Morphological and Genetic Diversity of the Fern Genus *Polystichum* Roth (Dryopteridaceae) in New Zealand.

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

Doctor of Philosophy
in
Plant Biology

at Massey University, Palmerston North,
New Zealand.

Leon Richard Perrie

December 2001

Abstract.

Two morphologically variable taxa in the fern genus *Polystichum* Roth (Dryopteridaceae) from New Zealand were investigated using a combination of morphological, cytological, and molecular (AFLP DNA-fingerprinting) analyses to test the null hypothesis that each constituted only a single evolutionary lineage. In this study, lineages for which there was prospective evidence (looking to the future; eg. inference of Specific Mate Recognition System, or SMRS, differentiation) of assortative fertilisation were recognised as distinct species. Lineages for which there was only retrospective evidence (looking to the past; eg. character state variation) of assortative fertilisation were recognised at the subspecific level.

Polystichum richardii (Hook.) J. Smith was shown to be an allopolyploid complex of four evolutionary lineages, with two tetraploid and two allo-octoploid lineages. The new combination *P. wawranum* (Szyszyl. in Wawra) comb. nov. is proposed for one of the tetraploids, with the name *P. oculatum* (Hook.) J.B. Armstr. reinstated for the other. The two octoploids, which are allopatric, are recognised as separate subspecies under the reinstated name *P. neozelandicum* Fée, of which the name *P. richardii* is a later synonym. The new combination *P. neozelandicum* subsp. *zerophyllum* (Colenso) comb. et stat. nov. is proposed for the southern octoploid lineage.

The same methodology confirmed that *P. silvaticum* (Colenso) Diels and *P. vestitum* (G. Forst.) C. Presl should be recognised as separate species, but did not indicate that morphologically 'divergent' plants from the Chatham Islands comprise a separate lineage from the remainder of *P. vestitum.* Consequently, *P. vestitum* is retained as a single, albeit morphologically variable species.

Also investigated was the genetic relationship of the New Zealand species of *Polystichum* to their geographically closest congeners from Australia and Lord Howe Island using AFLP DNA-fingerprinting and DNA sequences from the chloroplast. These data are consistent with the hypothesis that the ecologically diverse New Zealand species of *Polystichum* were derived from a single trans-Tasman disjunction event (between New Zealand, and Australia/Lord Howe Island), with long-distance dispersal implicated rather than vicariance.

Acknowledgements.

My initial thanks must be to my supervisors, Mark Large, Peter Lockhart, and Patrick Brownsey, without whom I would not have had the fantastic opportunity to tackle this work. Their guidance kept me on track, and from each I have learnt a great deal.

Personal financial assistance during my time at Massey came from a Massey Doctoral Scholarship, a William Georgetti Scholarship, a Freemason's Postgraduate Scholarship, a J.P. Skipworth Scholarship, and funding from the Foundation for Research, Science and Technology through Te Papa's program of Biosystematics of vascular plants (Contract MNZX0001).

My molecular 'skills' are indebted to Richard Winkworth and Trish McLenachan. I also thank Karen Stöckler, Carmel Gillman, and Robbie Lewis for help in the laboratory, and Lorraine Berry at MUSEQ. I am also appreciative of advice and support from several others here at Massey with evolutionary interests, namely the encyclopaedic Matt Phillips, David Penny, Barbara Holland, Ant Poole, Dan Jeffares, Nicole van Maltzahn, Vernon Trainor, Abby Harrison, Mike Hendy, Dave Lambert, and the Molecular Ecology group. David Barrington (University of Vermont) provided insights into *Polystichum* and access to unpublished *rbcL* sequence data. Ray Cranfill (University of California) provided primer sequences for the *rps4-trnS* region.

Life in IMBS was made much easier by assistance from Paul Hocquard, Mike Wilson (damn computers), David McSweeney, Liz Nickless (microscopes), and Catherine Norman.

I must thank the many people who have collected samples for me; John Armstrong, D. Bergstrom, Tony Dugdale, Lloyd Esler, Roland Forster, Reijel Gardiner, David Glenny, David Havell, Sheryl Hamilton, Dave King, Members of the Nelson Fern Society, Merilyn Merret, W. Misiak, Barbara Parris, Brian Rance, Chrissy Ryan, John Smith-Dodsworth, J.C. Stahl, Steve Wagstaff, Carol West, Gina Williams, Alan Wiltshire, Maureen Young, and especially, Elizabeth Brown for Australian and Lord Howe Island samples, Geoff Walls for fantastic help on the Chatham Islands, and Bruce Clarkson for introducing me to *Polystichum richardii*. I am also grateful to staff from the Department of Conservation for assistance with collection permits, and to the curators of the AK, CHR, WAIK, and WELT herbaria.

I thank my family for their support during my university years. And finally, I must acknowledge Lara Shepherd for her love, support, tolerance and understanding. I am sure that seldom has anyone had so many trips interrupted by their partner 'needing' to look at the scales of any *Polystichum* plants encountered as Lara has had to endure over the last few years.

Table of Contents.

Chapter One: Introduction.

Chapter Two: Species as Prospective and Retrospective Evolutionary Lineages.

Chapter Three: Materials and Methods.

Chapter Four: The Dissolution of *Polystichum richardii* (Hook.) J. Smith: the *P. neozelandicum* Fée Complex.

Chapter Five: *Polystichum vestitum* (G. Forst.) C. Presl on the Chatham Islands.

Chapter Six: Origins of the New Zealand *Polystichum* Species as Inferred from Genetic Relationships.

Chapter Seven: Summary, Conclusions, and Discussion.

List of Tables.

List of Boxes.

List of Figures.

Chapter One

Introduction.

1.1 The Genus *Polystichum* Roth.

The genus *Polystichum* (from the Greek *polys*, 'many,' and *stichos*, 'a row or line' – an illusion to the rows of sori) was established by Roth in 1799 for twelve species of ferns, some of which are now regarded as only distantly related. Indeed, only the generic type, *P. lonchitis* (L.) Roth, and *P. aculeatum* (L.) Roth are still retained in the genus (Farr et al. 1979). Since its initial description the circumscription of *Polystichum* has changed greatly and often. W.J. Hooker (1863), for instance, reduced *Polystichum* to a section of the genus *Aspidium* Sw. (actually a later synonym of *Tectaria* Cav.). This view was widely accepted, and many taxa now ascribed to *Polystichum* were first described in the genus *Aspidium*, including many of the New Zealand taxa. Diels (1899) and Christensen (1905) reinstated *Polystichum* at the generic level, although their broad definitions encompassed species now ascribed to distinct genera such as *Rumohra* Raddi and *Lastreopsis* Ching.

Indeed, the exact circumscription of *Polystichum* is still contested today, with debate centred on whether several small segregates like *Cyrtomium* C. Presl and *Phanerophlebia* C. Presl should be subsumed within *Polystichum* or treated as separate genera (Yatskievych et al. 1988, Kramer 1990, Yatskievych 1996). Ferns of the genus *Polystichum* are characterised by their terrestrial habit, fastigiate fronds with inequilateral pinnae, and (when present) peltate indusia (Barrington 1989). The latter character state distinguishes *Polystichum* from the closely related genera *Dryopteris* Adans., *Arachniodes* Blume, *Ctenitis* C. Chr., and *Lastreopsis*, which have reniform indusia (when present). These genera, together with *Polystichum*, the extensively creeping, often epiphytic *Rumohra*, and numerous small segregates, constitute a (probably monophyletic) 'dryopteroid' assemblage of the large and diverse family Dryopteridaceae Ching. This 'dryopteroid' group is largely equivalent to the subfamily Dryopteridoideae of Kramer (1990), but with the inclusion of *Elaphoglossum* Schott ex J. Sm., and the exclusion of *Tectaria* Cav. plus segregates.

No recent study has focused explicitly on the relationships of the Dryopteridaceae *s.l.*, whose circumscription Smith (1995 p.120) considered the "subject of perhaps the greatest disagreement among fern systematists." Stevenson and Loconte (1996 p.438) acknowledged the Dryopteridaceae as the "largest family of leptosporangiate ferns," but included only four of its genera in their cladistic analysis of fern morphology. The study by Hasebe et al. (1995; also see Pryer et al. 1995) of *rbcL* sequence variation included more representatives from the Dryopteridaceae and indicated that, in its common broad circumscription (eg. Brownsey et al. 1985, Kramer 1990), it was polyphyletic. For instance, the 'athryoid' ferns (eg. *Athyrium* Roth, *Diplazium* Sw., and *Deparia* Hook. et Grev.) may be more closely related to the Thelypteridaceae Pichi-Serm., while the 'dryopteroid' (see above) assemblage were most allied to a group comprising 'oleandroid' (eg. *Oleandra* Cav., and *Arthropteris* J. Sm. ex Hook. f.), 'davalloid' (eg. *Davallia* Sm., and *Nephrolepis* Schott) and 'polygrammatoid' (eg. *Grammitis* Sw., and *Polypodium* L.) taxa.

Within *Polystichum* itself, scales from the stipe, rachis and lamina have often been found to provide taxonomically useful character states (Wagner 1979, Barrington 1989). Indeed, the infrageneric classification of Japanese *Polystichum* by Daigobo (1972) was based largely on the 'microscales' occurring at the abaxial terminus of lamina veinlets. However, Wagner (1979) has questioned the utility of a hierarchical, intra-generic classification for *Polystichum*. This is because reticulate evolution, in the form of allopolyploidy, is thought to be common (Sleep & Reichstein 1967, Wagner 1979, Barrington 1985, Soltis et al. 1991), and is believed to occur even between species not considered closely related (Barrington 1990).

In that many allopolyploid species are widespread, combined with the often-frequent formation of (usually sterile) F_1-hybrids (eg. Mayer & Mesler 1993; see also Mullenniex et al. 1999), species' boundaries in *Polystichum* have often been obscured (Barrington 1985, Kramer 1990, Roux 2000). Further compounding the global understanding of *Polystichum* is the lack of detailed taxonomic study in many parts of its distribution. This is manifested in Kramer's (1990) rather broad estimate of 180-230 species worldwide (for *Polystichum* including *Cyrtomium* and *Phanerophlebia*). The distribution of *Polystichum* is subcosmopolitan, being most common and speciose in the

mountains of tropical and warm-temperate areas, sparse (in terms of species numbers) in temperate lowlands, and rare or absent in tropical lowlands (Kramer 1990). Barrington (1989) lists Latin America and eastern Asia as the two principal centres of diversity.

1.2 *Polystichum* in New Zealand.

Recent works (eg. Brownsey et al. 1985, Brownsey 1988, Brownsey & Smith-Dodsworth 1989, 2000) have recognised seven species of *Polystichum* in New Zealand. The three species *P. proliferum* (R. Br.) C. Presl, *P. setiferum* (Forssk.) Woyn., and *P. lentum* (D. Don) T. Moore are all considered adventive (from Australia, Europe and the Himalayas, respectively). None of these species is widespread in New Zealand, and their proliferous bulbils easily distinguish them from New Zealand's native species (see Brownsey 1988 for further details).

The four native species are all considered endemic to New Zealand. Two of the endemic species, *P. richardii* (Hook.) J. Smith and *P. vestitum* (G. Forst.) C. Presl, are more common and widespread than the others. They are, seemingly, also morphologically more variable, and are the respective foci of Chapters Four and Five of this thesis. *P. silvaticum* (Colenso) Diels is unquestionably allied to *P. vestitum*, and consequently is also discussed in Chapter Five.

The remaining endemic species, *P. cystostegia* (Hook.) J.B. Armstr., is not explicitly considered in this thesis, although its relationship to the other New Zealand species is discussed in Chapter Six. It is one of the few ferns to occur in the alpine regions of New Zealand, where it favours rocky habitats. *P. cystostegia* is relatively common throughout the high-altitude areas of the South Island, but in the North Island it is restricted to Mount Taranaki. It is also found on Stewart Island, the Auckland Islands and Campbell Island. *P. cystostegia* can be distinguished from the other New Zealand species by its inflated indusia, large orange-brown scales, and its alpine habitat (*P. vestitum* extends into the alpine zone, but only sparsely so).

1.3 Thesis Objectives and Structure.

The primary objective of this thesis was to investigate species boundaries within the widespread and morphologically variable *P. richardii* and *P. vestitum* using a combination of morphological and molecular analyses. Studies purporting to delimit species should, by necessity, outline the criteria used. Consequently, the issue of species delimitation is discussed in Chapter Two, together with an outline of the approach adopted for this study. The morphological, cytological and molecular methodology used in this thesis to implement this approach to species delimitation is described in Chapter Three.

The results and consequent taxonomic revision from the investigation of morphological and molecular variation in *P. richardii* are reported in Chapter Four (a paper, Perrie et al. 2000, describing earlier work with this taxon is provided in Appendix One). Similarly, Chapter Five presents the results of a parallel study in *P. vestitum.* Chapter Six places these results within the context of the overall relationships between all of the *Polystichum* ferns from the south-west Pacific region (New Zealand, Australia, and Lord Howe Island) as inferred from a molecular investigation.

Chapter Seven summarises the major findings of this thesis. In addition, a revised morphological key to the New Zealand species of *Polystichum* is presented, and possible directions for future work are outlined.

To facilitate cross-referencing each chapter is divided into numbered sections. For instance, Section 4.3.2 denotes sub-part 2 of part 3 of Chapter Four. The appendices are similarly numbered and subdivided.

The details of all samples collected for this study and analysed in some way (ie. morphological, molecular, cytological and/or distribution mapping) are provided in Appendix 2.1. Each such sample is denoted with a unique collection code in the format *tXxxN* (eg. *vPoh7*, *rKar12*; see Appendix 2.1 for an explanation of the collection code). Specimens of all samples included in the molecular and cytological analyses, together with representative specimens from the morphological analysis will be lodged in the

AK, CHR, MPN, and WELT herbaria. Specimens from AK, CHR and WELT used in the construction of distribution maps are listed in Appendix 2.2.

Herbarium abbreviations follow Holmgren et al. (1990). Authority abbreviations follow Brummitt and Powell (1992).

1.4 Nomenclatural Changes and Lectotypifications.

The results presented in this thesis will require several changes in nomenclature. These are the transfer of *Aspidium wawranum* Szyszyl. in Wawra to the genus *Polystichum*, and the establishment of an epithet at the subspecific level under *Polystichum neozelandicum* Fée. For clarity and also continuity with future publications, the new combinations of *P. wawranum* (Szyszyl. in Wawra) comb. nov. and *P. neozelandicum* Fée subsp. *zerophyllum* (Colenso) comb. et stat. nov. are used throughout this thesis. However, inasmuch as this thesis constitutes an unpublished body of work, the usage here of these new combinations does not, nor is it intended to, constitute their valid publication. Rather they should be viewed as recommendations that have yet to be properly established.

The lectotypification of *Aspidium richardii* Hook., *Aspidium oculatum* Hook., *A. zerophyllum* Colenso, *Polypodium vestitum* G. Forst., *Polystichum venustum* Hombr., *A. pulcherrimum* Colenso, *A. perelegans* Colenso and *A. waikarense* Colenso is discussed. Again, the conclusions reached within this context should be viewed as unpublished recommendations.

Chapter Two

Species as Prospective and Retrospective Evolutionary Lineages.

2.1 Species as Evolutionary Lineages.

2.1.1 An Evolutionary Basis for the Description of Biodiversity.

The variation in the biological world can be partitioned in any number of ways, the resultant units of which are often equated with the taxonomic category of 'species.' For instance, it is well appreciated that morphological variation is discontinuous (eg. Dobzhansky 1941, Schemske 2000, Turelli et al. 2001 p.336). Living organisms cluster into morphologically similar groups, which are in turn dissimilar from other such groups; each such cluster could be recognised as a species. A similar case could be made for ecological or genetic variation. It is also possible to partition the living world according to much more esoteric criteria (eg. assigning a given individual to a species depending on what day it was first encountered) (Ridley 1996).

These different criteria do not necessarily result in congruent partitions (Mishler & Donoghue 1982, Ridley 1996, Shaw 1998). Therefore, given the range of possibilities and the potential for conflict, what aspect of the biological world should be emphasised in the formal naming, description and classification (ie. the taxonomic scheme) of its constituent entities? Such naming and description is essential for the study of natural diversity, as it facilitates communication of both the entities themselves and of phenomena pertaining to them (eg. Mayr 1982 p.297, Stuessy 1990).

It is now routine for the classification of higher-level biological entities (ie. genera, families etc.) to be explicitly based on their (reconstructed) evolutionary history (Ridley 1986, 1996). This emphasis on evolutionary history entails several advantages, one of which is of course the better facilitation of evolutionary study! (Donoghue & Cantino 1988). More notably, however, is the presumption of a single history of life, or one 'true' phylogeny. Evolution, be it bifurcating or reticulate, has occurred only once (Hull

1999). This represents a real, unambiguous, 'objective' property of life, and provides an external standard by which to evaluate alternative hypotheses (Donoghue & Cantino 1988, Ridley 1996). Schemes based on morphological and/or ecological similarity, although common in traditional taxonomy, lack such external reference points. Similarity can be measured in multiple, often equally-valid ways (producing different results), and is therefore ambiguous, or 'subjective' (Ridley 1996).

It would also seem advantageous for classification purposes if the 'species' category was similarly reflective of evolutionary history, with the ensuing gain of making species delimitation also objective in the sense described above. A minimum requirement of entities to be ascribed to the species category could then be that they constitute distinct (separate and independent) evolutionary lineages. This usage of evolutionary 'independence,' or 'separation,' in this chapter is made explicitly in a genealogical context, such that the genomes related primarily by divergent means could be considered to represent evolutionary independent, or separate lineages.

Requiring, at a minimum, that species be separate evolutionary lineages would place the species category on the divergent side of the divergent-reticulate boundary of interrelationships between organisms. This appears to be one of the few viewpoints broadly agreed upon by protagonists in the 'species debate' (see de Queiroz 1998). Indeed, de Queiroz (1998 p.60) has contended that "all modern species definitions either explicitly or implicitly equate species with segments of population level evolutionary lineages." He termed this apparent consensus, the 'General Lineage Concept of Species,' and viewed the disparity between modern concepts as a reflection of the different criteria used to recognise lineages.

As noted above, separate lineage status might be regarded as a minimum, but not necessarily sufficient, requirement for a group of organisms to be categorised as a 'species.' This point is discussed further in Section 2.4, and relates, at least in part, to the conceptual distinction made below (Section 2.1.4) between sexually-outcrossing and non-sexually-outcrossing organisms. A species will then, by definition, comprise one or more lineages, but not all lineages need necessarily be categorised as a species. Delimiting species could then be viewed as a two step process: (1) delimit separate evolutionary lineages; and then (2) delimit these evolutionary lineages into species taxa.

The partitioning of the living world into lineages will not encapsulate all biological variation. While some characters (including morphological, genetic and ecological) will exhibit variation congruent with lineage boundaries, with this congruence facilitating lineage identification, other characters may of course vary within lineages. Such intra-lineage variation is not necessarily less 'interesting' than inter-lineage variation. Nevertheless, it is the latter that this chapter will focus on, given the view outlined above that delimiting evolutionary lineages provides an objective basis for the subsequent taxonomic categorisation of species.

2.1.2 Species Concepts Terminology.

There is no shortage of species concepts. Mayden (1997; see also Hey 2001), for instance, listed a total of 22, to which several others can subsequently be added (eg. Levin 2000). Over twenty years ago, Wiley (1978 p.17) wrote that "in all probability more paper has been consumed on the questions of the nature and definition of the species than any other subject in evolutionary and systematic biology." This quote may well still hold true today, especially with the recent publication of several books (eg. Claridge et al. 1997, Wilson 1999, Wheeler & Meier 2000) and an entire issue of the journal *Trends in Ecology & Evolution* (eg. Turelli et al. 2001) devoted to the topic.

The relevant literature is certainly vast, and the sheer number of different concepts, meanings and ideas appears to be a significant source of confusion and disagreement in the debate over 'what is a species?' For instance, aspects of the different 'phylogenetic' concepts are often, but seemingly inadvertently, blended together to yield some sort of confused 'hybrid' for which there is no previous literature background (eg. Avise 1994 p.253, Mallet 1995, Vrba 1995). Paterson (1981 p.113) has pointed out that "when an author glides imperceptibly from talking of species under one concept to talking of species in accordance with another and distinct concept, a subtle kind of nonsense is generated, which is exceedingly difficult to detect."

In an attempt to surmount such conflation, de Queiroz (1998) has proposed a standardised terminology. However, for the sake of continuity with the remainder of the literature, the traditional names of several popular concepts are retained. Nevertheless, de Queiroz's (1998 p.65) caveat that "general adjectives such as

'biological,' 'evolutionary,' and 'phylogenetic' ... can be applied to almost all modern species definitions, yet they are most often used to designate small subsets of them," is noted. Such generalised terms are denoted in this chapter with inverted commas.

2.1.3 The 'Evolutionary Species Concepts'.

Some species concepts, namely the 'Evolutionary Species Concepts' (eg. Simpson 1961, Wiley 1978; see de Queiroz 1998 p.67), expressly equate the species category with evolutionary lineages. A recent example is the "characterisation" of Wiley and Mayden (2000 p.73): "An evolutionary species is an entity composed of organisms that maintains its identity from other such entities through time and over space and that has its own independent evolutionary fate and historical tendencies."

In the sense of de Queiroz (1998), Templeton's 'Cohesion Species Concept' is also an 'evolutionary species concept,' in that it defines a species as "an evolutionary lineage whose boundaries arise from the genetic and ecological forces that create cohesive reproductive communities" (Templeton 2001 p.779). Van Valen's 'Ecological Species Concept' – "a species is a lineage (or a closely related set of lineages) which occupies an adaptive zone minimally different from that of any other lineage in its range and which evolves separately from all lineages outside its range" (Van Valen 1976 p.233) – can also be considered an 'evolutionary species concept,' in that it is first and foremost a description of a lineage (de Queiroz 1998).

'Evolutionary species concepts' explicitly emphasise the extension of species as lineages through time (de Queiroz 1998), and implicitly, that species may be held together not only by gene flow but also by developmental, genetic, and ecological constraints (Templeton 1989). However, these concepts are, by themselves, practically impotent, a point recognised by even their proponents (or at least some of them). With no associated criteria for delimiting lineages (or independent evolutionary entities), they lack operationality (Mayden 1997); that is, the ability to be implemented.

This does not mean, however, that they cannot lead to testable consequences (Wiley & Mayden 2000, Templeton 2001). Criteria inherent in other concepts can be used to infer the presence of separate and independent lineages. Mayden (1997) viewed the 'Evolutionary Species Concept' as the primary species concept because of its greater

theoretical significance, but stated that it required bridging concepts to permit the recognition of entities compatible with its intentions.

2.1.4 Sexuality (or lack of...) and the Pluralism of a Lineage Concept.

The idea that species should be distinct evolutionary lineages is applicable to all forms of life, and it therefore has broad generality (Mayden 1997; 'universality' in the parlance of Hull 1997, 1999). This is in contrast to gene-flow orientated concepts (eg. the 'Biological Species Concept' and the 'Recognition Species Concept'), which are applicable only to Sexually Outcrossing (SO) organisms and not Non-Sexually-Outcrossing[1,2] (NSO) organisms. This dichotomy is an important one. In SO organisms lineage independence is countered by reticulation but engendered by divergence. However, the relationships between NSO organisms can only be divergent, such that every NSO organism might be considered an independent lineage.

Consequently, although both SO and NSO organisms may form evolutionary lineages and are thus monistic (or 'acting' in the same manner; see Hull 1997, 1999) in this sense, the way in which they do so is quite different. In SO organisms, lineage formation might be held to occur when relationships between two groups of organisms change from being predominantly reticulate to predominantly divergent. In contrast, lineage formation in NSO organisms accompanies individual propagation. This is pertinent to the point made previously (and discussed further in Section 2.4) that status as a separate lineage might be considered a minimum, but not necessarily sufficient, requirement for categorisation as a 'species.' Otherwise, every single NSO organism, each as an 'independent evolutionary lineage,' might be regarded as a separate species, which is a proposition that most taxonomists would regard as impractical.

Thus, SO and NSO lineages are not identical (Paterson 1981, Masters & Spencer 1989, Baum & Donoghue 1995, Wiley & Mayden 2000). Not only do they form in different ways, but they must be delimited using different criteria and, given that the recognition of every NSO organism as a species would not be practically desirable, they must be accorded taxonomic status in a different manner. In this sense then, the species

[1] The definition here of NSO organisms includes both truly asexual organisms, and sexually-selfing organisms whose genetic-exchange dynamics are more akin to asexual organisms than to SO organisms (Templeton 1989).

[2] As discussed here, this distinction is most applicable to organisms in which reticulation is engendered by meiotic recombination rather than lateral transfer (eg. multi-cellular eucaryotic organisms, rather than procaryotic organisms; see Lan & Reeves 2000 for a somewhat analogous discussion of the latter).

category of any taxonomic scheme encompassing both NSO and SO organisms is necessarily pluralistic (see Mishler & Brandon 1987); that is, the entities accorded to the species category are not strictly comparable.

In this chapter a clear distinction will often be made between SO and NSO organisms (as above). However, it must be emphasised that this is done only for the sake of clarity, and not because it reflects some absolute distinction between the SO and NSO way of reproduction in nature (Templeton 1989, Mishler & Theriot 2000a). Some groups of organisms may be predominantly NSO, but exhibit infrequent SO, and vice versa. Consequently, the ontological differences between SO and NSO organisms cannot simply be resolved by having a separate taxonomic scheme for each because they are not mutually exclusive categories. Similarly, while the approaches to lineage delimitation in SO and NSO organisms are conceptually distinct, in practice they should be taken as being more or less applicable to a group of organisms depending on their predominant mode of reproduction.

2.1.5 Pattern and Process.

An enduring theme throughout the species literature is whether pattern (eg. Nixon & Wheeler 1990, Davis & Manos 1991, Luckow 1995, Goldstein & DeSalle 2000, Wheeler & Platnick 2000c) or process (eg. Schemske 2000) should be given precedence. A simple answer to this debate is neither; pattern and process are so intimately related that both must be considered to render outcomes that are biologically sensible.

A more comprehensive answer is beyond the scope of this chapter, but in brief: processes produce patterns, which in turn beget more processes, *ad infinitum* (de Queiroz & Donoghue 1990, Templeton 2001). This interaction need not only be linear, in that lower order processes may feedback on higher order patterns. Although patterns, in being the physical manifestation of matter, can be directly observed, processes cannot be. Processes are conceptual hypotheses, inferred to be responsible for changes in patterns (in this sense, 'change' also includes the engendering of non-randomness, or the countering of entropy). It is the observation of such changes in pattern that allow the presence of a particular process to be inferred. Although a given change in pattern may conceivably be caused by any number of different processes, some (or even one)

are usually considered more plausible than the others (with such consideration of increased plausibility itself resulting from the scientific 'process').

2.1.6 A Temporal Perspective of Lineages.

If lineages are evolutionarily independent, or separate, groups of individuals, the delimitation of lineages requires the inference of processes (eg. assortative fertilisation) that engender such separation. These processes might be inferred from contemporary (ie. in the present time) observations of 'changes' in pattern(s) (eg. non-random mating behaviour) between two groups of individuals. The inference of such processes could in turn be used to infer that the two groups are independent lineages in a *prospective* sense. That is, they will constitute two separate lineages in the future (if not already).

Alternatively, some 'changes' in pattern(s) (eg. non-random character state variation) may suggest that processes engendering evolutionary independence have been acting in the past[3], irrespective of whether it can be determined they are acting in the present. Groups exhibiting such patterns could be delimited as independent lineages in a *retrospective* sense. That is, it can be inferred that they have acted as separate lineages in the past.

Consequently, lineages may be delimited on a prospective and/or retrospective basis. Prospective delimitation could be criticised for making claims of future knowledge, which is of course unknowable (see O'Hara 1993). However, the same limitation applies to retrospective delimitation, as processes inferred to be operating in the past will not necessarily be so in the present or future (O'Hara 1993). Therefore, lineage delimitation should always be viewed as a hypothesis, defensible by contemporary data but not necessarily so at other points in time.

2.2 Processes Engendering Lineage Separation.

What engenders the 'evolutionary independence' of separate lineages? For NSO organisms this is a 'default' outcome of their purely divergent relationships to one

[3] Although the observation of these patterns is contemporary (by necessity, except where a detailed fossil record is available), the changes to them are believed to have occurred in the past. That is, changes to the pattern are not actually observed in the present time.

another. But for SO organisms, what processes engender primarily divergent rather than reticulate relationships?

There would seem to be many such processes and they are commonly listed as isolating (eg. Dobzhansky 1970 p.314) or cohesion (Templeton 1989 table 2) 'mechanisms.' However, following Williams (1966), Paterson (1981, 1986, 1988; also see Butlin 1987a, de Queiroz 1998, Harrison 1998) has pointed out that 'mechanisms' is a theory laden term, as it implies that the characters engendering isolation and/or cohesion were selected for that principal function. This is certainly not necessarily (if ever) the case for 'isolating' mechanisms (see Paterson 1985). de Queiroz (1998 table 5.2) has provided a recasting of Dobzhansky's isolating 'mechanisms,' using the neutral term of 'barrier.'

Isolating barriers can be partitioned according to whether they act before or after fertilisation; ie. pre-fertilisation barriers and post-fertilisation barriers (which are akin to pre-zygotic and post-zygotic barriers, respectively).

2.2.1 Post-Fertilisation Barriers.

2.2.1.1 Post-Fertilisation Barriers and Species Concepts.

Post-fertilisation barriers are important components of some species concepts. A good example is the 'Biological Species Concept,' whose essence is espoused in Mayr's 1942 (p.120) definition: "Species are groups of actually or potentially interbreeding natural populations, which are reproductively isolated from other such groups." Under the 'Biological Species Concept,' groups of SO individuals that do not (actually or potentially) exchange genes are delimited as species, without regard to whether the barriers to gene-flow are acting pre- or post-fertilisation. This emphasis on reproductive isolation between groups of SO individuals, no matter how it is engendered, led Paterson (1978) to 'rename' the 'Biological Species Concept' as the 'Isolation Species Concept.'

Although 'Ecological Species Concepts' (eg. Van Valen 1976 p.233) are generally lineage-based, they emphasise the importance of ecologically-based processes that bring about natural selection, rather than gene-flow, in engendering the separation of evolutionary lineages (de Queiroz 1998). Ecological barriers operating pre- and post-

fertilisation are included. Templeton's (1989, 2001) 'Cohesion Species Concept' is also lineage-based but includes both reproductive and ecological factors. Again, barriers operating both pre- and post-fertilisation are included.

2.2.1.2 Diversity of Post-Fertilisation Barriers.

Post-fertilisation barriers may have a basis in both reproduction and/or ecology. For instance, 'hybrids' may be of lowered fertility and/or fitness (mediated via the processes leading to natural selection), respectively. Because gene-flow can only occur between two groups of SO individuals via 'hybridisation' between them, a reduction in fertility and/or fitness of any 'hybrids' constitutes a barrier to gene-flow. These post-fertilisation barriers may range in effect from a partial reduction to absolute, where the fertility and/or fitness of 'hybrids' is zero.

