum	CATTG---GGCAAATCCCTTGC--CACAGTTAGCCAAAACCATTTCTTGTGCCAAATTTACCCCACGTACCTTTGGCAAGCTTGCCATATCTACATCAAATATCAATGCTTCTTGACCTCCGTTGCTTTACATTCGACTG
EK544	R. suaveolens	CATTG---GGCAAATCCCTTGC--CACAGTTAGCCAAAACCATTTCTTGTGCCAAATTTACCCCAAGTACCTTCGGCAAACTTGCCATATCAACATCAAATATCAATGCTTCTTGACCTCCGTCGCTTTACATCCAACTG
EK548	R. jasminiflorum	CATTG---GGCAAATCCCTTGC--CACAGTTAGCCAAAACCATTTCTTGTGCCAAATTTACCCCAAGTACCTTTGGCAAACTTGCCATATCAACATCAAATATCAATGCTTCTTGACCTCCGTTGCTTTACATCCAACTG
EK558	R. praetervisum	CATTG---GGCAAATCCCTTGC--CACAGTTAGCCAAAACCATTTCTTGTGCCAAATTTAACCCAAGTACCTTTGGCAAGCTTGCCATATCAACATCAAATATCAATGCTTCTTGACCTCCGTTGCTTTACATCCAACTG
EK565	R. superbum	CATTG---GGCAAATCCCTTGC--CACAGTTAGCCAAAACCATTTCTTGTGCCAAATTTACCCCAAGTACCTTTGGCAAACTTGCCATATCAACATCAAATATCAATGCTTCTTGACCTCCGTTGCTTTACATCCAACTG
EK574	R. vitis-idaea	CATTG---GGCAAATCCCTTGC--CACAGTTAGCCAAAACCATTTCTTGTGCCAAATTTACCCCAAGTACCTTTGGCAAACTTGCCATATCAACATCAAATATCAATGCTTCTTGACCTCCGTCGCTTTACATCCAACTG
EK579	R. citrinum	CATTG---GGCAAATCCCTTGC--CACGGTTAGCCAAAACCATTTCTTGTGCCAAATTTACCCCAAGTACCTTTGGCAAACTTGCCATATCAACATCAAATATCAATGCTTCTTGACCTCCGTCGCTTTACATCCAACTG
EK581	R. santapaui	CATTG---GGCAAATCCCTTGC--CACAGTTAGCCAAAAATCATTTCTTGTGCCACATTTACCCCAAGTACCTTTGGCAAACTTGCCATATCTACATCAAATATCGATGCTTCTTGACCTCCGTCGCTTTACATCCAACTG
EK583	R. stapfianum	CATTG---GGCAAATCCCTTGC--CACAGTTAGCCAAAACCATTTCTTGTGCCAAATTTACCCTAAGTACCTTTGGCAAACTTGCCACATCGACATCAAATATCAATGCTTCTTGACCTCCGTTGCTTTACATCCAACTG
EK588	R. superbum	CATTG---GGCAAATCCCTTGC--CACAATTTGCCAAAACCATTTCTTGTGCCAAATTTACCCGAAGTACCTTTGGCAAACTTGCCATATCAACATCAAATATCAATGCTTCTTGACCTCCGTTGCTTTACATCCAACTG
EK590	R. jasminiflorum	CATTG---GGCAAATCCCTTGC--CACAGTTAGCCAAAACCATTTCTTGTGCCAAATTTACCCCAAGTACCTTTGGCAAACTTGCCATATCAACATCAAATATCAATGCTTCTTGACCTCCGTCGCTTTACATCCAACTG
EK591	R. blackii	CATTG---GGCAAATCCCTTGC--CACAGTTAGCCAAAATCATTTCTTGTGCCAAATTTTACCCAAGTACCTTTGGCAAACTTGCCATATCAACATCAAATATCAATGCTTCTTGACCTCCGTTGCTTTACATCCAACTG
EK592	R. blackii	CATTG---GGCAAATCCCTTGC--CACAGTTAGCCAAAACCATTTCTTGTGCCAAATTTACCCCAAGTACCTTTGGCAAACTTGCCATATCTACATCAAATATCAATGCTTCTTGACCTCCGTCGCTTTACATTCAACTG
EK600	R. kochii	CATTG---GGCAAATCCCTTGC--CACAGTTAGCCAAAACCATTTCTTGTGCCAAATTTACCCCAAGTACCTTTGGCAAACTTGCCATATCAACATCAAATATCGATGCTTCTTGACCTCCGTCGCTTTACATCCAACTG
EK602	R. adinophyllum	CATTG---GGCAAATCCCTAGC--CACAGCTAGCCAAAACCATTTCTTGTGCCAAATTTACCCCAAGTACCTTTGGCAAACTTGCCATATCTACATCAAATATCGATGCTGCTTGACCTCCGTTGCTTTACATTCGACTG
EK608	R. arfakianum	CATTG---GGCAAATCCCTTGC--CACAGTTAGCCAAAACCATTTCTTGTGCCAAATTTACCCCAAGTACCTTTGGCAAACTTGCCATATCAACATCAAATATCAATGCTTCTTCACCTCCGTTGCTTTACATCCAACTG
EK614	R. solitarium	CATTG---GGCAAAACCCTTGC--CACAGTTAGCCAAAACCATTTCTTGTGCCACATTTACCCCAAGTACCTTTGGCAAACTTGCCATATCAACATCAAATATCAATGCTTCTTGACCTCCGTCGCTTTACATCCAACTG
EK616	R. superbum	CATTG---GGCAAATCCCTTGC--CACAGTTAGCCAAAACCATTTCTTGTGCCAAATTTACCCCAAGTACCTTTGGCAAACTTGCCATATCAACATCAAATATCAATGCTTCTTGACCTCCGTCGCTTTACATCCAACTG
EK617	R. solitarium	CATTG---GGCAAATCCCTTGC--CACAGTTAGCCAAAACCATTTCTTGTGCCAAATTTACCCCAAGTACCTTTGGCAAACTTGCCATATCAACATCAAATATCAATGCTTCTTGACCTCCGTCGCTTTACATCCAACTG
EK618	R. rarum	CATTG---GGCAAATCCCTTGC--CCCAGTTAGCCAAAACCATTTCTTGTGCCAAATTTACCCCAATTACCTTTGGCAAACTTGCCTTATCTACATCAAATATCAATGCTTCTTGACCTCTGTTGCTTTACATCCAACTG
EK626	R. carringtoniae	CATTG---GGCAAATCCCTTGC--CACAGTTAGCCAAAACCATTTCTTGTGCCAAATTTACCCCAAGTACCTTTGGCAAACTTGCCATATCAACATCAAATATCAATGCTTCTTGACCTCCGTCGCTTTACATCCAACTG
EK628	R. zoelleri	CATTG---GGCAAATCCCTTGC--CACAGTTAGCCAAAACCATTTCTTGTGCCAAATTTACCCCAAGTACCTTTGGCAAACTTGCCATATCAACATCAAATATCAATGCTTCTTGACCTCCGTCGCTTTACATCCAACTG
EK629	R. culminicola	CATTG---GGCAAATCCCTTGC--CACAGTTAGCCAAAACCATTTCTTGTGCCAAATTTACCCCAAGTACCTTTGGCAAACTTGCCATATCTACATCAAATATCAATGCTTCTTGACCTCCGTCGCTTTACATCCGACTG
EK632	R. commonae	CATTG---GGCAAATCCCTTGC--CACAGTTAGCCAAAACCATTTCTTGTGCCAAATTTACCCCAAGTACCTTTGGCAAACTTGCCATATCAACATCAAATATCAATGCTTCTTGACCTCCGTCGCTTTACATCCAACTG
EK633	R. commonae	CATTG---GGCAAATCCCTTGC--CACAGTTAGCCAAAACCATTTCTTGTGCCAAATTTACCCCAAGTACCTTTGGCAAACTTGCCATATCAACATCAAATATCAATGCTTCTTGACCTCCGTCGCTTTACATCCAACTG
EK635	R. gracilentum	CATTG---GGCAAATCCCTTGC--CACAGTTAGCCAAAACCATTTCTTGTGCCGAATTTACCCCAAGTACCTTTGGCGAGCTTGCCATATCAACATCAAATATCGATGCTTCTTGACCTCCGTCGCTTTACATCCAACTG
EK637	R. commonae	CATTG---GGCAAATCCCTTGC--CACAGTTAGCCAAAACCATTTCTTGTGCCAAATTTACCCCAAGTACCTTTGGCAAACTTGCCATATCAACATCAAATATCAATGCTTCTTGACCTCCGTCGCTTTACATCCAACTG
EK638	R. caliginis	CATTG---GGCAAATCCCTTGC--CACAGTTAGCCAAAACCATTTCTTGTGCCAAATTTACCCCAAGTACCTTTGGCAAACTTGCCATATCAACATCAAATATCAATGCTTCTTGACCTCCGTCGCTTTACATCCAACTG
EK639	R. herzogii	CATTG---GGCAAATCCCTTGC--CACAGTTAGCCAAAACCATTTCTTGTGCCAAATTTACCCCAAGTACCTTTGGCGAGCTTGCCATATCTACATCAAATATCGATGCTTCTTGACCTCCGTCGCTTTACATCCAACTG
EK644	R. laetum	CATTG---GGCAAATCCCTTGC--CACAGTTAGCCAAAACCATTTCTTGTGCCAAATTTACCCCAAGTACCTTTGGCAAACTTGCCATATCAACATCAAATATCAATGCTTCTTGACCTCCGTCGCTTTACATCCAACTG
EK645	R. jasminiflorum	CATTG---GGCAAATCCCTTGC--CACAGTTAGCCAAAATCATTTCTTGTGCCAAATTTTACCCAAGTACCTTTGGCAAACTTGCCATATCAACATCAAATATCAATGCTTCTTGACCTCCGTTGCTTTACATCCAACTG
EK647	R. rutenii	CATTG---GGCAAATCCCTTGC--CACAGTTAGCCAAAACCATTTCTTGTGCCAAATTTTACCCAAGTACCTTTGGCAAACTTGCCATATCAACATCAAATATCAATGCTTCTTGACCTCCGTTGCTTTACATCCAACTG
EK648	R. laetum	CATTG---GGCAAATCCCTTGC--AACAGTTAGCCAAAACCATTTCTTGTGCCAAATTTACCCCAAGTACCTTTGGCAAACTTGCCATATCAACATCAAATATCAATGCTTCTTGACCTCCGTCGCTTTACATCCAACTG
EK649	R. dielsianum	CATTG---GGCAAATCCCTTGC--CACAGTTAGCCAAAACCATTTCTTGTGCCAAATTTACCCCAAGTACCTTTGGCAAACTTGCCATATCTACATCAAATATCGATGCTTCTTGACCTCCGTCGCTTTACATTCAACTG
EK651	R. superbum	CATTG---GGCAAATCCCTTGC--CACAGTTAGCCAAAACCATTTCTTGTGCCAAATTTACCCCAAGTACCTTTGTCAAACTTGCCATATCAACATCAAATATCAATGCTTCTTGACCTCCGTCGCTTTACATCCAACTG
EK657	R. majus	CATTG---GGCAAATCCCTTGC--CACAGTTAGCCAAAACCATTTCTTGTGCCAAATTTACCCCAAGTACCTTTGGCAAACTTGCCATATCAACATCAAATATCAATGCTTCTTGACCTCCGTCGCTTTACATCCAACTG
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EK664	R. yongii	CATTG---GGCAAATCCCTTGC--CACAGTTAGCCAAAACCATTTCTTGTGCCAAATTTAACCCAAGTACCTTTGGCAAACTTGCCATATCTACATCAAATATCGATGCTTCTTGACCTCCGTTGCTTTACATCCAACTG
EK666	R. asperum	CATTG---GGCAAATCCCTTGC--CACAGTTAGCCAAAACCATTTCTTGTGCCAAATTTACCCCAAGTACCTTTGGCAAACTTGCCATATCTACATCAAATATCGATGCTTCTTGACCTCCGTCGCTATACATCCAACTG
EK667	R. radians	CATTG---GGCAAATCCCTTGC--CACAGTTAGCCAAAACCATTTCTTGTGCCAAATTTTACCCAAGTACCTTTGGCAAACTTGCCATATCAACATCAAATATCGATGCTTCTTGACCTCCGTTGCTTTACATCCGACTG
EK671	R. wilkiei	CATTG---GGCAAATCCCTTGC--CACAGTTAGCCAAAACCATTTCTTGTGCCAAATTTACCCCAATTACCTTTGGCAAACTTGCCTTATCAACATCAAATATCAATGCTTCTTGACCTCTGTTGCTTTACATCCAACTG
HF001	R. konori	CATTG---GGCAAATCCCTTGC--CACAGTTAGCCAAAACCATTTCTTGTGCCAAATTTACCCCAAGTACCTTTGTCAAACTTGCCATATCAACATCAAATATCAATGCTTCTTGACCTCCGTCGCTTTACATCCAACTG

