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**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

**Doctor of Philosophy
in
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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 *rpb2i* 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 *rpb2i* 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 *Rhododendron* 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 *Rhododendron* 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.

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هُوَ الَّذِي خَلَقَ لَكُمْ مِمَّا 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

Fayaz, A (2008) **Assessing the role of New Zealand in the *ex situ* conservation of vireya rhododendrons.** *Bulletin of the New Zealand Rhododendron Association* **96**: 28–30.

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

atpB – 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

matK – 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

rbcL – ribulose 1,5-bisphosphate carboxylase/oxygenase (RuBisCo) large subunit

rDNA – ribosomal DNA

RI – Retention Index

rpb2i – 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

trnF – transfer RNA Phenylalanine

trnK – transfer RNA Lysine

trnL – 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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