2.2.1.3 Negative Heterosis.

Post-fertilisation barriers are important components of many modern species concepts (see above). However, as highlighted by Paterson and his colleagues (eg. Paterson 1978, 1981, 1985, 1988, Lambert & Paterson 1984, White et al. 1990), it is questionable what role, if any, they should play in delimiting species (or lineages) of SO organisms. If only post-fertilisation barriers inhibit gene-flow between two groups of sympatric SO individuals, these groups will either merge or one will go extinct, depending on the severity of the fitness reduction in hybrids between the groups (Paterson 1978, Lambert et al. 1984, Spencer et al. 1987, Masters & Spencer 1989). These scenarios can be collectively termed the 'problem[4] of negative heterosis.'

If the fertility/fitness of the hybrids is zero, then one of the groups (most likely that with fewest individuals) will become extinct via gamete swamping. That is, some individuals from the numerically smallest group will fertilise with individuals from the more numerous group, remembering that the only barriers are operating post-fertilisation. Such events do not reproduce more individuals of the smallest group (but rather hybrids with zero fitness/fertility), whose numbers will then progressively reduce in a positive feedback loop. Alternatively, if the hybrid fertility/fitness is above zero, the groups will merge, with genes being exchanged between them (via the hybrids) and

[4] A 'problem' for the thinking of biologists (particularly those who do not consider it), rather than a value judgement about the process itself.

the cause of the barrier will eventually be eliminated. The outcome is the same whether the post-fertilisation barriers are reproductive or ecological in origin.

Given the above 'problem of negative heterosis,' what utility, if any, are post-fertilisation barriers, be they related to fertility and/or fitness, for delimiting lineages? Groups of SO organisms with only post-fertilisation barriers between them are simply not expected to be able to persist as two differentiated entities if in sympatry. Of course, such groups may survive in allopatric 'refugia.' However, if two groups of SO organisms are existing in sympatry there must be pre-fertilisation barriers to gene-flow between them. That is, they must be fertilising assortatively (ie. intra-group fertilisation occurs more often than expected by chance). Post-fertilisation barriers alone do not give rise to separate evolutionary lineages.

2.2.1.4 Polyploidy as a Post-Fertilisation Barrier.

The demonstration of differences in ploidy level (ie. number of chromosome sets) is often considered sufficient evidence in itself to recognise the two (or more) levels as separate species. Indeed, polyploidy is commonly regarded as an example of 'instantaneous speciation' (eg. Coyne et al. 1988, Ridley 1996, Ramsey & Schemske 1998, Johannesson 2001, Turelli et al. 2001). This is because, if hybridisation subsequently occurs between the different ploidy levels, gene-flow is unlikely as the resultant hybrids will be sterile because of meiotic irregularities (eg. a diploid × tetraploid cross will produce a sterile triploid). In the parlance of the 'Biological Species Concept,' the ploidy levels are reproductively isolated. However, this is a post-fertilisation barrier, with zero hybrid fertility (Paterson 1988). Therefore, in the case of a newly arisen tetraploid, it is likely to be swamped with gametes from its diploid progenitor population, such that it leaves no (or few, which will be faced with the same problem of gamete swamping) fertile tetraploid offspring (Paterson 1981). This specific, ploidy-level example of the 'problem of negative heterosis' has been referred to as 'minority cytotype exclusion' (see Vogel et al. 1999).

Polyploids are commonly documented in nature, so at least some must survive their initial 'problem of negative heterosis,' either by 'escaping' to allopatry (see above) and/or the (incidental) acquisition of pre-fertilisation barriers which engender assortative fertilisation. Such acquisition of novel characteristics may even accompany,

incidentally, the actual polyploid event (Levin 1983, Husband & Schemske 2000, Keller & Gerhardt 2000; see Schwarzbach et al. 2001 in the context of homoploid hybridisation).

In summary, a difference in ploidy level is a post-fertilisation barrier, which, by itself should not be used to delimit groups of SO organisms as prospective lineages, as such groups are unable to co-exist in sympatry without the subsequent acquisition of pre-fertilisation barriers.

2.2.2 Pre-Fertilisation Barriers.

2.2.2.1 The 'Recognition Species Concept.'

The 'Biological Species Concept', the 'Cohesion Species Concept,' and the 'Ecological Species Concepts,' discussed above, do not discriminate pre- and post-fertilisation barriers. Under their guidance, what is considered important is whether or not there is separation, whatever its cause. However, the utility of post-fertilisation for delimiting lineages has been questioned above.

In contrast, the Recognition Species Concept emphasises pre-fertilisation barriers (although only those pertaining to the reproductive system) to the complete exclusion of those acting post-fertilisation[5]. Developed initially by Paterson (eg. 1981, 1982a, 1985), the Recognition Species Concept derives from the observation that SO organisms possess adaptations to ensure syngamy, or fertilisation. The subset of these adaptations that are involved in signalling between mating partners, such that they 'recognise' each other as mates, constitutes their Specific Mate Recognition System (SMRS; Paterson 1985). Species (at least SO species) can then be regarded as the "... most inclusive population of individual biparental organisms which share a common fertilisation system" (Paterson 1985 p.25). SMRSs mediate assortative fertilisation between (locally) sympatric lineages.

Because of the co-adaptation between the signals and receivers of males and females, a strong constraint on independent change in either the male or female will exist, and

[5] Paterson and colleagues commonly distinguish pre- and post- ***mating*** barriers, but because assortative fertilisation may be operative post-mating, but pre-fertilisation (eg. Arnold 1997, Howard et al. 1998, Price et al. 2000), the distinction in this chapter is made between pre- and post-*fertilisation* barriers.

SMRSs are expected to be subject to strong stabilising selection (Lambert et al. 1982, Lambert & Paterson 1984, Paterson 1985, 1989). Given a surfeit of potential mates, deviation in the SMRS of one individual will render it 'unrecognisable' as a mate (at least with respect to those individuals with 'typical' SMRSs), such that it is unlikely to produce offspring and the deviation will be lost.

It was consequently predicted (Lambert & Paterson 1982, Masters & Spencer 1989) that there should be less geographic variation in components of an SMRS than in other phenotypic features of a widespread lineage. This prediction was shown to hold for a global study of *Drosophila melanogaster* (Henderson & Lambert 1982; see also Gray & Cade 2000 for American *Gryllus* crickets). However, some studies have reported geographic variation in SMRSs, particularly in the context of 'reproductive character displacement' (see Section 2.2.3.4; see Lambert & Paterson 1984 for a critique of some such studies).

Levin (2000 p.123) cites several investigations of inter-populational mating success amongst plants. These varied in finding no correlation with geographic distance, which would accord with the prediction of geographic stability of the SMRS, to reporting a negative correlation. However, at least some of these studies (eg. Bartholomew et al. 1973, which found no correlation; Nordenskiold 1971, Raven & Raven 1976, which both found a negative correlation) were concerned only with hybrid fertility and did not assess SMRS variation. Experiments explicitly addressing geographic variation in the SMRS of different plant groups could be very informative, especially if carried out within a population genetic and/or phylogenetic framework. Such studies may be more amenable to plant groups (eg. angiosperms) where gender is easier to manipulate (so as to rule out selfing) than it is in ferns. Also of considerable interest is the molecular dissection of the SMRS in several diverse taxonomic groups, especially the protein interactions involved in gamete recognition (Palumbi 1998, 1999, Wheeler et al. 2001, Swanson & Vacquier 2002). This field is still in its infancy, but is likely to provide vital insights into SMRS operation.

2.2.2.2 The SMRS as a System of Preference.

The operation of SMRSs in engendering assortative fertilisation does not appear to be absolute. In many instances, occasional hybrids are known between lineages that would otherwise be inferred to have incompatible SMRS. Such events, although usually rare, indicate that seemingly divergent SMRSs can be compatible, at least in some

between lineages that do not hybridise under natural settings (Lambert et al. 1987, Paterson 1989, Gray & Cade 2000, Meier & Willmann 2000, Wolf et al. 2001), and also, in both plants and animals, by 'sperm-competition' studies (Darwin 1900, Carney et al. 1994, 1996, Rieseberg et al. 1995, Emms et al. 1996, Arnold 1997, Klips 1999, Wolf et al. 2001). The latter experiments indicate that heterospecific sperm may often complete nearly 100% fertilisation in the absence of conspecific sperm. But, when con- and heterospecific sperm are applied in mixture, conspecific sperm will usually engender a much greater proportion of fertilisation events than its numerical proportion would suggest. That is, conspecific sperm can out compete heterospecific sperm, even when heavily outnumbered. Or, in terms of the SMRS, it may be possible for the female component to be fertilised by the sperm of multiple lineages, but she may preferentially 'recognise' sperm of her own lineage such that only it engenders fertilisation.

This preference aspect of SMRSs may be more important in plants. Whilst in mobile animals, components of the SMRS act to bring together (spatially and temporally) females and males of the same SMRS, plants are sessile and male gamete dispersal is not targeted (angiosperms with very high-fidelity pollinators may be an exception). In natural settings the reproductive organs of a sessile female are likely to be awash with a range of sperm of different SMRSs. While many of these different male SMRSs may be compatible (in the sense that they can engender fertilisation), sperm with the preferred SMRS will preferentially engender fertilisation, even if outnumbered. If preferred-SMRS sperm is absent, fertilisation will be by compatible but non-preferred SMRS sperm, and will produce a 'hybrid.' The absence of preferred-SMRS sperm may result from chance alone, but will be more likely in small and/or peripheral populations.

It is also possible that SMRS operation is environmentally contingent. That is, outside the approximate habitat in which the SMRS arose it may fail to mediate the assortative fertilisation that it did otherwise (Lambert et al. 1987, Paterson 1989). In the above context, this may be manifested in a loss of preference.

The above discussion is consistent with the observation of increased hybridisation in ecotonal and ecologically disturbed areas (eg. Brownsey 1977a, b, Barrington 1993, Arnold 1997, Schemske 2000). In these circumstances, populations may be small with

sperm of the preferred-SMRS then more likely to be absent, and/or individuals may be surviving outside their 'typical' habitats, such that SMRS-preference is lost.

For a pair of lineages where the hybridisation is restricted to a relatively small area of overlap, and in which the hybrids happen to retain some degree of fertility/fitness, SMRS-preference may lead to a stable, 'genetic-sink,' hybrid-zone in the region of overlap. Hybrids, when formed, would presumably have the SMRS of neither parent. Therefore, hybrid males would be unlikely to engender fertilisation of females of either parental lineage. However, female hybrids might be fertilised by males of either parental lineage or by hybrid males. The hybrid zone will thus act like a 'sink,' with genes flowing in, but not out.

The observations of increased hybridisation in ecotonal and ecologically disturbed areas are also consistent with an increased fitness of hybrids in these regions (eg. Arnold 1997). But, as discussed in Section 2.2.1.2, selection on hybrids operates post-fertilisation. Therefore, for separate evolutionary lineages of SO individuals that occur sympatrically over large areas, differential ecological selection is unlikely to be the only barrier engendering their separation (assuming that they really are indeed separate evolutionary lineages; see Section 2.2.2.5).

2.2.2.3 Using SMRS-Differentiation to Delimit Lineages Prospectively.

If the SMRS-preference of two groups of SO individuals is known to differ, they could be prospectively delimited as separate evolutionary lineages. That is, it can be inferred they will be evolutionarily independent in the future (even if they are not already), because they will fertilise assortatively even if in sympatry.

For some organisms, particularly those with largely visual SMRSs (eg. the courtship dances of some birds), this kind of assessment might be possible from *in situ* observation, even when the groups being considered are allopatric (see Lambert et al. 1987). However, for most organisms the study of SMRS compatibility, in a preferential context, will require experimental study. This involves testing for assortative fertilisation (ie. preferential fertilisation by con-group sperm in the presence of hetero-group sperm; Arnold 1997) and not just the ability to cross! This is because many lineages can be induced to hybridise in the laboratory when they do not do so

naturally, especially in the absence of conspecific mates (Lambert et al. 1987, Paterson 1989, Mallet 1995, Arnold 1997, Meier & Willmann 2000).

If SMRSs are environmentally contingent, laboratory experimentation may be confounded by an inability to adequately replicate 'natural' conditions (Lambert et al. 1987, Paterson 1989, Mallet 1995, Meier & Willmann 2000). This experimental approach also requires that self-fertilisation be ruled out. While this may not be a problem for the study of most vertebrates and arthropods (which tend to be dioecious), or even for many angiosperms (where many flowers are unisexual, or where hermaphroditic, emasculation can be relatively easily achieved by anther removal), controlling the gender of other organisms, such as ferns, would be less straight-forward. Small, free-living, hermaphroditic gametophytes represent the sexual phase of most ferns. Controlling their gender (ie. to force dioecy upon them so to rule out self-fertilisation) is possible by manipulating experimental conditions (see Lloyd 1974), but would be technically challenging.

2.2.2.4 Assortative Fertilisation by Allopatry.

Differentiated SMRSs are not the only effectors of assortative fertilisation. Assortative fertilisation will occur between allopatric populations (by definition), even if they share the same SMRS. If two populations remain allopatric for a significant period of time then, even if their respective SMRSs do not diverge, they will become distinct evolutionary lineages (Turelli et al. 2001).

Theory predicts that two populations will diverge by drift alone if, on average, less than one individual is exchanged between the populations per generation (Wright 1931; see Rieseberg & Burke 2001). Many studies (eg. Ehrlich & Raven 1969), particularly in plants (reviewed in Levin 1993; but also see Rieseberg & Burke 2001), have documented that the range of effective gene flow is relatively small. Even in 'good dispersers,' such as ferns, most propagules fall close to the parent with long-distance dispersal events being very rare over the time-scale of a few generations (Peck et al. 1990). While even occasional such events may bring about rapid and far-reaching range expansion during large-scale succession (eg. glacial/inter-glacial transition; Clark et al. 1998), it is questionable how much gene-flow they engender in a climactic community. This is because in an established population, an outside propagule is likely to be

numerically swamped by those from the population itself. Consequently, a wide-spread 'species' will probably exhibit a metapopulation pattern of gene-flow (eg. Cain et al. 2000 p.1220), and even contain populations (or sets of populations) which are effectively allopatric.

2.2.2.5 Assortative Fertilisation (or not) by Ecological Differentiation.

Ecological (or niche) differentiation may bring about assortative fertilisation even in the absence of SMRS differentiation. However, the circumstances in which this might happen are restrictive (see also Templeton 1981); these are outlined below. As described in Section 2.2.1.2, ecological differentiation operates post-fertilisation, such that two groups with only ecological barriers between them cannot co-exist in sympatry as separate, independent evolutionary lineages. The 'problem of negative heterosis' applies equally to ecological barriers as it does to those of a reproductive origin.

Ecological differentiation may engender allopatry. Such allopatry may be in the broad sense (see Section 2.2.2.4), or only local (ie. 'micro-allopatry'). Micro-allopatry may be brought about if fertilisation is targeted to the preferred niche, and is thus assortative between the differentiated habitats. This might be the case for motile animals, which may preferentially select some habitats over others. A change to which habitat is 'preferred' effectively engenders prospective assortative fertilisation (see Section 2.2.3.2), assuming the preference is of high fidelity.

Plants, however, are not able to 'choose' their habitat, as seed/spore-propagule dispersal is largely non-targeted or direction-less with respect to habitat variation. Nor is fertilisation in plants generally targeted to one habitat over another. Where two ecologically differentiated habitats are in close proximity to one another (relative to the dispersal of the sperm-propagule), sperm from plants adapted to one habitat may fertilise plants adapted to the other environment (remembering that the putative groups are differentiated only ecologically, and have no SMRS differentiation)[6]. If such trans-

[6] Fertilisation between ecologically differentiated groups may occur regularly in seed-plants, where the sperm-propagule is pollen, whose survival (at least to the point of sperm release) is likely to be equivalent in both habitats. However, for ferns and bryophytes it would require that gametophytes survive in non-parental habitats (ie. that the selection engendering ecological differentiation is acting only on the sporophyte, which is unlikely for bryophytes where the gametophyte is the dominant phase of the life-cycle). Alternatively, habitat differentiation could be within the relatively limited range that fern and bryophyte sperm can disperse.

habitat fertilisation occurs, there are two possible outcomes depending on the fitness/fertility of the hybrids.

If the hybrids have zero fitness/fertility, then one of the parental groups will go extinct, with the rarer more likely to do so, unless the habitats of the parental groups are large enough to act as allopatric 'refugia.' That is, if individuals of the rarer group exist beyond the effective range of the sperm-propagule of the commoner group, then the former will, by default, fertilise assortatively, and 'escape' in effective allopatry the 'problem of negative heterosis.'

Alternatively, if the hybrids are fit in at least some part of the environment and retain some degree of fertility, they might mediate gene flow, via back-crossing, between the ecologically differentiated (but not SMRS-differentiated) groups, at least of loci unlinked to those under selection (see Martinsen et al. 2001). Indeed, genetic loci not under selection might introgress relatively freely between groups of quite different niche occupation, including those with concomitant differences in morphology (with the morphological differences either adaptive themselves, or linked to genetic variation adaptive in the respective niches), but which do not show SMRS differentiation. Such groups, although ecologically (and possibly morphologically) quite distinct, could hardly be considered separate, independent evolutionary lineages[7]. Further, the variation (ie. the gene or gene-complex) facilitating ecological differentiation will itself only survive the 'problem of negative heterosis' if the habitat it is adaptive in is large enough to provide an allopatric 'refugium.'

The above discussion is only general. How much gene-flow occurs between groups which are ecologically-, but not SMRS-, differentiated, will depend on case-by-case details, and may itself be dynamic within the setting of a changing environment. For instance, if ecological differentiation is (incidentally) mediated by loci throughout the genome, then very little of the genome may be unlinked to selection, such that there is

---

[7] The European oaks, *Quercus petraea* (Matt.) Liebl. and *Q. robur* L., might have been considered to represent such a situation. Several molecular studies, although detecting substantial polymorphism, failed to find significant genetic differentiation congruent with these ecologically and morphologically discrete taxa (eg. Bodenes et al. 1997). However, a recent study has reported substantial genetic congruence across their sympatric range (Muir et al. 2000), indicating that these taxa do indeed represent separate evolutionary lineages.

minimal introgression. Or, if the habitat in which hybrids are fit is very small, so few hybrids may survive such that minimal gene-flow is mediated, and the parental groups remain largely evolutionary independent.

2.2.3 'Speciation' or the Formation of New Lineages.

In light of the above discussion of processes engendering assortative fertilisation, and thus the evolutionary independence of lineages, it may be pertinent to discuss how new lineages might form. The formation of new species is commonly termed 'speciation' in the literature. The following discussion will be placed in this context, but it will be re-emphasised that 'lineage' and 'species' need not necessarily be equivalent.

2.2.3.1 The Origin of New SMRSs.

As noted previously, SMRSs are expected to be under strong stabilising selection, as independent deviation in the SMRS of one individual will render it 'unrecognisable' as a mate (Lambert et al. 1982, Lambert & Paterson 1984, Paterson 1985, 1989). How then does SMRS divergence occur? Paterson (1989; also Lambert & Paterson 1984) has contended that change in the SMRS is expected to only take place in small, isolated populations, where the reciprocal fixation of new alleles in both males and females is more likely to occur. Other authors also predict that speciation is most likely (or even, only possible) in relatively localised populations (eg. Levin 1993, Rieseberg & Brouillet 1994; see Mayr 1982 p.601-606 for discussion). Such restriction is effectively a genetic 'bottleneck,' such that incipient species are likely to be genetically depauperate (at loci throughout the genome) when compared to their progenitor (or the ancestral population).

It is conceivable that such SMRS change may occur by drift alone (in small populations), although the processes leading to natural selection (including 'sexual' selection; eg. Gray & Cade 2000, Panhuis et al. 2001, Turelli et al. 2001) may commonly be involved. For instance, if a (small) population was restricted to an environment outside its normal niche, the fertilisation system character states of its constituent individuals may be 'destabilised' (Lambert & Paterson 1982, 1984, Paterson 1985, 1989). Directional selection might then select for SMRS character states more effective in the new environment (assuming more effective states are available), producing a new SMRS differentiated from that of the progenitor population. In such

scenarios, concomitant selection on non-SMRS characters in the new environment may mean that 'speciation' often involves congruent change in both niche and SMRS (Paterson 1985, 1989).

It is important to emphasise that speciation, in the above context of SMRS differentiation, is "an incidental *effect*, resulting from the adaptation of the characters of the fertilisation system, among others, to a new habitat, or way of life" (Paterson 1985 p.26; original italics). That is, although the processes leading to natural selection may be involved in SMRS differentiation, their effects are incidental (Paterson 1981, 1982a, 1985, 1993, Lambert & Paterson 1984; see also Darwin 1868 p.188, Mayr 1976 p.129-134 who similarly considered speciation as "incidental"). Others have seen a more direct view for the processes leading to natural selection in 'ensuring,' rather than 'engendering,' the separation of two groups. Dobzhansky (1976 p.104), for instance, viewed species as "adaptive devices through which the living world has deployed itself to master a progressively greater range of environments and ways of living." Similarly, Mayr[8] (1942 p.284) stated "speciation is thus an adaptive process toward the most efficient utilisation of the environment." However, to believe that the processes leading to natural selection are directly involved in speciation (ie. causing speciation so that two groups can be separate) is teleological, with overtones of group-selection and directed- or designed-evolution (see Paterson 1981, 1982a, 1982b, 1985, Paterson & Macnamara 1984).

2.2.3.2 Allopatry and Sympatry.

The origin of new lineages ('speciation') is commonly held to occur in allopatry (regardless of the size of the population involved). Turelli et al. (2001 p.330), for instance, hold that allopatric speciation is "pervasive," while suggesting that sympatric speciation is "far less common." Via (2001 p.387) has recently reviewed sympatric speciation in animals, listing the "most probable examples," of which there are only a few. Most, if not all, of these cases (eg. the well-known apple maggot fly *Rhagoletis pomonella*; Bush 1969, Feder 1998) appear to involve a change in habitat (eg. host plant) preference. The habitat preference of the 'incipient species' had diverged from that of the progenitor, and with host-fidelity restricting fertilisation to the preferred

[8] Paterson (1978 p.28) has pointed out that Mayr was somewhat "ambivalent" or ambiguous as to whether the processes leading to natural selection were directly involved in speciation.

habitat, assortative fertilisation in 'micro-allopatry' was engendered. Such divergence may have been incidental, although models of divergent natural and/or sexual selection are favoured, possibly without good reason, by many authors (eg. Johannesson 2001, Turelli et al. 2001, Via 2001; and references therein).

Polyploidy is often cited as an example of instantaneous, sympatric speciation, but as outlined in Section 2.2.1.4, because of the 'problem of negative heterosis,' a polyploid event should not *per se* be equated with 'speciation.'

Allochronic divergence can occur in sympatry (Simon et al. 2000, Cooley et al. 2001, Ritchie 2001), but the separation of such incipient lineages temporally could be considered analogous to the spatial context of allopatry.

2.2.3.3 Ecological Differentiation.

Ecological differentiation alone (ie. without SMRS differentiation) cannot engender assortative fertilisation, except in the restrictive circumstances described in Section 2.2.2.5. For the most part, these necessitate that a largely allopatric (or at least, micro-allopatric) distribution is facilitated by the ecological differentiation, unless the organisms concerned target fertilisation to only their 'preferred' niche (eg. motile animals).

2.2.3.4 'Speciation' by Reinforcement.

Speciation by reinforcement[9] is a popular model of speciation in some parts of the evolutionary community. Championed by Dobzhansky (eg. 1941, 1970), this model holds that pre-fertilisation barriers will evolve to 'protect the integrity' of groups returning to a situation of sympatry (or parapatry) after being allopatric and separated initially only by post-fertilisation barriers. Although these initial post-fertilisation barriers are often considered only in terms of reproduction, they may also be of ecological origin (Kirkpatrick 2001, Schluter 2001).

[9] Reinforcement in the broad sense, encompassing instances where post-fertilisation barriers are partial or absolute. Butlin (1987b) designated these alternatives as 'reinforcement' (*s.s.*) and 'reproductive character displacement,' respectively.

Turelli et al. (2001) cite the biogeographic study of Barraclough and Vogler (2000) as suggesting that reinforcement is not as common a route to speciation as strictly allopatric divergence. They then go on to state that the former has "spawned a much richer theoretical literature because its plausibility is less obvious ... and it is *appealing to believe* that *natural selection* can play a *direct role* in creating new species" (Turelli et al. 2001 p.338; italics added). But as noted in Section 2.2.3.1, a direct role for the processes leading to natural selection in speciation is a teleological one.

Several studies have observed that populations sympatric with a sibling species show greater discrimination against mating with that species than populations allopatric with it (reviewed in Noor 1999, see critique of such studies in Lambert & Paterson 1984). This phenomenon has been termed 'reproductive character displacement' (Butlin 1987b, Noor 1999, Turelli et al 2001), and two possible explanations have been given. One is the model of reinforcement discussed above, and the other is of differential fusion or extinction (Coyne & Orr 1989, Noor 1999 citing Alan Templeton pers. comm.; see also Templeton 1981 p.30). This latter idea holds that lineages observed in sympatry today are a non-random sample of all lineages that have come into contact with one another, with lineages lacking strong mating discrimination having fused or gone extinct.

In their comparative study of pre- and post-fertilisation barriers in closely related species of *Drosophila*, Coyne and Orr (1989) found only pre-fertilisation barriers to be enhanced in sympatry. They contended that this was not consistent with the differential fusion/extinction hypothesis, which they considered to predict "that both pre- and post-zygotic [=fertilisation] isolation will be stronger in sympatry, because *any* factor that reduces gene flow should inhibit fusion or extinction" (Coyne & Orr 1989 p.376; original italics). This has led subsequent authors (eg. Turelli et al. 2001) to indicate that reinforcement alone is responsible for observed instances of reproductive character displacement. That is, if differential fusion/extinction cannot explain reproductive character displacement, then reinforcement must be plausible. However, this conclusion is invalid, because the prediction of Coyne and Orr (1989) cited above is not correct. Post-fertilisation barriers do not inhibit extinction, but can actually mediate it via the 'problem of negative heterosis.' From other but related reasoning, Noor (1999) also concludes that the differential fusion/extinction hypothesis should not be ruled out as an explanation for reproductive character displacement.

Spencer et al. (1986), amongst others (eg. Paterson 1978, Lambert et al. 1984, Spencer et al. 1987, Masters & Spencer 1989), criticised the plausibility of the reinforcement model because it was difficult to reconcile with population genetics theory, namely the 'problem of negative heterosis.' As outlined previously, if two sympatric groups of SO individuals are 'isolated' only by post-fertilisation barriers, as postulated by the reinforcement model, then they will either fuse (if hybrid fertility/fitness is greater than zero) or one, most likely the rarer, will go extinct (if hybrid fertility/fitness is zero), with these outcomes likely to occur faster than the (incidental) evolution of pre-fertilisation barriers.

Evaluation of the reinforcement model has ranged from improbable on the basis of theoretical (see above), laboratory (eg. Paterson 1978, Harper & Lambert 1983), and field (eg. Lewis 1961, Barton & Hewitt 1985) studies, through to at least possible, even if only in theory (eg. Turelli et al. 2001 box 4 and references therein). For instance, Turelli et al. (2001) cite the study of Coyne and Orr (1989) as empirically indicating that reinforcement is plausible. However, as discussed above, the dismissal by the latter study of the differential fusion/extinction hypothesis is not justifiable. That is, their observations do not necessarily require the invocation of reinforcement. Turelli et al. (2001 p.341) also signal out the study of Liou and Price (1994) as a "theoretical treatment – using assumptions different from those of Spencer et al. [1986] – " which supported the plausibility of reinforcement. However, Liou and Price (1994 p.1452) concluded "reinforcement requires quite stringent conditions," one of which was that "there is sufficient initial divergence between the two populations in their mate recognition systems." That is, 'reinforcement' only occurred when pre-fertilisation barriers, engendering assortative fertilisation, were already in existence. This condition is not consistent with the original postulation of the reinforcement hypothesis that involved populations separated only by post-fertilisation barriers.

2.2.4 Summary of Processes Engendering Lineage Independence.

Only when groups of SO organisms fertilise assortatively can they co-exist sympatrically as separate evolutionary lineages. Assortative fertilisation is not engendered by post-fertilisation barriers, but by pre-fertilisation barriers. SMRS differentiation is perhaps the most intuitive factor bringing about assortative

fertilisation, but allopatry can also be responsible. Only when ecological differentiation engenders allopatry (or micro-allopatry) can it contribute to assortative fertilisation.

The inference of contemporary processes engendering assortative fertilisation will often be very difficult, such that the direct assessment of pre-fertilisation barriers will rarely be straightforward. For instance, few organisms have SMRSs that are amenable to study without intensive experimentation, and even then some may prove intractable. Given the difficulty of directly tracking propagule dispersal, determining whether two populations are effectively allopatric, in a prospective sense, will also be difficult. Consequently, in many cases, a prospective judgement of whether two groups of SO organisms constitute independent lineages will be problematic.

2.3 The Retrospective Delimitation of Separate Evolutionary Lineages.

An alternative to the difficulty of assessing the prospective separation of two groups is retrospective delimitation. Do contemporary patterns indicate that processes engendering assortative fertilisation have been acting in the past? That is, in a retrospective sense, are two groups of SO organisms separate lineages?

The contemporary patterns useful for assessing retrospective separation will usually be character state variation. Character state variation can be analysed in a number of ways, with these different approaches embodied in the diversity of the more 'pattern-orientated' species concepts.

An appropriate null hypothesis for the retrospective delimitation of groups of SO organisms is that a 'single evolutionary lineage is present.' The interpretation of character state variation can then be performed in the context of whether this null hypothesis can be rejected. (The alternative, where the null hypothesis is 'each SO organism represents a separate evolutionary lineage' would be much harder to reject with a retrospective approach.)

Box 2.1. *Similarity Species Concepts.*

- the 'Morphological' Species Concept: "Species are the smallest groups that are consistently and persistently distinct, and distinguishable by ordinary means" (Cronquist 1978 p.15; see also Stuessy 1990 p.171-172).

- the Phenetic Species Concept: "a) the smallest (most homogenous) cluster that can be recognised upon some given criterion as being distinct from other clusters, or b) a phenetic group of a given diversity somewhat below the subgenus category" (Sneath & Sokal 1973 p.365), or "a set of organisms not more than *x* phenetic distance units apart" (Ridley 1996 p.401).

2.3.1 Similarity.

Similarity, or lack of it, forms the heart of several species concepts, including the 'Morphological'[10] Species Concept, and the Phenetic Species Concept (see Box 2.1). The 'Morphological' Species Concept seems akin to that employed by many practising taxonomists (eg. many of the taxonomic studies in the *New Zealand Journal of Botany*), while the Phenetic Species Concept is associated with the 'school' of Numerical Taxonomy. The Phenetic Species Concept explicitly involves a non-evolutionary approach to the classification of biodiversity (Ridley 1986, 1996), while the same might be considered implicit in many examples of the 'Morphological' Species Concept.

In numerical taxonomy many characters are measured, with each character representing a dimension of space. The distance (a measure of dissimilarity) in hyperspace between the individuals from which the characters have been measured is found. These distances are then used to aggregate, or cluster, the individuals, with those least distant (ie. least dissimilar) being clustered first. A phenetic species could then be defined as in Box 2.1.