HF004	R. hellwigii	CATTG---GGCAAATCCCTTGC--CACAGTTAGCCAAAACCATTTCTTGTGCCAAATTTACCCCAAGTACCTTTGGCAAACTTGCCATATCTACATCAAATATCGATGCTTCTTGACCTCCGTTGCTTTACATCCAACTG
HF012	R. gardenia	CATTG---GGCAAATCCCTTGC--CACAGTTAGCCAAAACCATTTCTTGTGCCAAATTTACCCCAAGTACCTTTGGCAAACTTGCCATATCAACATCAAATATCAATGCTTCTTGACCTCCGTCGCTTTACATCCAACTG
HF014	R. rousei	CATTG---GGCAAATCCCTTGC--CACAGTTAGCCAAAACCATTTCTTGTGCCAAATTTTACCCAAGTACCTTTGGCAAACTTGCCATATCAACATCAAATATCAATGCTTCTTGACCTCCGTTGCTTTACATCCAACTG
HF016	R. cruttwellii	CATTG---GGCAAATCCCTTGC--CACAGTTAGCCAAAACCATTTCTTGTGCCAAATTTACCCCAAGTACCTTTGGCAAACTTGCCATATCAACATCAAATATCGATGCTTCTTGACCTCCGTCGCTTTACATCCAACTG
HF021	R. javanicum	CATTG---GGCAAATCCCTTGC--CACAGCTAGCCAAAACCATTTCTTGTGCCAAATTTACCCCAAGTACCTTTGGCAAACTTGCCATATCTACATCAAATATCAATGCTTCTTGACCTCCGTTGCTTTACATCCGACTG
HF023	R. dielsianum	CAGTG---GGCAAATTCCTTGC--CACAGTTAGCCAAAACCATTTCTTGTGCCAAATTTACCCCAAGTACCTTTGGCAAGCTTGCCATATCTCCATCAAATATGGATGCTTCTTGACCTCCGTTGCATTACATTTGACTG
HF028	R. truncicola	CATTG---GGCAAATCCCTTGC--CACAGTTAGCCAAAACCATTTCTTGTGCCAAATTTACCCCAAGTACCTTTGGCAAACTTGCCATATCTACATCAAATATCAATGCTTCTTGACCTCCGTCGCTTTACATTCAACTG
HF030	R. lochiae	CATTG---GGCAAATCCCTTGC--AACAGTTAGCCAAAACCATTTCTTGTGCCAAATTTACCCCAAGTACCTTTGGCAAACTTGCCATATCAACATCAAATATCAATGCTTCTTGACCTCCGTCGCTTTACATCCAACTG
HF031	R. curviflorum	CATTG---GGCAAATCCCTTGC--CACAGTTAGCCAAAACCATTTCTTGTGCCAAATTTACCCCAAGTACCTTTGGCAAACTTGCCATATCTACATCAAATATCAATGCTTCTTGACCTCCGTCGCTTTACATTCAACTG
HF032	R. armitii	CATTG---GGCAAATCCCTTGC--CACAGTTAGCCAAAACCATTTCTTGTGCCAAATTTACCCCAAGTACCTTTGGCAAACTTGCCATATCTACATCAAATATCGATGCTTCTTGACCTCCGTTGCTTTACATTCGACTG
HF036	R. pleianthum	CATTG---GGCAAATCCCTTGC--CACAGTTAGCCAAAACCATTTCTTGTGCCAAATTTACACCACGTACCTTTGGCAAACTTGCCATATCACCATCAAATATCGATGCTTCTTGACCTCCGTCGCTTTACATTTGACTG
HF043	R. burttii	TAATG---GGCAAATCCCTTGC--CACAGTTAGCCAAAACCATTTCTTGTGCCAAATTTACCCCAAGTACCTTTGGCAAACTTGCCATATCAACATCAAATATCAATGCTTCTTGACCTCCGTTGCTTTACATCCACCTG
HF050	R. emarginatum	CAGTG---GGAAAATTGCTTGC--CACAGTTAGCCAAAACCATTTCTTGTGCCAAATTTACCCTACGTACCTTTGGCGAGCTTGCCATATCAACATCAAATATCAATGCTTCTTGACCTCCGTTGCTTTACATCCAACTG
HF051	R. leucogigas	CATTG---GGCAAATCCCTTGC--CACAGTTAGCCAAAACCATTTCTTGTGCCAAATTTACCCCAAGTACCTTTGGCAAACTTGCCATATCAACATCAAATATCAATGCTTCTTGACCTCCGTCGCTTTACATCCAACTG
HF053	R. pubigermen	CATTG---GGCAAATCCCTTGC--TACAGTTAGCCAAAACCATTTCTTGTGCCAAATTTACCCCAATTACCTTTGGCAAACTTGCCATATCTACATCAAATATCAATGCTTCTTGACCTCCGTTGCTTTACATCCGACTG
HF062	R. commonae	CATTG---GGCAAATCCCTTGC--CACAGTTAGCCAAAACCATTTCTTGTGCCAAATTTACCCCAAGTACCTTTGGCAAACTTGCCATATCAACATCAAATATCAATGCTTCTTGACCTCCGTCGCTTTACATCCAACTG
HF066	R. laetum	CATTG---GGCAAATCCCTTGC--AACAGTTAGCCAAAACCATTTCTTGTGCCAAATTTACCCCAAGTACCTTTGGCAAACTTGCCATATCAACATCAAATATCAATGCTTCTTGACCTCCGTCGCTTTACATCCAACTG
HF067	R. villosulum	CATTG---GGCAAATCCCTTGC--CACAGTTAGCCAAAACCATTTCTTGTGCGACATTTACCCCAAGTACCTTTGGCAAACTTGCCATATCAACATCAAATATCAATGCTTCTTGACCTCCGTCGCTTTACATCCAACTG
HF068	R. aurigeranum	CATTG---GGCAAATCCCTTGC--CACAGTTAGCCAAAACCATTTCTTGTGCCAAATTTACCCCAAGTACCTTTGGCAAACTTGCCATATCAACATCAAATATCAATGCTTCTTGACCTCCGTCGCTTTACATCCAACTG
HF070	R. celebicum	CATTG---GGCAAATCCCTTGC--CACAGTTAGCCAAAACCATTTCTTGTGCCAAATTTACCCCAAGTACCTTTGGCAAACTTGCCATATCTACATCAAATATCAATGCTTCTTGACCTCCGTCGCTTTACATTCAACTG
HF072	R. kawakamii	CATTG---GGCAAATCCCTTGC--CACAGTTAGCCAAAACCATTTCTTGTGCCAAATTTACCCCAAGTACCTTTGGCAAACTTGCCACATCAACATCAAATATCAATGCTTCTTGACCTCCGTTGCTTTACATCCAACTG
HF077	R. viriosum	CATTG---GGCAAATCCCTTGC--CACAGTTAGCCAAAACCATTTCTTGTGCCAAATTTACCCCAAGTACCTTTGGCAAACTTGCCATATCAACATCAAATATCAATGCTTCTTGACCTCCGTCGCTTTACATCCAACTG
HF082	R. suaveolens	CATTG---GGCAAATCCCTTGC--CACAGTTAGCCAAAACCATTTCTTGTGCCAAATTTACCCCAAGTACCTTTGGCAAGCTTGCCATAACAACATCAAATATCGATGCTTCTTGACCTCCGTTGCTTTACATCCAACTG
HF090	R. loranthiflorum	CATTG---GGCAAATCCCTTGC--CACAGTTAGCCAAAACCATTTCTTGTGCCAAATTTACCCCAAGTACCTTTGGCAAACTTGCCATATCAACATCAAATATCAATGCTTCTTGACCTCCGTCGCTTTACATCCAACTG
HF091	R. verticillatum	CATTG---GGCAAATCCCTTGC--CACAGTTAGCCAAAACCATTTCTTGTGCCAAATTTACCCCAAGTACCTTTGGCAAACTTGCCATATCAACATCAAATATCAATGCTTCTTGACCTCCGTCGCTTTACATCCAACTG
HF092	R. orbiculatum	CATTG---GGCAAATCCCTTGC--CACAGTTAGCCAAAACCATTTCTTGTGCCAAATTTACCCCAAGTACCTTTGGCAAACTTGCCATATCAACATCAAATATCAATGCTTCTTGACCTCCGTCGCTTTACATCCAACTG
HF093	R. sumatranum	CATTG---GGCAAATCCCTTGC--CACAGTTAGCCAAAACCATTTCTTGTGCCAAATTTACCCCAAGTACCTTTGGCAAACTTGCCATATCAACATCAAATATCAATGCTTCTTCACCTCCGTCGCTTTACATCCAACTG
HF094	R. luraluense	CATTG---GGCAAATCCCTTGC--CACAGTTAGCCAAAACCATTTCTTGTGCCAAATTTACCCCAAGTACCTTTGGCAAACTTGCCATATCAACATCAAATATCAATGCTTCTTGACCTCCGTCGCTTTACATCCAACTG
HF097	R. zollingeri	CATTG---GGCAAATCCCTTGC--CACAGTTAGCCAAAACCATTTCTTGTGCCAAATTTACCCCAAGTACCTTTGGCAAACTTGCCATATCTACATCAAATATCAATGCTTCTTGACCTCCGTCGCTTTACATTCAACTG
HF100	R. tuba	CATTG---GGCAAATCCCTTGC--CACAGTTAGCCAAAACCATTTCTTGTGCCAAATTTACCCCAAGTACCTTTGGCAAACTTGCCATATCAACATCAAATATCGATGCTTCTTGACCTCCGTCGCTTTACATCCAACTG
HF101	R. lowii	CATTG---GGCAAATCCCTTGC--CACAGTTAGCCAAAACCATTTCTTGTGCCAAATTTACCCCAAGTACCTTTGGCAAACTTGCCATATCAACATCAAATATCAATGCTTCTTGACCTCCGTCGCTTTACATCCAACTG
HF135	R. impositum	CATTG---GGCAAATCCCTTGC--CACAGTTAGCCAAAACCATTTCTTGTGCCAAATTTACCCCAAGTACCTTTGGCAAACTTGCCATATCAACATCAAATATCAATGCTTCTTGACCTCCGTCGCTTTACATCCAACTG
HF137	R. luraluense	CATTG---GGCAAATCCCTTGC--CACAGTTAGCCAAAACCATTTCTTGTGCCAAATTTACCCCAAGTACCTTTGGCAAACTTGCCATATCTACATCAAATATCGATGCTTCTTGACCTCCGTCGCTTTACATCCAACTG
HF139	R. jasminiflorum	CATTG---GGCAAATCCCTTGC--CACAGTTAGCCAAAACCATTTCTTGTGCCAAATTTACCCCAAGTACCTTTGGCAAACTTGCCATATCAACATCAAATATCAATGCTTCTTGACCTCCGTCGCTTTACATCCAACTG
HF145	R. x planecostatum	CATTG---GGCAAATCCCTTGC--CACAGTTAGCCAAAACCATTTCTTGTGCCAAATTTACCCCAAGTACCTTTGGCAAACTTGCCATATCTACATCAAATATCAATGCTTCTTGACCTCCGTCGCTTTACATTCAACTG
HF147	R. rushforthii	CATTG---GGCAAATCCCTTGC--CACAGTTAGCCAAAACCATTTCTTGTGCCAAATTTACCCCAAGTACCTTTGGCAAACTTGCCACATCAACATCAAATATCAATGCTTCTTGACCTCCGTTGCTTTACATCCAACTG

Acc. Num.	Taxon	701 840
2002-018	R. acrophilum	TAGAAAAATGTGTAGCTGTGCTGAAATAGAATCATCTCCATAAGCAAAAAAAGT-------GGTAAAATAACTCTATGGCGAGCTAATTATTAGCAATGTCACAAACCTGTGTAGGTGTTCTTGTGCTTGTAATCTC-TT
99-0330	R. dielsianum	TAGAAAAATGGGTAGCTGTGCTGAAATAGAATCATCTCCATAAGCAAAAAAAGT-------GGTAAAATAACTCTATGGCGAGCTAATTATTAGCAATGTCACCAACCTGTGTAGGTGTTCTTGTGCTTGTAATCTC-TT
EI153	R. jasminiflorum	TAGAAAAATGTGTAGCTGTGCTGAAATAGAATCATCTCCATAAGCAAAAAAAGT-------GGTAAAATAACTCTATGGCGAGCTAATTATTAGCAATGTCACAAACCTGTGTAGGTGTTCTTGTGCTTGTAATCTC-TT
EI158	R. majus	TAGAAAAATGTGTAGCTGTGCTGAAATATAATCATCTCAATAAGCAAAAAAAGT-------GGTAAAATAACGCTATGGCGAGCTAATTATTAGCAATGTCACAAACCTGTGTAGGTGTTCTTGTGCTTGTAATCTC-TT
EI169	R. gardenia 'Odyssey'	TAGAAAAATGTGTAGCTGTGCTGAAATAGAATCATCTCCATAAGCAAAGAAAGT-------GGTAAAATAACTCTATGGCGAGCTAATTATTAGCAATGTCACAAACCTGTGTAGGTGTTCTTGTGCTTGTAATCTC-TT
EK507	R. lochiae	TAGAAAAATGTGTAGCTGTGCTGAAATAGAATCATCTCCATAAGCAAAAAAAGT-------GGTAAAATAACTCTATGGCGAGCTAATTATTAGCAATGTCACAAACCTGTGTAGGTGTTCTTGTGCTTGTAATCTC-TT
EI192	R. luraluense	TAGAAAAATGTGTAGCTGTGCTGAAATAGAATCATCTCCATAAGCAAAAAAAGT-------GGTAAAATAACTCTATGGCGAGCTAATTATTAGCAATGTCACAAACCTGTGTAGGTGTTCTTGTGCTTGTAATCTC-TT
EK525	R. bagobonum	TAGAAAAATGTGTAGCTGTGCTGAAATAGAATCATCTCCATAAGCGAAAAAAGT-------GGTAAAATAACTCTATGGCGAGCTAATTATTAGCAATGTCACAAACCTGTGTAGGTGTTCTTGTGCTTGTAATCTC-TT
EK536	R. alborugosum	TAGAAAAATGGGTAGCTGTGCTGAAATAGAATCATCTCCATAAGCAAAAAAAGT-------GGTAAAATAACTCTATGGCGAGCTTTTTATTAGCAATGTCACCAACCTGTGTAGGTGTTCTTGTGCTTGTAATCTC-TT
EK544	R. suaveolens	TAGAAAAATGTGTAGCTGTGCTGAAATAGAATCATCTCCATAAGCAAAAAAAGT-------GGTAAAATAACTCTATGGCGAGCTAATTATTAGCAATGTCACAAACCTGTGTAGGTGTTCTTGTGCTTGTAATCTC-TT
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EK565	R. superbum	TAGAAAAATGTGTAGCTGTGCTGAAATAGAATCATCTCAATAAGCAAAAAAAGT-------GGTAAAATAACTCTATGGCGAGCTAATTATTAGCAATGTCACAAACCTGTGTAGGTGTTCTTGTGCTTGTAATCTC-TT
EK574	R. vitis-idaea	TAGAAAAATGTGTAGCTGTGCTGAAATAGAATCATCTCCATAAGCAAAAAAAGT-------GGTAAAATAACTCTATGGCGAGCTAATTATTAGCAATGTCACAAACCTGTGTAGGTGTTCTTGTGCTTGTAATCTC-TT
EK579	R. citrinum	TAGAAAAATGTGTAGCTGTGCTGAAATAGAATCATCTCCATAAGCAAAAA---TGACATACGGTGAAATAACTCTATGGCGAGCTAATTATTAGCAATGTCACAAACCTGTGTAGGTGTTCTTGTGCTTGTAATCTC-TT
EK581	R. santapaui	TAGAAAAATGTGTAGCTGTGCTGAAATAGAATCATCTCAATAAGCAAAAAAAGT-------GGTAAAATAACTCTATGGCGAGCTAATTATTAGCAATGTCACAAACCTGTGTAGGTGTTCTTGTGCTTGTAATCTC-TT
EK583	R. stapfianum	TACAAAAATGTGTAGCTGAGCTGAAATAGAATCGTCTCAATAAGCAAAAAAAGT-------GGTAAAATAACTCTATGGCAAGCTAATTGTTAGCAATGTCACAAACCTGTGTAGGTGCTCTTGTGCTCGTAATCTC-TT
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EK590	R. jasminiflorum	TAGAAAAATGTGTAGCTGTGCTGAAATAGAATCATCTCCATAAGCAAAAAAAGT-------GGTAAAATAACTCTATGGCGAGCTAATTATTAGCAATGTCACAAACCTGTGTAGGTGTTCTTGTGCTTGTAATCTC-TT
EK591	R. blackii	TAGAAAAATGTGTAGCTGTGCTGAAATATAATCATCTCAATAAGCAAAAAAAGT-------GGTAAAATAACGCTATGGCGAGCTAATTATTAGCAATGTCACAAACCTGTGTAGGTGTTCTTGTGCTTGTAATCTC-TT
EK592	R. blackii	TAGAAAAATGTGTAGCTGTGCTGAAATAGAATCATCTCCATAAGCAAAAAAAGT-------GGTAAAATAACTCTATGGCGAGCTAATTATTAGCAATGTCACAAACCTGTGTAGGTGTTCTTGTGCTTGTAATCTC-TT
EK600	R. kochii	TAGAAAAATGTGTAGCTGTGCTGAAATAGAATCATCTCCATAAGCAAAAAAAGT-------GGTAAAATAACTCTATGGCGAGCTAATTATTAGCAATGTCACAAACCTGTGTAGGTGTTCTTGTGCTTGTAATCTC-TT
EK602	R. adinophyllum	TAGAAAAATGTGTAGCTGTGCTGAAATAGAATCATCTCCATAAGCAAAAAAAGT-------GGTAAAATAACTCTATGGCGAGCTAATTATTAGCAATGTCACAAACCTGTGTAGGTGTTCTTGTGCTTGTAATCTC-TT
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EK616	R. superbum	TAGAAAAATGTGTAGCTGTGCTGAAATAGAATCATCTCCATAAGCAAAAAAAGT-------GGTAAAATAACTCTATGGCGAGCTAATTATTAGCAATGTCACAAACCTGTGTAGGTGTTCTTGTGCTTGTAATCTC-TT
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EK626	R. carringtoniae	TAGAAAAATGTGTAGCTGTGCTGAAATAGAATCATCTCCATAAGCAAAAAAAGT-------GGTAAAATAACTCTATGGCGAGCTAATTATTAGCAATGTCACAAACCTGTGTAGGTGTTCTTGTGCTTGTAATCTC-TT
EK628	R. zoelleri	TAGAAAAATGTGTAGCTGTGCTGAAATAGAATCATCTCCATAAGCAAAAAAAGT-------GGTAAAATAACTCTATGGCGAGCTAATTATTAGCAATGTCACAAACCTGTGTAGGTGTTCTTGTGCTTGTAATCTC-TT
EK629	R. culminicola	TAGAAAAATGTGTAGCTGTGCTGAAATAGAATCATCTCCATAAGCAAAAAAAGT-------GGTAAAATAACTCTATGGCGAGCTAATTATTAGCAATGTCACAAACCTGTGTAGGTGTTCTTGTGCTTGTAATCTC-TT
EK632	R. commonae	TAGAAAAATGTGTAGCTGTGCTGAAATAGAATCATCTCCATAAGCAAAAAAAGT-------GGTAAAATAACTCTATGGCGAGCTAATTATTAGCAATGTCACAAACCTGTGTAGGTGTTCTTGTGCTTGTAATCTC-TT
EK633	R. commonae	TAGAAAAATGTGTAGCTGTGCTGAAATAGAATCATCTCCATAAGCAAAAAAAGT-------GGTAAAATAACTCTATGGCGAGCTAATTATTAGCAATGTCACAAACCTGTGTAGGTGTTCTTGTGCTTGTAATCTC-TT
EK635	R. gracilentum	TAGAAAAATGTGTAGCTGTGCTGAAATAGAATCATCTCCATAAGCAAAAAAAGT-------GGTAAAATAACTCTATGGCGAGCTAATTATTAGCAATGTCACAAACCTGTGTAGGTGTTCTTGTGCTTGTAATCTC-TT
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EK638	R. caliginis	TAGAAAAATGTGTAGCTGTGCTGAAATAGAATCATCTCCATAAGCAAAAAAAGT-------GGTAAAATAACTCTATGGCGAGCTAATTATTAGCAATGTCAC-AACCTGTGTAGGTGTTCTTGTGCTTGTAATCTC-TT
EK639	R. herzogii	TAGAAAAATGTGTAGCTGTGCTGAAATAGAATCATCTCCATAAGCAAAAAAAGT-------GGTAAAATAACTCTATGGCGAGCTAATTATTAGCAATGTCACAAACCTGTGTAGGTGTTCTTGTGCTTGTAATCTC-TT
EK644	R. laetum	TAGAAAAATGTGTAGCTGTGCTGAAATAGAATCATCTCCATAAGCAAAAAAAGT-------GGTAAAATAACTCTATGGCGAGCTAATTATTAGCAATGTCACAAACCTGTGTAGGTGTTCTTGTGCTTGTAATCTC-TT
EK645	R. jasminiflorum	TAGAAAAATGTGTAGCTGTGCTGAAATATAATCATCTCAATAAGCAAAAAAAGT-------GGTAAAATAACGCTATGGCGAGCTAATTATTAGCAATGTCACAAACCTGTGTAGGTGTTCTTGTGCTTGTAATCTC-TT
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EK649	R. dielsianum	TAGAAAAATGGGTAGCTGTGCTGAAATAGAATCATCTCCATAAGCAAAAAAAGT-------GGTAAAATAACTCTATGGCGAGCTAATTATTAGCAATGTCACAAACCTGTGTAGGTGTTCTTGTGCTTGTAATCTC-TT
EK651	R. superbum	TAGAAAAATGTGTAGCTGTGCTGAAATAGAATCATCTCCATAAGCAAAAAAAGT-------GGTAAAATAACTCTATGGCGAGCTAATTATTAGCAATGTCACAAACCTGTGTAGGTGTTCTTGTGCTTGTAATCTC-TT
EK657	R. majus	TAGAAAAATGTGTAGCTGTGCTGAAATAGAATCATCTCCATAAGCAAAAAAAGT-------GGTAAAATAACTCTATGGCGAGCTAATTATTAGCAATGTCACAAACCTGTGTAGGTGTTCTTGTGCTTGTAATCTC-TT
EK658	R. majus	TAGAAAAATGTGTAGCTGTGCTGAAATAGAATCATCTCCATAAGCAAAGAAAGT-------GGTAAAATAACTCTATGGCGAGCTAATTATTAGCAATGTCACAAACCTGTGTAGGTGTTCTTGTGCTTGTAATCTC-TT
EK664	R. yongii	TAGAAAAATGGGTAGCTGTGCTGAAATAGAATCATCTCAATAAGCAAAAAAAGT-------GGTAAAATAACTCTATGGCGAGCTAATTATTAGCAATGTCACAAACCTGTGTAGGTGTTCTTGTGCTTGTAATCTC-TT
EK666	R. asperum	TAGAAAAATGTGTAGCTGTGCTGAAATAGAATCATCTCCATAAGCAAAAAAAGT-------GGTAAAATAACTCTATGGCGAGCTAATTATTAGCAATGTCACAAACCTGTGTAGGTGTTCTTGTGCTTGTAATCTC-TT
EK667	R. radians	TAGAAAAATGTGTAGCTGTGCTGAAATAGAATCATCTCAATAAGCAAAAAAAGT-------GGTAAAATAACTCTATGGCGAGCTAATTATTAGCAATGTCACAAACCTGTGTAGGTGTTCTTGTGCTTGTAATCTC-TT
EK671	R. wilkiei	TAGAAAAATGTGTAGCTGTGCTGAAATAGAATCATCTCAATAAGCAAAAAAAGT-------GGTAAAATAACTCTATGGCGAGCTAATTATTAGCAATGTCACAAACCTGTGTAGGTGTTCTTGTGCTTGTAATCTC-TT
HF001	R. konori	TAGAAAAATGTGTAGCTGTGCTGAAATAGAATCATCTCCATAAGCAAAAAAAGT-------GGTAAAATAACTCTATGGCGAGCTAATTATTAGCAATGTCACAAACCTGTGTAGGTGTTCTTGTGCTTGTAATCTC-TT