A problem for using similarity in constructing classifications is that there are different ways in which to measure similarity, and that these different measures can produce different classifications (Ridley 1986, 1996). This makes the resultant classifications

[10] Another misleading name; the real essence of the 'Morphological' Species Concept is its utility of the 'similar enough' criterion, and not morphological characters *per se*, which can of course provide data to be interpreted in light of other criteria.

arbitrary, as a classification based on any given similarity measure cannot be justified over numerous other (but different) classifications based on other similarity measures. The choice of a particular similarity measure is subjective.

Further, classifications produced by similarity measures are unlikely in many situations to reflect evolutionary history (particular its cladogenetic aspect[11]; that is, the relative recency of common ancestry between divergent evolutionary entities), although, as classifications of overall similarity, this was never their intended purpose. But can similarity be used to delimit evolutionary lineages (the 'basal' units themselves, rather than the cladogenetic relationships between them)? It cannot when it is implemented under the premise that individuals 'similar enough' to meet some criterion are delimited together as the same species, while individuals 'dissimilar enough' so that they do not met the same criterion are regarded as different species. This idea has a long historical association with the delimitation of biological diversity. Organic variation was thought to be manifested about 'standard' or 'idealised' forms of different organisms, the 'types.' A 'species' was then a group of individuals considered sufficiently similar to the type (the 'Typological Species Concept;' Ridley 1996).

Current evolutionary theory dismisses the existence of these 'idealised' types; "evolution does not make any variants in a population more typical or more real than others" (Ridley 1996 p.402). However, although variation in the natural world is acknowledged, much species delimitation continues to be based on the concept of 'dissimilar enough.' This approach then begs the question; 'just what level of dissimilarity is sufficient for individuals to be considered separate species?' which is of course subjective, and can be the cause of much disagreement (Hull 1999).

Lambert & Paterson (1982) concluded that speciation (their perspective was in terms of SMRS divergence) was not accompanied by a set level of morphological and/or genomic divergence. They found for close relatives little correlation between morphological and genomic divergence. Two separate evolutionary lineages may show

[11] Classifications that reflect only the cladogenetic aspect of history can be considered more objective than those attempting to encompass both cladogenesis and anagenesis. This is principally because assessment of the latter is based on the 'amount of similarity' between groups, which is subjective (see Ridley 1986).

a greater morphological and/or genomic similarity to one another than the average similarity between individuals of a single, but more variable evolutionary lineage. Similarity *per se*, then, should not (because it cannot) be used to identify evolutionary lineages. However, the pattern of similarity can be informative, a point noted by Mallet (1995 p.296), in that the "patterns of ... discrete genetic differences, rather than the discreteness itself, [can be used] to reveal genotypic clusters." Mallet's (1995 p.296) 'Genotypic Cluster Definition' of species held them to be "identifiable genotypic clusters ... recognised by a deficit of intermediates, both at single loci (heterozygote deficits) and at multiple loci (strong correlations or disequilibria between loci that are divergent between clusters"). Mallet's definition is very similar to some 'Phenetic' concepts, although with an explicit evolutionary infusion. In an evolutionary context, such 'clusters' across multiple characters for two groups of SO organisms are only expected when the said groups have been (retrospectively) fertilising assortatively.

It should be emphasised that the above discussion does not advocate the use of similarity for the reconstruction of cladogenetic relationships between separate evolutionary lineages (at least without an explicit model of evolutionary change). Rather, in some contexts (see Sections 2.3.5.3 & 3.2.2) the pattern of similarity (but not similarity *per se*) might be employed to test the null hypothesis that only a single evolutionary lineage is present within a group of SO organisms.

2.3.2 The 'Phylogenetic' Approach.

Phylogenetics can be broadly construed as the reconstruction of evolutionary relationships between taxa. A common phylogenetic method is that of Cladistics. Cladistics emphasises only the cladogenetic aspect of evolutionary history; that is, the relative recency of common ancestry between the evolving entities (Ridley 1986, 1996). Cladistic-based phylogenetics has given rise to several so-called 'Phylogenetic Species Concepts,' which are in fact quite different from one another (Baum 1992, Baum & Donoghue 1995, de Queiroz 1998). Before these are introduced, some of the basic tenants of cladistic-phylogenetics will be discussed.

As articulated by Hennig (1966), cladogenetic descent relationships can be inferred from the observed distribution pattern of character states if two conditions are satisfied (Davis & Nixon 1992; see also Ridley 1986, Mishler & Theriot 2000a). Firstly, the

descent system must be hierarchical (that is, divergent or bifurcating), and secondly, all descendants of a common ancestor must retain all of the ancestor's characters, either in the original or a transformed state. If these conditions are satisfied, then synapomorphies can be used to infer the presence of monophyletic groups. Synapomorphies are a subset of the observed character states; they are those which are homologous (identical by common descent rather than convergence), derived (the 'apomorphic' state, rather than the ancestral or 'plesiomorphic' state) and shared (occur in more than one taxa/sample). Monophyletic groups constitute the inferred descent relationships. Hennig (1966) defined a monophyletic group as one containing an ancestral species and all of its descendant species.

The *a priori* identification of apomorphic character states (ie. the 'polarisation' of character states) is rarely straightforward, and even when possible different characters may indicate conflicting relationships (conflict between characters is discussed further in Section 3.4.4.6). If the descent system is indeed hierarchical, conflict between 'true' synapomorphies is not possible (by definition). Thus, conflict indicates that shared, but convergently acquired character states (homoplasy) have been erroneously inferred to represent a synapomorphy. Conflict in a cladistic context is usually mediated by parsimony. This is based on the assumption that evolutionary change is improbable, such that the most plausible hypothesis of relationships is that requiring the least inference of evolutionary change (Ridley 1996).

Parsimony analysis can be used to find the bifurcating pattern of relationships requiring the minimum inference of evolutionary change even without *a priori* polarisation of the character states. This produces an unrooted tree. If this is rooted somehow (eg. by outgroup analysis), the inferred character state transformations are effectively polarised. From the rooted tree, a group containing an ancestral species (represented in the tree as an internal node) and all of its descendant species might be inferred to be a monophyletic group. Character states supporting such groups might in turn be inferred, *a posteriori*, to be synapomorphies. However, it is possible for a 'monophyletic' group containing an ancestor and all of its descendants to be recovered from such tree-based analyses for which there is no corresponding synapomorphy (see Goldstein et al. 2000).

The above conditions for linking character state variation to cladogenetic descent relationships hold for higher taxa (eg. genera) and for NSO organisms, whose interrelationships are divergent. However, they do not hold for reticulating systems (ie. SO organisms) where introgression means that derived character states cease to be necessarily indicative of close relationship (Nixon & Wheeler 1990, Baum 1992, Luckow 1995). Hence, although the method of parsimony analysis can always be performed (and will return a hierarchical pattern of relationship), phylogenetic interpretation of the result is logically dependant on the underlying descent system being hierarchically based (Davis & Nixon 1992, Brower 1999, Goldstein et al. 2000). At the 'species level,' *a priori* knowledge of whether the relationships of interest are divergent or reticulate is usually lacking (Vrana & Wheeler 1992); indeed, it is often exactly this that is being tested (Goldstein & DeSalle 2000, Goldstein et al. 2000).

2.3.3 The Monophyletic Perspective.

By its very definition, Hennig's concept of monophyly (monophyly *s.s.*, where a monophyletic group is "a *group of species* descended from a single ('stem') *species*, and which includes all *species* descended from this stem *species*;" Hennig 1966 p.73; italics added) cannot be used as a criterion to delimit species (Wheeler & Nixon 1990, Nixon & Wheeler 1990, Luckow 1995, Goldstein & DeSalle 2000). This was regarded as only a semantic problem by some phylogeneticists (eg. Mishler & Donoghue 1982, Donoghue 1985, Mishler & Brandon 1987, de Queiroz & Donoghue 1990, Baum 1992; see also Mishler & Theriot 2000a, 2000b, 2000c), and was circumvented by broadening the concept of monophyly (monophyly *s.l.*), such that a monophyletic group was "a group that contains all and only descendants of a common ancestor, originating in a single event" (Mishler & Brandon 1987 p.409).

However, while the *Monophyly Criterion* (de Queiroz 1998) was considered a necessary requirement for species delimitation, it was not in itself deemed sufficient, as monophyletic groups could be recognised at biological levels of organisation much lower (and higher) than where species were traditionally delimited (eg. Mishler & Donoghue 1982, Donoghue 1985, Mishler & Brandon 1987, Baum 1992, Baum & Donoghue 1995). Species definitions based on the monophyly criterion consequently also included ranking criteria: "A species is the least inclusive taxon recognised in a classification, into which organisms are grouped because of monophyly (usually, but

not restricted to, the presence of synapomorphies), that is ranked as a species because it is the smallest 'important' lineage deemed worthy of formal recognition, where 'important' refers to the action of those processes that are dominant in producing and maintaining lineages in a particular case" (Mishler & Brandon 1987 p.406).

The major weakness of this 'simplistic' monophyly perspective is that, no attempt is made to verify that the underlying descent pattern is hierarchical before synapomorphies are used to infer monophyletic groups. Because of introgression, individuals may exhibit a derived character state without constituting a separate evolutionary lineage.

2.3.4 The Diagnostic Perspective.

Some cladistic-phylogeneticists hold that the underlying descent pattern must be demonstrated as hierarchical (or divergent) before phylogenetic interpretation can be logically carried out from a cladistic analysis (eg. Wheeler & Nixon 1990, Nixon & Wheeler 1990, Davis & Nixon 1992, Goldstein and DeSalle 2000, Goldstein et al. 2000, Wheeler & Platnick 2000a, b, c). (Some also advocate their own terminology, which is reinterpreted here[12].) Their initial step is to identify 'diagnostic' species, the relationships between which are strictly divergent. Definitions of 'diagnostic' species include species as "the smallest diagnosable cluster of individual organisms within which there is a parental pattern of ancestry and descent" (Cracraft 1983 p.170) and "the smallest aggregation of populations (sexual) or lineages (asexual) diagnosable by a unique combination of character states in comparable individuals (semaphoronts)" (Nixon & Wheeler 1990 p.218).

Evidence for 'diagnostic' species and divergent relationships comes from diagnostic characters (cf. the *Diagnostic Criterion*; de Queiroz 1998). These are characters whose (two) states are alternatively fixed between (two) groups of individuals (Nixon & Wheeler 1990, Davis & Nixon 1992). The cause of such fixation, whether it be due to assortative fertilisation (for SO organisms), selection, or drift, is generally not considered important, at least by proponents of the diagnostic criterion. Rather, they

[12] 'Characters,' 'fixed characters,' and 'unfixed characters' are commonly termed 'attributes,' 'characters,' and 'traits' by advocates of the Diagnostic Perspective (eg. Nixon & Wheeler 1990, Davis & Nixon 1992).

Box 2.2. *The Operation of Population Aggregation Analysis (PAA).*

PAA operates by aggregating populations not diagnosed by alternatively-fixed character state differences. Such aggregated populations are then compared with other populations (or aggregated populations), and are further aggregated if they cannot be diagnosed. Populations or aggregated populations which can be diagnosed from all other populations or aggregated populations are regarded as separate 'diagnostic' species. It is possible that two populations diagnosable from one another may subsequently be regarded as conspecific if the states for which they are alternatively fixed are both present in a third population.
Note : although the procedure of PAA was initially described by Davis & Manos (1991), the name PAA was first adopted by Davis & Nixon (1992).

Box 2.3. *Doyle's 'Field For Recombination' Approach.*

Proposed by Doyle (1995), the *Field For Recombination* Approach is an 'individual-based' analogue of the 'population-based' Population Aggregation Analysis, hence avoiding the difficulty of having to delimit a population. Basically (for SO organisms), heterozygous individuals constitute evidence that the shared alleles are part of the same gene-pool because they are capable of recombining, and are therefore part of the same Field For Recombination (FFR). The FFRs from each locus are combined to give a multi-locus FFR. This approach does not appear to have been implemented, probably because of the difficulty of obtaining multi-allelic character state (eg. DNA sequence) data from numerous nuclear loci. Further, it suffers from the problems of 'too little' variation, such that any shared ancestral alleles will link taxa, and 'too much' variation, such that the diagnosed FRR might not extend beyond a single heterozygous individual (Doyle 1995).

consider it sufficient if fixation of alternate character states can be demonstrated, as this indicates an absence of reticulation between two groups (Davis & Nixon 1992, Luckow 1995). Such groups, being divergently related, could be retrospectively delimited as separate evolutionary lineages.

The identification of diagnostic characters is, however, not necessarily straightforward (de Queiroz & Donoghue 1990). One advocated approach is *Population Aggregation Analysis* (PAA; Davis & Nixon 1992; see Davis & Manos 1991 for an implementation of PAA). The operation of PAA is described in Box 2.2, while Box 2.3 describes a population-based analogue. By grouping individuals into populations, PAA allows population polymorphisms to be inferred such that non-identical individuals can be assigned to the same species (Davis & Nixon 1992). In the absence of such an initial

grouping criterion all (non-identical) individuals could be assumed to represent different species (Davis & Manos 1991). However, in many ways, PAA shifts the operational difficulty of delimiting 'species' to that of delimiting 'populations' (Davis & Nixon 1992, Mishler & Theriot 2000b). While this may be relatively simple when organisms are discretely distributed, it is certainly not the case when distributions are more or less continuous[13].

A further problem with PAA is that if two sympatric species are inadvertently ascribed to the same population, it will not be able to discern them as different even if they are separated by diagnostic characters (Davis & Manos 1991). And perhaps most importantly, as pointed out by Brower (1999), because PAA only focuses on fixed characters congruent with the *a priori* designated populations, it ignores contradictory characters such that more parsimonious interpretations of the data will be missed. To counter this problem Brower (1999) proposed the method of Cladistic Haplotype Aggregation (CHA), which uses parsimony analysis of character state data not to infer phylogeny, but rather to represent parsimonious patterns of empirical grouping that corroborate or reject specific *a priori* hypotheses of 'diagnostic species' boundaries. Basically, *a priori* designated groups of individuals are supported as 'diagnostic species' if the edge corresponding to this split is recovered in the cladogram.

Another approach, which relies on concordance between characters to detect the boundary between divergent and reticulate relationships, and which does not necessarily require *a priori* designations, is discussed in the next section.

2.3.5 A Concordant Perspective.

The respective proponents of the Monophyletic and Diagnostic Perspectives have engaged with one another in a protracted and often somewhat bitter debate over the merits (or otherwise) of each approach. The Diagnostic Perspective has been criticised because it may result in the delimitation of 'non-monophyletic species,' or groups characterised only by shared ancestral character states (symplesiomorphies) (eg. Mishler

[13] For instance, what constitutes the 'population' to be analysed?: all of the individuals at the bottom of the valley; on the valley sides; in the river catchment; over the entire mountain range, in which case some of the individuals from the 'population' of the mountain range might be geographically closer to individuals of other 'populations' (of neighbouring landforms) than they are to other members of their own 'population.'

& Brandon 1987, de Queiroz & Donoghue 1990, Baum 1992, Baum & Donoghue 1995). The Monophyletic Perspective has been criticised because synapomorphies cease to be indicative of monophyletic groups in reticulating systems (eg. Nixon & Wheeler 1990, Davis & Nixon 1992).

Both of these problems largely stem from the sentiment that lineage delimitation based on one character, whether it has a synapomorphic state in one group or is diagnostic between groups, is sufficient (eg. Mishler & Brandon 1987, Davis & Nixon 1992, DeSalle & Vogler 1994, Baum & Donoghue 1995, Zink & McKitrick 1995; see Avise & Ball 1990 for discussion). Similarly, implementation of Templeton's 'Cohesion Species Concept' involves testing a series of null hypotheses with only a single gene tree (Templeton 1994, 1998a, 1998b, 2001, Templeton et al. 1995, 2000).

This 'one character' approach, although informative in some contexts (eg. range expansion; see Templeton et al. 1995), has several, interrelated problems when used to delimit lineages or species. Templeton (1998a p.394) himself acknowledges that "one major limitation of this approach is that it is basically a single-locus analysis. As a result, both evolutionary stochasticity and locus-specific evolutionary forces such as natural selection may erode power or even mislead the investigator."

These problems, which will be discussed further below, are overcome if concordance is sought amongst multiple, independent characters. This has been suggested by proponents of both the Monophyletic (eg. Baum & Shaw 1995; see Box 2.4 for a discussion of their Genealogical Species Concept) and Diagnostic (eg. Sites & Crandall 1997; see also Grady & Quattro 1999) Perspectives, and seems implicit in Mallet's (1995) 'Genotypic Cluster Definition' (see Section 2.3.1).

The advantages of seeking concordance across multiple characters over the 'one character' approach when attempting to delimit primarily divergent relationships from those which are primarily reticulate are best illustrated by Coalescent Theory.

2.3.5.1 Coalescent Theory.

Coalescent Theory is a phylogenetically-oriented subdiscipline of population genetics dealing with the branching and sorting of gene alleles through time (Hudson 1990,

Box 2.4. *The Genealogical Species Concept.*

The Genealogical Species Concept of Baum and colleagues (eg. Baum & Shaw 1995, Baum & Donoghue 1995; see also Baum 1992, Shaw 1998) is based on the concordant partitioning of individuals across multiple, independent c-gene (see Section 2.3.5.1) phylogenies. It holds that "species [are] basal, exclusive groups of organisms" where a "group of organisms is exclusive if their genes coalesce more recently within the group than between any member of the group and any organisms outside the group" (Baum & Shaw 1995 p.291, p.296). They state that the lowest level at which exclusive groups emerge should approximate the boundary between predominantly reticulate and predominantly divergent organismal relationships. However, they admit uncertainty in how concordance across different gene phylogenies might be assessed. Further, they do not account for the potential 'problem' of having to simultaneously place heterozygous individuals in more than one part of a given gene phylogeny (Doyle 1995).

Avise & Wollenberg 1997). The alleles of any particular non-recombining region of DNA (a coalescent-gene or 'c-gene,' which is not necessarily equivalent to a functional gene; Hudson 1990) will be divergently related to one another (Maddison 1995). Consequently, cladistic analysis of allele sequence variation can be used to reconstruct the cladogenetic relationships between the alleles (Avise & Wollenberg 1997). The absence of recombination in asexual organisms means their entire genome is a single c-gene (Baum & Shaw 1995, Doyle 1995). Therefore, phylogenetic reconstruction of sequence variation from different 'functional' genes should be in broad agreement with one another, and that any, and indeed all, 'functional' gene phylogenies should reflect the divergent relationships between the asexual organisms studied (allowing of course that not all cladogenetic relationships are marked by a mutation).

In sexual organisms, recombination engenders the genealogical independence of different c-genes from one another, and from organism level relationships. Consequently, in a reticulating system there is little expectation that any gene phylogenies should be congruent with one another, or with organism level relationships (Baum & Shaw 1995, Doyle 1995). This is at the heart of the 'one character' problem. In a reticulating system, any given character is likely to partition individuals, whether it be by 'synapomorphies' or 'diagnoses,' into groups different to those from other characters, and none of these non-congruent partitioned groups can be necessarily

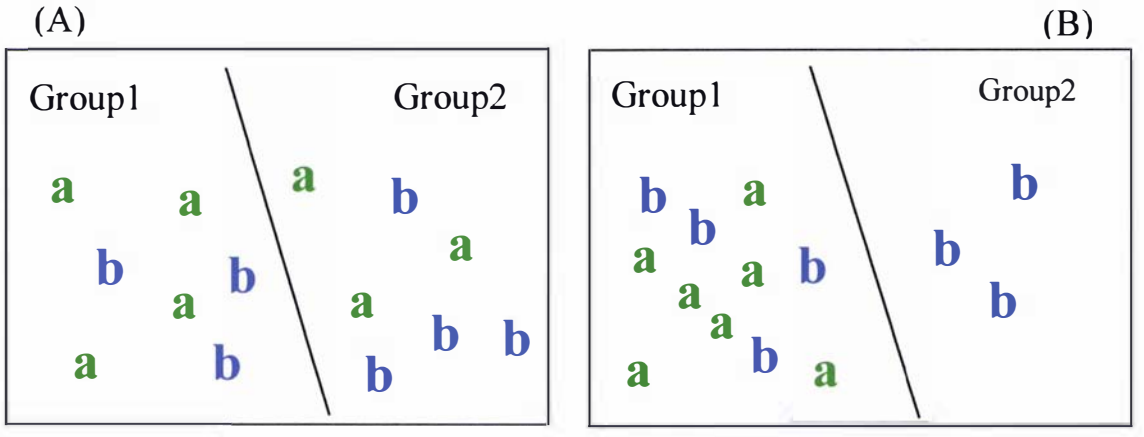


Figure 2.1. 'Paraphyly' or 'Polyphyly' From the Sundering of a Reticulating Group.
When the constituent individuals of a reticulating system are sundered into two disjoint groups, it is unlikely than any given character will be 'diagnostic' for that partitioning. As with this example, where individuals may have state 'a' or 'b' (but not both) of a character, the initial pattern of relationship is more likely to be 'polyphyletic' (eg. A), or 'paraphyletic' (eg. B). (Note that the use here of the terms 'polyphyletic' and 'paraphyletic' differs somewhat from their typical usage.)
(A) could be considered a 'polyphyletic' situation, where individuals of each group are more closely related (with respect to their allele state) to some individuals in the other group than they are to all other members of their own group.
(B) could be considered a 'paraphyletic' situation, where the individuals in Group 1 with allele 'b' are more closely related to the individuals of Group 2 than they are to the remaining individuals (those with allele 'a') of their own group.

expected to reflect organism level relationships (Avise & Ball 1990, Doyle 1995, Maddison 1995). As stated by Avise and Wollenberg (1997 p.7752), with respect to reticulating systems, "any approach that promulgates clade diagnosis on the basis of synapomorphs at only one or a few genes makes little sense."

2.3.5.2 The Temporal Progression of Concordance.

When a reticulating system is sundered into two groups, it is unlikely that any given character will reflect, or be 'diagnostic' for, this partitioning. Instead, a 'polyphyletic' or 'paraphyletic' pattern of relationship is likely, with the latter becoming more likely when one of the sundered groups is small (see Figure 2.1). If the two groups remain separated (so that there is no gene-flow between them), lineage-sorting, which is the chance extinction of character states in one group but not the other, will result in the alternative fixation of states so that the character becomes diagnostic for the two groups (Avise & Ball 1990, Avise & Wollenberg 1997).

This lineage-sorting mediated progression from initial 'polyphyly,' through 'paraphyly,' to alternate state fixation and 'diagnosis' will occur independently across different characters. Because lineage-sorting is a stochastic process, the attainment of alternative fixation will occur at different times for different characters, even with all other things being equal (Avise & Ball 1990, Baum & Shaw 1995). Further, characters under divergent selection will progress faster than those that are neutral, while those under selection for balanced polymorphism will be slower (Baum & Shaw 1995).

2.3.5.3 Analysing Concordance and the Null Hypothesis.

If a set of organisms is partitioned into two groups concordantly across multiple, independent characters, the implication is that they are, in a retrospective sense, separate evolutionary lineages in that they must have been fertilising assortatively for such concordance to occur (Avise & Ball 1990). Further, with multiple 'diagnostic' characters, some are likely to be synapomorphic in one group and others in the second group. This 'concordant' approach does not necessarily require *a priori* hypotheses of the presence of specific groups. Rather, if analysis of character state variation leads to the rejection of the null hypothesis (that 'a single evolutionary lineage is present'), the detection of the groups therein delimited can be considered an 'emergent' property of the analysis.

Concordance is probably most easily appreciated for the kinds of qualitative characters typically associated with a 'diagnostic' or 'synapomorphic' approach. However, quantitative characters, which are usually analysed by similarity or distance measures, can also be assessed for concordance (see Section 3.2.2). In this context, for both qualitative and quantitative characters, it is the pattern of character state variation that is emphasised (and not the distance between character states).

If two characters partition a set of samples in exactly the same way (eg. congruent diagnostic characters), they might be considered 'absolutely' concordant. However, even when there is little or no absolute concordance in a data set, there may be enough characters that are 'almost' concordant with one another to still be strongly indicative of retrospective assortative fertilisation (Figure 2.2). Such almost-concordance might stem from incomplete lineage sorting, or reflect a breakdown in absolute-concordance because of (limited) introgression or homoplasy.

Individuals	Characters
A	11111101111
B	11110111111
C	11111111110
D	10111111111
E	11111111011
F	00100000000
G	00000001000
H	00000100000
I	00000000010
J	00010000000

Figure 2.2. Absolute and Almost Concordance.
The character highlighted in blue partitions the individuals {A,B,C,D,E} from individuals {F,G,H,I,J}. No other character is absolutely-concordant with the character highlighted in blue, but the others are all 'almost' concordant with it. The state variation across these characters fairly clearly supports the partitioning {A,B,C,D,E}{F,G,H,I,J}, and may be indicative that these two groups have been fertilising assortatively.

Similarly, there are multiple reasons why characters might be discordant in the way they partition a sample set (eg. incomplete lineage sorting, introgression, homoplasy). Even with the infinite separation of two sundered groups, some characters might remain discordant either through balancing selection (Baum & Shaw 1995, Doyle 1995) or because of idiosyncrasies of the lineage-sorting phenomenon (see Avise & Wollenberg 1997 fig.3, and the second complete paragraph on p.7751; also Nichols 2001).

The results of Chen and Li (2001), in one of the first studies of its kind, illustrate this point (and also the 'danger' of using a one-character approach). They studied sequence divergence at 53 autosomal non-coding loci (to give 53 independent genomic characters) between a human, a chimpanzee, a gorilla, and an orangutan. The phylogeny of these four species is reasonably well-established (ie. humans and chimps are closest relatives), and the divergence between them is relatively old (c. 5 million years). Nevertheless, only 31 (58%) of the loci supported the expected phylogeny under neighbour-joining analysis, with 23% and 19% of the loci supporting the two other topologically possible (but 'incorrect') arrangements of taxa. Under parsimony analysis, only 45% of the loci supported the expected topology, although only 17% supported the 'incorrect' topologies (38% provided no resolution). In either case, while discordance is present, the strongest signal in this data is by far that for the expected phylogeny (which is overwhelmingly supported in concatenated analyses).

The prospect of any discordance has led some (eg. Doyle 1995) to reject the concordant approach (at least as espoused by Baum & Shaw 1995) outright. This is overly conservative. Discordance itself does not indicate the null hypothesis (ie. that only one lineage is present) is correct. Rather, it means that it cannot be rejected, at least given the available data. Balancing selection, homoplasy, or shared ancestral states will not result in the improper rejection of the null hypothesis, although they may mask the presence of separate lineages (so the null hypothesis is not rejected when it 'should' be). However, even with the presence of these 'misleading' phenomena, any concordance between other characters can lead to the rejection of the null hypothesis.

Divergent selection, however, may result in the rejection of the null hypothesis when only one lineage is really present. For instance, two 'independent' morphological characters might be differentially adaptive (or each linked to variation that is) in two different habitats. Concordance between the characters under divergent selection would give the impression of retrospective assortative fertilisation, even if there had in actuality been none, such that neutral characters, if assayed, exhibited no concordance (see Section 2.2.2.5).

For the above reason, when concordance is between characters often associated with (divergent) selection, and particularly when it is associated with ecological/allopatric separation, rejection of the null hypothesis should be tentative until characters that are more likely to be neutral can also be assessed. This caveat may seem to down play the role of morphological characters in delimiting evolutionary lineages. In practice the initial suggestion that more than one lineage may be present will usually be based on morphological variation. However, rejection of the null hypothesis might be considered stronger for analyses incorporating characters that are likely to be neutral (eg. many molecular characters; Givnish & Sytsma 1997).

Methods for assessing character concordance (allowing for both 'absolute' and 'almost' concordance) for morphological and AFLP DNA-fingerprinting data are developed and discussed in Chapter Three. It should be emphasised that the general approach of seeking concordance is not new to taxonomy; it simply reflects 'good' traditional

taxonomic practice! However, character concordance in this study is explicitly couched within an evolutionary framework.

2.3.5.4 The Extent of Concordance.

The extent of concordance required for delimiting separate lineages remains a qualitative matter (Avise & Ball 1990, Baum & Shaw 1995, Sites & Crandall 1997). Obviously, the more characters which are concordant and the greater the degree of concordance (ie. 'absolute' rather than 'almost'), the more robust the inference of retrospective assortative fertilisation may be. This in turn leads to a more robust rejection of the null hypothesis (that states only a single lineage is present), and a stronger inference that two (or more) separate evolutionary lineages are present. (Additional properties of the sample set that may also impact on these decisions are discussed in Section 2.3.6)

Nevertheless, there is no threshold value of concordance above which the null hypothesis can be 'confidently' rejected, at least in the absence of an explicit statistical framework. It might be questioned that, given the qualitative nature of the concordant approach (ie. how much concordance is enough?), why advocate it? Primarily, its strength lies in providing a theoretically-justifiable (ie. objective) inference 'chain' by which observable patterns of character variation can be linked to the determination of whether separate evolutionary lineages are present (or at least, detectable).

To summarise, concordant partitioning across multiple, independent characters is only expected when the therein partitioned groups are fertilising assortatively, and have been doing so for such time that lineage sorting has engendered that concordance. This then allows inference of separate, retrospectively-independent evolutionary lineages. Although the inference chain itself is robust, the qualitative nature of its constituent links (ie. firstly, how much concordance is present? and secondly, is the degree of recovered concordance sufficient to recognise multiple lineages?) necessitates that its resultant conclusions must be inherently qualitative. These conclusions should be regarded as hypotheses, some more supported than others, and always subject to corroboration or refutation by future analyses.

2.3.6 The Spatial Context of Retrospective Lineage Delimitation.

Studies seeking to test the null hypothesis that 'a single lineage is present' amongst a set of SO individuals should sample (individuals and characters) as extensively as possible. Otherwise, concordance, for instance, may be recovered where it does not really exist (eg. concordant partitioning may be recovered between populations at the extreme ends of a lineage's range that is not apparent when intermediate populations are included).

Particular caution should be exercised when dealing with small peripheral populations (irrespective of whether the methodology emphasises concordance, diagnosis, or monophyly). Because of the 'founder effect' these may be relatively homogeneous and, akin to the duplicate analysis of a single sample, are likely to be recovered with strong concordant partitioning. In that such concordantly-partitionable peripheral isolates may represent (1) recent founding events[14] which may (1.1) go extinct; (1.2) be joined by other colonists (engendering reticulation and a lack of concordance); or (1.3) go on to form a long-separated lineage, as well as (2) long-separated lineages, it may not be desirable to recognise all of them as distinct lineages[15]. Consequently, any rejection of the null hypothesis is likely to be more robust when the inferred lineages are both widespread, and especially when they are sympatric.

2.3.7 Retrospectively-Undelimitable Lineages.

When changes occur that result in the prospective separation of two groups of SO organisms (ie. SMRS differentiation), it is likely that the only characters concordant with such partitioning will be those engendering the separation (ie. those which have undergone the changes that have resulted in differentiation of the SMRS) (see Section 2.3.5.2). Such lineages will be missed by retrospectively orientated methods (unless they happen to pick up the character state changes involved in SMRS differentiation). Retrospective delimitation is only likely to detect those lineages that have been separated for sufficient time such that lineage sorting has engendered character state

[14] Peripheral populations formed from recent founding events will assumedly be characterised by relatively little genetic variation.