HF004	R. hellwigii	TAGAAAAATGTGTAGCTGTGCTGAAATAGAATCATCTCCATAAGCAAAAAAAGT-------GGTAAAATAACTCTATGGCGAGCTAATTATTAGCAATGTCACAAACCTGTGTAGGTGTTCTTGTGCTTGTAATCTC-TT
HF012	R. gardenia	TAGAAAAATGTGTAGCTGTGCTGAAATAGAATCATCTCCATAAGCAAAAAAAGT-------GGTAAAATAACTCTATGGCGAGCTAATTATTAGCAATGTCACAAACCTGTGTAGGTGTTCTTGTGCTTGTAATCTC-TT
HF014	R. rousei	TAGAAAAATGTGTAGCTGTGCTGAAATAGAATCATCTCAATAAGCAAAAAAAGT-------GGTAAAATAACTCTATGGCGAGCTAATTATTAGCAATGTCACAAACCTGTGTAGGTGTTCTTGTGCTTGTAATCTC-TT
HF016	R. cruttwellii	TAGAAAAATGTGTAGCTGTGCTGAAATAGAATCATCTCCATAAGCAAAAAAAGT-------GGTAAAATAACTCTATGGCGAGCTAATTATTAGCAATGTCACAAACCTGTGTAGGTGTTCTTGTGCTTGTAATCTC-TT
HF021	R. javanicum	TAGAAAAATGTGTAGCTGTGCTGAAATAGAATCATCTCCATAAGCAAAAAAAGT-------GGTAAAATAACTCTATGGCGAGCTAATTATTAGCAATGTCACAAACCTGTGTAGGTGTTCTTGTGCTTGTAATCTC-TT
HF023	R. dielsianum	TAGAAAAATGGGTAGCTGTGCTGAAATAGAATCATCTCCATAAGCAAAAAAAGT-------GGTAAAATAACTCTATGGCGAGCTAATTATTAGCAATGTCACAAACCTGTGGAGGTGTTCTTGTGCTTGTAATCTC-TT
HF028	R. truncicola	TAGAAAAATGTGTAGCTGTGCTGAAATAGAATCATCTCCATAAGCAAAAAAAGT-------GGTAAAATAACTCTATGGCGAGCTAATTATTAGCAATGTCACAAACCTGTGTAGGTGTTCTTGTGCTTGTAATCTC-TT
HF030	R. lochiae	TAGAAAAATGTGTAGCTGTGCTGAAATAGAATCATCTCCATAAGCAAAAAAAGT-------GGTAAAATAACTCTATGGCGAGCTAATTATTAGCAATGTCACAAACCTGTGTAGGTGTTCTTGTGCTTGTAATCTC-TT
HF031	R. curviflorum	TAGAAAAATGTGTAGCTGTGCTGAAATAGAATCATCTCCATAAGCAAAAAAAGT-------GGTAAAATAACTCTATGGCGAGCTAATTATTAGCAATGTCACAAACCTGTGTAGGTGTTCTTGTGCTTGTAATCTC-TT
HF032	R. armitii	TAGAAAAATGTGTAGCTGTGCTGAAATAGAATCCACTCCATAAGCAAAAAAAGT-------GGTAAAATAACTCTATGGCGAGCTTATTATTAGCAATGTCACCAACCTGTGTAGGTGTTCTTGTGCTAGTAATCTC-TT
HF036	R. pleianthum	TAGAAAAATGTGTAGCTGTGCTGAAATAGAATCATCTCCATAAGCAAAGAAAGT-------GGTACAATACCTCTATGGCGAGCTTATTATTAGCAATGTCACCAACCTGTGTAGGTGTTCTTGTGCTTGTAATCTC-TT
HF043	R. burttii	TAGAAAAATGTGTAGCTGTGCTGAAATAGAATCATCTCAATAAGCAAAAAAAGT-------GGTAAAATAACTCTATGGCGAGCTAATTATTAGCAATGTCACAAACCTGTGTAGGTGTTCTTGTGCTTGTAATCTC-TT
HF050	R. emarginatum	TAGAAAAATGTGTAGCTGTGCTGAAATAGAATCGTCTCAATAAGCAAAAAAAGT-------GGTAAAATAACTCTATTGCAAGCTAATTGTTAGCAATGTCACAAACCTGTGTAGGTGTTCTTGTGCTTGTAATCTC-TT
HF051	R. leucogigas	TAGAAAAATGTGTAGCTGTGCTGAAATAGAATCATCTCCATAAGCAAAAAAAGT-------GGTAAAATAACTCTATGGCGAGCTAATTATTAGCAATGTCACAAACCTGTGTAGGTGTTCTTGTGCTTGTAATCTC-TT
HF053	R. pubigermen	TAGAAAAATGTGTAGCTGTGCTGAAATAGAATCATCTCAATAAGCAAAAAAAGT-------GGTAAAATAACTCTATGGCGAGCTTATTATTAGCAATGTCACAAACCTGTGTAGGTGTTCTTGTGCTTGTATTCTC-TT
HF062	R. commonae	TAGAAAAATGTGTAGCTGTGCTGAAATAGAATCATCTCCATAAGCAAAAAAAGT-------GGTAAAATAACTCTATGGCGAGCTAATTATTAGCAATGTCACAAACCTGTGTAGGTGTTCTTGTGCTTGTAATCTC-TT
HF066	R. laetum	TAGAAAAATGTGTAGCTGTGCTGAAATAGAATCATCTCCATAAGCAAAAAAAGT-------GGTAAAATAACTCTATGGCGAGCTAATTATTAGCAATGTCACAAACCTGTGTAGGTGTTCTTGTGCTTGTAATCTC-TT
HF067	R. villosulum	TAGAAAAATGTGTAGCTGTGCTGAAATAGAATCATCTCCATAAGCAAAAAAAGT-------GGTAACATAACTCTATGGCGAGCTTATTATTAGCAATGTACCAAACCTGTGTAGGTGTTCTTGTGCTTGTAATCTC-TT
HF068	R. aurigeranum	TAGAAAAATGTGTAGCTGTGCTGAAATAGAATCATCTCCATAAGCAAAAAAAGT-------GGTAAAATAACTCTATGGCGAGCTAATTATTAGCAATGTCACAAACCTGTGTAGGTGTTCTTGTGCTTGTAATCTC-TT
HF070	R. celebicum	TAGAAAAATGTGTAGCTGTGCTGAAATAGAATCATCTCCATAAGCAAAAAAAGT-------GGTAAAATAACTCTATGGCGAGCTAATTATTAGCAATGTCACAAACCTGTGTAGGTGTTCTTGTGCTTGTAATCTC-TT
HF072	R. kawakamii	TAGAAAAATGTGTAGCTGTGCTGAAATAGAATCGTCTCAATAAGCAAAAAAAGT-------GGTAAAATAACTCTATGGCAAGCTAATTGTTAGCAATGTCACAAACCTGTGTAGGTGCTCTTGTGCTTGTAATCTC-TT
HF077	R. viriosum	TAGAAAAATGTGTAGCTGTGCTGAAATAGAATCATCTCCATAAGCAAAAAAAGT-------GGTAAAATAACTCTATGGCGAGCTAATTATTAGCAATGTCACAAACCTGTGTAGGTGTTCTTGTGCTTGTAATCTC-TT
HF082	R. suaveolens	TAGAAAAATGTGTAGCTGTGCTGAAATAGAATCATCTCAATAAGCAAAAAAAGT-------GGTAAAATAACTCTATGGCGAGCTAATTATTAGCAATGTCACAAACCTGTGTAGGTGTTCTTGTGCTTGTAATCTC-TT
HF090	R. loranthiflorum	TAGAAAAATGTGTAGCTGTGCTGAAATAGAATCATCTCCATAAGCAAAAAAAGT-------GGTAAAATAACTCTATGGCGAGCTAATTATTAGCAATGTCACAAACCTGTGTAGGTGTTCTTGTCCTTGTAATCTC-TT
HF091	R. verticillatum	TAGAAAAATGTGTAGCTGTGCTGAAATAGAATCATCTCCATAAGCAAAAAAAGT-------GGTAAAATAACTCTATGGCGAGCTTATTATTAGCAATGTCACAAACCTGTGTAGGTGTTCTTGTGCTTGTAATCTC-TT
HF092	R. orbiculatum	TAGAAAAATGTGTAGCTGTGCTGAAATAGAATCATCTCCATAAGCAAAAAAAGT-------GGTAAAATAACTCTATGGCGAGCTAATTATTAGCAATGTCACAAACCTGTGTAGGTGTTCTTGTGCTTGTAATCTC-TT
HF093	R. sumatranum	TAGAAAAATGTGTAGCTGTGCTGAAATAGAACCATCTCAATAAGCAAAAAAAGT-------GGTAAAATAACTCTATGGCGAGCTAATTGTTAGCAATGTCACAAACCTGTGTAGGTGTTCTTGTGCTTGTAATCTC-TT
HF094	R. luraluense	TAGAAAAATGTGTAGCTGTGCTGAAATAGAATCATCTCCATAAGCGAAAAAAGT-------GGTAAAATAACTCTATGGCGAGCTAATTATTAGCAATGTCACAAACCTGTGTAGGTGTTCTTGTGCTTGTAATCTC-TT
HF097	R. zollingeri	TAGAAAAATGTGTAGCTGTGCTGAAATAGAATCATCTCCATAAGCAAAAAAAGT-------GGTAAAATAACTCTATGGCGAGCTAATTATTAGCAATGTCACAAACCTGTGTAGGTGTTCTTGTGCTTGTAATCTC-TT
HF100	R. tuba	TAGAAAAATGTGTAGCTGTGCTGAAATAGAATCATCTCCATAAGCAAAAAAAGT-------GGTAAAATAACTCTATGGCGAGCTAATTATTAGCAATGTCACAAACCTGTGTAGGTGTTCTTGTGCTTGTAATCTC-TT
HF101	R. lowii	TAGAAAAATGTGTAGCTGTGCTGAAATAGAATCATCTCCATAAGCAAAAAAAGT-------GGTAAAATAACTCTATGGCGAGCTAATTATTAGCAATGTCACAAACCTGTGTAGGTGTTCTTGTGCTTGTAATCTC-TT
HF135	R. impositum	TAGAAAAATGTGTAGCTGTGCTGAAATAGAATCATCTCCATAAGCAAAAAAAGT-------GGTAAAATAACTCTATGGCGAGCTAATTATTAGCAATGTCACAAACCTGTGTAGGTGTTCTTGTGCTTGTAATCTC-TT
HF137	R. luraluense	TAGAAAAATGTGTAGCTGTGCTGAAATAGAATCATCTCCATAAGCGAAAAAAGT-------GGTAAAATAACTCTATGGCGAGCTAATTATTAGCAATGTCACAAACCTGTGTAGGTGTTCTTGTGCTTGTAATCTC-TT
HF139	R. jasminiflorum	TAGAAAAATGTGTAGCTGTGCTGAAATAGAATCATCTCCATAAGCAAAAAAAGT-------GGTAAAATAACTCTATGGCGAGCTAATTATTAGCAATGTCACAAACCTGTGTAGGTGTTCTTGTGCTTGTAATCTC-TT
HF145	R. x planecostatum	TAGAAAAATGTGTAGCTGTGCTGAAATAGAATCATCTCCATAAGCAAAAAAAGT-------GGTAAAATAACTCTATGGCGAGCTTATTATTAGCAATGTCACAAACCTGTGTAGGTGTTCTTGTGCTTGTAATCTC-TT
HF147	R. rushforthii	TAGAAAAATGTGTAGCTGTGCTGAAATAGAATCGTCTCAATAAGCAAAAAAAGT-------GGTAAAATAACTCTATGGCAAGCTAATTGTTAGCAATGTCACAAACCTGTGTAGGTGCTCTTGTGCTTGTAATCTC-TT