[15] The more stringent criteria of reciprocal 'genomic monophyly' could be used to distinguish between isolates of recent origin and those separated from the 'main' population for a long time. Only in the latter instance, after a period of lineage sorting will concordant partitioning between the two groups be characterised by 'reciprocal' synapomorphies (ie. synapomorphies in the progenitor population as well as the derivative population).

patterns that are congruent with the lineages themselves. It is likely that young lineages will only be delimitable by prospective means. Given the empirical difficulty of the prospective approach (see Section 2.2.4), it is quite probable that young lineages will often go undetected.

2.3.8 Lineage Permanence.

That current data may indicate two groups of SO organisms are separate lineages does not necessarily mean that this will always be the case. For instance, a physical (and/or ecological) barrier that has engendered allopatry between two groups for a long period, such that they are retrospectively delimitable as separate lineages, may break down. If there is no SMRS differentiation, the two groups now in parapatry or sympatry may introgress genes, such that a reanalysis might be unable to reject the null hypothesis that only 'a single lineage is present.' Lineages delimited by retrospective means are, thus, not necessarily permanently separate entities (see Baum & Shaw 1995).

Species concepts based on reproductive criteria (eg. Spencer et al. 1986, Masters & Spencer 1989, Meier & Willmann 2000; see also Avise & Ball 1990) often consider the entities they (prospectively) delimit as irreversibly separate. Lineages prospectively delimitable by SMRS differentiation may be more 'permanent' than those whose delimitation stems from their allopatry, notwithstanding that parallel acquisition of compatible SMRSs is at least conceivable.

2.3.9 Lineage Integration: Individuals and Classes.

A distinction is often made in the species literature between 'species as individuals' (ie. integrated and cohesive entities with a restricted spatiotemporal location) and 'species as classes' (ie. spatiotemporally unrestricted sets with defining characteristics) (see Mishler & Donoghue 1982). Some authors (reviewed in Mishler & Donoghue 1982) have argued that species are (and/or must be) 'individuals,' in that they are the most inclusive entities that are 'actively evolving.' This would make them fundamentally different from higher taxa like genera and families, which might be viewed simply as the passive end products of evolution.

Except for loci under strong selection (Rieseberg & Burke 2001), levels of effective gene flow may be insufficient to engender the 'integration' of a lineage, especially if

widespread, such that its constituent organisms could hardly be considered to be collectively evolving as a unit (Ehrlich & Raven 1969). Rather, the organisms of widespread lineages may owe their 'similarity' (ie. shared SMRS, morphology, ecology etc.) more to common descent and/or stabilising selection rather than cohesive gene-flow (Mishler & Donoghue 1982, Lambert & Paterson 1984), all the while their genealogical relationships are 'decaying,' or becoming increasingly divergent.

Prospectively, that organisms may share the same SMRS merely demonstrates that there is the potential for gene-flow to engender 'integration,' but not necessarily that they are integrated. In a retrospective context, the non-rejection of the null hypothesis that 'a single lineage is present' does not demonstrate that a single 'cohesive' lineage is indeed present, but rather that the available data does not suggest the presence of two (or more) lineages.

It is quite possible that a group of SO organisms delimited as a single lineage might actually contain subsets which are evolving independently and are divergently related, but which lineage sorting has yet to accrue sufficient character concordance to allow their separate delimitation. Because of this (strong) possibility that lineage integration is absent, lineages might be considered to have at least some properties of 'classes' such that they cannot be true 'individuals,' but instead represent some sort of 'hybrid' between the two (Mishler & Donoghue 1982, Mishler & Brandon 1987).

Distinct evolutionary lineages reside, by definition, on the divergent side of the divergent-reticulate boundary of interrelationships between SO organisms. In that distinct lineages can be placed as close as possible to this boundary, their ranking within the descent hierarchy might be considered objective (in that the boundary is real; Baum & Shaw 1995). This is in contrast to higher taxa (eg. genera and families) whose absolute positions are not fixed to any objective property (see Avise & Johns 1999 for a suggestion that would at least make the ranking of higher taxa consistent across the different forms of life).

2.4 The Taxonomic Delimitation of Lineages.

As discussed in Sections 2.2 and 2.3, respectively, separate evolutionary lineages might be delimited using prospective (eg. SMRS differentiation) and/or retrospective evidence (eg. character concordance). Basing the species category of taxonomy (ie. the 'taxonomic species') on separate evolutionary lineages gives it 'objectivity,' in that because evolution has only occurred once it provides an external reference on which to evaluate competing hypotheses (see Section 2.1.1)

The taxonomic scheme, however, might be construed as having two principal functions. Firstly, it should accurately reflect the evolutionary history of biodiversity, as this gives the resultant scheme objectivity (see Section 2.1.1). Secondly, it must also be practical, in that there is little use in having a descriptive scheme of biodiversity, which although highly objective, is unusable and inaccessible. Unfortunately, these two functions can be in conflict with one another, in that objectivity (ie. the reflection of evolutionary history) is bought at the price of usability, and vice versa. Consequently, it might be construed that the taxonomic scheme be 'as accurate a representation of evolutionary history as possible, while still retaining usefulness.'

An example of this conflict is NSO organisms, where each and every organism can be considered a separate lineage (in a genealogical evolutionary sense). To recognise each NSO lineage (= each NSO organism) as a separate species would produce a truly impractical and unwieldy taxonomic system. Every separate lineage cannot be a separate species, at least in NSO organisms. Pragmatism is necessary, and the delimitation of taxonomic species in NSO organisms is discussed in Section 2.4.2.

In contrast, reticulation between SO organisms binds them into higher order groupings that can be delimited at the boundary between primarily reticulate and divergent relationships in an objective manner (in that the boundary is real). Such delimitation identifies separate evolutionary lineages, and these in turn need to be delimited into the taxonomic scheme. It can be re-emphasised that post-fertilisation barriers (eg. hybrid sterility, ecological differentiation), which do not engender the separation of evolutionary lineages *per se* (although ecological differentiation might engender

(micro-) allopatry, which in turn engenders assortative fertilisation), are not involved in delimiting lineages, or in delimiting the lineages into taxonomic categories.

2.4.1 The Delimitation of SO Lineages as Taxonomic Species.

Every lineage of SO organisms, whether delimited by prospective and/or retrospective evidence, could be recognised as a separate taxonomic species. This is certainly a defensible position. However, a more conservative alternative is that separate lineages of SO organisms should be recognised as subspecies or species, with the latter category reserved only for lineages for which there is direct or indirect evidence of prospective separation. That is, not all lineages of SO organisms need be automatically recognised at the specific level (which accords with Templeton eg. 2001).

Direct evidence of prospective separation includes the demonstration of SMRS differentiation (see Section 2.2). However, even without direct demonstration, prospective separation can be indirectly inferred for groups of SO organisms that are retrospectively delimitable and exist in sympatry. Two groups of SO organisms cannot remain evolutionarily separate (ie. concordantly-partitionable) in sympatry unless they are fertilising assortatively. Such inference (1) assumes that the groups have been sympatric long enough for the 'problem of negative heterosis' to 'act' (if in fact assortative fertilisation does not occur), and (2) is stronger the greater the sympatry.

Lineages for which there is (direct) evidence for prospective separation and/or which are retrospectively delimitable and sympatric could be referred to the species category. A lineage for which there is no (direct) evidence for prospective separation but which is retrospectively delimitable and allopatric could be (conservatively) recognised at the subspecific level.

2.4.1.1 The Varietal Category in SO Organisms.

'Obvious' character state variation, be it adaptive or neutral, may often occur within a single lineage. That is, an easily observable character may be polymorphic, but variation in other characters is not concordant with it. The varietal category could be based on such 'obvious' polymorphic characters. However, such delimitation is subjective, tantamount to emphasising taxonomically state variation in only one of numerous non-concordant characters, any one of which could equally be used to base a

variety on. Basing a variety on every single non-concordant character would of course be impractical. But, the emphasis at the varietal level of particularly 'obvious' polymorphic characters (although discordant with lineage boundaries) could be used to signal taxonomically that the individuals ascribed to the varieties, although obviously different, do *not* represent separate evolutionary lineages which, as above, would be recognised as species or subspecies (for similar reasoning see Hedren et al. 2001).

The value of recognising such varieties would really be determined by their utility to the end-users of the resultant classification. Because each character (subjectively) emphasised partitions a minimum of two varieties, taxonomic emphasis of two discordant characters would require the recognition of four varieties, three characters would require eight varieties, etc. If a lineage exhibits variation in several obvious, but discordant characters it may be more pragmatic to simply recognise it as a 'polymorphic' species (or subspecies) rather than delimit numerous varietal taxa, which because of their sheer numbers may individually be of little use.

Further, if the states of the character on which a varietal partition is based are discrete, the assignment of any given individual will be straightforward, but this will not necessarily be the case for continuously varying characters (see Figure 2.3). Individuals with intermediate states may be quite difficult, or impossible, to categorise. In such instances, varietal categories might serve more as 'typological' signposts of the characters' extreme states, rather than facilitating the practical categorisation of all individuals.

2.4.1.2 'Cryptic' SO Lineages.

It may also be difficult to assign individuals to a particular lineage (species or subspecies), especially when the (prospective or retrospective) evidence used to delimit the lineage is visually cryptic (eg. chemical aspects of the SMRS, molecular character variation). However, there is a critical difference between varieties and lineages (and therefore between varieties, and species and subspecies), at least in SO organisms. Lineages of SO organisms, in that they represent divergently related groups of individuals, exist independently of our ability to recognise them. Varieties, however, are (at least as defined in Section 2.4.1.1) the artificial constructs of a taxonomist

(A) Qualitative Characters

	D	E	F	G	H	I	J	K	L
W	1	1	1	1	1	0	0	0	0
X	1	0	1	1	0	1	0	1	0
Y	0	0	0	1	1	0	1	1	1
Z	1	0	0	0	1	1	1	0	0

(B) Quantitative (Continuous) Characters

	D	E	F	G	H	I	J	K	L
W	1	2	3	4	5	6	7	8	9
X	4	7	2	6	1	3	9	8	5
Y	3	5	8	6	4	9	2	1	7
Z	9	6	5	1	3	2	8	4	7

Figure 2.3. Partitioning with Qualitative and Quantitative Characters.
The ease of assignment of a given individual under a particular varietal partition may well depend on whether the partition is based on a qualitative (A) or quantitative (B) character. In both (A) and (B), W-Z represent different characters and D-L different individuals. Character W has been 'emphasised' to base the varietal partition on. The assignment of individuals in (A) is straightforward, dependant only on whether they have state '1' or '0' at Character W. In (B), if states '1' and '9' represent the extremes of the character state continuum on which the varietal partition is based, to which variety does Individual H belong (its state of '5' for Character W is equally close to '1' and '9')?

emphasising the variation in one character over the discordant variation in numerous other characters.

Practical difficulties in assigning a given individual to a particular category might be used as an argument against the recognition of varietal taxa. After all, their 'purpose' is one of utility. However, if *lineages* can be delimited using only cryptic characters, a strong argument can still be made for formally naming them as separate species (or subspecies) as this acknowledges their existence, even if their morphological similarity is such to necessitate their amalgamated recognition in the field as a species aggregate.

2.4.2 The Taxonomic Delimitation of NSO Organisms.

Each NSO organism can be considered a separate evolutionary lineage because of their purely divergent relationships. The taxonomic recognition (as species or subspecies) of each NSO lineage (= each NSO organism) would of course be impractical. How then, should NSO organisms be taxonomically delimited?

Given that the bifurcating, divergent relationships between NSO organisms are analogous to those between higher taxa, an initial requirement might be that NSO species should be monophyletic. NSO taxa assumedly have a single origin, the

'ancestor,' which together with all of its descendants would constitute a monophyletic group (the NSO reproduction could be considered the 'synapomorphy'). However, if such monophyletic groups of NSO organisms are 'obviously' phenotypically and/or ecologically variable it may be desirable to represent this biodiversity by more than one taxonomic category. If a subset of a NSO taxon is synapomorphic for some 'obvious' character, such than the remainder of the group is rendered paraphyletic, individuals with the derived state might be recognised at the varietal level. Alternatively, if the 'obvious' variation is alternatively fixed across reciprocally monophyletic lineages, it might be justifiable to recognise two NSO species.

Sometimes a strict adherence to the monophyletic criterion in NSO organisms may not be practical. For instance, if one SO lineage has given rise to multiple NSO lineages, it might well be impractical to recognise each as a separate species (see Hedren et al. 2001 for a discussion of taxonomic practicality in the analogous situation of where polyploids have had numerous origins). In no small way will taxonomic delimitation of NSO organisms be driven by pragmatism.

2.4.3 Summary of Taxonomic Delimitation.

Basing the taxonomic system explicitly on evolution lends it objectivity; it provides an external reference on which competing hypotheses can be evaluated. Consequently, there is much value in basing the 'species' category on evolution by requiring that species be (demonstrably) separate evolutionary lineages. Even so, this does not preclude subjectivity completely from the taxonomic 'process.'

For instance, how much character concordance is 'enough' to delimit a lineage? Should all delimited lineages be recognised as species? How should the taxonomy of NSO organisms be treated? These questions have no objective answers, and there is certainly room for the 'taxonomist's prerogative.' Because of such subjectivity, studies pertaining to delimit taxonomic entities, indeed any kind of biological entity, should be explicit in the data they have employed and in the interpretations they have made. The delimitation of entities constitutes a hypothesis that is subject to future corroboration or refutation.

In many ways, a comprehensive description of biological patterns and an understanding of the processes engendering them could be considered more important than what the patterns are called. For instance, the knowledge that *Polystichum neozelandicum* subsp. *neozelandicum* and subsp. *zerophyllum* (see Chapter Four) appear to be two separate evolutionary lineages that are allopatric, morphologically very similar, and seemingly both allopolyploids, is probably more important than whether they are recognised at the subspecific or specific level.

Names too are of course very important. They greatly facilitate succinct communication, but this succinctness comes with the price of biological imprecision. That separate evolutionary lineages can be delimited might be considered monistic, but the engendering of these lineages is pluralistic. Further, because of the differences by which lineages are engendered in NSO and SO organisms, the taxonomic delimitation of lineages is necessarily pluralistic (if it is to retain any degree of practicality). Even with the objective foundation of evolution, the only equivalence between entities of the same taxonomic category may be just that: their delimitation to the same taxonomic category.

Chapter Three

Materials and Methods.

3.1 Sampling.

Samples collected in the field for the molecular analyses constituted three to four primary pinnae stored in small, 'snap-lock,' plastic bags, along with c.20 g of anhydrous silica gel (6-8 mesh, BDH). This ensured rapid desiccation of the tissue (Chase & Hills 1991), preserving it in a state from which high-molecular weight DNA could still be extracted after long-term storage (> three years at -20°C, or room temperature if in unlit conditions). Young, soft, but unfurled, fronds were targeted preferentially for samples intended for molecular work, as such tissue was found to be the easiest to extract DNA from, and also to provide the greatest yields.

Herbarium specimens were collected to accompany all 'molecular' samples, and to represent additional sampled plants. Fronds with ripe sporangia were targeted preferentially for herbarium specimens. When available, two to three sporangia-bearing primary pinnae were placed in paper envelopes, providing a 'spore' sample. Fronds were pressed and dried for long-term storage.

Several whole plants were collected and grown in the shade-house facility in the Ecology compound at Massey University. Once established and producing new (fertile) fronds, these provided a ready source of material for cytological analysis (Section 3.3).

Full collection details of all samples included in the morphological, cytological and molecular analyses of this study are given in Appendix 2.1. Specimens of all samples included in the molecular and cytological analyses, together with representative specimens from the morphological analysis will be lodged in WELT, MPN, AK, and CHR. Type specimens will be annotated accordingly.

3.2 Morphological Methodology.

3.2.1 Measurements.

Morphological characters were investigated as described below. Details of characters found to be useful in differentiating taxa are further described in the relevant chapters: Chapter Four for the *Polystichum neozelandicum* complex, and Chapter Five for *P. vestitum*.

FRONDS & PRIMARY PINNAE: These were measured to the nearest millimetre using a ruler. Length and width measurements were made on the longest primary pinnae from both sides of the frond, and averaged. The (often enlarged) basal secondary pinnae were excluded when measuring the maximum width of the longest primary pinnae.

RACHIS SCALES: Length and width measurements of rachis scales were made at x40 magnification. For plants of the *P. neozelandicum* complex, five of the larger scales from around the stipe-rachis junction were measured. These were removed intact with tweezers, and mounted in a solution of 50:50 glycerol (BDH):water. For *P. vestitum* plants, five of the larger scales from the mid-rachis were also measured in addition to those from the stipe-rachis junction. Measurements from these different positions were analysed separately. The rachis scales of *P. vestitum* are usually bicolorous, with a central dark-brown area surrounded on all sides by a light-brown area. A quantitative measurement of the proportion of the total scale area occupied by the dark centre was *estimated* by calculating the ratio of the length × width product of the dark centre (at its mid-length) to the same product for the whole scale. Measurements from each scale were averaged to give one value (ie. the mean) for each character for each individual. The occurrence and length of marginal projections on the rachis scales were qualitatively assessed at x40 magnification.

INDUSIA: Ten randomly selected indusia were removed from each frond, using tweezers under a dissecting microscope, and mounted in a solution of 50:50 glycerol:water. The

maximum diameter of each indusium was measured at x100 magnification. A quantitative assessment of the percentage of the indusium surface area occupied by the dark central area was calculated using a measurement made of it in the same plane as the measurement of the maximum diameter of the indusium.

SPORANGIA: These were removed in bulk using tweezers and mounted in a solution of 50:50 glycerol:water. The number of indurated annulus cells was counted at x100 magnification, with counts made from ten randomly selected sporangia for each frond.

SPORES: These were transferred from spore envelopes (Section 3.1) using flame-cleaned tweezers to a mountant solution of 50:50 glycerol:water. Maximal length and width measurements, to the nearest micrometre, of spore exine and perine were made from 30 spores per individual at x1000 magnification.

3.2.2 Analysis of Morphological Data.

All analysis (calculation, graphing, etc.) of the morphological data was performed using the program SPSS 10.1.0 (SPSS 2000).

Concordant partitioning across morphological characters was used to (retrospectively) test the null hypothesis that 'a single evolutionary lineage was present.' The underlying principle is most easily visualised for qualitative characters. For instance, with two binary-state characters (ie. character X with states X1 & X2, and character Y with states Y1 & Y2), concordant partitioning can be inferred if all samples, when tabulated for their state for each character, fall into two of the quadrants which are on the diagonal to one another (the 'two-diagonal-quadrant' condition) (Table 3.1). Such concordant partitioning across two independent characters would be unlikely to arise between two groups of individuals unless they were fertilising assortatively (ie. unless there were two separate lineages present).

Table 3.1. Concordant Partitioning in Qualitative Characters.

Examples of when concordant partitioning is present (A, B), and when it is not (C, D), for two qualitative characters, 'X' and 'Y,' each with two states (ie. 'X1' & 'X2,' 'Y1' & 'Y2'). Individuals are denoted by lowercase letters.

(A) Individuals have either characters states X1 & Y1 or X2 & Y2. Both characters partition the sample set in the same way; {a b c d e f g h i j}{k l m n o p q r s t}. That is, they are concordant in their partitioning.
(B) Individuals have either characters states X1 & Y2 or X2 & Y1. Both characters partition the sample set in the same way; {a b c d e f g h i j}{k l m n o p q r s t}. That is, they are concordant in their partitioning.
(C) Individuals have either character state X1 or X2, but all have Y1. The two characters do not partition the sample set concordantly (character X partitions the sample set as; {a b c d e f g h i j}{k l m n o p q r s t}, but character Y does not partition the sample set at all).
(D) Individuals exist with all possible combinations of character states (ie. X1 & Y1, X1 & Y2, X2 & Y1, X2 & Y2). Character X partitions the sample set as {a b c d e f g h i j}{k l m n o p q r s t}, but character Y partitions the sample set as {a b c d e f k l m n}{g h i j o p q r s t}. Their partitioning of the sample set is not concordant.

(A)

	X1	X2
Y1	a b c d e f g h i j	
Y2		k l m n o p q r s t

(B)

	X1	X2
Y1		k l m n o p q r s t
Y2	a b c d e f g h i j	

(C)

	X1	X2
Y1	a b c d e f g h i j	k l m n o p q r s t
Y2		

(D)

	X1	X2
Y1	a b c d e f	k l m n
Y2	g h i j	o p q r s t

The same principle can be applied to quantitative characters. When two quantitative characters, which exhibit concordant partitioning of the sample set, are plotted on a two-dimensional scatter-plot, samples will fall into two (not necessarily equal) quadrants of the graph which are diagonally opposite one another. This approach has the advantage in that the axes represent variation in observed characters. Consequently, the presence

of concordant partitioning between two (or more) independent characters may be related to assortative fertilisation. This is not the case for other methods such as Principal Components Analysis (PCA) (eg. Perrie et al. 2000), where the axes represent composite variation across multiple characters. For two putatively separate groups, all that can be concluded from PCA analysis is whether or not they lie in different regions of multidimensional space, the cause of which may be variation in several characters, or only one.

Concordant partitioning may be 'absolute,' where all individuals can be assigned to one or other of two diagonal quadrants, or it may be 'almost,' where most but not all individuals fall into two diagonal quadrants. Figure 3.1 illustrates the range of concordant partitioning, from 'absolute' to none.

Inference of assortative fertilisation might be strongest with 'absolute' concordant partitioning across independent characters, but 'almost' concordance may still be highly indicative (depending on how many samples 'violate' the two quadrant diagonal condition, and other properties of the sample set). 'Almost' concordance may be recovered between two separate lineages for a number of reasons, including hybridisation or simply that the states of the characters may not have reached alternate fixation.

This latter reason means that, while the recovery of concordant partitioning across independent characters can be used to infer the presence of assortative fertilisation (and then, in turn, the presence of separate lineages), the absence of concordant partitioning does not necessarily imply that only one lineage is present. (For instance, *P. silvaticum* and *P. vestitum* show marked overlap in variation between a number of characters, eg. 'number of primary pinnae' and 'length of longest primary pinna' (data not shown), but other characters concordantly partition them strongly, supporting their separation; see Chapter Five.) This represents another advantage of the concordant approach over PCA and other similar methods, in that the inclusion in PCA of 'randomly'-varying

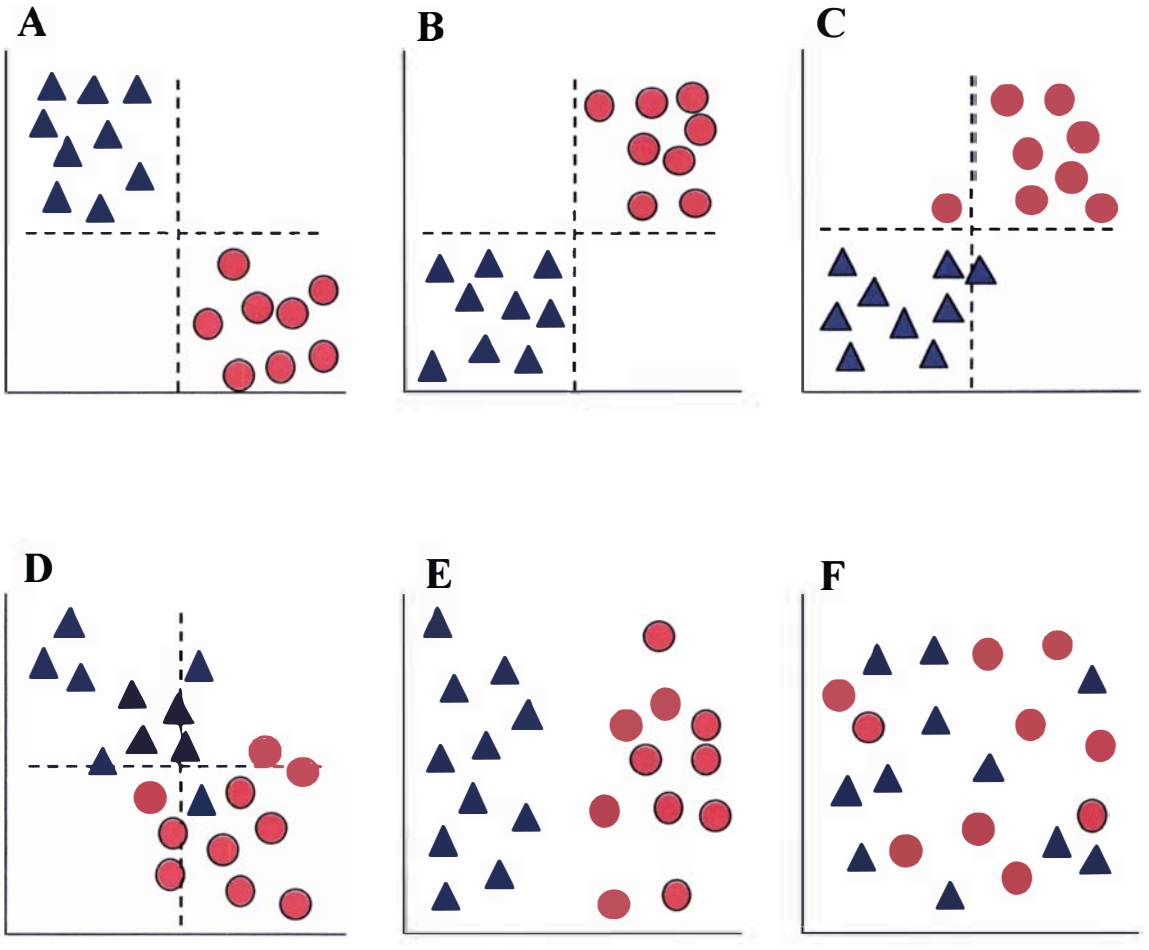


Figure 3.1. Concordant Partitioning With Quantitative Characters.
Examples of differing degrees of concordant partitioning between two quantitative characters on two-dimensional scatter-plots. (A) and (B): 'absolute' concordance, with no violation of the two diagonal quadrant condition. (C): strong 'almost' concordance. (D): weak 'almost' concordance. (E): no concordance, with partitioning by only one character. (F): no concordance, with variation in both characters essentially 'random.'

characters can actually mask concordantly-varying characters, such that lineages (concordantly partitioned by some characters) are distributed randomly in the PCA hyperspace. This is a problem because without a direct analysis of character concordance, it is impossible to determine *a priori* which characters are 'random' and which are 'concordant.'

The more independent characters that concordantly partition two groups the stronger the inference of assortative mating. The approach outlined above is limited to pair-wise comparison of characters, with visual inspection of these two dimensions as to whether

concordant partitioning is present. However, the principle is extendable to the simultaneous assessment of three characters (although visual determination of concordant partitioning is more difficult in three dimensions, at least with the available tools), or even more. Hyper-dimensional assessment of concordant partitioning (four or more characters assessed simultaneously) might be possible computationally, and may even be an emergent property of the data, although its development is beyond the scope of this project.

3.3 Cytological Methodology.

Material for cytological analysis was collected from plants grown in the shadehouse facility in the Ecology compound of Massey University. In New Zealand species of *Polystichum* the outer sporangia in a given sorus mature earlier than those more internal, such that a range of developmental stages are present in a maturing sorus. Sori bearing full-size but still white sporangia were targeted. Pinnae segments bearing such sori were removed with scissors about midday (11am-2pm), and fixed for c.20 hours in a 3:1 solution of absolute ethanol and glacial acetic acid.

After fixation, the pinnae were rinsed in water (to remove the fixative solution, which otherwise interfered with the subsequent staining procedure), and blotted briefly on a paper towel to remove excess water. Under a dissecting microscope, 15-25 sporangia were isolated on a glass slide from the remainder of the tissue. These were allowed to air dry for several minutes.

Acetocarmine solution was added to the sporangia, and then left to stain for c.3 minutes. A flat-ended aluminium rod was then used to pulverise the sporangia until most of the tissue was single-cellular. A cover-slip was placed over the material, with additional acetocarmine added if necessary. The slide was gently heated over an ethanol-wicker flame for 10-20 seconds. The slide (with cover-slip) was then held between two pieces

of filter-paper, and the preparation squashed manually by placing the thumb over the position of the cover-slip and pressing as hard as possible.

Slides of the squashed material were scanned at x100 magnification for chromosomes. Digital images of countable chromosome preparations at diakinesis were recorded at x1000 (with oil immersion) using a Zeiss Axiophot microscope, with a JVC 3-CCD colour video camera and a Silicon Graphics Indy computer. For some images, the Nomarski setting of the Zeiss Axiophot microscope was employed to maximise the contrast between individual, but layered, pairs of homologous chromosomes.

3.4 Molecular Methodology.

3.4.1 DNA Extraction.

DNA was extracted from silica-gel dried, or freshly collected (< 1 hour) tissue material using a protocol modified from Doyle and Doyle (1990). The tissue was ripped into small fragments using clean tweezers and placed into a 1.6 mL microcentrifuge tube until it was one-quarter to a third full. The tissue was then promptly snap-frozen by suspending the microcentrifuge tube, uncapped, in liquid nitrogen for approximately one minute.

The frozen tissue was then ground into a fine powder using a 'grinding' tool made from a glass pasteur pipette (Volac). These tools were made by heating the pipette at the position where it becomes narrowed in a blue-hot bunsen flame. As the glass melted, the thinner section was discarded, and the molten end of the thicker section was moulded in the flame to create a small bulb that, once cooled, would comprise the grinding surface of the tool. One grinding tool was prepared for each tissue sample.

Several iterations of freezing and grinding were often necessary to completely grind the tissue to a fine powder, particularly when it was sourced from older (and tougher) fronds. When the tissue had been ground to a fine powder, 600 μL of CTAB extraction

buffer (2% w/v CTAB (cetyl- trimethyl-ammonium bromide, Sigma), 1% w/v PVP (polyvinyl-pyrrolidone, Sigma), 1.4 M sodium chloride, 100 mM Tris-HCl pH 8.0, and 20 mM EDTA (ethylenediaminetetra-acetic acid, BDH)) was added to the tube, which was then capped and agitated to thoroughly mix the contents. The tube was then placed in a heating block at 65°C, where it was incubated for 40 minutes with occasional gentle agitation.

At the completion of incubation, 600 µL of chloroform was added to the tube, and the contents thoroughly mixed by vigorous agitation. The tube was then centrifuged briefly (until the rotor force reached 5000 x g), such that the heavier organic phase (chloroform and tissue proteins) was sequestered at the bottom of the tube, and the tissue fragments compacted at the interface. The lighter aqueous phase (containing the DNA) was then transferred to a new 1.6 mL microcentrifuge tube, using a pipette with a wide-bored tip (ie. a normal 1 mL tip with approximately 5 mm cut from the narrow end with a scalpel blade) to mimimise mechanical shearing of the DNA.

To the collected aqueous solution, 600 µL of isopropanol was added. The tube was then inverted once to gently mix the contents, and left to stand on ice for c.5-10 minutes. During this time, if the extraction of high molecular weight DNA was successful, the DNA would precipitate in white spools at about half the depth of the solution.

Precipitated DNA was transferred, using a pipette with a wide-bored tip (see above), to a new 1.6 mL microcentrifuge tube containing c.800 µL 80% ethanol. The tube was gently inverted so as to wash the DNA precipitate in the ethanol solution (removing protein and carbohydrate contaminants). This ethanol wash was then repeated, with agitation becoming progressively more vigorous.