Acc. Num.	Taxon	841 945
2002-018	R. acrophilum	GACAGATCAAACCTAGAGT----------TAAGCTAAAAATTGTTTGTTGTTGCTCCTAGGTGGACAACATCAGCAAAGCCCTTCACAAATGCGG
99-0330	R. dielsianum	GACAGATCAAACCTAGAGT----------TAAGCTAAAAATTGTTTGTTGTTGCTCCTAGGTGGACAACATCAGCAAAGCCCTTCACAAATGCGG
EI153	R. jasminiflorum	GACAGATCAAACCTAGAGT----------TAAGCTAAAAATTGTTTGTTGTTGCTCCTAGGTGGACAACATCAGCAAAGCCCTTCACAAATGCGG
EI158	R. majus	GACAGATCATACCTAGAGT----------TAAGCTAAAAATTGTTTGTTGTTGCTCCTAGGTGGACAACATCAGCAAAGCCCTTCACAAATGCGG
EI169	R. gardenia 'Odyssey'	GACAGATCAAACCTAGAGT----------TAAGCTAAAAATTGTTTGTTGTTGCTCCTAGGTGGACAACATCAGCAAAGCCCTTCACAAATGCGG
EK507	R. lochiae	GACAGATCAAACCTAGAGT----------TAAGCTAAAAATTGTTTGTTGTTGCTCCTAGGTGGACAACATCAGCAAAGCCCTTCACAAATGCGG
EI192	R. luraluense	GACAGATCAAACCTAGAGT----------TAAGCTAAAAATTGTTTGTTGTTGCTCCTAGGTGGACAACATCAGCAAAGCCCTTCACAAATGCGG
EK525	R. bagobonum	GACAGATCAAACCTAGAGT----------TAAGCTAAAAATTGTTTGTTGTTGCTCCTAGGTGGACAACATCAGCAAAGCCCTTCACAAATGCGG
EK536	R. alborugosum	GACAGATCAAACCTAGAGT----------TAAGCTAAAAATTGTTTGTTGTTGCTCCTAGGTGGACAACATCAGCAAAGCCCTTCACAAATGCGG
EK544	R. suaveolens	GACAGATCAAACCTAGAGT----------TAAGCTAAAAATTGTTTGTTGTTGCTCCTAGGTGGACAACATCAGCAAAGCCCTTCACAAATGCGG
EK548	R. jasminiflorum	GACAGATCAAACCTAGAGT----------TAAGCTAAAACTTGTTTGTTGTTGCTCCTAGGTGGACAACATCAGCAAAGCCCTTCACAAATGCGG
EK558	R. praetervisum	GACAGATCATACCTAGAGT----------TAAGCTAAAAATTGTTTGTTGTTGCTCCTAGGTGGACAACATCAGCAAAGCCCTTCACAAATGCGG
EK565	R. superbum	GACAGATCAAACCTAGAGT----------TAAGCTAAAAATTGT----TGTTGCTCCTAGGTGGACAACATCAGCAAAGCCCTTCACAAATGCGG
EK574	R. vitis-idaea	GACAGATCAAACCTAGAGT----------TAAGCTAAAAATTGTTTGTTGTTGCTCCTAGGTGGACAACATCAGCAAAGCCCTTCACAAATGCGG
EK579	R. citrinum	GACAGATCAAACCTAGAGT----------TAAGCTAAAAATTGTTTGTTGTTGCTCCTAGGTGGACAACATCAGCAAAGCCCTTCACAAATGCGG
EK581	R. santapaui	GACAGATCAAACCTAGAGT----------TAAGCTAAAAATTGTTTGTTGTTGCTCCTAGGTGGACAACATCAGCAAAGCCCTTCACAAATGCGG
EK583	R. stapfianum	GACAGAACAAACCTAGAGTTTCCTTGAGAGCATAAATTGTAAGCTAAAAATTGTTTGTTGTTGCTCCTAGGTGGACAACATCAGCAAAGCCCTTCACAAATGCGG
EK588	R. superbum	GACAGATCAAACCTAGAGT----------TAAGCTAAAAATTGTTTGTTGTTGCTCCTAGGTGGACAACATCAGCAAAGCCCTTCACAAATGCGG
EK590	R. jasminiflorum	GACAGATCAAACCTAGAGT----------TAAGCTAAAAATTGTTTGTTGTTGCTCCTAGGTGGACAACATCAGCAAAGCCCTTCACAAATGCGG
EK591	R. blackii	GACAGATCATACCTAGAGT----------TAAGCTAAAAATTGTTTGTTGTTGCTCCTAGGTGGACAACATCAGCAAAGCCCTTCACAAATGCGG
EK592	R. blackii	GACAGATCAAACCTAGAGT----------TAAGCTAAAAATTGTTTGTTGTTGCTCCTAGGTGGACACCATCAGCAAAGCCCTTCACAAATGCGG
EK600	R. kochii	GACAGATCAAACCTAGAGT----------TAAGCTAAAAATTGTTTGTTGTTGCTCCTAGGTGGACACCATCAGCAAAGCCCTTCACAAATGCGG
EK602	R. adinophyllum	GACAGATCAAACCTAGAGT----------TAAGCTAAAAATTGTTTGTTGTTGCTCCTAGGTGGACAACATCAGCAAAGCCCTTCACAAATGCGG
EK608	R. arfakianum	GACAGATCAAACCTAGAGTTTCCTTGAGAGCATAAATTGTAAGCTAAAAATTGTTTGTTGTTGCTCCTAGGTGGACAACATCAGCAAAGCCCTTCACAAATGCGG
EK614	R. solitarium	GACAGATCAAACCTAGAGT----------TAAGCTAAAAATTGTTTGTTGTTGCTCCTAGGTGGACAACATCAGCAAAGCCCTTCACAAATGCGG
EK616	R. superbum	GACAGATCAAACCTAGAGT----------TAAGCTAAAAATTGTTTGTTGTTGCTCCTAGGTGGACAACATCAGCAAAGCCCTTCACAAATGCGG
EK617	R. solitarium	GACAGATCAAACCTAGAGT----------TAAGCTAAAAATTGTTTGTTGTTGCTCCTAGGTGGACTACATCAGCAAAGCCCTTCACAAATGCGG
EK618	R. rarum	GACAGATCAAACCTAGAGT----------TAAGCTAAAAATTGTTTGTTGTTGCTCCTAGGTGGACAACATCAGCAAAGCCCTTCACAAATGCGG
EK626	R. carringtoniae	GACAGATCAAACCTAGAGT----------TAGGCTAAAAATTGTTTGTTGTTGCTCCTAGGTGGACAACATCAGCAAAGCCCTTCACAAATGCGG
EK628	R. zoelleri	GACAGATCAAACCTAGAGT----------TAAGCTAAAAATTGTTTGTTGTTGCTCCTAGGTGGACAACATCAGCAAAGCCCTTCACAAATGCGG
EK629	R. culminicola	GACAGATCAAACCTAGAGT----------TAAGCTAAAAATTGTTTGTTGTTGCTCCTAGGTGGACAACATCAGCAAAGCCCTTCACAAATGCGG
EK632	R. commonae	GACAGATCAAACCTAGAGT----------TAAGCTAAAAATTGTTTGTTGTTGCTCCTAGGTGGACAACATCAGCAAAGCCCTTCACAAATGCGG
EK633	R. commonae	GACAGATCAAACCTAGAGT----------TAAGCTAAAAATTGTTTGTTGTTGCTCCTAGGTGGACAACATCAGCAAAGCCCTTCACAAATGCGG
EK635	R. gracilentum	GACAGATCAAACCTAGAGT----------TAAGCTAAAAATTGTTTGTTGTTGCTCCTAGGTGGACAACATCAGCAAAGCCCTTCACAAATGCGG
EK637	R. commonae	GACAGATCAAACCTAGAGT----------TAAGCTAAAAATTGTTTGTTGTTGCTCCTAGGTGGACAACATCAGCAAAGCCCTTCACAAATGCGG
EK638	R. caliginis	GACAGATCAAACCTAGAGT----------T-AGCTAAAAATTGTTTGTTGTTGCTCCTAGGTGGACAACATCAGCAAAGCCCTTCACAAATGCGG
EK639	R. herzogii	GACAGATCAAACCTAGAGT----------TAAGCTAAAAATTGTTTGTTGTTGCTCCTAGGTGGACAACATCAGCAAAGCCCTTCACAAATGCGG
EK644	R. laetum	GACAGATCAAACCTAGAGT----------TAAGCTAAAAATTGTTTGTTGTTGCTCCTAGGTGGACAACATCAGCAAAGCCCTTCACAAATGCGG
EK645	R. jasminiflorum	GACAGATCATACCTAGAGT----------TAAGCTAAAAATTGTTTGTTGTTGCTCCTAGGTGGACAACATCAGCAAAGCCCTTCACAAATGCGG
EK647	R. rutenii	GACAGATCAAACCTAGAGT----------TAAGCTAAAAATTGTTTGTTGTTGCTCCTAGGTGGACAACATCAGCAAAGCCCTTCACAAATGCGG
EK648	R. laetum	GACAGATCAAACCTAGAGT----------TAAGCTAAAAATTGTTTGTTGTTGCTCCTAGGTGGACAACATCAGCAAAGCCCTTCACAAATGCGG
EK649	R. dielsianum	GACAGATCAAACCTAGAG----------TAAGCTAAAAATTGTTTGTTGTTGCTCCTAGGTGGACAACATCAGCAAAGCCCTTCACAAATGCGG
EK651	R. superbum	GACAGATCAAACCTAGAGT----------TAAGCTAAAAATTGTTTGTTGTTGCTCCTAGGTGGACTACATCAGCAAAGCCCTTCACAAATGCGG
EK657	R. majus	GACAGATCAAACCTAGAGT----------TAAGCTAAAAATTGTTTGTTGTTGCTCCTAGGTGGACAACATCAGCAAAGCCCTTCACAAATGCGG
EK658	R. majus	GACAGATCAAACCTAGAGT----------TAAGCTAAAAATTGTTTGTTGTTGCTCCTAGGTGGACAACATCAGCAAAGCCCTTCACAAATGCGG
EK664	R. yongii	GACAGATCAAACCTAGAGT----------TAAGCTAAAAATTGTTTGTTGTTGCTCCTAGGTGGACAACATCAGCAAAGCCCTTCACAAATGCGG
EK666	R. asperum	GACAGATCAAACCTAGAGT----------T-AGCTAAAACTTGTTTGTTGTTGCTCCTAGGTGGACACCATCAGCAAAGCCCTTCACAAATGCGG
EK667	R. radians	GACAGATCAAACCTAGAGT----------TAAGCTAAAAATTGTTTGTTGTTGCTCCTAGGTGGACAACATCAGCAAAGCCCTTCACAAATGCGG
EK671	R. wilkiei	GACAGATCAAACCTAGAGT----------TAAGCTAAAAATTGTTTGTTGTTGCTCCTAGGTGGACAACATCAGCAAAGCCCTTCACAAATGCGG
HF001	R. konori	GACAGATCAAACCTAGAGT----------TAAGCTAAAAATTGTTTGTTGTTGCTCCTAGGTGGACAACATCAGCAAAGCCCTTCACAAATGCGG
HF004	R. hellwigii	GACAGATCAAACCTAGAGT----------TAAGCTAAAAATTGTTTGTTGTTGCTCCTAGGTGGACAACATCAGCAAAGCCCTTCACAAATGCGG

HF012	R. gardenia	GACAGATCAAACCTAGAGT----------TAAGCTAAAAATTGTTTGTTGTTGCTCCTAGGTGGACAACATCAGCAAAGCCCTTCACAAATGCGG
HF014	R. rousei	GACAGATCAAACCTAGAGT----------TAAGCTAAAAATTGTTTGTTGTTGCTCCTAGGTGGACAACATCAGCAAAGCCCTTCACAAATGCGG
HF016	R. cruttwellii	GACAGATCAAACCTAGAGT----------TAAGCTAAAAATTGTTTGTTGTTGCTCCTAGGTGGACAACATCAGCAAAGCCCTTCACAAATGCGG
HF021	R. javanicum	GACAGATCAAACCTAGAGT----------TAAGCTAAAAATTGTTTGTTGTTGGTCCTAGGTGGACAACATCAGCAAAGCCCTTCACAAATGCGG
HF023	R. dielsianum	GACAGATCAAACCTAGAG----------TAAGCTAAAAATTGTTTGTTGTTGCTCCTAGGTGGACAACATCAGCAAAGCCCTTCACAAATGCGG
HF028	R. truncicola	GACAGATCAAACCTAGAGT----------TAAGCTAAAAATTGTTTGTTGTTGCTCCTAGGTGGACAACATCAGCAAAGCCCTTCACAAATGCGG
HF030	R. lochiae	GACAGATCAAACCTAGAGT----------TAAGCTAAAAATTGTTTGTTGTTGCTCCTAGGTGGACAACATCAGCAAAGCCCTTCACAAATGCGG
HF031	R. curviflorum	GACAGATCAAACCTAGAGT----------TAAGCTAAAACTTGTTTGTTGTTGCTCCTAGGTGGACACCATCAGCAAAGCCCTTCACAAATGCGG
HF032	R. armitii	GACAGATCCAACCTAGAGT----------TAAGCTATAACTTGTTTGTTGTTGCTCCTAGGTGGACAACATCAGCAAAGCCCTTCACAAATGCGG
HF036	R. pleianthum	GACAGATCAAACCTAGAGT----------TAAGCTAAAAATTGTTTGTTGTTGCTCCTAGGTGGACAACATCAGCAAAGCCCTTCACAAATGCGG
HF043	R. burttii	GACAGATCAAACCTAGAGT----------TAAGCTAAAAATTGTTTGTTGTTGCTCCTAGGTGGACAACATCAGCAAAGCCCTTCACAAATGCGG
HF050	R. emarginatum	GACAGATCAAACCTAGAGTTTCCTTGAGAGCATAAATTGTAAGCTAAAAATTGTTTGTTGTTGCTCCTAGGTGGACAACATCAGCAAAGCCCTTCACAAATGCGG
HF051	R. leucogigas	GACAGATCAAACCTAGAGT----------TAAGCTAAAAATTGTTTGTTGTTGCTCCTAGGTGGACAACATCAGCAAAGCCCTTCACAAATGCGG
HF053	R. pubigermen	GACAGATCAAACCTAGAGT----------TAGGCTAAAAATTGTTTGTTGTTGCTCCTAGGTGGACAACATCAGCAAAGCCCTTCACAAATGCGG
HF062	R. commonae	GACAGATCAAACCTAGAGT----------TAAGCTAAAAATTGTTTGTTGTTGCTCCTAGGTGGACAACATCAGCAAAGCCCTTCACAAATGCGG
HF066	R. laetum	GACAGATCAAACCTAGAGT----------TAAGCTAAAAATTGTTTGTTGTTGCTCCTAGGTGGACAACATCAGCAAAGCCCTTCACAAATGCGG
HF067	R. villosulum	GACAGATCAAACCTAGAGT----------TAAGCTAAAAATTGTTTGTTGTTGCTCCTAGGTGGACAACATCAGCAAAGCCCTTCACAAATGCGG
HF068	R. aurigeranum	GACAGATCAAACCTAGAGT----------TAAGCTAAAAATTGTTTGTTGTTGCTCCTAGGTGGACACCATCAGCAAAGCCCTTCACAAATGCGG
HF070	R. celebicum	GACAGATCAAACCTAGAGT----------TAAGCTAAAAATTGTTTGTTGTTGCTCCTAGGTGGACAACATCAGCAAAGCCCTTCACAAATGCGG
HF072	R. kawakamii	GACAGATCAAACCTAGAGTTTCCTTGAGAGCATAAATTGTAAGCTAAAAATTGTTTGTTGTTGCTCCTAGGTGGACAACATCAGCAAAGCCCTTCACAAATGCGG
HF077	R. viriosum	GACAGATCAAACCTAGAGT----------TAAGCTAAAAATTGTTTGTTGTTGCTCCTAGGTGGACAACATCAGCAAAGCCCTTCACAAATGCGG
HF082	R. suaveolens	GACAGATCAAACCTAGCGT----------TAAGCAAAAAATTGTTTGTTGTTGCTCCTAGGTGGACAACATCAGCAAAGCCCTTCACAAATGCGG
HF090	R. loranthiflorum	GACAGATCAAACCTAGAGT----------TAAGCTAAAAATTGTTTGTTGTTGCTCCTAGGTGGACAACATCAGCAAAGCCCTTCACAAATGCGG
HF091	R. verticillatum	GACAGATCAAACCTAGAGT----------TAAGCTAAAAATTGTTTGTTGTTGCTCCTAGGTGGACAACATCAGCAAAGCCCTTCACAAATGCGG
HF092	R. orbiculatum	GACAGATCAAACCTAGAGT----------TAAGCTAAAAATTGTTTGTTGTTGCTCCTAGGTGGACAACATCAGCAAAGCCCTTCACAAATGCGG
HF093	R. sumatranum	GACTGATCAAACCTAGAGT----------TAAGCTAAAAATTGTTTGTTGTTGCTCCTAGGTGGACAACATCAGCAAAGCCCTTCACAAATGCGG
HF094	R. luraluense	GACAGATCAAACCTAGAGT----------TAAGCTAAAAATTGTTTGTTGTTGCTCCTAGGTGGACAACATCAGCAAAGCCCTTCACAAATGCGG
HF097	R. zollingeri	GACAGATCAAACCTAGAGT----------TAAGCTAAAAATTGTTTGTTGTTGCTCCTAGGTGGACAACATCAGCAAAGCCCTTCACAAATGCGG
HF100	R. tuba	GACAGATCAAACCTAGAGT----------TAAGCTAAAAATTGTTTGTTGTTGCTCCTAGGTGGACAACATCAGCAAAGCCCTTCACAAATGCGG
HF101	R. lowii	GACAGATCAAACCTAGAGT----------TAAGCTAAAAATTGTTTGTTGTTGCTCCTAGGTGGACAACATCAGCAAAGCCCTTCACAAATGCGG
HF135	R. impositum	GACAGATCAAACCTAGAGT----------TAAGCTAAAAATTGTTTGTTGTTGCTCCTAGGTGGACAACATCAGCAAAGCCCTTCACAAATGCGG
HF137	R. luraluense	GACAGATCAAACCTAGAGT----------TAAGCTAAAAATTGTTTGTTGTTGCTCCTAGGTGGACAACATCAGCAAAGCCCTTCACAAATGCGG
HF139	R. jasminiflorum	GACAGATCAAACCTAGAGT----------TAAGCTAAAAATTGTTTGTTGTTGCTCCTAGGTGGACAACATCAGCAAAGCCCTTCACAAATGCGG
HF145	R. x planecostatum	GACAGATCAAACCTAGAGT----------TAAGCTAAAAATTGTTTGTTGTTGCTCCTAGGTGGACAACATCAGCAAAGCCCTTCACAAATGCGG
HF147	R. rushforthii	GACAGATCAAACCTAGAGTTTCCTTGAGAGCATAAATTGTAAGCTAAAAATTGTTTGTTGTTGCTCCTAGGTGGACAACATCAGCAAAGCCCTTCACAAATGCGG