The ethanol was then tipped from the tube (with the DNA pelleted by gentle centrifugation if necessary), which was left uncapped (but covered by foil) to allow the

DNA to air dry. When liquid could no longer be seen in the tube, TE (10 mM Tris-HCl, 1 mM EDTA pH 8.0) buffer was added with the exact volume depending on the amount of DNA present (usually 30-50 µL of TE, but occasionally as much as 80 µL). The tube was then left uncapped (but covered by foil) for an additional c.1 hour to allow any remaining ethanol to evaporate. The DNA was resuspended by gentle agitation, and by overnight incubation in a fridge (to better ensure complete re-suspension). 4 µL of the extraction from each sample was then treated to remove RNA, by incubating it at 65°C for 2 minutes with 1.5 µL of 10x loading buffer (see section 3.4.2.1) containing 5 ng/µL RNAse A. This was then electrophoresed on a 1% w/v agarose gel (see Section 3.4.2.1) to provide a qualitative assessment of the quantity of the DNA present, as well as allowing confirmation that high molecular weight DNA was present. The remaining solution of extracted DNA was then stored at –80°C until required.

3.4.2 DNA Electrophoresis.

Electrophoresis of DNA was performed on two types of gel matrix, agarose and polyacrylamide.

3.4.2.1 Agarose Gels.

Electrophoresis of extracted and restricted DNA, and of single-locus PCR products, was conducted using 1% w/v agarose gels: 1% w/v Seakem LE agarose (FMC BioProducts) in 1× TAE buffer (40 mM Tris acetate, 1mM EDTA pH 8.0). 3% w/v agarose gels were used to check the selective amplification PCR step of the AFLP protocol. Before loading on the gel, samples were combined with c.1 µL of 10x loading buffer (27.5% w/v Ficoll Ty 400 (Pharmarcia), 0.44% w/v bromophenol blue (Serva) and 0.44% w/v xylene cyanol (Sigma)). A lane containing 400 ng of 1 Kb Plus DNA Ladder (Gibco BRL) was always run as a positive control. Electrophoresis was conducted at 5-6 V/cm in 1× TAE buffer, for 50-70 minutes. DNA samples were then visualised by ethidium bromide fluorescence on an UV transilluminator (wavelength 302 nm, UVP Incorporated), with a digital image captured using a video camera (Panasonic) and ImagePC (Scion) software.

3.4.2.2 Polyacrylamide Gels.

AFLP profiling and single-locus genotyping (ie. the characterisation of size differences between PCR products) were carried out on silver-stained denaturing-polyacrylamide gels. The glass plates used to make these gels were, prior to each use, thoroughly cleaned with detergent and then repeatedly rinsed with water to remove any soapy residue. They were then dried with paper towels, and one surface of each plate (the surface that was to be internal) was washed twice with c.2 mL of 80% ethanol.

To the cleaned surface of the shorter plate was added 2 mL of bind-silane solution (0.5% v/v glacial acetic acid, 0.05% v/v Bind-Silane (Pharmarcia), in 95% ethanol). This was applied thoroughly to the entire cleaned surface using a Kimwipe (Kimberly-Clark), and allowed to dry for 4-5 minutes. Excess bind-silane was then removed from the plate's surface by four vigorous washes of 2 mL of 95% ethanol with a Kimwipe.

The repellant RAIN-X® was applied to the cleaned surface of the longer plate according to the manufacturer's instructions. This was then dried using a Kimwipe.

The plates were assembled with a pair of 0.4 mm spacers (Gibco BRL) separating their internal surfaces. Extreme care was taken to avoid the internal surfaces of the plates coming into contact, and to avoid the transfer of residue from one plate to the other. The assembled plates and spacers were then clamped together using a S2 casting boot (Gibco BRL).

The polyacrylamide (PAA) gel solution was prepared as follows: 10% v/v Long Ranger gel solution (FMC BioProducts) for the 5% PAA gels used for AFLP profiling (or 16% v/v Long Ranger gel solution for the 8% PAA gels used for single-locus genotyping), 8 M urea, and 1× TBE buffer (90 mM Tris-borate and 1 mM EDTA pH 8.0) in a total volume of 70 mL. This solution was filtered through 2 pieces of Whatman 1 filter paper. Just prior to pouring the gel, 350 µL of 10% w/v ammonium persulphate

(Sigma) and 35 μL NNN'N'-tetramethylethylenediamine (TEMED, BDH) were added to the filtered solution, and mixed in with gentle agitation.

A 60 mL syringe was used to slowly dispense the gel solution between the glass plates. With the solution in place between the plates, a pair of shark-tooth combs (5.7 mm point-to-point spacing, Gibco BRL) were inserted in reverse orientation to form the sample well. These combs were clamped in place with several large bulldog clips. The gel was then left to polymerise for c.1-1.5 hours. It was then either used immediately, or stored overnight at 4°C with a glad-wrap seal.

Immediately prior to use, the casting boot and bulldog clips were removed and the glass plate-gel assembly placed into an S2 electrophoresis apparatus (Gibco BRL). Both of the buffer tanks were filled with approximately 600 mL of 1× TBE buffer. The combs were removed, and a 25 mL syringe was used to flush excess urea and any PAA fragments or air bubbles from the sample well. The gel was then pre-run at 55 W for 45 minutes. The sample well was again flushed to remove urea, and the shark-tooth combs inserted so that their teeth were just embedded within the gel.

Each sample (3.5 μL for AFLP profiling, 3 μL for single-locus genotyping) to be loaded on a PAA gels was mixed with 1-1.5 μL of formamide loading dye (98% v/v formamide, 10 mM EDTA, 0.05% w/v bromophenol blue, and 0.05% w/v xylene cyanol) in clean 0.2 mL reaction tubes. This mixture was then denatured at 94°C for four minutes in a PCR machine, before being rapidly cooled to 4°C. Samples were subsequently kept on ice until loaded.

Each well was flushed before a sample was loaded into it; loading was accomplished using a flat 0.37 mm gel pipette tip. 4 μL of a preparation of 100 base-pair (b.p.) DNA Ladder was loaded on either side of the sample set (Gibco BRL; 1 μL in 99 μL of formamide loading dye, and denatured as above. The remainder of the ladder preparation was stored at -20°C). Electrophoresis was then carried out at 40 W for

approximately three hours, until the bromophenol blue dye front was c.5 cm from the bottom of the PAA gel.

Following electrophoresis, the DNA on the PAA gel was visualised by silver-staining (Promega 1998). The glass plates were separated by levering a scalpel blade between them. The short glass plate, to which the PAA gel had adhered, was transferred to a developing tray containing 4 L 10% v/v acetic acid, and gently agitated on a mechanical shaker for two hours. This removed the urea from the gel matrix. The gel was then transferred to a clean tray and washed, with gentle agitation, for c.5 minutes in 2 L Milli–Q water (MQ H_2O). This step was repeated an additional two times, until the acetic acid had been removed from the gel matrix (confirmed when water sheeted smoothly off the gel's surface, rather than forming rivulets).

The gel was then placed in a clean tray containing 3 L of stain solution (6 mM silver nitrate ($AgNO_3$, BDH) and 0.15% v/v formaldehyde (37%, BDH)), and gently agitated for one hour. Then the gel was briefly rinsed (for approximately three seconds) in 2.5 L of chilled MQ H_2O, and promptly transferred to a clean tray containing 2 L of chilled developing solution (280 mM anhydrous sodium carbonate (Na_2CO_3, AnalaR, BDH), with 0.16% v/v formaldehyde (37%, BDH) and 50 μM sodium thiosulphate ($Na_2S_2O_3$, BDH) added immediately prior to use). The gel was initially agitated vigorously by hand to disperse the brown precipitate that would otherwise form, and then gentle mechanical agitation was continued. When banding patterns first became apparent on the gel, it was quickly transferred to another clean tray containing an additional 2 L of chilled developing solution. Mechanical agitation was continued until the AFLP profiles (or single-locus PCR products) were satisfactorily developed, at which point c.2 L of chilled 10% v/v acetic acid was added to the tray as a fixative to stop the reaction. The solutions were vigorously mixed, and left for 3-5 minutes until the bubbling of CO_2 had ceased. The gel was then rinsed in water to remove the acetic acid, and stored upright in an operating fume-cupboard to dry overnight.

3.4.3 Single Locus PCR Analyses.

3.4.3.1 PCR Protocol.

PCR of single locus regions was carried out in 0.2 mL reaction tubes (Sorenson BioScience). Each reaction mixture typically contained 1× Q solution (Qiagen), 1× PCR buffer (Tris-HCl, KCl, $(NH_4)_2SO_4$, 1.5 mM $MgCl_2$; Qiagen), 250 µmol of each deoxy-nucleosidetriphosphate (dNTP; Boehringer Mannheim), 10 pmol of each primer, 1 U of *Taq* DNA polymerase (5 U/µL; Qiagen), and 1 µL of diluted genomic DNA as template (with dilutions typically varying between ×0.1 and ×0.01, providing approximately 5-50 ng of template DNA), in a final amplification volume of 20 µL (MQ H_2O added as necessary). A negative control, containing no template DNA, was included with all reaction sets. Primer oligonucleotides were sourced from Life Technologies.

The thermocycling of PCR reactions was performed in either a MJ Research PTC-200 DNA Engine or a MJ Research PTC-150 Minicycler. Both machines were equipped with heated lid attachments to prevent evaporation of the reaction mixture, such that the addition of oil to the PCR reactions was not required. Following thermocycling, 3 µL of each amplified reaction was electrophoresed on a 1% w/v agarose gel, as described in Section 3.4.2.1, to determine the efficacy of the amplification (and to check the negative control).

Two single locus regions, both from the chloroplast, were investigated for variation; the *rbcL* gene, and the *rps4-trnS* spacer.

THE *RBCL* LOCUS. Previous studies of the *rbcL* locus in ferns are discussed in Section 6.1. *Polystichum*-specific external and internal primers were designed from a consensus sequence of existing *Polystichum* accessions of the *rbcL* locus in Genbank (Accession numbers U05938 & U30832). These primers are detailed in Appendix 3.1.1. The thermocycling profile used was: initial denaturation of 94°C for 2 minutes; 36 cycles of 94°C for 1 minute, 55°C for 1 minute, and 72°C for 1 minute; final extension of 72°C for 5 minutes; indefinite hold at 4°C.

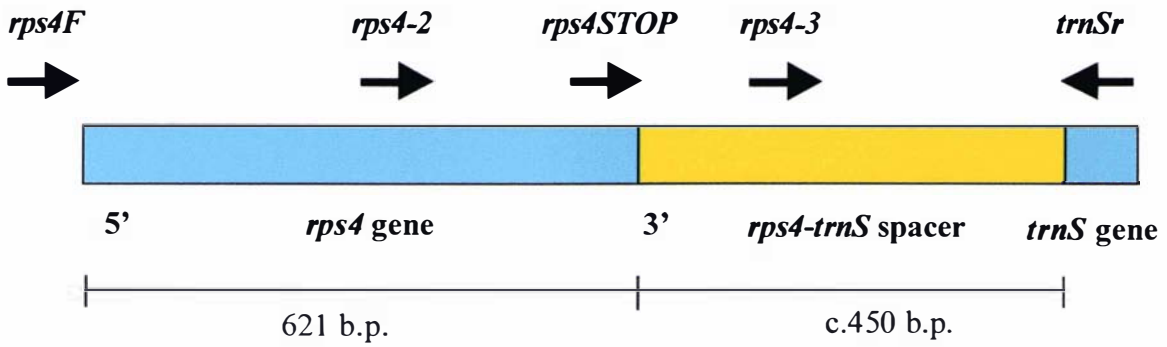


Figure 3.2. Primer Map of the *rps4* Region.
Map of primers for the *rps4* gene and the *rps4-trnS* spacer region. Primer position and orientation are denoted with arrows. Indel events in the spacer region means the base-pair (b.p.) size indicated above is only approximate.

THE *RPS4-TRNS* SPACER. This locus is described further in Section 6.1. Sequences of two external primers, which amplify the entire *rps4* gene and *rps4-trnS* spacer region in the fern genus *Blechnum* L., were supplied by Ray Cranfill (University of California, Berkley). These primers were found to amplify relatively poorly (unspecifically) in New Zealand *Polystichum*. However, sufficient sequence of the *rps4* gene was obtained such that novel, specific primers could be designed to facilitate efficient amplification of the *rps4-trnS* spacer region. Primers for this locus are detailed in Appendix 3.1.1, and their approximate positions relative to the locus are mapped in Figure 3.2.

For genotyping, the *rps4-trnS* spacer region was amplified using the primer combination *rpsSTOP* and *trnSr* with the following thermocycling profile: initial denaturation of 94°C for 2 minutes; 38 cycles of 94°C for 1 minute, 50°C for 1 minute, and 72°C for 45 seconds; final extension of 72°C for 5 minutes; indefinite hold at 4°C. For sequencing, the *rps4-trnS* spacer region (together with c.200 b.p. of the 3' end of the *rps4* gene) was typically amplified using the primer combination *rps4-2* and *trnSr* with the following thermocycling profile: initial denaturation of 94°C for 2 minutes; 38 cycles of 94°C for 1 minute, 58°C for 1 minute, and 72°C for 1 minute; final extension of 72°C for 5 minutes; indefinite hold at 4°C.

3.4.3.2 Sequencing of Single Locus PCR Products.

PCR products successfully amplified for DNA-sequencing were purified using the CONCERT Rapid PCR Purification System (Gibco BRL). The manufacturer's instructions were followed except that the tubes were allowed to stand for c.5 minutes (rather than 1 minute) before the final elution, and that 40 μL (rather than 50 μL) of warmed TE was used to elute the products. 2 μL of each purified sample was electrophoresed on a 1% w/v agarose gel (see Section 3.4.2.1), and the concentration of PCR product estimated using the Low DNA Mass Ladder (Gibco BRL).

The purified PCR products were sequenced in both directions by automatic sequencing, using the ABI PRISM™ Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin Elmer). Each reaction was prepared in a 0.2 mL reaction tube containing 4 μL of Terminator Ready Reaction Mix (Perkin-Elmer), 1.6 ρmol of primer, and 25-50 ng of purified PCR product, with sufficient MQ H_2O to give a final reaction volume of 10 μL. Bi-directional sequencing of the *rbcL* locus employed all six *rbcL* primers listed in Appendix 3.1.1 as sequencing primers. Sequence for approximately 95% of the *rps4-trnS* spacer region could be obtained using *rps4-2* and *trnSr* (see Appendix 3.1.1) as sequencing primers. To get the full sequence, with the remaining c.20 b.p. at the *trnS* end of the spacer, required an extra sequencing reaction using the primer *rps4-3*. The automatic sequencing reactions were performed in either a MJ Research PTC-200 DNA Engine or a MJ Research PTC-150 Minicycler, using the following thermocycling profile: 25 cycles of 96°C for 10 seconds, 50°C for 5 seconds, and 60°C for 4 minutes; indefinite hold at 4°C (with ramping between temperature steps limited to 1°C/second).

Automatic sequencing products were subsequently purified by ethanol precipitation in a 1.6 mL microcentrifuge tube. 1 μL of 3 M sodium acetate pH 5.2 and 25 μL of ice-chilled (c. 0°C) absolute ethanol were added to each automatic sequencing reaction, and mixed by gentle agitation. The tubes were then stood on ice for approximately 7 minutes, followed by centrifugation at 13 000 × g for 15 minutes at 4°C. The supernatant was then carefully removed using an automatic pipette, and 700 μL of ice-

cold 80% ethanol added to the tube, which was repeatedly inverted to wash the ethanol over the pellet. The tube was then centrifuged again at 13 000 × g for 15 minutes at 4°C. The supernatant was decanted, and as much ethanol was removed as possible by capillary action with pipette tips, with care taken not to disturb the pelleted products. The uncapped tubes were left to air-dry until there were no visible signs of liquid remaining. The tubes were then capped and stored at 4°C until ready for sequencing. Sequencing was performed by the Massey University Sequence Analysis Facility (MUSEQ), using an Applied Biosystems 373A DNA Sequencing System.

3.4.3.3 Analysis of the Sequence Data.

The output files from MUSEQ for the different sequencing reactions for each sample were compiled to give a consensus sequence covering the entire locus. These sequences were aligned with the program ClustalX 1.8 (Thompson et al. 1997), with the resultant alignment checked and edited manually. This was a relatively straight-forward process, with the alignment largely unambiguous, because of the low level of sequence difference found between samples. The program Splitstree 2.4 (Huson 1997, 1998) was used to analyse and represent the data, with the split-decomposition method (see Huson 1998, Lockhart et al. 2001) implemented under a parsimony-criterion (although the results here are not discernibly different under a distance-criterion). Because of the low complexity of the sequence data (with only two-dimensional conflict), all inferable mutation events could be represented in the splits-graphs.

Several concepts pertinent to the analysis of sequence data (eg. 'splits,' 'edges') are discussed in Section 3.4.4.6.

3.4.3.4 rps4-trnS *Spacer Genotyping.*

Sequencing of the *rps4-trnS* spacer region detected size polymorphism, due to insertion and/or deletion ('indel') mutation events, between *P. wawranum* and *P. oculatum*, and again between *P. silvaticum* and *P. vestitum*. These size polymorphisms were assayed by genotyping on PAA gels with a larger sample set analysed than what would have

been possible (financially) with direct sequencing. The PCR for this genotyping is described in Section 3.4.3.1, with the resultant PCR products electrophoresed and visualised on silver-stained 8% denaturing-PAA gels as described in Section 3.4.2.2.

3.4.4 AFLP Analyses.

AFLP is a DNA fingerprinting technique said to combine the replicability of RFLP (Restriction Fragment Length Polymorphism) with the sensitivity of PCR (in that relatively low amounts of template DNA are required) (Mueller & Wolfenbarger 1999). AFLP was originally described by Vos et al. (1995), and was recently reviewed by Mueller & Wolfenbarger (1999). The great advantage of this technique is that it simultaneously assays variation at multiple, independent (but anonymous) loci. Not only does this allow resource-efficient detection of genetic variation at very low taxonomic levels (ie. good resolution), but it can provide data from multiple, independent characters, allowing an assessment of genomic-level relationships between individuals. AFLP is commonly assumed to represent an abbreviation of 'Amplified Fragment Length Polymorphism.' However, Vos et al. (1995 p.4413) specifically cautioned that "the name AFLP ... should not be used as an acronym, because the technique will display presence or absence of restriction fragments rather than length differences."

3.4.4.1 Restriction of Genomic DNA.

The initial step in AFLP analysis is the digestion of template (high molecular weight) genomic DNA with restriction enzymes. For each sample to be analysed, 300-600 ng of genomic DNA was restricted in a total volume of 25 μL containing 4 U *Mse*I (New England Biolabs), 10 U *Eco*RI (Roche), 50 mM potassium acetate (Sigma), 10 mM magnesium acetate (Sigma), and 10 mM Tris-HCl pH 7.5. Restriction reactions were incubated at 37°C for three hours, with occasional agitation, before being heated to 70°C for 15 minutes to denature the restriction enzymes. To confirm complete digestion, 5 μL of the restriction reaction was electrophoresed on a 1% w/v agarose gel (see section 3.4.2.1).

3.4.4.2 Ligation of Adapter-Linkers to the Digested DNA.

Adapter-linkers were ligated on to the 'sticky-ends' of the digested DNA to form target primer sites for the subsequent PCR steps. The adapter-linkers were prepared as follows; *Eco*RI linker: 500 ρmol *Eco*RI adapter I oligonucleotide, 500 ρmol *Eco*RI adapter II oligonucleotide, and 45% v/v TE (10 mM Tris-HCl, 1mM EDTA pH 8.0) in a total volume of 100 μL; *Mse*I linker: 5 nmol *Mse*I adapter I oligonucleotide, 5 nmol *Mse*I adapter II oligonucleotide, and 45% v/v TE (10 mM Tris-HCl, 1mM EDTA pH 8.0) in a total volume of 100 μL. The sequences of these oligonucleotides are detailed in Appendix 3.1.2. These linker mixtures were then incubated at 94°C for 4 minutes, before being allowed to slowly cool to room temperature.

*Eco*RI and *Mse*I linkers were ligated on to the digested DNA of each sample in a 10 μL reaction containing 2.5 ρmol *Eco*RI linker, 25 ρmol *Mse*I linker, 0.5 Weiss U T4 ligase (Gibco BRL), 1× ligation buffer (Gibco BRL), and 2-6 μL of digested DNA (usually 5 μL, but adjusted so that equivalent amounts of digested DNA were ligated for all samples). These ligation mixtures were agitated, gently centrifuged, and incubated at 4°C overnight. The following day, the ligation mixtures were used as templates for the AFLP PCR steps, or stored at -80°C until use.

3.4.4.3 The AFLP PCR Steps.

PCR for AFLP fingerprinting is performed in two steps; an initial 'pre-amplification' and a subsequent 'selective-amplification.' In a sense, both steps are selective in that they target only a subset of all possible templates for amplification (Vos et al. 1995, Gibco BRL: AFLP Analysis System I Instruction Manual). Primers used at the 'pre-amplification' step are designed to the adapter-linker sequences but have an additional one-base overhang, so that only some (approximately $1/4 \times 1/4 = 1/16$, assuming equal base frequencies) of the linkered-digestion products are amplified. Similarly, the primers used at the 'selective' amplification step have three-base overhangs, so that for each reaction only a further subset of the pre-amplification products is re-amplified.

Such amplification of a subset of all possible templates is necessary to reduce the complexity of the resultant profiles (Vos et al. 1995).

Pre-amplifications were carried out in a total volume of 20 μL containing 1× Q solution (Qiagen), 1× PCR buffer (Qiagen), 250 μmol of each dNTP (Boehringer Mannheim), 10 ρmol of *Eco*RI pre-amplification primer (see Appendix 3.1.2 for primer sequences), 10 ρmol of *Mse*I pre-amplification primer, 1 U of *Taq* DNA polymerase (Qiagen), and 1 μL of undiluted ligation-reaction products. Any additional dilution of the ligation-reaction products was found to introduce inconsistency into the resultant profiles. Thermocycling was performed in a MJ Research PTC-200 DNA engine with the following profile: 20 cycles of 94˚C for 30 seconds, 56˚C for 1 minute, 72˚C for 1 minute; indefinite hold at 4˚C (with ramping between temperature steps limited to 1˚C/second).

Selective-amplifications were carried out in a total volume of 20 μL containing 1× PCR buffer (Qiagen), 50 mM $MgCl_2$ (Qiagen), 250 μmol of each dNTP (Boehringer Mannheim), 10 ρmol of *Eco*RI selective primer (see Appendix 3.1.2 for primer sequences), 10 ρmol of *Mse*I selective primer, 1 U of *Taq* DNA polymerase (Qiagen), and 1 μL of a 1/50 dilution of pre-amplification products. Trials indicated that profile consistency was robust across a range of pre-amplification product dilutions (at least 1/10 to 1/100). Thermocycling was performed in a MJ Research PTC-200 DNA engine with the following profile: an initial denaturation at 94˚C for 2 minutes; 6 cycles of 94˚C for 30 seconds, 65˚C for 30 seconds, 72˚C for 1 minute; 6 cycles of 94˚C for 30 seconds, 60˚C for 30 seconds, 72˚C for 1 minute; 24 cycles of 94˚C for 30 seconds, 56˚C for 30 seconds, 72˚C for 1 minute; final extension of 72˚C for 5 minutes; indefinite hold at 4˚C (with ramping between temperature steps limited to 1˚C/second).

To test the efficacy of the AFLP PCR before PAA gel electrophoresis, 7 μL of selective amplification product was electrophoresed on a 3% w/v agarose gel (see Section

3.4.2.1). Consistent smearing between 100-1000 b.p. (usually with at least some emergent bands) indicated successful amplification in all samples.

3.4.4.4 Electrophoresis, and Profile Visualisation and Scoring.

AFLP PCR products were profiled by electrophoresis on denaturing PAA gels and visualised with silver staining (see Section 3.4.2.2). Once the gel had dried, the profiles were scored over a light-box. A band at a given size position in any sample was assumed to represent an independent character, and was scored as present or absent in all samples. Qualitative differences in band intensity were not scored. The resultant binary data matrix was converted into NEXUS format for analysis.

3.4.4.5 Quality of AFLP Data.

Trialling with independent, duplicate extractions (of samples from both the *P. neozelandicum* complex and *P. vestitum*) indicated that AFLP profiles produced from the above protocol were highly reproducible (> 99%). This is consistent with other studies (Mueller & Wolfenbarger 1999 p.392, and references therein) which have reported an overall error rate (including mis-priming and scoring error) of generally less than 2%. However, it was noted during trialling that dilution of the ligation reaction products before use as template for the pre-amplification step (over and above the dilution of the 1 μL of ligation product in the 20 μL total volume of the pre-amplification step; Section 3.4.4.3) resulted in marked inconsistencies between the resultant profiles of duplicate extractions. Transitions between other stages (ie. restriction → ligation, pre-amplification → selective amplification) were not so susceptible to the dilution factor; in fact, trialling indicated they were quite robust (over dilutions of almost an order of magnitude for the former, and at least two orders of magnitude for the latter).

Character independence is a requirement of any study seeking to infer relationships from the states of multiple characters. If two 'characters' are not, in fact, independent, then treating them separately equates to counting (or weighting) the same character twice, and

is certainly not appropriate for determining the presence of concordant partitioning between characters (a single character is always concordant with itself). Several studies (Maughan et al. 1996, Maheswaran et al. 1997, Liu et al. 1998) have reported that the AFLP characters are generally inherited in a Mendelian fashion, indicating that they are independent, and that they are spread throughout the nuclear genome (eg. Nilsson et al. 1999). The assumption of AFLP character independence is probably particularly robust in the case of New Zealand *Polystichum*. The high chromosome numbers ($n \geq 82$) ensures that any given pair of AFLP characters are likely to be segregating on different chromosomes.

The assumption of independence will be violated in the case of characters with co-dominant alleles; that is, alleles which produce bands of different size. In the absence of knowledge that such bands (at least those detectable within the size limits of the AFLP procedure; c.100-2000 b.p.) are sourced from the same locus, they will be scored as separate characters. Although signalling the same split, the banding patterns of co-dominant 'characters' will be the complement of one another (ie. {1 1 1 1}{0 0 0 0} & {0 0 0 0}{1 1 1 1}). Studies have found co-dominant AFLP characters amongst all polymorphic AFLP markers at frequencies between 4 - 15% (Mueller & Wolfenbarger 1999 p.392, and references therein). Consideration of the AFLP data sets recorded for this study indicates that most banding-patterns (splits) occur only once (see Chapters Four, Five, & Six). This suggests that, although their presence cannot be ruled out, co-dominant 'characters' do not appear to be a prevalent feature of these data sets.

The majority of AFLP characters can therefore be assumed to represent a single genomic locus, which in different individuals either produces a band of a single (assayable) size, or it does not. That is, there are 'band-presence' alleles and 'band-absence' alleles. However, the bands from individuals homozygous for the 'band-presence' allele are usually indistinguishable from those from individuals heterozygous for the 'band-presence' and 'band-absence' alleles (but see Piepho & Koch 2000). Consequently,

such AFLP characters are said to be 'dominant' (Mueller & Wolfenbarger 1999), or, at least, the 'band-presence' allele is dominant over the 'band-absence' allele.

Because the nuclear genome is much bigger (Lewin 1994) and has a higher average mutation rate (Wolfe et al. 1987, Palmer 1992) than the organelle (ie. chloroplast and mitochondrial) genomes, most polymorphic AFLP characters will be of nuclear origin. Consequently, it can be considered that each dominant AFLP character is equivalent to one microsatellite locus, or one nuclear sequence locus (assuming the latter represents a 'c-gene' in the sense of Doyle 1995; see Section 2.3.5.1). Because of their inability to distinguish heterozygotes from homozygotes for the 'band-presence' allele, AFLP characters are genealogically naive compared to microsatellite or nuclear sequence loci. Nevertheless, that AFLP characters can distinguish homozygotes for the 'band-absence' allele from heterozygotes or homozygotes for the 'band-presence' allele still makes them informative.

Another weakness of AFLP characters is that, with only two possible states (ie. band is either present or absent), they are prone to homoplasy (where a character state has two or more independent origins), and certainly more so than microsatellite or nuclear characters, which typically (have at least the potential to) exhibit a much larger number of states. However, the AFLP marker system has the great advantage that many more characters can be assayed than is currently technically possible for microsatellite markers or independent sequencable loci. (Note that a sequence locus of even large numbers of conjoint and linked bases constitutes only one genomic character). Consequently, although a given AFLP character will generally be less informative (in terms of both information content, and in an accurate depiction of its own genealogy) than any particular microsatellite or (nuclear) sequence locus, the former system derives great phylogenetic strength from sheer numbers of characters assayable. As indicated by both Farris (1983 p.12-14) and Brower et al. (1996 p.433), even if individual characters are relatively 'poor,' as long as any homoplasy is random with respect to any phylogenetic signal, it can still be possible to recover signal from the data set as a whole.

For characters with finite numbers of states, homoplasy generally becomes increasingly prevalent with increasing evolutionary 'distance' between the samples being compared (Givnish & Sytsma 1997). With only two possible states, this problem is especially likely to apply to AFLP characters. Between particularly divergent taxa, the sharing of states might be reduced to the level of chance. Indeed, Mueller and Wolfenbarger (1999; also O'Hanlon & Peakall 2000) indicated that AFLP would be most useful for investigating relationships between closely related lineages. However, it should be kept in mind that while homoplasy may obscure phylogenetic signal, it will not produce strong misleading signal as long as it occurs randomly with respect to any phylogenetic signal. The randomness of homoplasy is probably a reasonable assumption for AFLP characters, but is known to be not always the case for other molecular marker systems (eg. Lockhart et al. 1998, 1999).

3.4.4.6 Splits and Edges.

Before discussing the properties and analysis of concordance in AFLP data sets, several useful 'phylogenetic' concepts will be introduced (see also Penny et al. 1992).

A 'split' is the bipartitioning of the sample set into two mutually-exclusive subsets (Huson 1998, Charleston & Page 1999). A polymorphic (binary-state) character can thus be considered to define a 'split' in the sample set from which it was assayed (Figure 3.3). A split might be described as 'internal' when both subsets it partitions contain at least two individual samples. Such internal splits are also said to be parsimony-informative. When one of the subsets of a split contains only one individual sample, it can be described as 'external' or parsimony-uninformative, or said to be a 'singleton-split.' For a sample set of n individuals, there are a possible $(2^{(n-1)} - 1)$ non-null splits, of which n are external splits and $(2^{(n-1)} - n - 1)$ internal splits.

Two splits considered in tandem may be described as either compatible or incompatible. Compatible splits can be represented on the same bifurcating (phylogenetic) tree, while incompatible splits cannot be. Incompatible splits describe competing hypotheses of

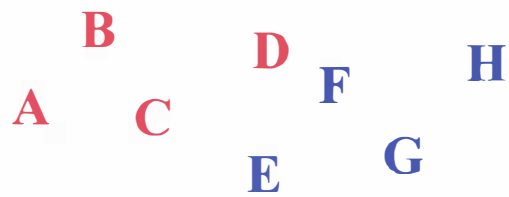


Figure 3.3. Splits From Binary Characters.
An example of a split defined by a binary character. For the above individuals (A, B, C, D, E, F, G, & H), the character 'colour,' with binary character states **red** and **blue**, defines the split **{A, B, C, D}{E, F, G, H}**.

relationship, and can be said to be in conflict with one another. More formally, two splits, $S_1 = \{A, B\}$ and $S_2 = \{C, D\}$ (where A, B, C & D represent subsets of the sample set), are incompatible (or in conflict) if none of their four intersections ($A \cap C$, $A \cap D$, $B \cap C$, $B \cap D$) are empty (Huson 1998). Only internal splits can be incompatible with one another.