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A4 Genbank Accessions

Table 45 DNA Sequences of Rhododendron taxa for the rpb2i intron 23 region. All the sequences downloaded from the nucleotide database of the National Centre for Biotechnology Information (http://www.ncbi.nlm.nih.gov/).

Taxon	Accession #	Reference
R. dielsianum	AY765583	Goetsch et al (2005)
R. radians	AY765589	Goetsch et al (2005)
R. herzogii	AY765595	Goetsch et al (2005)
R. konori	AY765601	Goetsch et al (2005)
R. crassifolium	AY765607	Goetsch et al (2005)
R. sororium	AY765613	Goetsch et al (2005)
R. asperulum	AY765619	Goetsch et al (2005)
R. santapaui	AY765625	Goetsch et al (2005)
R. vaccinioides	AY765631	Goetsch et al (2005)
R. leptothrium	AY765925	Goetsch et al (2005)
R. vaseyi	AY765961	Goetsch et al (2005)
R. albiflorum	AY765979	Goetsch et al (2005)
R. tuba	GU445771	Goetsch et al (2011)
R. lochiae	GU445772	Goetsch et al (2011)
R. solitarium	GU445773	Goetsch et al (2011)
R. superbum	GU445774	Goetsch et al (2011)
R. inconspicuum	GU445775	Goetsch et al (2011)
R. luraluense	GU445776	Goetsch et al (2011)
R. laetum	GU445777	Goetsch et al (2011)
R. culminicola	GU445778	Goetsch et al (2011)
R. christi	GU445779	Goetsch et al (2011)
R. yelliotii	GU445780	Goetsch et al (2011)
R. zoelleri	GU445781	Goetsch et al (2011)
R. rarum	GU445782	Goetsch et al (2011)
R. gracilentum	GU445783	Goetsch et al (2011)
R. pulleanum	GU445784	Goetsch et al (2011)
R. rubineiflorum	GU445785	Goetsch et al (2011)
R. commonae	GU445786	Goetsch et al (2011)
R. carringtoniae	GU445787	Goetsch et al (2011)
R. inundatum	GU445788	Goetsch et al (2011)
R. leucogigas	GU445789	Goetsch et al (2011)
R. loranthiflorum	GU445790	Goetsch et al (2011)
R. saruwagedicum	GU445791	Goetsch et al (2011)
R. jasminiflorum	GU445793	Goetsch et al (2011)
R. suaveolens	GU445794	Goetsch et al (2011)

R. rutenii	GU445795	Goetsch et al (2011)
R. salicifolium	GU445796	Goetsch et al (2011)
R. edanoi	GU445797	Goetsch et al (2011)
R. orbiculatum	GU445798	Goetsch et al (2011)
R. stapfianum	GU445799	Goetsch et al (2011)
R. maxwellii	GU445801	Goetsch et al (2011)
R. yongii	GU445802	Goetsch et al (2011)
R. burttii	GU445803	Goetsch et al (2011)
R. rousei	GU445804	Goetsch et al (2011)
R. pauciflorum	GU445805	Goetsch et al (2011)
R. album	GU445806	Goetsch et al (2011)
R. aequabile	GU445807	Goetsch et al (2011)
R. sumatranum	GU445808	Goetsch et al (2011)
R. citrinum	GU445809	Goetsch et al (2011)
R. eymae	GU445810	Goetsch et al (2011)
R. lowii	GU445811	Goetsch et al (2011)
R. lagunculicarpum	GU445812	Goetsch et al (2011)
R. vanvuurenii	GU445813	Goetsch et al (2011)
R. alternans	GU445814	Goetsch et al (2011)
R. celebicum	GU445815	Goetsch et al (2011)
R. renschianum	GU445817	Goetsch et al (2011)
R. correoides	GU445818	Goetsch et al (2011)
R. madulidii	GU445819	Goetsch et al (2011)
R. arenicola	GU445820	Goetsch et al (2011)
R. rhodopus	GU445821	Goetsch et al (2011)
R. multicolor	GU445822	Goetsch et al (2011)
R. zollingeri	GU445823	Goetsch et al (2011)
R. javanicum	GU445824	Goetsch et al (2011)
R. rarilepidotum	GU445825	Goetsch et al (2011)
R. robinsonii	GU445827	Goetsch et al (2011)
R. williamsii	GU445828	Goetsch et al (2011)
R. pseudobuxifolium	GU445829	Goetsch et al (2011)
R. sarcodes	GU445830	Goetsch et al (2011)
R. bagobonum	GU445831	Goetsch et al (2011)
R. fallacinum	GU445832	Goetsch et al (2011)
R. malayanum	GU445833	Goetsch et al (2011)
R. himantodes	GU445834	Goetsch et al (2011)
R. apoanum	GU445835	Goetsch et al (2011)
R. nanophyton	GU445836	Goetsch et al (2011)
R. quadrasianum	GU445837	Goetsch et al (2011)
R. ericoides	GU445838	Goetsch et al (2011)
R. gaultheriifolium	GU445839	Goetsch et al (2011)

R. meliphagidum	GU445840	Goetsch et al (2011)
R. retusum	GU445842	Goetsch et al (2011)
R. adinophyllum	GU445843	Goetsch et al (2011)
R. perakense	GU445844	Goetsch et al (2011)
R. emarginatum	GU445845	Goetsch et al (2011)
R. euonymifolium	GU445846	Goetsch et al (2011)
R. kawakamii	GU445847	Goetsch et al (2011)
R. rushforthii	GU445848	Goetsch et al (2011)

A5 Microsatellite Data

Table 46 Fragment length dataset generated from 11 microsatellite markers for 192 accessions of vireya taxa.

			GA117		GA102		DD042		DC046				RM3D2		GA211		DC049				DD113		DD095		DC027	
#	PFR#	Taxon	L1a	L1b	L1a	L1b	L1a	L1b	L1a	L1b	L2a	L2b	L1a	L1b	L1a	L1b	L1a	L1b	L2a	L2b	L1a	L1b	L1a	L1b	L1a	L1b
1	1978/102	*R. jasminiflorum* ssp. *jasminiflorum*					218	218	172	174															214	216
2	1983/059	*R. culminicola* var. *angiense*											116	116	78	78									204	206
3	1983/063	*R. dianthosmum*			180	180	207	217							150	150									206	206
4	1983/60	*R. dielsianum*																							214	238
5	1987/048	*R.* × *sheilae*																							230	230
6	1994/373	*R. javanicum* ssp. *gracile*							162	164															202	206
7	1997/087	*R. rushforthii*			180	180																			234	234
8	1999/286	*R. culminicola* var. *culminicola*											140	144			174	174			130	142				
9	1999/307	*R. javanicum* ssp. *teysmannii*			180	180	223	233	162	162															202	214
10	1999/308	*R. vaccinioides*			180	180	201	217																	202	206
11	1999/318	*R. javanicum* ssp. *brookeanum*			180	180	195	245																	200	202
12	1999/382	*R. emarginatum*			180	180			139	141															212	212
13	2002/018	*R. acrophilum*			176	176	223	225	150	165					245	261	172	172							208	210
14	87/041	*R. leptanthum*			180	180																			208	220
15	94/333	*R. polyanthemum*			180	180	221	242	155	174					231	233	164	164							216	216
16	99/330	*R. dielsianum*			180	180	251	253																	226	234
17	994/336	*R. polyanthemum*			180	180	226	228	172	174							164	164							218	218
18	EI153	*R. jasminiflorum* ssp. *jasminiflorum*	47	47			215	215	144	144							105	105	170	174					198	206
19	EI154	*R. jasminiflorum* ssp. *jasminiflorum*	47	47			215	215	140	144							105	105	170	174					198	206
20	EI164	*R. yelliotii*	47	47			207	207	160	162															198	204
21	EI186	hybrid	47	47			207	212	140	157							156	156							200	206
22	EI187	*R. konori*	40	45			220	220	162	164																
23	EK514	*R. macgregoriae* (orange form)	47	47			205	205	166	170			112	112	116	116							148	148		
24	EK517	*R. quadrasianum*	47	47																					194	200
25	EK522	*R. crassifolium*	45	47			215	220	164	166					218	222									200	206
26	EK523	*R. maxwellii*	47	47			215	215	146	146															182	186
27	EK525	*R. bagobonum*					206	211	162	162					231	245									204	204
28	EK526	*R. stenophyllum* ssp. *angustifolium*					207	207	153	153															202	220
29	EK527	*R. fallacinum*	47	47					156	160					249	271									172	176
30	EK531	*R. fallacinum*	41	47					150	150					223	225									180	184
31	EK537	*R. ericoides*	47	47			200	217	166	174					190	208									200	206
32	EK540	*R. rugosum*					212	220	144	146					233	245									228	230
33	EK542	*R. micromalayanum*					200	200	154	156					164	271	166	166							218	222
34	EK543	*R. burtii*			180	180																				
35	EK544	*R. suaveolens*					210	210	144	146					104	139									212	220
36	EK547	*R. javanicum* ssp. *brookeanum* var. *kinabaluense*					212	217	146	148															202	202
37	EK548	*R. jasminiflorum* ssp. *jasminiflorum*	47	47					140	144							105	105	170	174						
38	EK553	*R. perakense*	47	47			212	212							241	243									198	206
39	EK555	*R. malayanum*	43	47					146	162							153	153							196	206
40	EK560	*R. crassifolium*	47	47			215	215							223	223									192	198
41	EK564	*R. luraluense*	43	47					150	154															200	206
42	EK565	*R. superbum*	47	47			207	215	159	159															44	44
43	EK568	*R. sp.*					212	212							108	108									206	210
44	EK568	*R. sp.*					212	215							113	125										
45	EK569	*R. viriosum*	43	47					163	163							156	156					292	292	208	208
46	EK570	*R. album*	47	47					148	150																
47	EK571	*R. retusum*	41	47																					112	112
48	EK572	*R. viriosum*					212	215									170	170							208	208
49	EK573	*R. lagunculicarpum*	47	47			200	207	148	148															196	202
50	EK576	*R. gracilentum*					210	210																		

			GA117		GA102		DD042		DC046				RM3D2		GA211		DC049				DD113		DD095		DC027	
#	PFR#	Taxon	L1a	L1b	L1a	L1b	L1a	L1b	L1a	L1b	L2a	L2b	L1a	L1b	L1a	L1b	L1a	L1b	L2a	L2b	L1a	L1b	L1a	L1b	L1a	L1b
51	EK577	*R. rhodopus*	47	47					140	146					233	243									200	208
52	EK578	*R. taxifolium*					212	215	165	165																
53	EK580	*R. taxifolium*					210	215	178	178															174	202
54	EK581	*R. santapaui*					205	220	178	178															198	198
55	EK582	*R. fallacinum*	47	47			197	197	150	156					215	221									164	168
56	EK584	*R. rarilepidotum*	47	47			197	205	154	162					226	311									196	202
57	EK585	*R. macgregoriae* (large form)	47	47			205	205	150	154			116	130	206	224	156	156	195	199						
58	EK588	*R. superbum*			180	180	207	217	156	158															206	206
59	EK589	*R. viriosum* (Mt Finnigan form)					212	215	160	162					179	327	170	170			160	160	376	376	208	208
60	EK590	*R. jasminiflorum* ssp. *oblongifolium*	47	47			215	215	144	146					188	190	105	105	170	174					196	206
61	EK591	*R. blackii*			180	180	220	231																	208	214
62	EK596	*R. arenicola*	47	68			212	217	148	148					229	229									196	202
63	EK597	*R. rhodopus*	47	47			217	217	150	152					233	239									190	196
64	EK601	*R. zollingeri*	42	47					146	148															200	210
65	EK603	*R. gracilentum*																							208	310
66	EK604	*R. viriosum*					197	197	160	162					132	138	170	170			222	222	292	376	208	208
67	EK605	*R. taxifolium*																								
68	EK606	*R. lochiae*	47	47					159	159			134	138			156	156			134	134			198	204
69	EK608	*R. arfakianum*	47	47			217	220	152	154															198	204
70	EK609	*R. christi* (Mt Miap form)	47	47			212	212	152	154															200	204
71	EK610	*R. christi*	47	47					152	156					232	232									198	204
72	EK611	*R. goodenoughii*	41	47			207	207	165	165					248	248									202	208
73	EK612	*R. jasminiflorum* ssp. *jasminiflorum*	41	47			215	215	140	144							105	105	170	174					198	206
74	EK613	*R. konori* (Edie Creek form)	47	47			207	207	154	158															202	210
75	EK614	*R. macgregoriae*					205	217	139	139			132	134	88	88					134	160	54	54		
76	EK615	*R. macgregoriae*	47	47			205	205	162	164							156	156					176	176	196	200
77	EK616	*R. superbum*	42	47			290	290	159	163															202	206
78	EK617	*R. solitarium* (Bulldog Rd form)							140	140															206	252
79	EK620	*R. viriosum* (Mt Finnegan form)					220	220	160	162					116	133	170	170			160	160			208	208
80	EK621	*R. gracilentum* (Mt Miap form)					205	205	146	146					94	110									216	218
81	EK628	*R. zoelleri*	47	52					148	148							156	156							84	88
82	EK629	*R. culminicola* var. *culminicola* (Bulldog Rd form)	47	47					136	152			120	132	146	146	152	152	182	200	142	142			204	210
83	EK630	*R. viriosum*	47	64			212	212							226	226	156	156							200	208
84	EK634	*R. macgregoriae* (red form)	47	47			205	205	150	156			100	107	48	52	156	156	198	202					196	200
85	EK635	*R. gracilentum*			180	180	208	210	164	164															218	218
86	EK639	*R. herzogii* (Mt Yakananda form)					197	197	158	158			138	140	99	116			186	186			86	184	204	204
87	EK641	*R. vanvuurenii*																								
88	EK642	*R. robinsonii*	47	47			220	220	151	159															196	202
89	EK643	*R. laetum*	47	47					147	147															122	160
90	EK644	*R. laetum*	47	49																					126	160
91	EK645	*R. jasminiflorum* ssp. *oblongifolium*	47	47					143	146					168	192	105	105	170	174					202	206
92	EK646	*R. rarilepidotum* (yellow form)	47	47																						
93	EK648	*R. laetum*	47	47			220	220	156	158					106	129										
94	EK649	*R. dielsianum* (labelled *R. bryophilum*)	47	47			210	210	162	164					230	241									196	202
95	EK650	*R. orbiculatum*					207	207																	216	216
96	EK651	*R. superbum*	47	47			220	220	156	158															202	206
97	EK652	*R. javanicum* ssp. *brookeanum*	45	47			215	215							194	194									198	204
98	EK653	*R. pudorinum*	47	47					136	136					229	239										
99	EK654	*R. inundatum*					216	220					120	150	81	83					156	156				
100	EK656	*R. jasminiflorum* ssp. *jasminiflorum*	47	47					144	144					224	224	105	105	170	174					198	206
101	EK659	*R. sumatranum*							146	146																
102	EK660	*R. arenicola*	47	47			200	207	146	148					251	251									196	202
103	EK661	*R. javanicum* ssp. *moultonii*	47	47			210	220							206	259									198	204
104	EK662	*R. quadrasianum* var. *rosmarinifolium*	47	47											218	236									130	200
105	EK663	*R. quadrasianum* var. *malindangense*	47	47			212	212	156	156					214	232									130	192
106	EK665	*R. rarilepidotum*	47	47			197	205	162	164					218	218									196	202