When a split is represented in an evolutionary tree, or more generally, an evolutionary graph, it can be described as an 'edge.' There are internal and external edges corresponding to internal and external splits.

3.4.4.7 Analysis of Concordant Partitioning in AFLP Data.

The AFLP data sets of this study were complex, with the recovery of large numbers of splits. By using the program Spectronet 1.0 (Langton 2001) to list all splits present in an AFLP data set, it was found that few splits occurred at any more than a few characters, and most occurred at only one character. That is, there was very little 'absolute' concordance, where a given split occurred at multiple characters. Further, any given internal split was typically conflicted by many others. There are several reasons for the inherent conflict and low levels of 'absolute' concordance in the AFLP data sets of this study. Firstly, they bridge the divergence-reticulation divide, and in reticulating systems there is little expectation for the state patterns of independent characters to be in agreement, or ('absolutely'-) concordant, with one another (see Section 2.3.5).

Secondly, even on the divergent side of the boundary, not all characters may have become concordant through lineage sorting (cf. insufficient time). And thirdly, homoplasy, which as discussed in Section 3.4.4.5 is probably common in AFLP data, can mask 'absolute' concordance (and in doing so may produce conflict). As shown in Table 3.2, the recovery of 'absolute' concordance from 'noisy' data systems is actually expected to be lowered with greater sampling. This is in accord with the finding of greater variance of edge lengths with increased taxon sampling (Charleston et al 1994, Waddell et al. 1994; see also Givnish & Sytsma 1997).

Figure 2.2 has already illustrated that even in the absence of 'absolute' concordance there can be sufficient presence of 'almost' concordance across multiple independent characters to be strongly indicative of the presence of assortatively-fertilising groups. To assay both 'almost' and 'absolute' concordance in the AFLP data sets, evolutionary graph-reconstructing methods were used which would only recover internal edges (and support them highly) when substantial (multi-character) signal for concordant partitioning (be it 'absolute,' 'absolute' & 'almost,' or 'almost' alone) was present in the data. Consequently, the results of these methods should be interpreted in terms of the strength of such signal (a continuum from very strong to absent) between the samples analysed, rather than as their literal phylogeny.

The recovery of internal edges with such methods indicates signal in the data for concordant partitioning across multiple independent characters. This is unlikely to have arisen unless the sample set encompasses two (or more) assortatively-fertilising groups, with lineage sorting between these groups engendering the concordance. The null hypothesis that a 'single lineage is present' may then be rejected. However, the non-recovery of internal edges between samples (such that they are left in an unresolved polytomy) does not necessarily indicate the presence of (on-going) reticulation. Rather, the null hypothesis cannot be rejected because the lineage sorting expected to accompany assortative fertilisation has not resulted in detectable concordant

Table 3.2. The Effect of Sampling on 'Absolute' Concordance.
Illustration with an artificial data set of how increased sampling can lead to the recovery of less 'absolute' concordance in analyses of the data set.
A) An initial sample set consisting of three individuals of taxon 'X' and three of taxon 'Y.' 12 of the 25 characters assayed are 'absolutely' concordant (marked with a * above the character column) with the split {All samples of X}{All samples of Y}. The corresponding edge under split-decomposition is recovered with 100% bootstrap support.
B) An expanded sample set containing an extra three individuals from each of X and Y. Of the same 25 characters, none of them are now 'absolutely' concordant with the split {All samples of X}{All samples of Y}. However, the corresponding edge under split-decomposition is still recovered with 97% bootstrap support.

A) Initial Sample Set.

	** * * * **** * **
X1	1001110001100110001100111
X2	1000110000100100001110110
X3	1000110101100100001100111
Z1	0100101011011011110000000
Z2	0110101011110011110100000
Z3	0110000011010011100101000

B) Expanded Sample Set.

X1	1001110001100110001100111
X2	1000110000100100001110110
X3	1000110101100100001100111
X4	1000110110100010001110010
X5	1001110000100010001110011
X6	0000110100110010101100111
Y1	0100101011011011110000000
Y2	0110101011110011110100000
Y3	0110000011010011100101000
Y4	0110101011010010111000010
Y5	0010010011111011110100010
Y6	0110000011111010100101000

partitioning (either because of on-going reticulation, or because lineage sorting has not been acting for sufficient time to engender detection of assortatively-fertilising groups).

The graph building methods of parsimony with bootstrap analysis, neighbour-joining with bootstrap, and split-decomposition with bootstrap were used to assay for any strongly supported (via concordant partitioning) internal edges in the data set. Distance-spectral analysis was also used, to assess the relative support for any given split in the data. These approaches are described below.

PARSIMONY & NEIGHBOUR-JOINING. Character state patterns can be represented as evolutionary graphs using a variety of methods (Swofford et al. 1996), of which two are 'maximum parsimony' (hereafter parsimony) and neighbour-joining. Both methods

reconstruct fully-bifurcating graphs, or 'trees,' and both were implemented for this study using the program PAUP* 4.08b (Swofford 2001).

Parsimony is an optimality method (Swofford et al. 1996), in that all possible trees, or bifurcating relationships of the samples, are evaluated to determine which requires inference of the least number of character state transformations when the observed character states are mapped on to each tree. That is, which tree (or trees) provides the most parsimonious representation of the observed data? Parsimony was implemented in PAUP* 4.08b with the 'heuristic' search option, and with the 'tree-bisection-reconnection swapping' algorithm and 'accelerated transformation' (ACCTRAN) optimisation in effect.

Neighbour-joining, in contrast, is an algorithmic method (Swofford et al. 1996) which operates by progressively clustering samples after conversion of the character-state data to a distance matrix (of pairwise distance comparisons between samples). Hamming (= p-distances = observed) distances were used in this study.

Both parsimony and neighbour-joining reconstruct fully resolved trees, with a maximum complement of internal edges recovered (for n samples a bifurcating tree may contain a maximum of n - 3 internal edges), even when variation in the input data is essentially random. However, when either are implemented with bootstrap-analysis only internal splits that are strongly supported in the data are recovered as internal edges in the resultant consensus tree (see Koopman et al. 2001 for a discussion in the context of AFLP data).

BOOTSTRAP ANALYSIS. This involves resampling the original character state data, with replacement, many times (eg. ×100 or ×1000) to create multiple, resampled 'pseudosample' data sets (of the same number of characters) (Swofford et al. 1996). Each of these pseudosample data sets is then analysed with the graph-building method (eg. parsimony, or neighbour-joining via a recalculated distance matrix) of interest. The

percentage of times a given edge is recovered in the graphs built from the pseudosample data sets is termed the bootstrap support (BS) for that edge (also for the corresponding split). The results of bootstrap analysis are usually depicted in a consensus tree in which only edges with greater than 50% BS are represented. Consequently, these consensus trees are not necessarily fully resolved (where internal edges are supported with $\leq$ 50% BS), with portions of the tree collapsed into polytomies.

For a given split, a low BS value can result when the number of supporting characters in the original data are low, such that they are missed in the resampling process, and/or because there are similarly-supported but conflicting splits in the original data (Lockhart et al. 2001). If two well-supported (in the original data), but incompatible splits are present, neither is likely to be recovered with high BS under parsimony or neighbour-joining, because both methods can only represent bifurcating relationships.

SPLIT-DECOMPOSITION. Split-decomposition (Bandelt & Dress 1992, Swofford et al. 1996, Huson 1998) is not restricted to reconstructing a bifurcating tree. For instance, two similarly supported but conflicting splits can be represented as a 'box' in the resultant splits-graph. However, the graphical representation of conflict by splits-decomposition is limited to only simple cases (ie. two or three dimensions). The hyper-dimensional conflict of splits in the AFLP data sets of this study far exceeds this limitation (ie. a given split may conflict with many others). Consequently, with complex data sets only splits with relatively high support/conflict ratios are represented in the splits-graph (those with isolation indices more than zero; see Lockhart et al. 2001), which thus does not depict all signal in the data set (Huson 1998, Lockhart et al. 2001). Such high support/conflict ratios for a given split may result from high support (from concordant partitioning across multiple characters) and/or low conflict (where incompatible splits occur at few characters) in the data. Bootstrap analysis was employed to identify which, if any, internal splits recovered in the splits-graph had high support in the data (and thus would have high BS values).

For this study, split-decomposition was implemented using the program Splitstree 2.4 (Huson 1997, 1998), using the criterion of parsimony. However, the results obtained therein differed little from those using a distance criterion. The operation of split-decomposition is detailed (non-mathematically) in Winkworth (2000) and Lockhart et al. (2001).

DISTANCE-SPECTRAL ANALYSIS. In addition to the above graph-building methods, spectral analysis (Swofford et al. 1996, Charleston & Page 1999) was used to assess the extent of concordant support for different splits in the AFLP data. Spectral analysis may be based directly on observed character state patterns ('s' or observed spectra). Observed spectra report the number of characters at which a split occurs. This is akin to assessing only 'absolute' concordance. Typically, the number of characters conflicting that split are also reported.

Alternatively, spectral analysis may be distance-based (hereafter 'distance-spectra'), where character state patterns are first converted into a matrix of pairwise distances (Hamming distances were used in this study). These are used to estimate generalised distances, which are in turn used to calculate the support and conflict for all splits via the Hadamard conjugation (Penny et al. 1996). Empirically, distance-spectra appear to account for both 'absolute' and 'almost' concordance. It has often been observed that distance-spectra have less associated variance than observed-spectra. This may be related to the finding that the variance of branch lengths inferred from a distance matrix is reduced in comparison to that of branch lengths inferred directly from character state data (Charleston et al 1994, Waddell et al. 1994).

The program Spectrum 2.0 (Charleston 1997, 1998) was used to calculate the distance-spectra. The results of a spectral analysis can be visualised as a 'spectrum,' or a histogram with support and conflict for each split plotted on the positive y-axis and negative y-axis respectively (eg. Lento et al. 1995, Lockhart et al. 1995). The conflict plotted on such graphs is usually normalised, such that the total conflict shown is

adjusted to equal the total support shown (Lento et al. 1995). Otherwise the conflict signal will usually dwarf the support signal, as while one character can only support one split (at least in character-spectra), 'its' split may conflict many other splits (and therefore many characters).

Although the relative support and conflict for splits corresponding to two different hypotheses may be compared, there is no absolute threshold above which a given split might be easily interpreted as indicative of phylogenetic divergence as opposed to 'random noise.' However, the pattern of support and conflict for splits recovered in the spectrum can be informative (see Charleston & Page 1999 p.259-260). For instance, if there is no concordant partitioning in the data set (ie. there is no 'phylogenetic' signal, with variation more or less randomly distributed amongst the samples), all splits will be recovered with similar (low) levels of support. If the data set encompasses two divergently related groups, separated by strong concordant partitioning, then the split corresponding to their partition may be strongly recovered, while other splits will have relatively much lower support. (See Figure 5.19, with the split partitioning *P. silvaticum* from *P. vestitum*, for a putative example; analysis of the AFLP data for *P. wawranum* and *P. oculatum*, with *P. neozelandicum* excluded, shows a similar pattern - not shown.) When there is signal for more than one (primarily-) divergent relationship in the data, multiple splits will be recovered with relatively high support (eg. Figure 4.21), although in such cases the contrast between highly supported and poorly supported splits is not always so clear (eg. Figure 6.5).

METHOD COMPARISON. Although the four methods outlined above operate very differently from one another, they produce highly congruent results in their analysis of the AFLP data. Internal edges recovered with high BS support (ie. > 90%) under one of the graph-reconstructing methods were always similarly recovered by the other two methods (with one exception, see Section 4.3.3.2). BS was generally higher under neighbour-joining, while split-decomposition (at least, as implemented) appeared most conservative, in that it failed to recover some edges reported, albeit with low BS (eg. 50-

65%), by the other methods. Further, highly-supported splits in the distance-spectra were recovered with high BS by the graph-building methods. This correlation has been reported previously (Lento et al. 1995, Lockhart et al. 1995).

It should be noted that the results of these analyses are emergent, in that the demonstration of support for a relationship is not dependant on its *a priori* postulation (cf. Cladistic Haplotype Analysis, see Section 2.3.4).

Chapter Four

The Dissolution of *Polystichum richardii* (Hook.) J. Smith: the *P. neozelandicum* Fée Complex.

4.1 Introduction

Polystichum richardii (Hook.) J. Smith (*sensu* Allan 1961, Crookes 1963, Brownsey et al. 1985, Brownsey 1988, Brownsey and Smith-Dodsworth 1989, 2000, and Perrie et al. 2000) has long been acknowledged as morphologically variable. Brownsey and Smith-Dodsworth (1989 p.131) described it as "very variable," Brownsey (1988 p.25) as "extremely polymorphic," and Brownsey (1981) cited it as an example of a polymorphic species. Some authors have questioned whether such variation results from the presence of more than one species. Crookes (1963), for instance, suggested that *P. richardii* was almost certainly a compound species, while Allan (1961) described it (along with the other species of *Polystichum* he listed) as an 'aggregate.' Clarkson (1991, pers. comm.) recently drew attention to the sympatric nature of some of this morphological variation on the North Island's east coast. His suggestion that more than one species was present led to the inception of the current study.

Samples were collected from plants referrable to *P. richardii* from throughout New Zealand. Character state variation was analysed to retrospectively test the null hypothesis that 'a single evolutionary lineage was present.' Morphological measurements were made from the frond, scales, indusia, and spores. Ploidy levels of the morphological groups identified therein were investigated using cytological analyses, and the genetic relationships of these groups were assessed using AFLP DNA-fingerprinting.

This work has shown that *P. richardii*, which was previously understood to be a single polymorphic species, actually constitutes an allopolyploid complex of four distinct evolutionary lineages. These are here recognised as three taxonomic species, with one of these species encompassing two subspecies. *P. richardii* is not a legitimate name for any of these taxa. Its basionym, *Aspidium richardii* Hook., and the earlier name *P.*

neozelandicum Fée are both based on material from the same lineage (see Webb & Edgar 1999 for notes on the hyphenation of the epithet *neozelandicum*). The precedence of *P. neozelandicum* over *P. richardii* is not yet generally recognised, but it is reinstated here as the correct name for a species comprising two allopatric, octoploid lineages. These two lineages are recognised as separate subspecies.

P. neozelandicum subsp. *neozelandicum* is found from Kawhia and the Bay of Plenty north into Northland. The new combination *P. neozelandicum* subsp. *zerophyllum* (Colenso) comb. et stat. nov. is adopted for the other octoploid lineage. *Aspidium zerophyllum* Colenso is based on material from this lineage, and the transfer of this epithet is considered preferable to the construction of a novel subspecific epithet under *P. neozelandicum*. *Polystichum neozelandicum* subsp. *zerophyllum* occurs from near Taupo south to Stewart Island, and also extends east to the Chatham Islands.

Both subspecies of *P. neozelandicum* appear to be allopolyploids between the two tetraploid species discussed below, but it is uncertain whether they arose from the same, or distinct, allopolyploid event(s). Both subspecies of *P. neozelandicum* are generally morphologically intermediate between their putative parents, although they have larger spores and their indusia always have obvious dark centres. Although the dark centre of the indusia of subsp. *neozelandicum* is generally larger than that found in subsp. *zerophyllum*, they are otherwise virtually morphologically indistinguishable from one another. Nevertheless, AFLP analysis indicates they are distinct evolutionary lineages.

The new combination *P. wawranum* (Szyszyl. in Wawra) comb. nov. is proposed for a lineage of tetraploid ferns common throughout much of the North Island, with hair-like rachis scales and indusia usually without an obvious dark centre.

The name *P. oculatum* (Hook.) J.B. Armstr. is reinstated for another lineage of tetraploid ferns found principally on the eastern side of the axial ranges of the lower North Island and upper South Island. This species has wide, often pentagonal rachis scales and indusia with an obvious dark centre.

Descriptions of these three species are given below, together with an identification key (Section 4.2). The morphological, cytological and AFLP analyses that support these

conclusions are fully described in Section 4.3, with taxonomic delimitation discussed in Section 4.4. As a group, these three species will be referred to as the *P. neozelandicum* complex (which is equivalent to *P. richardii sensu* Allan 1961, Brownsey 1988, Brownsey & Smith-Dodsworth 1989, 2000), *neozelandicum* being the first published epithet of those employed here.

4.2 Taxonomy.

4.2.1 Key to Taxa of the *Polystichum neozelandicum* Complex.

1 Rachis scales appearing hair-like to the naked eye (scales from the stipe-rachis junction < 130 µm wide at mid-length); indusia often lacking an obvious dark centre ..... **1. *P. wawranum***

Rachis scales obviously scale-like to the naked eye (scales from the stipe-rachis junction > 130 µm wide at mid length); indusia always with an obvious dark centre 2

2 Scales from the stipe-rachis junction > 750 µm (and usually > 1000 µm) wide at their mid-length, usually pentagonal; spores small (exine length 36-48 µm × width 27-36 µm) .... **2. *P. oculatum***

Scales from the stipe-rachis junction < 650 µm wide at their mid-length, generally acicular-lanceolate; spores large (exine length 46-58 µm × width 36-45 µm) 3

3 Dark centre of indusia usually occupying > 30% of surface area, often much more so; from the Bay of Plenty and Kawhia northwards ..... **3a. *P. neozelandicum*** subsp. ***neozelandicum***

Dark centre of indusia usually occupying < 30% of surface area; from Taranaki, Taupo, and the Urewera Ranges southwards ..... **3b. *P. neozelandicum*** subsp. ***zerophyllum***

4.2.2 Taxonomic Descriptions.

Fronds, scales from the stipe-rachis junction, indusia and spores of representative samples of each taxon are compared in Figures 4.1, 4.2, 4.3, and 4.4, respectively. Analysis of these and other morphological characters is discussed more fully in Section 4.3.2. Note that the ranges for quantitative characters given in the following descriptions are based on 5th and 95th percentiles. The distribution of each taxon is mapped in Figure 4.5. Details of samples collected for this study are given in Appendix 2.1.1. Redesignated specimens from the AK, CHR, and WELT herbaria are listed in Appendix 2.2.1.

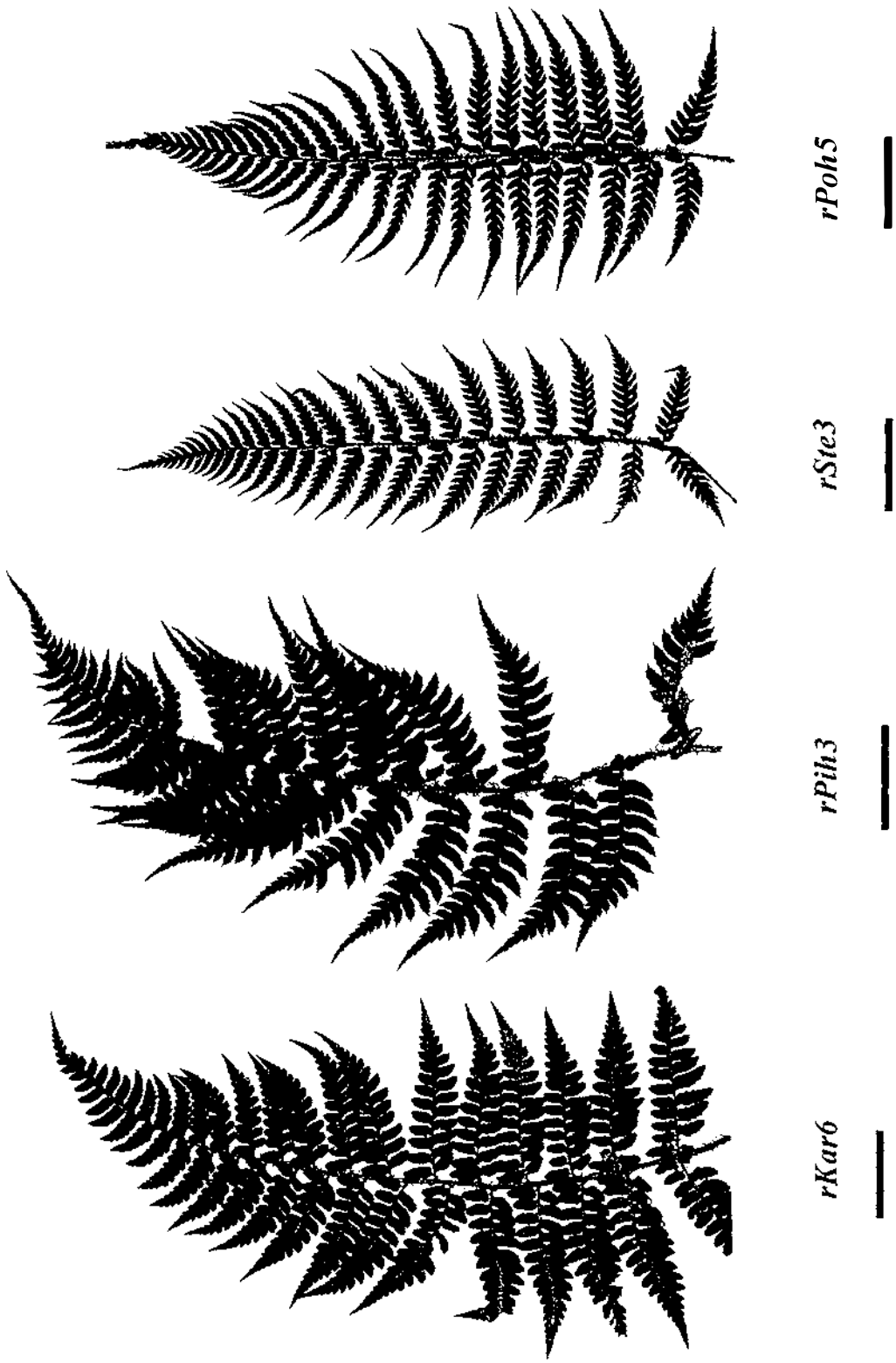


Figure 4.1. Representative fronds of *Polystichum wawranum.*
Sample details are given in Appendix 2.1.1. Fronds at different scales; scale bars indicate 5 cm. Note that some pinnae have been removed for molecular and/or spore analyses.

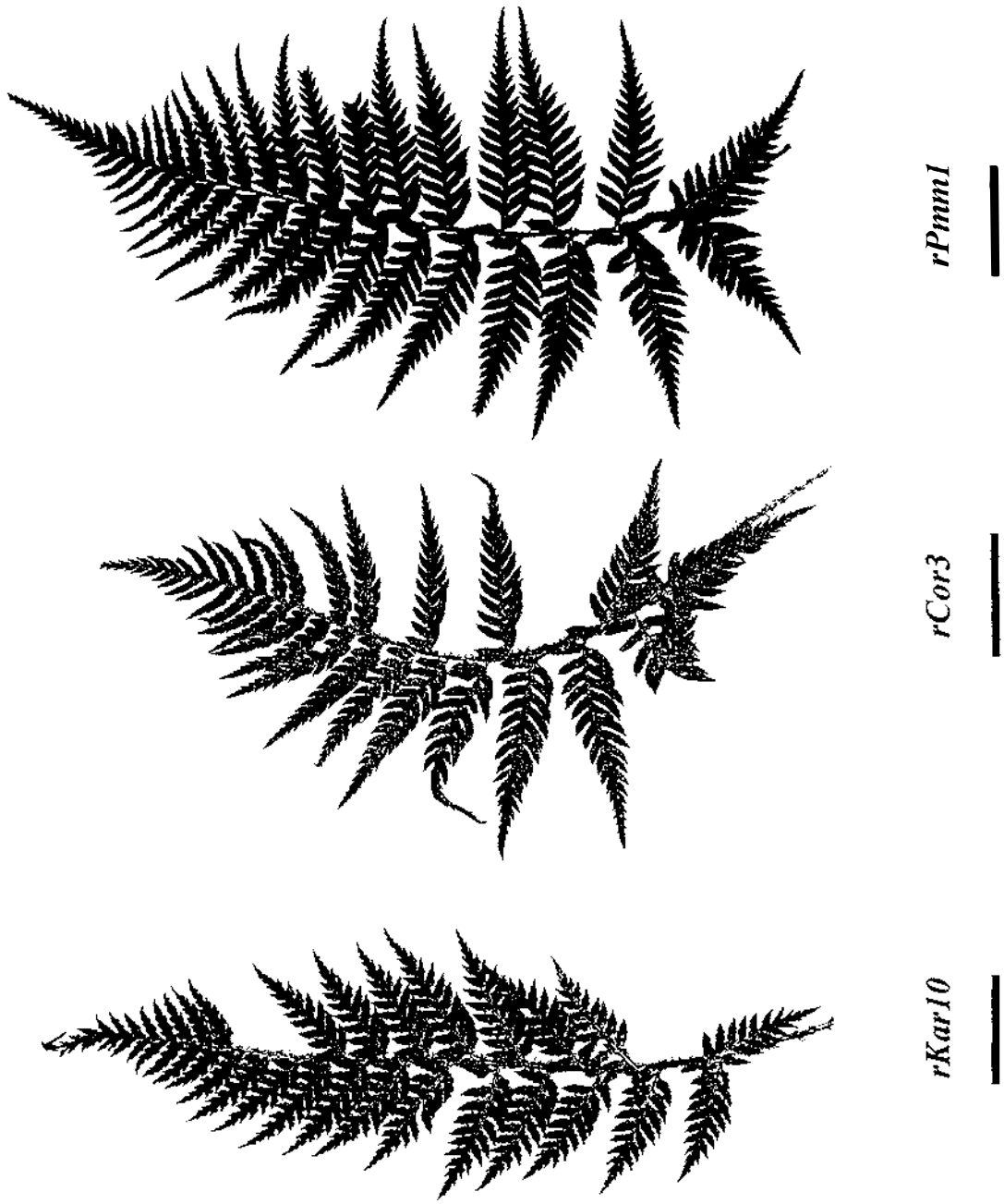


Figure 4.1 (continued). Representative fronds of *Polystichum neozelandicum* subsp. *neozelandicum*.
Sample details are given in Appendix 2.1.1. Fronds at different scales; scale bars indicate 5 cm. Note that some pinnae have been removed for molecular and/or spore analyses.

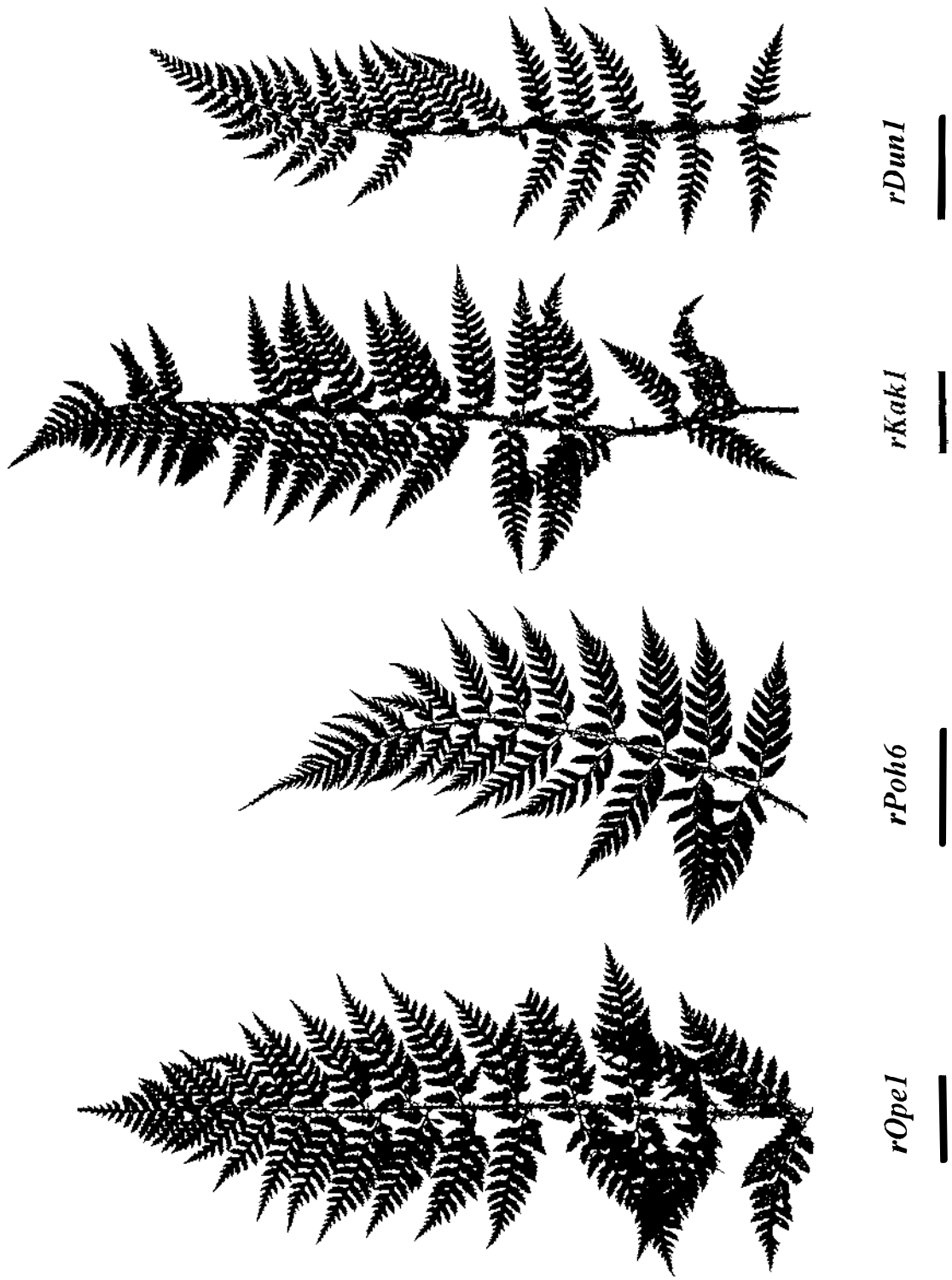


Figure 4.1 (continued). Representative fronds of *Polystichum neozelandicum* subsp. *zerophyllum.*

Sample details are given in Appendix 2.1.1. Fronds at different scales; scale bars indicate 5 cm. Note that some pinnae have been removed for molecular and/or spore analyses.