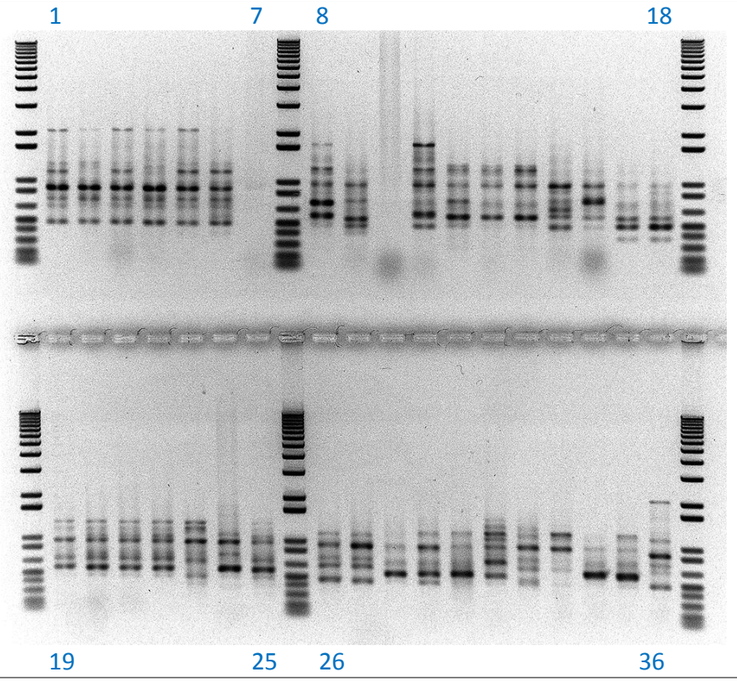
			GA117		GA102		DD042		DC046				RM3D2		GA211		DC049				DD113		DD095		DC027	
#	PFR#	Taxon	L1a	L1b	L1a	L1b	L1a	L1b	L1a	L1b	L2a	L2b	L1a	L1b	L1a	L1b	L1a	L1b	L2a	L2b	L1a	L1b	L1a	L1b	L1a	L1b
107	EK666	*R. asperum*			180	180	206	215																	204	204
108	EK668	*R. longiflorum*	47	47					162	164					235	237									200	206
109	EK669	*R. acrophilum*	47	47			217	217	162	165															202	210
110	EK670	*R. leptanthum* (syn: *R. warianum*)	47	47					165	168															202	210
111	EK671	*R. wilkiei*	47	47			217	217	157	160					223	243									198	204
112	EK675	*R. retusum*	42	47			200	217	154	156					200	202									206	206
113	EK677	*R. macgregoriae* (?)											126	136									54	54		
114	HF002	*R. archboldianum*							160	166			130	136	72	75	165	169					86	120	210	214
115	HF003	*R. archboldianum*					212	212	161	167			128	134	157	157	166	166			130	142			210	214
116	HF004	*R. hellwigii*																							206	206
117	HF005	*R. rugosum*					220	220	144	146															226	228
118	HF006	*R. superbum*																								
119	HF007	*R. tuba*																							206	210
120	HF010	*R. superbum*			180	180	225	249	156	163															204	206
121	HF011	*R. orbiculatum*																							222	224
122	HF011	*R. orbiculatum*			180	180	209	225																	224	224
123	HF012	*R. gardenia* 'Odyssey'																								
124	HF013	*R. wilkiei*																							204	204
125	HF014	*R. rousei*			180	180																			208	208
126	HF015	*R. multinervium*							160	160															208	208
127	HF016	*R. cruttwellii*																								
128	HF017	*R. christi* (small form)																							206	206
129	HF019	*R. phaeochitum*							144	150															206	206
130	HF021	*R. javanicum* ssp. *teysmannii*			180	180	205	221	150	198					231	233									202	204
131	HF022	*R. phaeochitum*							128	150	242	254													206	206
132	HF023	*R. dielsianum*							160	162															210	214
133	HF024	*R. scabridibracteum*			180	180	215	225	146	146															210	210
134	HF026	*R. perakense*			180	180	187	215	63	63					241	243									198	198
135	HF027	*R. crassifolium* × *stenophyllum*			180	180	223	242	146	160					197	199									202	230
136	HF028	*R. truncicola*			180	180	211	217	146	160															204	216
137	HF029	*R. lochiae* 'Highlander'					200	200	154	156					82	82	170	170					376	376	208	208
138	HF030	*R. lochiae* 'Baby Bells'							146	154	204	238					170	170					292	376	208	210
139	HF031	*R. curviflorum*			180	180	209	215	146	155															206	208
140	HF032	*R. armitii*			180	180	198	198																	214	214
141	HF033	*R. maxwellii*					212	212																		
142	HF034	*R. rhodoleucum*																							190	190
143	HF035	*R. kochii*			180	180	154	223							189	191									200	200
144	HF036	*R. pleianthum*			180	180	213	217	160	166															210	210
145	HF037	*R. lanceolatum*					221	231							165	184									222	228
146	HF038	*R. searleanum*					203	249																	208	208
147	HF040	*R. multicolor*					207	207																	202	204
148	HF041	*R.* × *coriifolium*					198	219							192	192									204	228
149	HF042	*R. inundatum*					208	220													134	134	66	86	208	208
150	HF043	*R. burtii*			180	180	223	231	156	168															224	226
151	HF045	*R. viriosum* (Mt Lewis form)																							206	206
152	HF046	*R. superbum*					216	220	156	158															202	206
153	HF047	*R. longiflorum*					215	215																	224	224
154	HF048	*R. christi* (red form)					210	210	150	152					196	227									204	204
155	HF049	*R. viriosum*					217	217	160	162							170	170					376	376	206	206
156	HF051	*R. leucogigas* 'Hunstein's Surprise'			180	180	215	219	139	146															204	208
157	HF053	*R. pubigermen*							148	150					122	138									208	210
158	HF054	*R. viriosum*					210	220	150	150					141	224	152	152			160	172	300	376	208	208
159	HF055	*R. inconspicuum*							139	139					82	155									204	204
160	HF056	*R. blackii*					212	212	153	153					290	322									212	214
161	HF057	*R. macgregoriae* (orange form)					205	205					122	132			170	170			116	200	120	176	192	212
162	HF059	*R. kawakamii*					205	205	150	150															232	240
163	HF060	*R. christianae*			180	180	260	260	156	168			116	134	71	82	154	154					122	126		

			GA117		GA102		DD042		DC046				RM3D2		GA211		DC049				DD113		DD095		DC027	
#	PFR#	Taxon	L1a	L1b	L1a	L1b	L1a	L1b	L1a	L1b	L2a	L2b	L1a	L1b	L1a	L1b	L1a	L1b	L2a	L2b	L1a	L1b	L1a	L1b	L1a	L1b
164	HF062	*R. commonae* (coral-pink form)			180	180	212	221	148	162															208	210
165	HF070	*R. celebicum*			180	180	182	182																	202	202
166	HF072	*R. kawakamii*							150	150					159	172									232	240
167	HF076	*R. gracilentum*					212	212	146	146															220	222
168	HF077	*R. viriosum* (Mt Finnegan form)							160	162					151	151	170	170					376	376	208	208
169	HF079	*R. macgregoriae*											142	144	81	81										
170	HF081	*R. suaveolens*							148	148															206	208
171	HF082	*R. stenophyllum* ssp. *stenophyllum*					200	200																	202	202
172	HF084	*R. cruttwellii*					210	215	158	160																
173	HF087	*R. majus*					205	220	150	152					164	289									212	214
174	HF088	*R. majus*																							214	214
175	HF089	*R. solitarium*					207	207	130	140															196	196
176	HF090	*R. loranthiflorum*					212	212	148	150															206	212
177	HF091	*R. verticillatum*					220	220	158	160					120	120									262	262
178	HF092	*R. orbiculatum*			180	180	227	238	146	174																
179	HF093	*R. sumatranum* (parent of HF086)			180	180	207	209	146	174															206	206
180	HF094	*R. luraluense*					207	207	152	154															206	206
181	HF095	*R. cruttwellii*													192	284										
182	HF096	*R. orbiculatum*					227	275	149	149					211	211									206	206
183	HF097	*R. zollingeri*					212	212																		
184	HF099	*R. sp.*					212	215	139	150															206	210
185	HF101	*R. lowii*			180	180	203	221	155	157															204	210
186	HF135	*R. impositum*					205	215							241	263										
187	HF137	*R. luraluense* (Solomon Is form)					212	212							81	103									188	188
188	HF138	*R. luraluense* (Solomon Is form)					205	215	150	154					132	220									206	206
189	HF139	*R. jasminiflorum* 'X'			180	180	215	219	148	162					247	249	166	166							210	216
190	HF145	*R.* × *planecostatum*					197	197							103	104									202	206
191	HF147	*R. rushforthii*					217	217																		
192	V112	*R. solitarium*			178	178	212	225	140	140															206	206

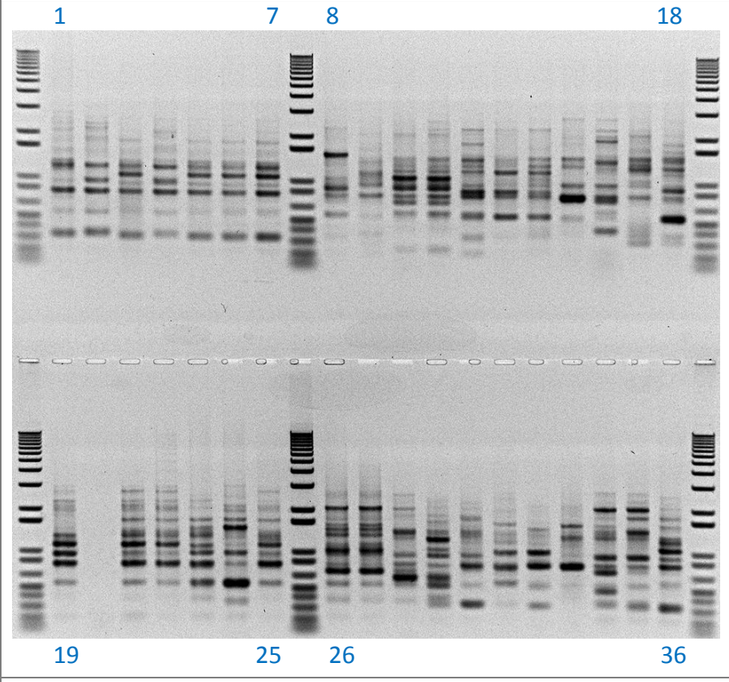
A6 RAPD Data

Photo 27 Gel electrophoresis photos obtained for the RAPD analyses. Lanes 1–36 correspond to the accessions EK548, EK590, EK612, EK645, EK656, EI153, EI154, EK657, EK658, EI157, EI158, EK591, EK592, EK593, EK613, EK619, EK618, EK655, EK572, EK589, EK604, EK620, EK630, EK507, EK569, EK574, EK575, EK609, EK610, EK616, EK651, EK603, EK621, EK614, EK617 and EK612. All the ladders are 1kb+.