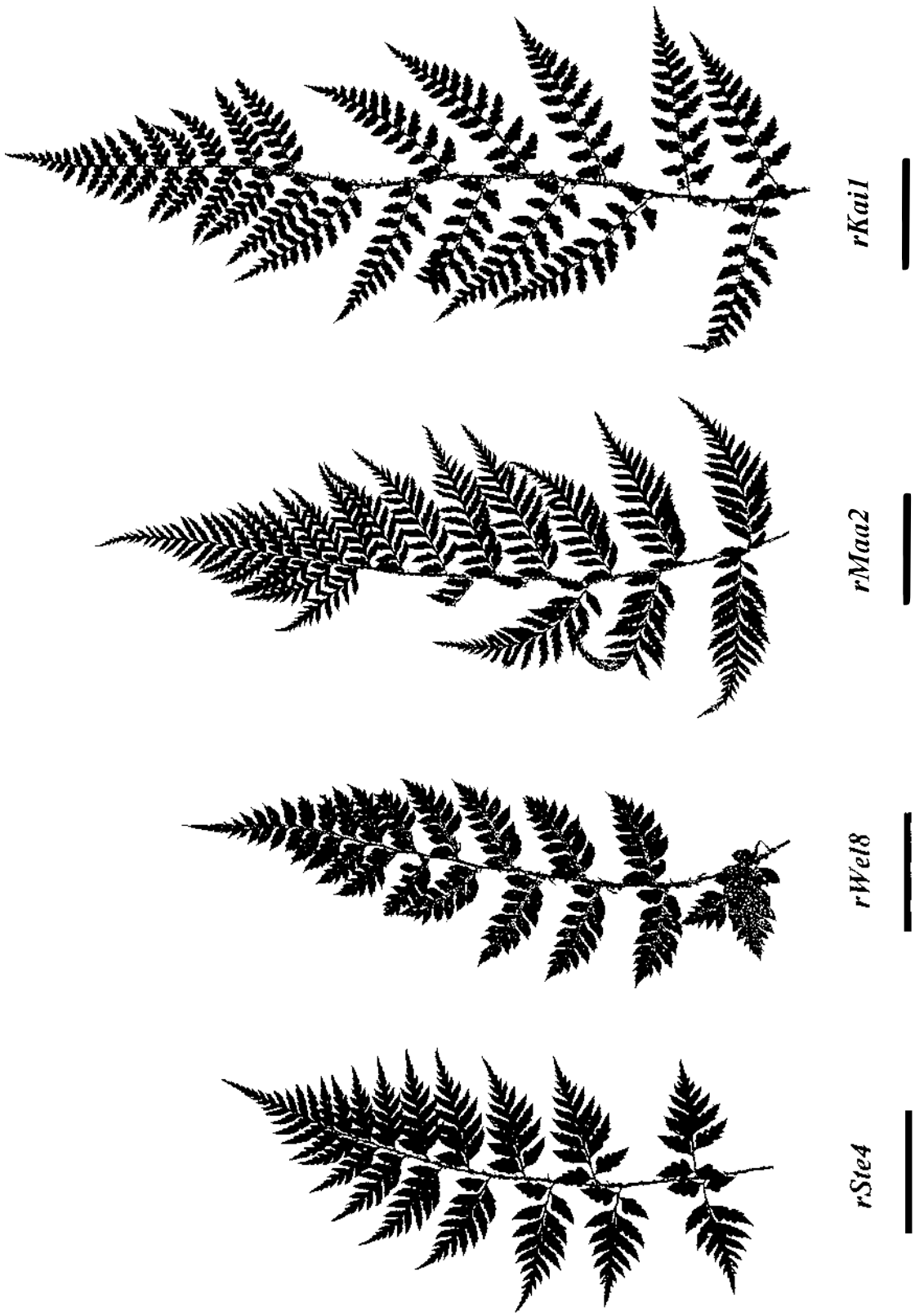


Figure 4.1 (continued). Representative fronds of *Polystichum oculatum.*
Sample details are given in Appendix 2.1.1. Fronds at different scales; scale bars indicate 5 cm. Note that some pinnae have been removed for further molecular and/or spore analyses.

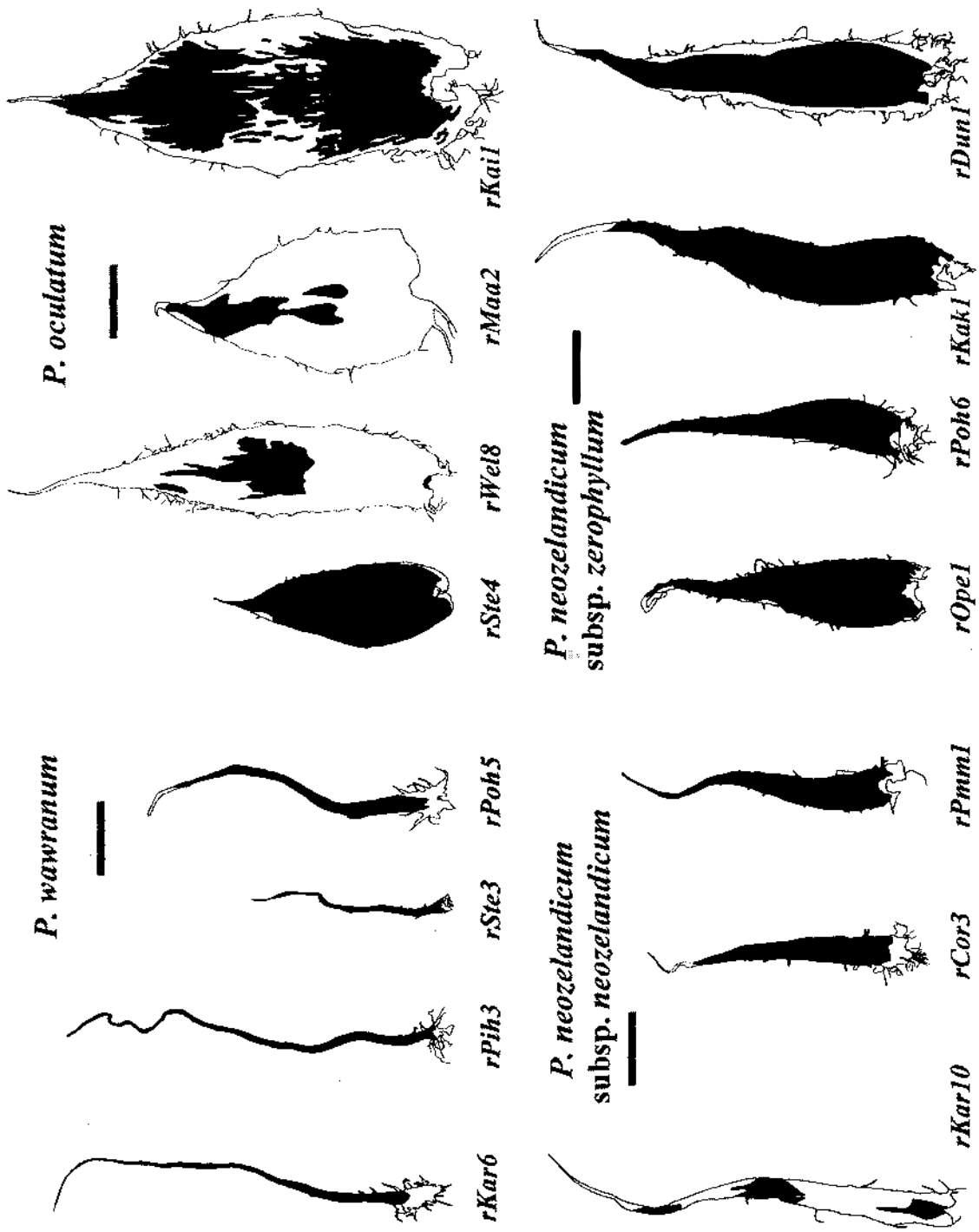


Figure 4.2. Stipe-Rachis Junction Scales from the *Polystichum neozelandicum* Complex.

Representative scales from the stipe-rachis junctions of *P. wawranum*, *P. oculatum*, *P. neozelandicum* subsp. *neozelandicum*, and *P. neozelandicum* subsp. *zerophyllum*. Sample details are given in Appendix 2.1.1. Scale bars indicate 1000 μm.

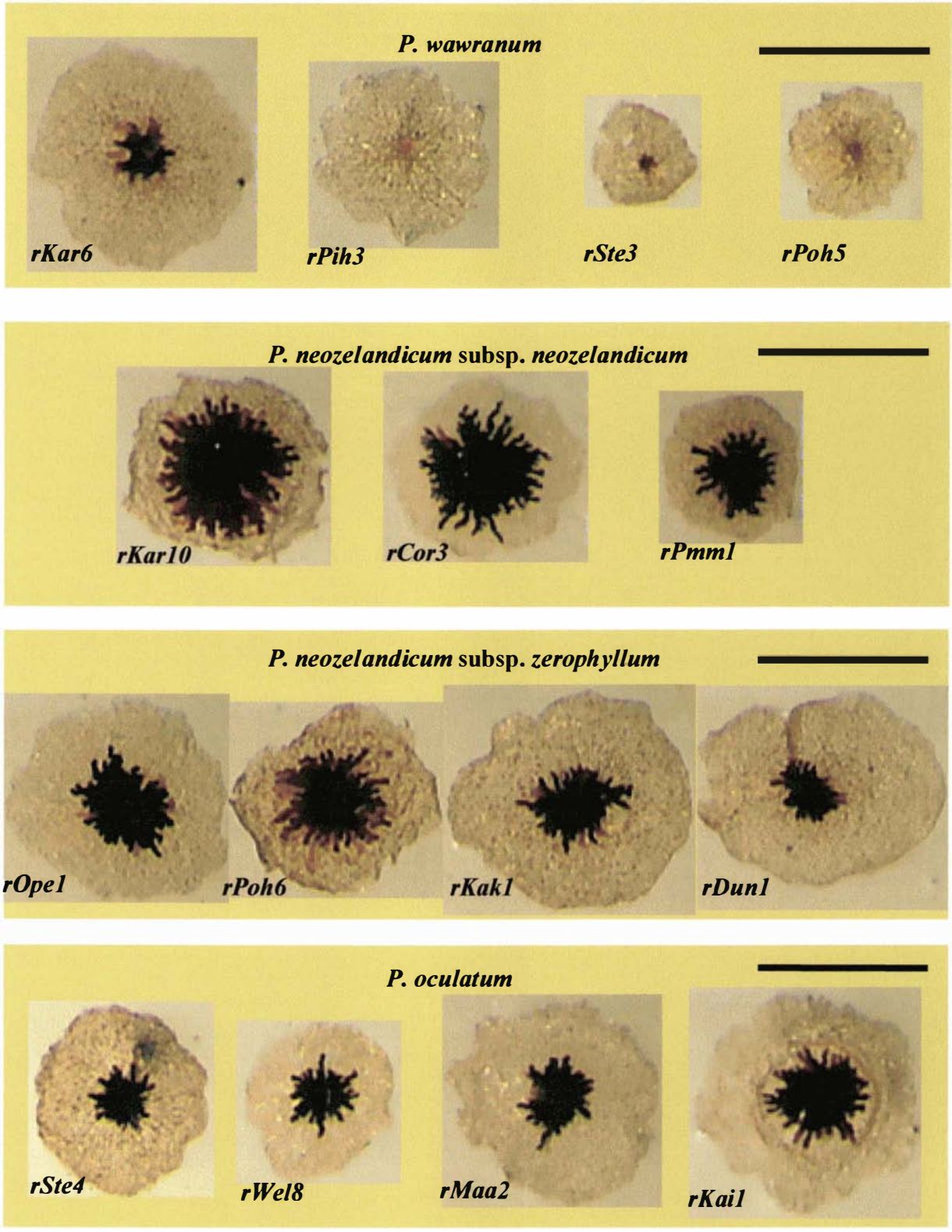


Figure 4.3. Indusia from the *Polystichum neozelandicum* Complex.
Representative indusia of *P. wawranum*, *P. neozelandicum* subsp. *neozelandicum*, *P. neozelandicum* subsp. *zerophyllum*, and *P. oculatum*. Sample details are given in Appendix 2.1.1. Scale bars indicate 1000 μm.

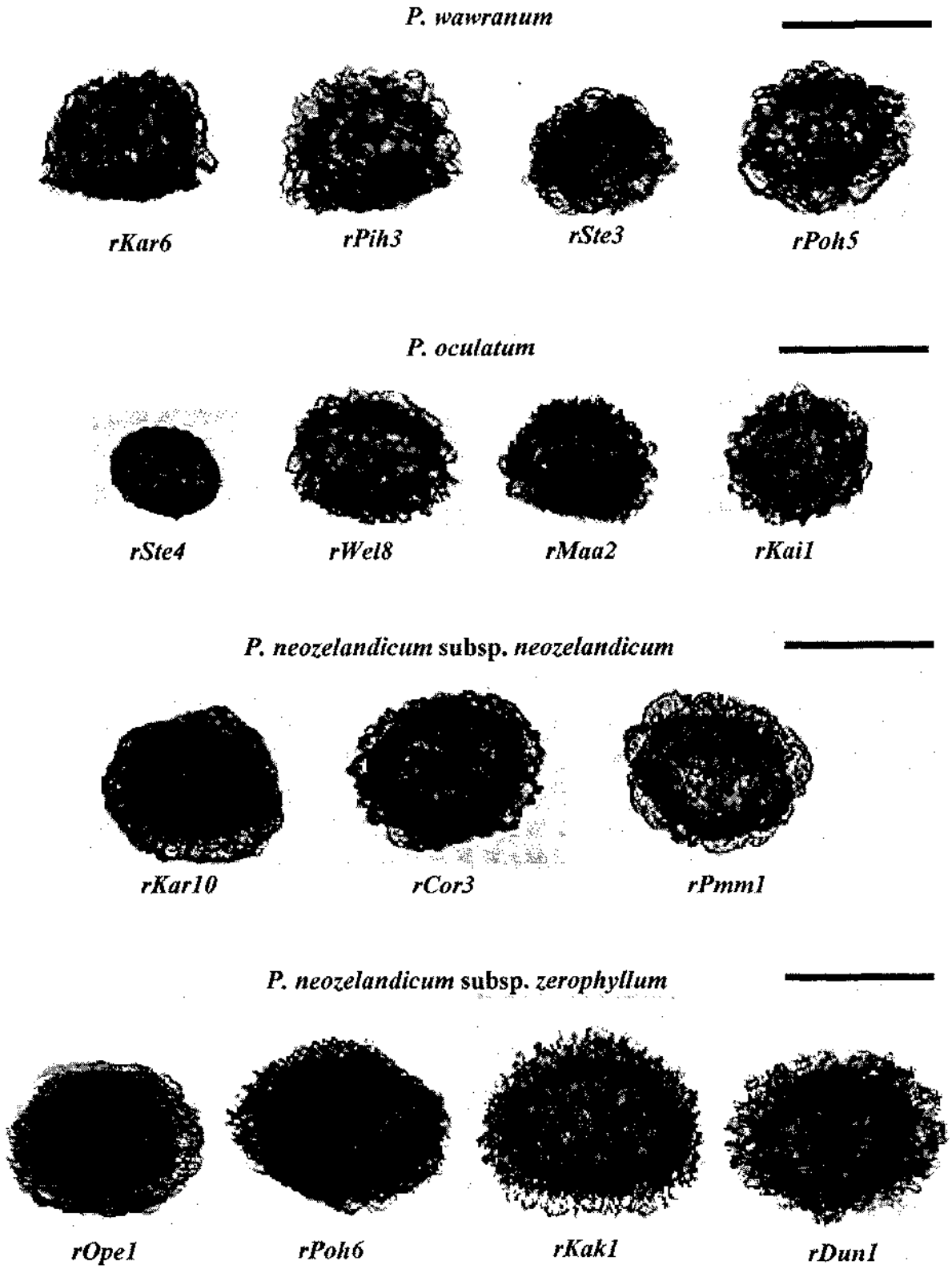


Figure 4.4. Spores from the *Polystichum neozelandicum* Complex.
Representative spores, in equatorial view, of *P. wawranum*, *P. oculatum*, *P. neozelandicum* subsp. *neozelandicum*, and *P. neozelandicum* subsp. *zerophyllum*. Sample details are given in Appendix 2.1.1. Scale bars indicate 50 µm.

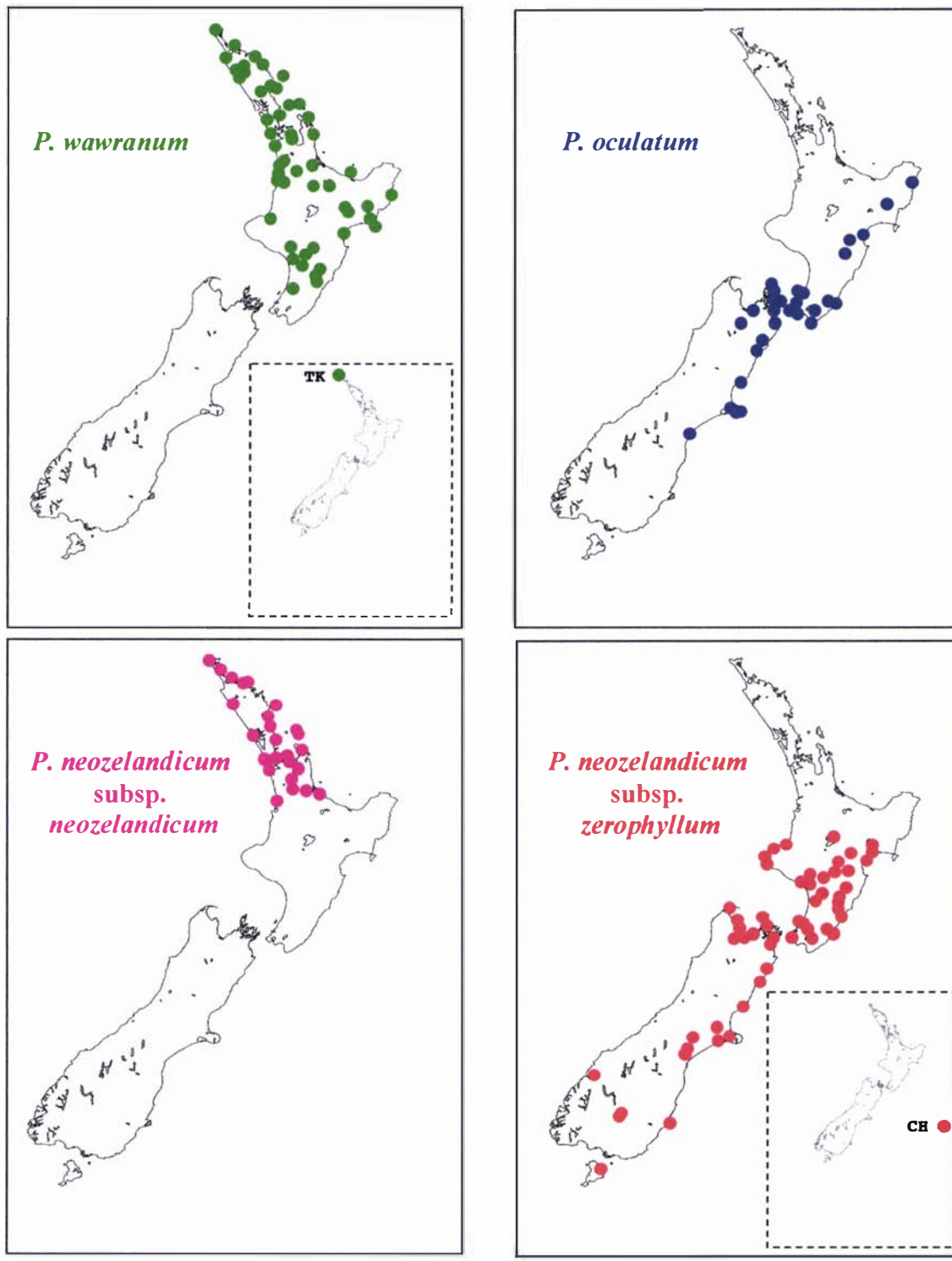


Figure 4.5. Distribution Maps for the *Polystichum neozelandicum* Complex.
Maps for *P. wawranum*, *P. oculatum*, *P. neozelandicum* subsp. *neozelandicum*, and *P. neozelandicum* subsp. *zerophyllum* compiled using collections made for this study and those of the herbaria AK!, CHR!, and WELT!. Details of individual collections are given in Appendices 2.1.1 and 2.2.1. Inserts: TK = Three Kings Islands; CH = Chatham Islands.

1. ***Polystichum wawranum*** (Szyszyl. in Wawra) comb. nov.

Aspidium wawranum Szyszyl. in Wawra, Itin. princ. Coburgi 126, t. 15 (1888) – as *A. wawraeanum*[1]. Type: Waitemata, New Zealand, H. Wawra No. 242, 1872-73; holotype in W (see Figure 4.6).

DESCRIPTION: **Rhizomes** short, erect. **Stipes** 150-550 mm long. Stipes and rachises densely scaly. **Scales** filiform, appearing hair-like to the naked eye; almost always widest at their base; those from the stipe-rachis junction almost always less than 130 μm wide at their mid-length; usually dark brown, but appearing black to the naked eye; apex long and tapering; margins often with protrusions, which are usually blunt; often densely fimbriate around base, so much so that in young fronds they appear to be underlain by dense white tomentum. **Lamina** 270-590 × 110-280 mm; bipinnate with the basal primary pinnae of some large fronds becoming tripinnate; varying in colour from olive green to blue-green, usually with primary and secondary costae dark blue. **Primary pinnae** in 18-35 pairs, the longest 55-140 × 13-35 mm. **Secondary pinnae** usually adnate, but becoming sessile to almost stalked towards the base of primary pinnae, particularly in basal primary pinnae; often with only sparse marginal toothing, sometimes almost entire but for apical point. **Sori** round. Indusia peltate, ± flat, ± round, with entire although often with undulate and/or scalloped margins; often deciduous with soral maturity, and sometimes almost fugaceous; central dark area usually insignificant (< 15% surface area). Number of annulus cells of sporangia 13-19, but most commonly 14-15. Spore exine 40-48 × 29-36 μm; length-width product 1160-1720 μm^2 (39 individuals, 24 populations).

CHROMOSOME NUMBER: Tetraploid; n = c.82, *rWan7*, Pungarehu, Wanganui; n = c.78, *rPoh1*, Totara Reserve, Manawatu (see Section 4.3.1).

HABITAT & DISTRIBUTION: Endemic; in the North Island from Cape Reinga to near Otaki in the west and Pahiatua in the east; also Three Kings Islands (Figure 4.5). Ranges from scrubby coastal rocks to montane forest. Usually grows in the margins of forest or scrub, on sloping substrates such as hillsides, banks between stream terraces, or road-cuttings, but sometimes under dense shade and/or on alluvial terraces.

[1] The epithet *wawraeanum* in the original publication has an incorrectly formed termination, and requires correction under ICBN Art. 60 and Rec. 60C. 1(c) (Phil Garnock-Jones pers. com.).

Figure 4.6. Holotype of *Aspidium wawranum* Szyszyl. in Wawra.
In W. Right-hand label reads "Coll. Dr. H. Wawra. No. 242. New Zeeland, Waitemata. 1872-73."
Left-hand label reads "*Aspidium Wawraeanum* nov. sp. det Dr. Ign. de Szyszylowicz."

NOTES: *P. wawranum* appears to be morphologically more variable in the northern part of its distribution, particularly in the extent of crenulation of the pinnae margins and in the size of the dark centre of the indusia. *P. wawranum* is likely to be confused only with *P. neozelandicum* subsp. *neozelandicum*, with the later being distinguished by its wider scales, larger dark centre of the indusia, and larger spores (see Section 4.3.2).

2. ***Polystichum oculatum*** (Hook.) J.B. Armstr., Trans. New Zealand Inst. 13: 364 (1881)

Aspidium oculatum Hook., Sp. Fil. 4: 24, t. 228 (1862); *Dryopteris oculata* (Hook.) Kuntze, Revis. Gen. Pl. 2: 813 (1891); *Polystichum richardii* var. *oculatum* (Hook.) C. Chr., Index Fil. 85, 280 (1905). Type: Wairarapa, W. Colenso, no date recorded; lectotype (here designated) in K (see Figure 4.7).

DESCRIPTION: **Rhizomes** short, erect. **Stipes** 90-300 mm long. Stipes and rachises moderately to only sparsely scaly. **Scales** large; often pentagonal, such that they are widest near their mid-length; those from the stipe-rachis junction greater than c.750 μm (usually greater than c.1000 μm) wide at their mid-length; pale brown to dark brown, sometimes bicolorous but never with a dark centre completely enclosed by a pale margin; apex often appearing quite blunt because of dehiscence of apical cell(s); almost always with marginal projections which often taper to cilia-like apices; underlain by smaller scales, including 'arachnioid' scales with fimbriate bases, but these only sparse, such that stipe and rachis never appear completely clothed in indumentum. **Lamina** 180-410 × 80-200 mm, bipinnate (with the lower primary pinnae of some large fronds being tripinnate); usually blue-green and almost concolorous with dark blue primary and secondary costae. **Primary pinnae** in 11-22 pairs, the longest 43-105 × 16-43 mm. **Secondary pinnae** stalked towards the base of primary pinnae, becoming sessile and adnate towards the apex of primary pinnae; never entire, with sharply pointed apices and usually additional marginal teeth and/or crenulations. **Sori** round. Indusia peltate, ± flat, ± round, with entire although often undulate and/or scalloped margins; persistent; central dark area always significant and obvious (usually > 10% surface

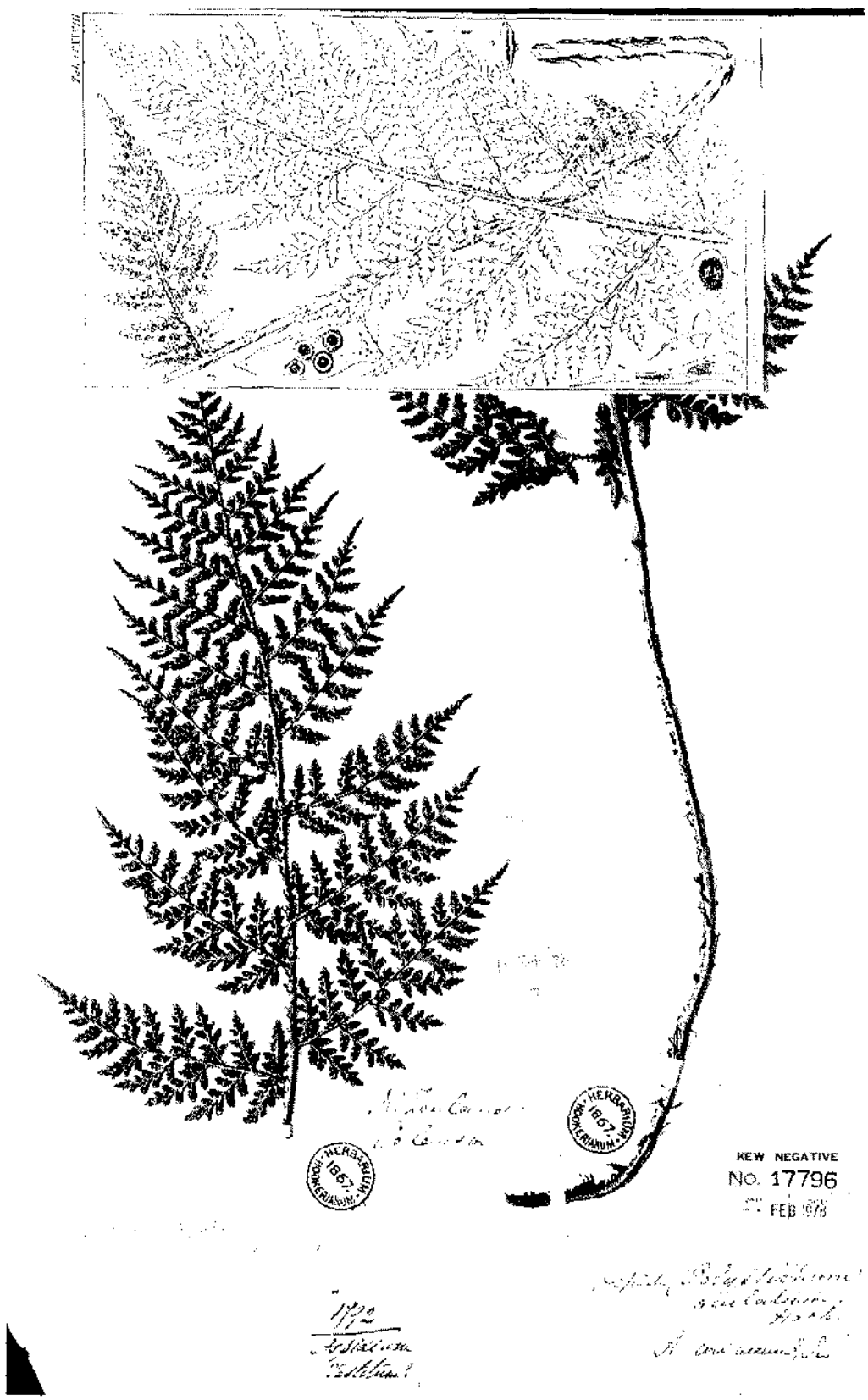


Figure 4.7. Lectotype of *Aspidium oculatum* Hook.
In K. Labelling reads "N. Zealand - Colenso" and "Wairarapa." Approximately x0.5 life-size.

area). Number of annulus cells of sporangia 15-21, but most commonly 17-19. Spore exine 36-48 × 27-36 μm; length-width product 970-1750 μm^2 (20 individuals, 13 populations).

CHROMOSOME NUMBER: Tetraploid; n = c.85, *rWel8*, Makara, Wellington (see Section 4.3.1).

Brownlie's (1958) count of n = 82 for '*P. richardii*' is thought to be from a plant belonging to *P. oculatum*. Although the voucher for his count has not been located, it is known to have been collected from "Lyttleton, Port Hills" (Brownlie 1958), where *P. oculatum* is the predominant taxon. Brownlie's count would appear to be the only one previously made from a plant of the *P. neozelandicum* complex (see Dawson et al. 2000 p.36).

HABITAT & DISTRIBUTION: Endemic; in the North Island from near East Cape down the eastern side of the axial ranges, also extending westward to Wellington and Kapiti Island; in the South Island from the Marlborough Sounds and Nelson, down the eastern coast to Banks Peninsula and extending southward to Timaru (Figure 4.5). Lowland forest and scrub margins, usually on sloping substrates such as hillsides and road-cuttings.

NOTES: Lectotypification of *Aspidium oculatum* Hook. W.J. Hooker (1863) listed two specimens with his original description of *Aspidium oculatum*; (Wairarapa) "Northern Island, Rev. W. Colenso" and (Akaroa) "Middle Island, Raoul." Both specimens, held in K, have been viewed and are consistent with the description in Hooker's protologue. However, the accompanying illustration (Hooker 1863 Tab. CCXXVIII) appears to be based on the specimen of Colenso, and it is consequently chosen as the lectotype of *A. oculatum* Hook. (see Figure 4.7). Although the scales remaining on this specimen are somewhat small (from the stipe-rachis junction c.350 μm wide at their mid-length), the morphology of its frond indicates that it belongs with the lineage recognised here.

Field identification. With their substantial geographic overlap and gross morphological similarity, *P. oculatum* might be confused with *P. neozelandicum* subsp. *zerophyllum*, but they can be distinguished by the narrower scales and larger spores of the latter (see

Section 4.3.2). The often stark contrast in colour between the primary costae (dark blue) and the remaining lamina (forest green) in *P. neozelandicum* subsp. *zerophyllum* compared to *P. oculatum*, where they are more similarly coloured (dark blue to blue-green), can be a useful initial field character. Hybrids may further complicate identification, although these can be recognised by their aborted spores (see Section 4.2.4).

3. ***Polystichum neozelandicum*** Fée, Mém. Soc. Sci. Nat. Strasbourg 5: 99 (1857)

Type: "N^{elle} Zélande", S. Mossman No. 617, 1854; holotype in P (see Figure 4.8).