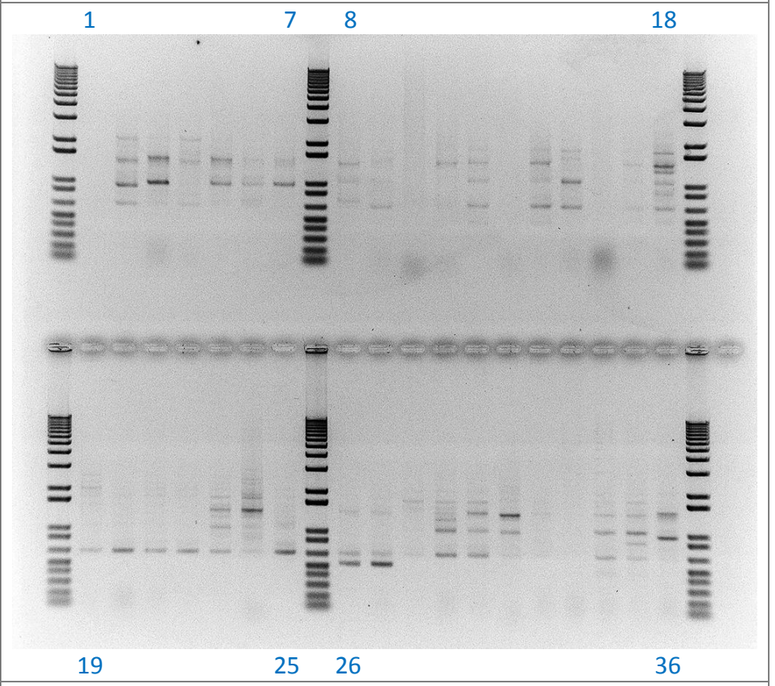
OPAN-14



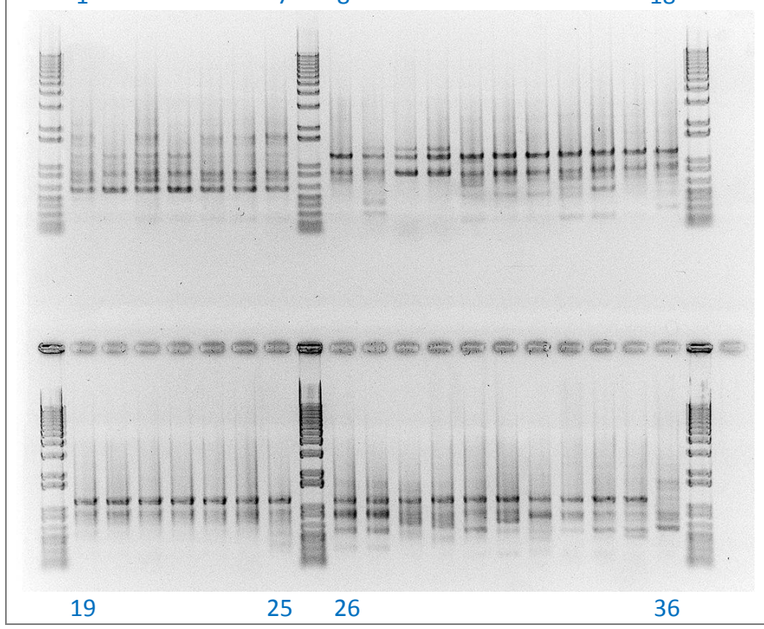
OPAN-20



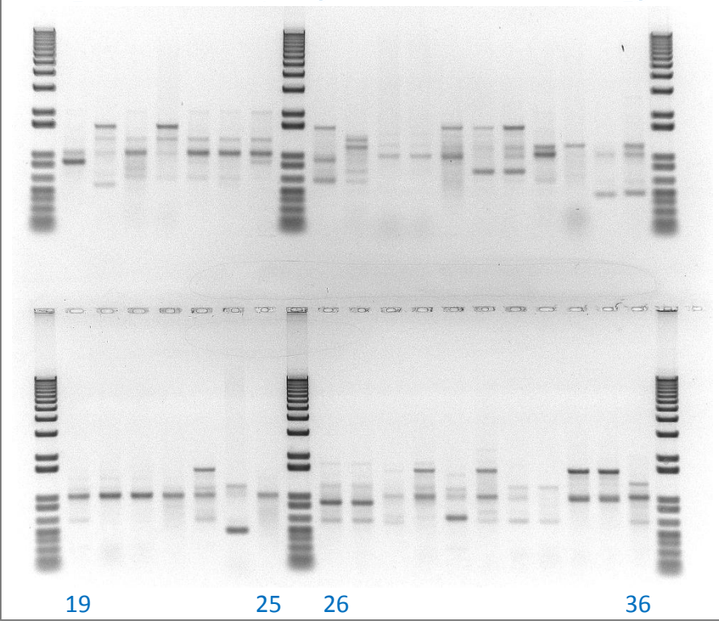
OPAR-08



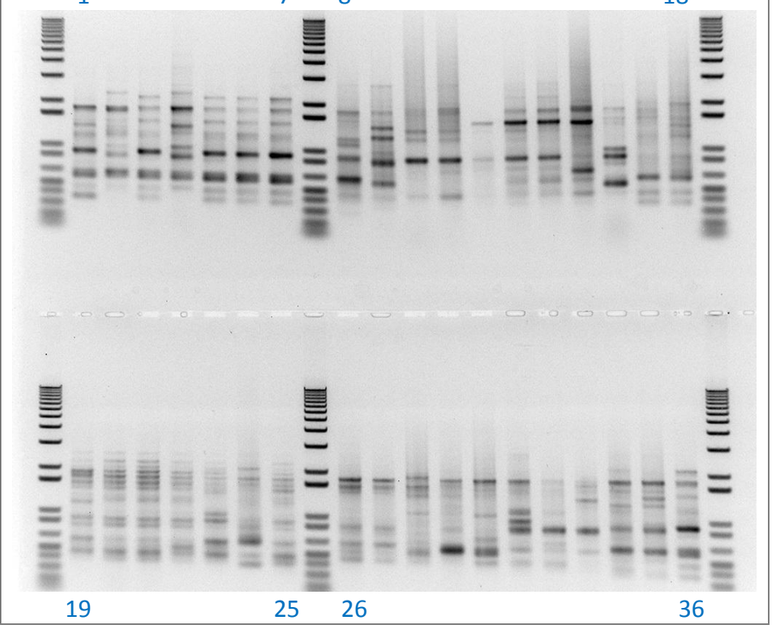
OPAX-12



OPAT-15



OPAX-20



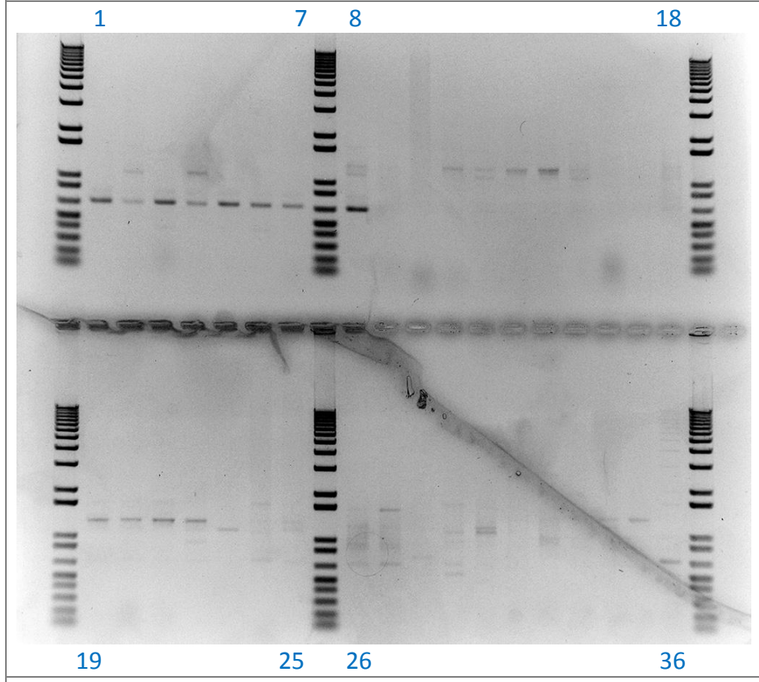
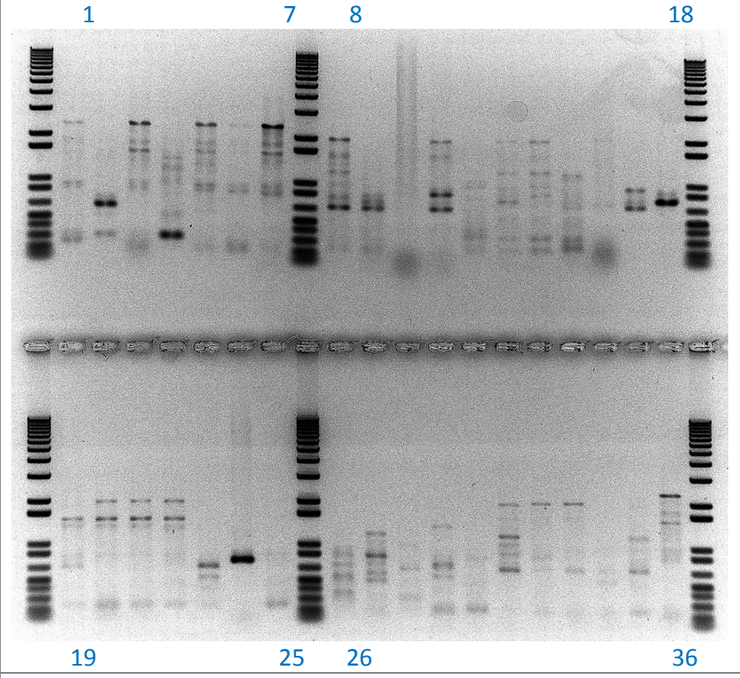
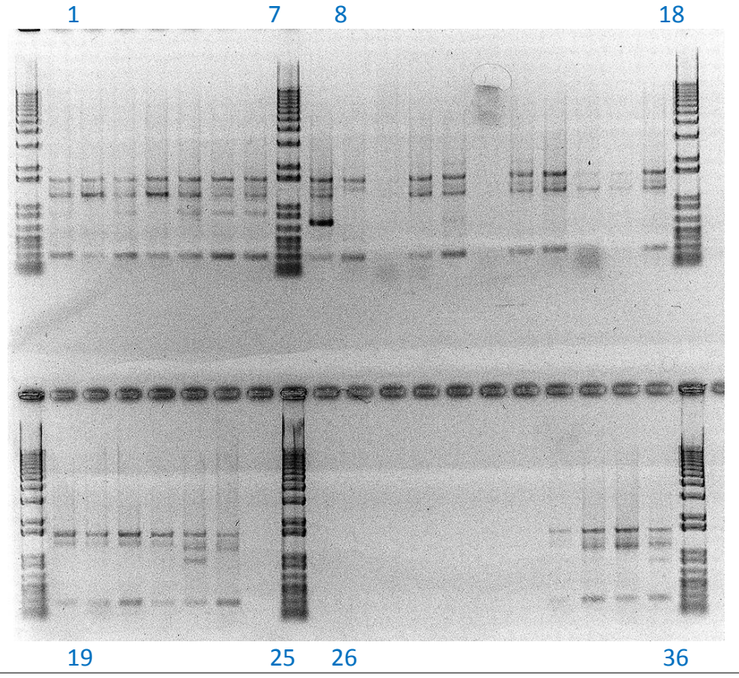
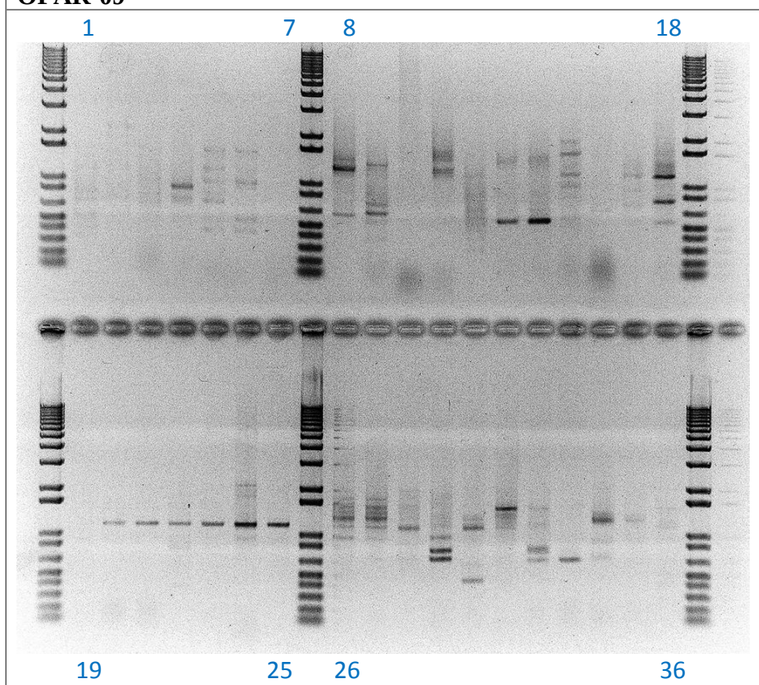
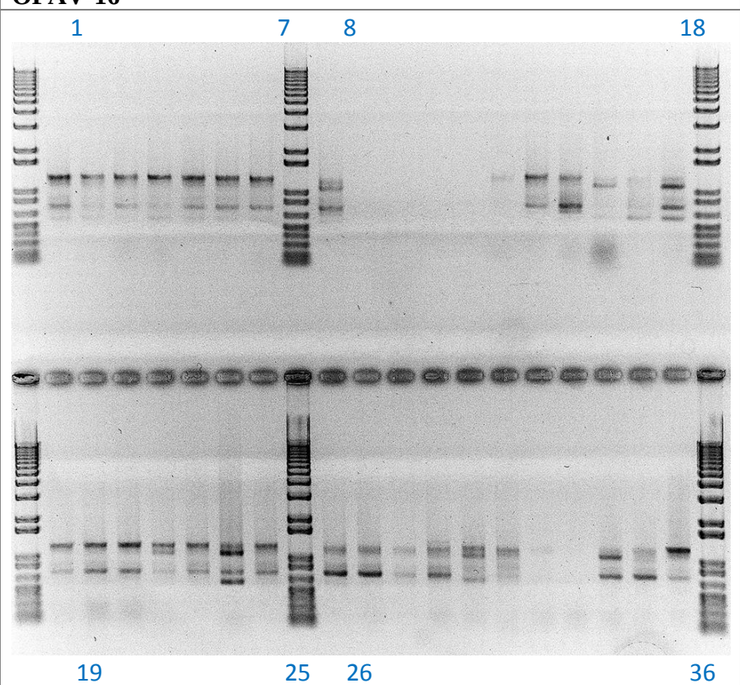
OPAR-10	OPAN-18	OPAB-13
		
OPAR-09	**OPAV-16**	
		

Table 47 Binary Data Table obtained from the RAPD analyses.

			OPAN-14																		OPAN-20																		OPAR-08								
Lane	PFR #	Taxon	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	1	2	3	4	5	6	7	8	9
1	EK548	*R. jasminiflorum* ssp. *jasminiflorum*	1	0	0	1	1	0	0	1	0	1	1	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	1	0	-	-	-	-	-	-	-	-	-
2	EK590	*R. jasminiflorum* ssp. *oblongifolium*	1	0	0	1	0	0	0	1	0	1	1	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	1	0	1	0	1	0	1	0	1	0	0	1	0
3	EK612	*R. jasminiflorum* ssp. *jasminiflorum*	1	0	0	0	1	0	0	1	0	1	1	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	1	0	0	0	1	0	1	0	1	0	0	0	0
4	EK645	*R. jasminiflorum* ssp. *oblongifolium*	1	0	0	0	0	0	0	1	0	1	1	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0
5	EK656	*R. jasminiflorum* ssp. *jasminiflorum*	1	0	0	0	1	0	0	1	0	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	1	0	0	0	1	0	1	0	1	0	0	0	0
6	EI153	*R. jasminiflorum* ssp. *jasminiflorum*	0	0	0	1	1	0	1	1	0	1	1	0	0	0	1	0	1	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	1	0	0	0	1	0	0	0	1	0	0	0	0
7	EI154	*R. jasminiflorum* ssp. *jasminiflorum*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0	0	0	0	1	0	1	0	0	1	0	0	0	0	1	0	0	0	0	1	0	0	1	0	0	0	0
8	EK657	*R. majus*	0	1	0	1	0	1	0	1	0	0	1	0	1	0	0	1	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0
9	EK658	*R. majus*	0	0	0	0	0	0	0	1	0	1	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
10	EI157	*R. majus*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0	0	0	0	0	0	1	0	1	0	0	0	0	1	0	0	1	-	-	-	-	-	-	-	-	-
11	EI158	*R. majus*	0	1	1	0	1	0	0	1	0	0	1	0	1	0	0	1	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	1	0	0	1	0	0	1	0	0	0	0	0	0	0
12	EK591	*R. blackii*	0	0	0	1	1	0	0	1	0	1	0	0	0	1	0	1	0	0	1	0	1	0	0	0	0	0	0	0	1	0	0	0	1	0	1	1	0	0	1	0	1	0	0	1	0	0
13	EK592	*R. blackii*	0	0	0	0	1	0	0	1	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	-	-	-	-	-	-	-	-	-	-
14	EK593	*R. blackii*	0	0	0	0	1	0	0	1	0	0	1	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	1	0	0
15	EK613	*R. konori* (Edie Creek form)	0	0	0	0	0	0	0	1	1	0	1	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0
16	EK619	*R. konori* (white form)	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	-	-	-	-	-	-	-	-	-
17	EK618	*R. rarum*	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-	-	-	-	-	-	-	-	-
18	EK655	*R. rarum*	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	1	1	1	1	0	0	1	0	0
19	EK572	*R. viriosum*	0	0	0	1	1	0	0	1	0	0	1	0	0	1	0	1	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0
20	EK589	*R. viriosum* (Mt Finnigan form)	0	0	0	1	1	1	0	1	0	0	1	0	0	1	0	1	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0	0	0	0	0	0	1	0
21	EK604	*R. viriosum*	0	0	0	1	0	0	1	1	0	0	1	0	0	1	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0
22	EK620	*R. viriosum* (Mt Finnigan form)	0	0	0	1	1	0	1	1	0	0	1	0	0	1	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0
23	EK630	*R. viriosum*	0	0	0	1	1	0	0	1	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	1	1	0	1	0	0	1	0
24	EK507	*R. viriosum* (Mt Finnigan form)	0	0	0	0	0	1	0	1	0	0	1	0	0	1	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	1	0	0	1	1	0	1	0	0	0	0
25	EK569	*R. viriosum*	0	0	0	1	0	0	1	1	0	0	0	1	0	0	1	1	1	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0
26	EK574	*R. vitis-idaea*	0	0	0	0	0	1	0	0	1	0	1	0	1	0	0	0	1	0	1	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0
27	EK575	*R. vitis-idaea*	0	0	0	0	0	1	0	0	1	0	1	0	1	0	0	0	1	0	1	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0
28	EK609	*R. christi* (Mt Miap form)	0	0	0	0	0	1	0	0	1	0	0	1	0	1	0	0	1	0	1	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0
29	EK610	*R. christi*	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	1	0	0
30	EK616	*R. superbum*	0	0	0	0	0	1	0	0	0	1	0	0	1	0	0	1	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	1	0	1	0	0	1	0	1	1	0	0	1	0	0
31	EK651	*R. superbum*	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	1	1	0	1	0	0	1	0
32	EK603	*R. gracilentum*	0	0	0	1	1	1	1	0	1	0	0	0	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0
33	EK621	*R. gracilentum* (Mt Miap form)	0	0	0	0	1	0	1	0	0	1	0	1	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	1	0	-	-	-	-	-	-	-	-	-
34	EK614	*R. solitarium*	0	0	0	0	0	1	0	0	0	1	0	1	0	0	1	0	0	1	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	-	-	-	-	-	-	-	-	-
35	EK617	*R. solitarium* (Bulldog Road form)	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	0	1	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	1	0	0	0	1	0	1	0	0	1	0

Lane	PFR #	Taxon	OPAX-12										OPAT-15										
			1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10	11
1	EK548	*R. jasminiflorum* ssp. *jasminiflorum*	1	0	1	1	1	1	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0
2	EK590	*R. jasminiflorum* ssp. *oblongifolium*	0	0	1	1	0	1	0	0	0	0	0	1	0	1	0	1	0	0	0	1	0
3	EK612	*R. jasminiflorum* ssp. *jasminiflorum*	1	0	1	1	1	1	0	0	0	0	0	0	0	1	0	1	0	1	1	0	0
4	EK645	*R. jasminiflorum* ssp. *oblongifolium*	0	0	1	1	0	1	0	0	0	0	0	1	0	1	0	1	0	0	1	0	0
5	EK656	*R. jasminiflorum* ssp. *jasminiflorum*	1	0	0	1	1	1	0	0	0	0	1	0	0	1	0	1	0	0	1	0	0
6	EI153	*R. jasminiflorum* ssp. *jasminiflorum*	1	0	0	1	1	1	0	0	0	0	1	0	0	1	0	1	0	0	1	0	0
7	EI154	*R. jasminiflorum* ssp. *jasminiflorum*	1	0	0	1	1	1	0	0	0	0	1	0	0	1	0	1	1	0	0	0	0
8	EK657	*R. majus*	0	0	1	1	0	0	0	0	0	0	0	1	0	0	0	0	1	0	1	0	0
9	EK658	*R. majus*	0	0	1	1	0	0	0	1	1	0	0	0	0	1	1	0	0	1	1	0	0
10	EI157	*R. majus*	0	1	1	1	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0
11	EI158	*R. majus*	0	1	1	1	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0
12	EK591	*R. blackii*	0	0	1	1	0	1	0	0	0	1	0	1	0	0	0	1	1	0	0	0	0
13	EK592	*R. blackii*	0	0	1	1	0	0	1	0	0	0	0	1	0	0	0	0	0	1	0	0	0
14	EK593	*R. blackii*	0	0	1	1	1	0	1	0	0	0	0	1	0	0	1	1	0	1	0	0	0
15	EK613	*R. konori* (Edie Creek form)	0	0	1	1	1	0	0	0	0	1	0	0	0	0	1	1	0	0	1	0	0
16	EK619	*R. konori* (white form)	0	0	1	1	1	1	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0
17	EK618	*R. rarum*	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1
18	EK655	*R. rarum*	0	0	1	1	1	0	0	0	0	0	0	0	0	0	1	1	0	0	1	0	1
19	EK572	*R. viriosum*	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0
20	EK589	*R. viriosum* (Mt Finnigan form)	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
21	EK604	*R. viriosum*	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
22	EK620	*R. viriosum* (Mt Finnigan form)	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
23	EK630	*R. viriosum*	0	0	1	1	0	0	0	0	0	0	0	1	0	0	1	1	0	0	1	0	0
24	EK507	*R. viriosum* (Mt Finnigan form)	0	0	1	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1
25	EK569	*R. viriosum*	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
26	EK574	*R. vitis-idaea*	0	0	1	1	0	1	0	1	0	0	0	1	0	0	1	0	1	0	1	0	0
27	EK575	*R. vitis-idaea*	0	0	1	1	0	1	0	1	0	0	0	1	0	0	1	0	1	0	1	0	0
28	EK609	*R. christi* (Mt Miap form)	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0
29	EK610	*R. christi*	0	0	1	0	1	0	0	0	0	0	0	1	1	0	1	1	0	0	0	0	0
30	EK616	*R. superbum*	0	0	1	1	0	1	0	0	0	0	0	0	0	1	1	1	0	0	1	0	0
31	EK651	*R. superbum*	0	0	1	1	1	1	0	0	1	0	1	0	1	0	0	1	0	1	1	0	0
32	EK603	*R. gracilentum*	0	0	1	1	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	0	0
33	EK621	*R. gracilentum* (Mt Miap form)	0	0	1	1	0	1	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0
34	EK614	*R. solitarium*	0	0	1	1	0	1	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0
35	EK617	*R. solitarium* (Bulldog Road form)	0	0	1	1	0	1	1	0	0	0	0	0	0	1	0	0	1	0	0	0	0

Lane	PFR #	Taxon	OPAX-20																				
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1	EK548	*R. jasminiflorum* ssp. *jasminiflorum*	0	0	0	1	0	1	0	1	0	0	0	1	0	0	0	0	1	0	0	0	1
2	EK590	*R. jasminiflorum* ssp. *oblongifolium*	1	0	0	1	0	0	0	1	0	0	1	0	1	0	0	0	1	0	0	0	0
3	EK612	*R. jasminiflorum* ssp. *jasminiflorum*	0	1	0	1	0	1	0	1	0	0	0	1	0	0	0	0	1	0	0	1	1
4	EK645	*R. jasminiflorum* ssp. *oblongifolium*	1	0	0	1	0	0	1	1	0	0	1	0	1	0	0	0	1	0	0	1	0
5	EK656	*R. jasminiflorum* ssp. *jasminiflorum*	0	1	0	1	0	1	0	1	0	0	0	1	0	0	0	0	1	0	0	1	1
6	EI153	*R. jasminiflorum* ssp. *jasminiflorum*	0	1	0	1	0	1	0	1	0	0	0	1	0	0	0	0	1	0	0	1	1
7	EI154	*R. jasminiflorum* ssp. *jasminiflorum*	0	1	0	1	0	1	0	1	0	0	0	1	0	0	0	0	1	0	0	1	1
8	EK657	*R. majus*	0	0	0	1	0	0	0	0	1	0	0	0	1	0	0	0	1	0	0	0	1
9	EK658	*R. majus*	0	0	0	1	0	0	1	0	1	0	1	0	0	1	1	0	0	1	0	0	1
10	EI157	*R. majus*	0	0	0	0	0	0	1	0	1	0	0	0	1	0	0	0	0	0	0	0	1
11	EI158	*R. majus*	0	0	0	1	0	0	0	0	1	0	0	0	1	0	0	1	0	0	0	0	1
12	EK591	*R. blackii*	0	0	0	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0
13	EK592	*R. blackii*	0	0	0	1	0	1	0	0	0	0	0	0	1	0	0	1	1	0	0	0	0
14	EK593	*R. blackii*	1	0	0	1	0	1	0	0	0	0	0	0	1	0	0	1	1	0	0	0	0
15	EK613	*R. konori* (Edie Creek form)	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	1	1	0	0	1	0
16	EK619	*R. konori* (white form)	0	0	0	1	1	1	0	0	0	0	1	0	1	1	1	0	0	1	0	0	0
17	EK618	*R. rarum*	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1
18	EK655	*R. rarum*	0	0	1	0	1	1	0	0	1	0	0	0	1	0	0	0	1	0	0	1	1
19	EK572	*R. viriosum*	0	0	1	1	1	0	0	0	1	0	0	1	1	0	1	0	1	0	0	1	0
20	EK589	*R. viriosum* (Mt Finnigan form)	1	0	1	1	1	1	0	1	0	0	0	1	0	0	1	0	1	0	0	1	0
21	EK604	*R. viriosum*	1	1	1	1	1	1	0	1	0	0	0	1	0	0	1	0	1	0	0	1	0
22	EK620	*R. viriosum* (Mt Finnigan form)	0	0	1	1	1	1	0	1	0	0	0	1	1	0	0	0	0	1	0	0	0
23	EK630	*R. viriosum*	0	0	1	1	1	1	0	0	0	0	1	1	0	0	0	0	1	0	0	1	0
24	EK507	*R. viriosum* (Mt Finnigan form)	0	1	0	1	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	1
25	EK569	*R. viriosum*	0	0	1	1	1	1	0	0	0	0	0	1	0	0	0	0	1	0	0	1	1
26	EK574	*R. vitis-idaea*	0	0	0	1	1	1	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0
27	EK575	*R. vitis-idaea*	0	0	0	1	1	0	0	0	0	0	0	0	0	1	0	0	1	0	1	0	0
28	EK609	*R. christi* (Mt Miap form)	0	0	1	0	1	1	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0
29	EK610	*R. christi*	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0
30	EK616	*R. superbum*	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	1	0	1	0	1	0
31	EK651	*R. superbum*	0	0	0	1	0	0	0	0	0	1	1	0	0	1	0	0	1	0	0	1	0
32	EK603	*R. gracilentum*	0	0	0	1	0	0	0	0	0	1	0	0	0	1	0	0	1	0	0	1	0
33	EK621	*R. gracilentum* (Mt Miap form)	0	0	0	0	1	0	1	0	0	0	0	0	0	1	0	0	1	0	0	0	0
34	EK614	*R. solitarium*	0	0	0	1	1	0	0	1	0	0	0	0	0	1	0	0	1	0	0	0	0
35	EK617	*R. solitarium* (Bulldog Road form)	0	0	0	1	1	0	0	1	0	0	0	0	0	1	0	0	1	0	0	0	0

			OPAR-10					OPAN-18								OPAB-13						OPAR-09									OPAV-16			
Lane	PFR #	Taxon	1	2	3	4	5	1	2	3	4	5	6	7	8	1	2	3	4	5	6	1	2	3	4	5	6	7	8	9	1	2	3	4
1	EK548	*R. jasminiflorum* ssp. *jasminiflorum*	1	0	0	0	0	1	0	0	0	0	0	0	1	1	1	0	1	0	0	-	-	-	-	-	-	-	-	-	1	1	0	0
2	EK590	*R. jasminiflorum* ssp. *oblongifolium*	1	0	1	0	0	0	0	0	1	1	0	0	0	1	1	0	1	0	0	-	-	-	-	-	-	-	-	-	1	0	0	0
3	EK612	*R. jasminiflorum* ssp. *jasminiflorum*	1	0	0	0	0	1	0	1	0	0	0	0	0	1	1	0	1	0	0	-	-	-	-	-	-	-	-	-	1	0	0	0
4	EK645	*R. jasminiflorum* ssp. *oblongifolium*	1	0	1	0	0	0	0	0	0	1	0	1	0	1	1	0	1	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0
5	EK656	*R. jasminiflorum* ssp. *jasminiflorum*	1	0	0	0	0	1	0	1	0	0	0	0	1	1	1	1	1	0	0	-	-	-	-	-	-	-	-	-	1	1	0	0
6	EI153	*R. jasminiflorum* ssp. *jasminiflorum*	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0	0	-	-	-	-	-	-	-	-	-	1	1	0	0
7	EI154	*R. jasminiflorum* ssp. *jasminiflorum*	1	0	0	0	0	1	0	1	0	0	0	0	1	1	1	1	1	0	0	-	-	-	-	-	-	-	-	-	1	1	0	0
8	EK657	*R. majus*	1	1	1	0	0	0	1	1	1	0	0	1	0	1	1	0	1	0	1	1	0	0	1	0	1	0	0	0	0	1	1	0
9	EK658	*R. majus*	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	1	0	0	0	0	0	1	0	0	0	1	0	-	-	-	-
10	EI157	*R. majus*	0	0	0	0	0	0	0	0	0	0	0	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	EI158	*R. majus*	0	1	0	0	0	0	1	1	1	0	1	0	0	1	1	0	1	0	0	1	0	0	0	0	1	0	0	0	-	-	-	-
12	EK591	*R. blackii*	0	1	1	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-
13	EK592	*R. blackii*	0	1	0	0	0	0	0	0	0	0	1	1	0	-	-	-	-	-	-	0	0	0	1	0	0	0	0	0	-	-	-	-
14	EK593	*R. blackii*	0	1	0	0	0	0	1	1	0	1	0	1	0	1	1	0	1	0	0	0	0	0	1	0	1	0	0	0	1	1	0	0
15	EK613	*R. konori* (Edie Creek form)	0	0	0	0	0	0	0	0	0	1	0	1	0	1	1	0	1	0	0	1	0	0	0	0	0	0	0	1	1	1	0	0
16	EK619	*R. konori* (white form)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	-	-	-	-	-	-	-	-	-	0	0	1	0
17	EK618	*R. rarum*	0	0	0	0	0	0	0	0	1	0	0	0	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0	0	1
18	EK655	*R. rarum*	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1	0	1	0	0	1	0	1	1	0	0	0	0	0	0	1	1	1
19	EK572	*R. viriosum*	0	1	0	0	0	0	0	1	0	0	0	0	0	1	0	0	1	0	0	-	-	-	-	-	-	-	-	-	1	1	0	0
20	EK589	*R. viriosum* (Mt Finnigan form)	0	1	0	0	0	0	1	1	0	1	0	0	0	1	0	0	1	0	0	1	0	0	0	0	0	0	0	0	1	1	0	0
21	EK604	*R. viriosum*	0	1	0	0	0	0	1	1	0	1	0	0	0	1	0	0	1	0	0	1	0	0	0	0	0	0	0	0	1	1	0	0
22	EK620	*R. viriosum* (Mt Finnigan form)	0	1	0	0	0	0	1	1	0	1	0	0	0	1	0	0	1	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0
23	EK630	*R. viriosum*	0	0	1	0	0	0	0	0	1	0	0	0	0	1	1	0	1	1	0	1	0	0	0	0	0	0	0	0	1	1	0	0
24	EK507	*R. viriosum* (Mt Finnigan form)	1	0	0	0	0	0	0	0	0	0	1	0	0	1	1	0	1	0	0	1	0	0	0	0	0	0	0	0	0	1	1	1
25	EK569	*R. viriosum*	1	0	1	0	0	0	0	0	0	1	0	0	0	-	-	-	-	-	-	1	0	0	0	0	0	0	0	0	1	1	0	0
26	EK574	*R. vitis-idaea*	1	0	0	0	0	0	0	0	0	0	0	0	0	-	-	-	-	-	-	0	0	0	0	0	0	0	1	0	0	1	0	0
27	EK575	*R. vitis-idaea*	1	0	0	1	0	0	0	0	0	0	0	1	1	-	-	-	-	-	-	0	0	0	0	0	1	0	1	1	0	1	0	0
28	EK609	*R. christi* (Mt Miap form)	0	0	0	0	0	0	0	0	0	0	0	0	0	-	-	-	-	-	-	1	0	0	0	0	0	0	0	0	-	-	-	-
29	EK610	*R. christi*	1	0	0	0	1	0	0	0	0	1	0	0	0	-	-	-	-	-	-	0	0	1	0	1	0	0	0	0	0	1	0	0
30	EK616	*R. superbum*	0	0	1	0	0	0	0	0	0	1	0	0	0	-	-	-	-	-	-	1	0	0	0	0	0	1	0	0	1	0	1	0
31	EK651	*R. superbum*	0	0	0	0	0	0	1	0	1	0	0	1	1	-	-	-	-	-	-	0	0	0	0	0	1	0	0	0	-	-	-	-
32	EK603	*R. gracilentum*	0	0	0	0	0	0	1	0	0	0	0	0	0	-	-	-	-	-	-	0	0	1	0	1	0	0	0	0	-	-	-	-
33	EK621	*R. gracilentum* (Mt Miap form)	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	1	0	0	0	0	-	-	-	-
34	EK614	*R. solitarium*	0	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0
35	EK617	*R. solitarium* (Bulldog Road form)	0	1	0	0	0	0	0	0	1	0	0	1	0	1	1	0	1	0	0	-	-	-	-	-	-	-	-	-	0	0	0	0

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