DESCRIPTION: **Rhizomes** short, erect. **Stipes** 100-420 mm long. Stipes and rachises moderately to densely scaly. **Scales** obviously scale-like to naked-eye; usually acicular-lanceolate; usually widest in the basal third of their length; those from the stipe-rachis junction usually 130-640 μm wide at their mid-length; mid to dark brown, often appearing black to the naked eye; apex tapering; margins almost always with projections which usually taper to cilia-like apices; underlain by smaller scales, including 'arachnioid' scales with fimbriate bases. **Lamina** 175-525 × 90-220 mm, bipinnate with the basal primary pinnae of some large fronds becoming tripinnate; usually forest green with primary and secondary costae dark blue. **Primary pinnae** in 11-25 pairs, the longest 45-120 × 5-38 mm. **Secondary pinnae** stalked towards the base of primary pinnae, becoming sessile and adnate towards the apex of primary pinnae; with sharply pointed apices and usually additional marginal teeth and/or crenulations. **Sori** round. Indusia peltate, ± flat, ± round, with entire although often undulate and/or scalloped margins; persistent; central dark area always significant and obvious (usually > 10% surface area). Number of annulus cells of sporangia 13-20, but most commonly 15-18. Spore exine 46-58 × 36-45 μm; length-width product 1660-2540 μm^2 (49 individuals, 32 populations).

Figure 4.8. Holotype of *Polystichum neozelandicum* Fée.
In P. Label reads "*Polystichum Coriaceum* Sw. N^{elle} Zélande. S. Mossman. 1854. N^{o}. 617."

3a. ***Polystichum neozelandicum*** Fée subsp. ***neozelandicum***

Aspidium richardii Hook., Sp. Fil. 4: 23, t. 222 (1862); *Polystichum richardii* (Hook.) J. Smith, Hist. Fil. 220 (1875); *Dryopteris richardii* (Hook.) Kuntze, Revis. Gen. Pl. 2: 813 (1891). Type: "Tangururu Bay [Whangaruru Bay, Bay of Islands], W. Colenso, no date recorded; lectotype (here designated) in K (see Figure 4.9).

Polystichum aristatum auct. non C. Presl (1836): Hook. f., Fl. New Zealand 2: 37, t.78 (1854).

DIAGNOSIS: Indusial central dark area often very large (> 30% surface area).

CHROMOSOME NUMBER: Octoploid; n = 164, *rPmm1*, Pukemokemoke, near Gordonton, Waikato (see Section 4.3.1).

HABITAT & DISTRIBUTION: Endemic; from Northland to Kawhia and the Bay of Plenty (Figure 4.5). Found on hillsides and banks, from coastal to lowland forest and scrub, usually in well-lit conditions.

NOTES: Lectotypification of *Aspidium richardii* Hook. W.J. Hooker (1863) listed three specimens with his original description of *Aspidium richardii*; "Northern Island, D'Urville," "Tangururu Bay [Whangaruru Bay, Bay of Islands], Colenso," and "Wyran River [Waikare River, Bay of Islands], Hook. fil." The D'Urville specimen has not been located, but the latter two (both in K) have been examined. They closely resemble one another and match the description in the protologue equally well. The accompanying illustration (Hooker 1863 Tab. CCXXII) is not an exact representation of either of these collections, but possibly draws more from the specimen of Colenso from Whangaruru Bay (see Figure 4.9), and consequently it is selected as the lectotype of *A. richardii* Hook.

Identity of *Polystichum aristatum* auct. non C. Presl (1836): Hook. f. (1854). J.D. Hooker (1855) appears to misapply the name "*Polystichum aristatum*, Presl" (≡*Polystichum aristatum* (G. Forst.) C. Presl = *Polypodium aristatum* G. Forst = *Arachniodes aristata* (G. Forst.) Tindale), as inspection of the accompanying illustration (Hooker 1855 table 78) strongly suggests that he is actually referring to the lineage here recognised as *P. neozelandicum* subsp. *neozelandicum*.

Figure 4.9. Lectotype of *Aspidium richardii* Hook.
In K. Label in lower-left hand corner reads: "On the E. Coast. Tangururu [Whangaruru] Bay. Sides of cliffs. Colenso."

Field identification. *Polystichum neozelandicum* subsp. *neozelandicum* might be confused with both *P. wawranum* (see notes under this species) and *P. neozelandicum* subsp. *zerophyllum*. In the latter instance the two taxa are allopatric, and the dark centre of the indusia of subsp. *neozelandicum* is usually larger than that found in subsp. *zerophyllum* (see Section 4.3.2).

3b. *Polystichum neozelandicum* Fée subsp. ***zerophyllum*** (Colenso) comb. et stat. nov.

Aspidium zerophyllum Colenso, Trans. New Zealand Inst. 29: 418 (1897); *Polystichum zerophyllum* (Colenso) C. Chr., Index Fil. 98 (1905), 589 (1906); *Polystichum aculaetum* (L.) Schott var. *zerophyllum* (Colenso) Domin, Biblioth. Bot. 20, 85: 56 (1913). Type: Dannevirke, W. Colenso (numbered '312'), no date recorded; lectotype (here designated) AK 139720 (see Figure 4.10).

DIAGNOSIS: Indusial central dark area moderately sized (10-30% surface area).

CHROMOSOME NUMBER: Octoploid; n = c.170, *rRkk1*, Ruakokoputuna, Wairarapa; n > 150, *rVin5*, Vinegar Hill, Rangitikei (see Section 4.3.1).

HABITAT & DISTRIBUTION: Endemic; in the North Island from Taranaki, Taupo and the southern Urewera Ranges southwards; in the South Island from Nelson and Marlborough through Canterbury and into Otago, although apparently uncommon in the south and absent from the central west coast; also Stewart Island and the Chatham Islands (Figure 4.5). Usually found on sloping substrates such as hillsides, banks between stream terraces, and road-cuttings, and usually in well-lit conditions. Ranges from the coast to lower montane forest and scrub.

NOTES: Lectotypification of *Aspidium zerophyllum* Colenso. Colenso's (1897) original description of *Aspidium zerophyllum* did not list any specimens, instead simply noting "Hilly woods south-west of Dannevirke; 1896: *W.C.*" W. Colenso collected numerous specimens consistent with the lineage here recognised as *P. neozelandicum* subsp. *zerophyllum*. There are six such specimens in AK and 13 in WELT (see Appendix

Figure 4.10. Lectotype of *Aspidium zerophyllum* Colenso.
AK 139720. "*Aspidium zerophyllum* Col. 312" is in W. Colenso's handwriting. The locality given on the T.F. Cheeseman herbarium label is "Hawkes Bay, probably [which has been crossed out], Dannevirke."

2.2.1), with most of these labelled "Dannevirke." However, the only specimen labelled with the epithet '*zerophyllum*' in Colenso's handwriting (see Goulding 1978) is AK 139720 (as "*Aspidium zerophyllum* Col."), and consequently it is selected as the lectotype of *Aspidium zerophyllum* Colenso (see Figure 4.10).

Field identification. Specimens of *Polystichum neozelandicum* subsp. *zerophyllum* might be confused with either *P. neozelandicum* subsp. *neozelandicum* or *P. oculatum* (see notes under these taxa).

4.2.3 Name of Uncertain Synonymy.

Aspidium coriaceum (Sw.) Sw. var. *acutidentatum* A. Rich., Essai Fl. New Zealand 71 (1832)

The above name was listed as synonymous with *P. richardii* (Hook.) J. Smith by Brownsey et al. (1985). The type specimen is believed to be held in P but it has not been available for examination. Its synonymy with the taxa recognised here is uncertain. However, the epithet *acutidentatum* has no priority at either the specific or the subspecific level.

4.2.4 Hybrids.

Hybrids between fern species often have aborted spores (eg. Brownsey 1977a, b, Lovis 1977, Brownsey 1985b, Haufler 1996, Vogel et al. 1998; but see Brownsey 1981, Mayer & Mesler 1993). Such a post-fertilisation barrier is assumedly due to the incidental accumulation of genomic incompatibilities (resulting in meiotic abnormality and spore abortion) between diverged evolutionary lineages.

Plants with aborted spores and intermediate morphology (eg. *rXvKak2*, *rXvKaw2*, *rXvPee2*; collection details of putative hybrids are given in Appendix 2.1.4) are believed to be hybrids between *P. neozelandicum* subsp. *zerophyllum* and *P. vestitum*. Such plants are often common where the two grow together, particularly in ecologically disturbed areas. The specimen *rXvBan9* is thought to be a hybrid between *P. oculatum* and *P. vestitum*. The specimens *rXvNap1* and *rXvNap2* may be hybrids between *P. vestitum* and either *P. neozelandicum* subsp. *zerophyllum* or *P. oculatum*, with the latter

more probable. Hybrids between *P. vestitum* and either *P. wawranum* or *P. neozelandicum* subsp. *neozelandicum* are not known with certainty.

Hybrids between the different lineages of the *P. neozelandicum* complex do not appear to be common. However, given the morphological similarity of these lineages, the frequency of their hybrids may have been underestimated. In particular, it would be very difficult to distinguish the tetraploid hybrid between *P. wawranum* and *P. oculatum* from the allo-octoploid *P. neozelandicum*. The specimen *rWeb1* is almost certainly a back-cross hybrid between *P. wawranum* and *P. neozelandicum* subsp. *zerophyllum*, with an intermediate morphology under multivariate analysis and aborted spores (Figure 4.11). The specimens *rNap13*, *rNap15*, *rNap16*, and *rNap18* may be hybrids from the other back-cross between *P. neozelandicum* subsp. *zerophyllum* and *P. oculatum*. All have aborted spores, but quantitative evidence of morphological intermediacy is less strong because the two putative parents are so similar.

While no direct analyses have been carried out, the involvement of *P. wawranum*, *P. oculatum* and *P. neozelandicum* subsp. *zerophyllum* in hybridisation with other lineages suggests that they all must have a sexually-outcrossing component to their respective breeding systems. The same is probably true for *P. neozelandicum* subsp. *neozelandicum*, although it is not known to hybridise with other lineages. (Note that *P.neozelandicum* subsp. *neozelandicum* has, like the other taxa, 64 spores per sporangium, indicating that it is not apomictic.)

4.2.5 Distributions.

P. wawranum, *P. oculatum*, and *P. neozelandicum* subsp. *zerophyllum* are broadly sympatric with one another over large areas (see Figure 4.5). Numerous instances of local sympatry between *P. neozelandicum* subsp. *zerophyllum* and either *P. wawranum* or *P. oculatum* are known, and listed in Table 4.1. Despite their broad sympatry, only one instance of local sympatry between *P. wawranum* and *P. oculatum* was recorded during this study (Table 4.1). However, Augustus Hamilton collected *P. wawranum*, *P. oculatum*, and *P. neozelandicum* subsp. *zerophyllum* all from "Petane," about 10 km north of Napier, in the early 1880's (see Table 4.1).

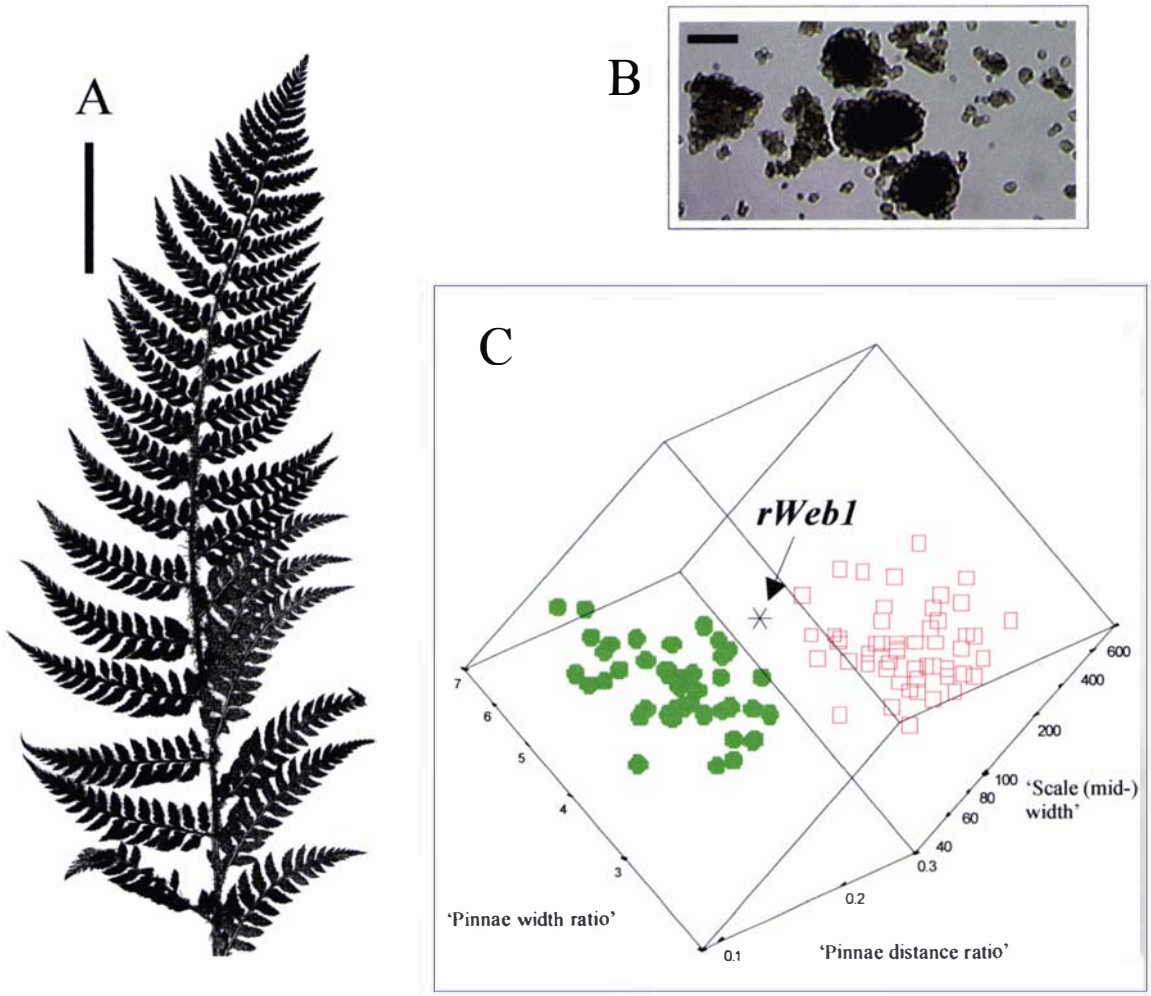


Figure 4.11. A Putative Hybrid, *rWeb1*.
Frond (A) and abnormally-developed spores (B) of the specimen *rWeb1* (✱), which is possibly a hybrid between *Polystichum wawranum* (●) and *P. neozelandicum* subsp. *zerophyllum* (□). The intermediate morphology of *rWeb1* between these two taxa is depicted in the three-dimensional scatter-plot shown in (C). The morphological characters are defined in Table 4.2. The scale bar in (A) indicates 5 cm, and that in (B) 50 μm. Collection details for *rWeb1* can be found in Appendix 2.1.4.

P. neozelandicum subsp. *neozelandicum* is allopatric with respect to both *P. neozelandicum* subsp. *zerophyllum* and *P. oculatum*, but is broadly sympatric with *P. wawranum* in the northern third of the North Island (see Figure 4.5). Several instances of 'local' sympatry between *P. neozelandicum* subsp. *neozelandicum* and *P. wawranum* have been recorded (see Table 4.1).

Table 4.1. 'Local' Sympatric Occurrences Between Taxa of the *Polystichum neozelandicum* complex.
Further details of specimens are given in Appendices 2.1.1 (samples collected for this study) and 2.2.1 (herbaria collections). '?' indicates distance between specimens not recorded.

Locality	Distance	Representative Specimens
P. wawranum and *P. oculatum*		
Pehiri, Gisborne	< 10 m	*rSte3 & rSte4*
P. wawranum and *P. neozelandicum* subsp. *neozelandicum*		
Karikari Peninsula, Northland	c.2 km	*rKar6 & rKar9*
Wenderholm, Auckland	< 100 m	*rAuc2 & rAuc1*
Great Barrier Island	?	AK 122317! & 122318!, *Frater et al.*
Te Henga (Bethells Beach), Waitakare	?	WELT P008167! & P008168!, *H. Dobbie.*
P. wawranum and *P. neozelandicum* subsp. *zerophyllum*		
Bellbird Bush, Napier	< 100 m	*rBel4 & rBel2*
Sutherlands Bush, Wanganui	< 2 m	*rTur6 & rTur7*
Lake Colenso, Ruahine Ranges	< 10 m	*rCol2 & rCol1*
Ruahine Dress Circle, Rangiwahia	< 2 m	*rDre1 & rDre2*
Totara Reserve, Pohangina	< 50 m	*rPoh5 & rPoh6*
Ngapaeruru Reserve, Dannevirke	< 100 m	*rNga3 & rNga6*
Coonoor	< 10 m	*rCoo2 & rCoo3*
Pongaroa Reserve, Pongaroa	< 10 m	*rPng1 & rPng2*
P. oculatum and *P. neozelandicum* subsp. *zerophyllum*		
Wakarara	< 2 m	*rWak5 & rWak1*
Wilkinson Track, Kapiti Island	< 5 m	*rKap1 & rKap2*
Carswell	< 10 m	*rCar1 & rCar4*
Castlepoint	< 10 m	*rCas1 & rCas2*
Ruakokoputuna	< 10 m	*rRkk2 & rRkk1*
Makara, Wellington	<10 m	*rWel9 & rWel10*
Whites Bay, Rarangi	< 100 m	*rMaa1 & rMaa4*
Napenape Reserve, Napenape	< 100 m	*rNap12 & rNap3*
Kaituna, Banks Peninsula	< 10 m	*rKai1 & rKai3*
Matai Valley, Nelson	?	CHR179030! (two specimens), *P. Wardle.*
Kenepuru, Marlborough Sounds	?	WELT P008160! (three specimens), *J.H. McMahon.*
P. wawranum, *P. oculatum* and *P. neozelandicum* subsp. *zerophyllum*		
Petane (Esk River), Napier	?	WELT P008145!, P008180A!; & P008180B!, P008140!; & P008138!, P008139!, *A. Hamilton*

4.3 Lineage Delimitation.

4.3.1 Cytological Analysis.

Substantial variation in spore size was observed in the plants studied. Principally, *P. neozelandicum* was found to have larger spores than either *P. wawranum* or *P. oculatum*; see Section 4.3.2. Because spore size is correlated with ploidy level

(Barrington et al. 1986), a cytological analysis was carried out. This confirmed the presence of two ploidy levels. Samples of *P. wawranum* and *P. oculatum* (both taxa with small spores) were found to be tetraploid with n = c.82 homologous pairs of chromosomes counted at diakinesis (the diploid chromosome number in *Polystichum* is n = 41, Kramer 1990). In contrast, samples of both subspecies of *P. neozelandicum* (both of which have large spores) were found to be octoploid with n = c.164 homologous pairs of chromosomes counted at diakinesis. Examples of the cell preparations used to make these counts are given in Figure 4.12.

Large-spored, and assumedly octoploid, plants occur sympatrically with small-spored, and assumedly tetraploid, plants over a large geographic area (Northland to Canterbury). The extensive sympatric coexistence of these different ploidy levels suggests that they fertilise assortatively, such that (at least) two species taxa could be recognised. Further, as detailed in the next section, the small-spored tetraploid plants fall into two distinctive morphological groups, which correspond to the species taxa recognised as *P. wawranum* and *P. oculatum*.

4.3.2 Morphological Analysis.

Morphological characters showing (at least some) differentiation between the taxa recognised above are defined in Table 4.2. Variation in these characters within and between taxa is summarised in Table 4.3, with box-plot summaries presented in Figure 4.13. Details of the samples included in these analyses are listed in Appendix 2.1.1, and Appendix Four provides a file of the morphological data ('Morph-NeoCom').

As discussed in Section 3.2.2, the partitioning of a sample set by two independent characters is only likely to be concordant if two (or more) separate lineages, or assortatively fertilising groups of individuals, are present. Two quantitative morphological characters can be considered to exhibit concordant partitioning if, in a scatterplot, the samples more or less are restricted to two diagonally-opposite quadrants of the graph. The greater this restriction, the stronger the partitioning.

P. wawranum and *P. oculatum* are morphologically very different from one another, being concordantly partitioned by a number of morphological characters. This is strongest for the character combination 'scale (mid-) width' and 'pinnae distance ratio'

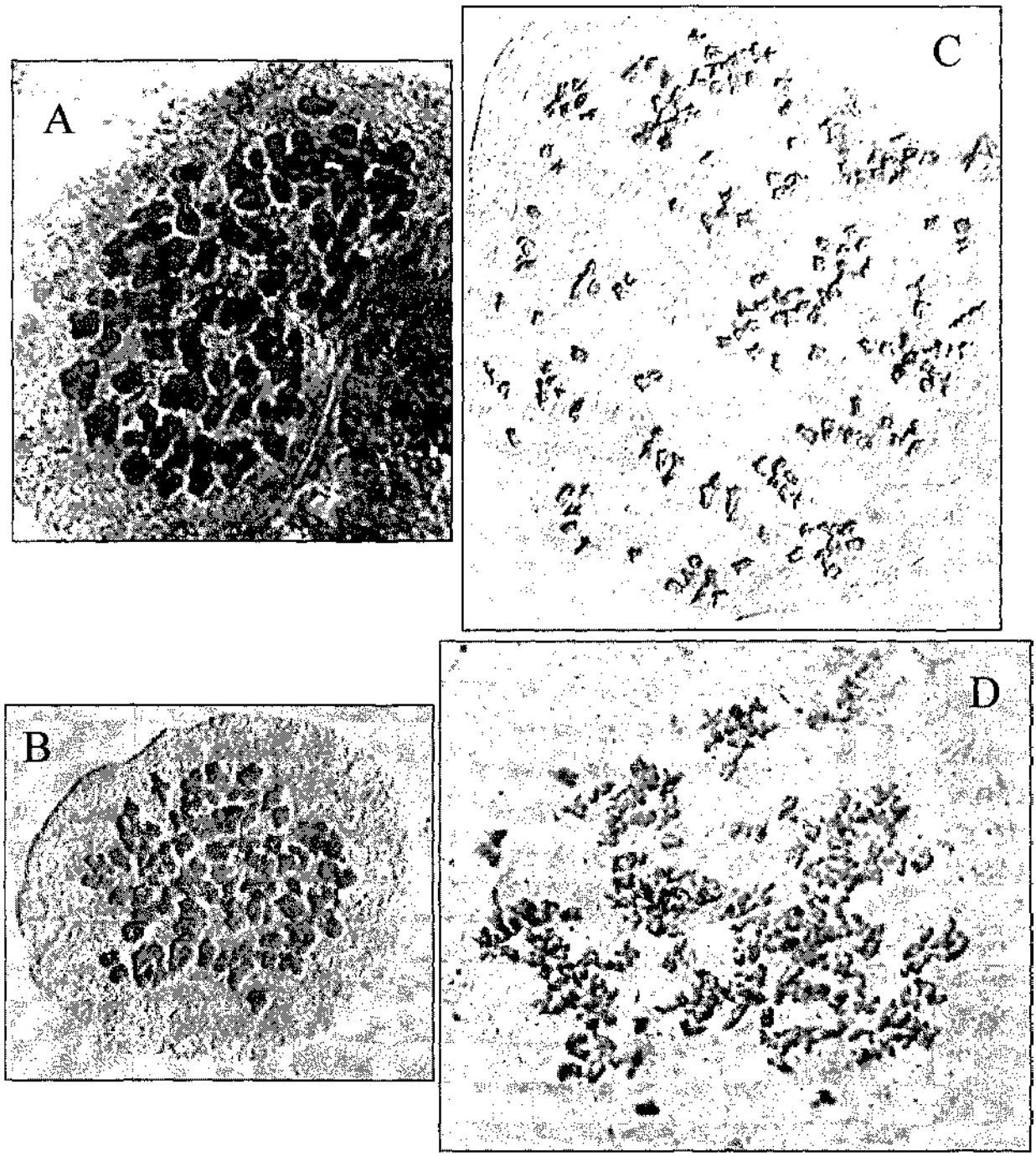


Figure 4.12. Chromosome Preparations.
Examples of the aceto-carimine preparations of diakinesis used to make chromosome counts, photographed at x1000 magnification. Sample details can be found in Appendix 2.1.1.

A) *P. wawranum*:	*rWan7*,	n = c.82,	tetraploid.
B) *P. oculatum*:	*rWel8*,	n = c.85,	tetraploid.
C) *P. neozelandicum* subsp. *neozelandicum*:	*rPmm1*,	n = 164,	octoploid.
D) *P. neozelandicum* subsp. *zerophyllum*:	*rRkk1*,	n = c.170,	octoploid.

Table 4.2. Definitions of Morphological Characters for Chapter Four.

Character Name	Character Definition
'Scale (mid-) width'	Average mid-length width of five scales from the stipe-rachis junction (μm).
'Scale (max-) width'	Average maximum width of five scales from the stipe-rachis junction (μm).
'Pinnae distance ratio'	Distance between the 2nd and 4th most basal primary pinnae, divided by the length of the rachis.
'Pinnae width ratio'	Length divided by width of the longest primary pinna.
'Annulus cells'	Average number of indurated cells counted in ten sporangia.
'Indusial dark centre'	Average percentage of surface area occupied by central dark area in ten indusia.
'Spore size'	Average product of spore exine length and width of 30 spores (μm^2).

Table 4.3. Distinguishing Characters for the *Polystichum neozelandicum* Complex. Morphological (and cytological) characters that distinguish *Polystichum wawranum*, *P. neozelandicum* subsp. *neozelandicum*, *P. neozelandicum* subsp. *zerophyllum*, and *P. oculatum*. The ranges given below for the quantitative characters are based on the 5th and 95th percentiles. Quantitative characters are defined in Table 4.2.

Character	*P. wawranum*	*P. neozelandicum* subsp. *neozelandicum*	*P. neozelandicum* subsp. *zerophyllum*	*P. oculatum*
Quantitative characters				
Scale (mid-) width	40-120 μm	135-340 μm	150-570 μm	770-2280 μm
Scale (max-) width	200-390 μm	290-650 μm	380-890 μm	1060-2550 μm
Pinnae distance ratio	0.097-0.154	0.140-0.203	0.144-0.211	0.194-0.275
Pinnae width ratio	3.2-6.1	2.5-3.7	2.3-3.7	2.0-3.5
Annulus cells	13.0-18.8	14.2-18.3	13.1-19.7	15.4-21.4
Indusial dark centre	1.0-17.1%	16.6-59.0%	6.1-29.7%	7.0-50.6%
Spore size	1160-1720 μm^2	1800-2270 μm^2	1670-2540 μm^2	980-1750 μm^2
Qualitative characters				
Scale shape	filiform (hair-like), widest at base	acicular-lanceolate, widest in basal third	acicular-lanceolate, widest in basal third	pentagonal (or almost so), often widest near mid-length
Ploidy level	tetraploid	octoploid	octoploid	tetraploid

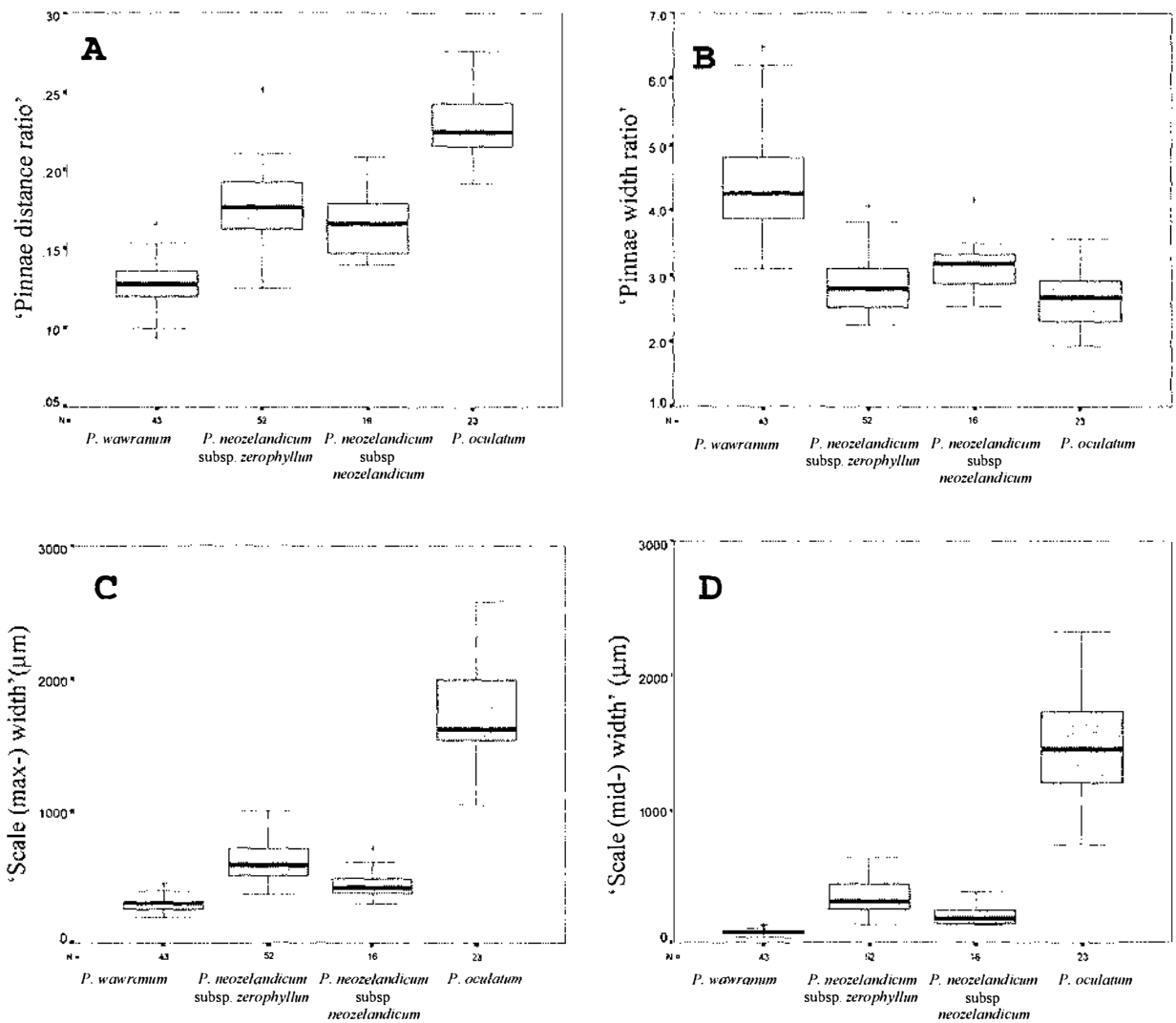


Figure 4.13. *Polystichum neozelandicum* Complex Morphological Box-plots.
Box-plot summaries of variation in morphological characters which show (at least some) differentiation between *P. wawranum*, *P. neozelandicum* subsp. *zerophyllum*, *P. neozelandicum* subsp. *neozelandicum*, and *P. oculatum*. 'N=' denotes the number of samples analysed; '+' & '×' denote outliers. Characters are defined in Table 4.2, and details of samples analysed can be found in Appendix 2.1.1.

(A) 'Pinnae distance ratio.'
(B) 'Pinnae width ratio.'
(C) 'Scale (max-) width.'
(D) 'Scale (mid-) width.'

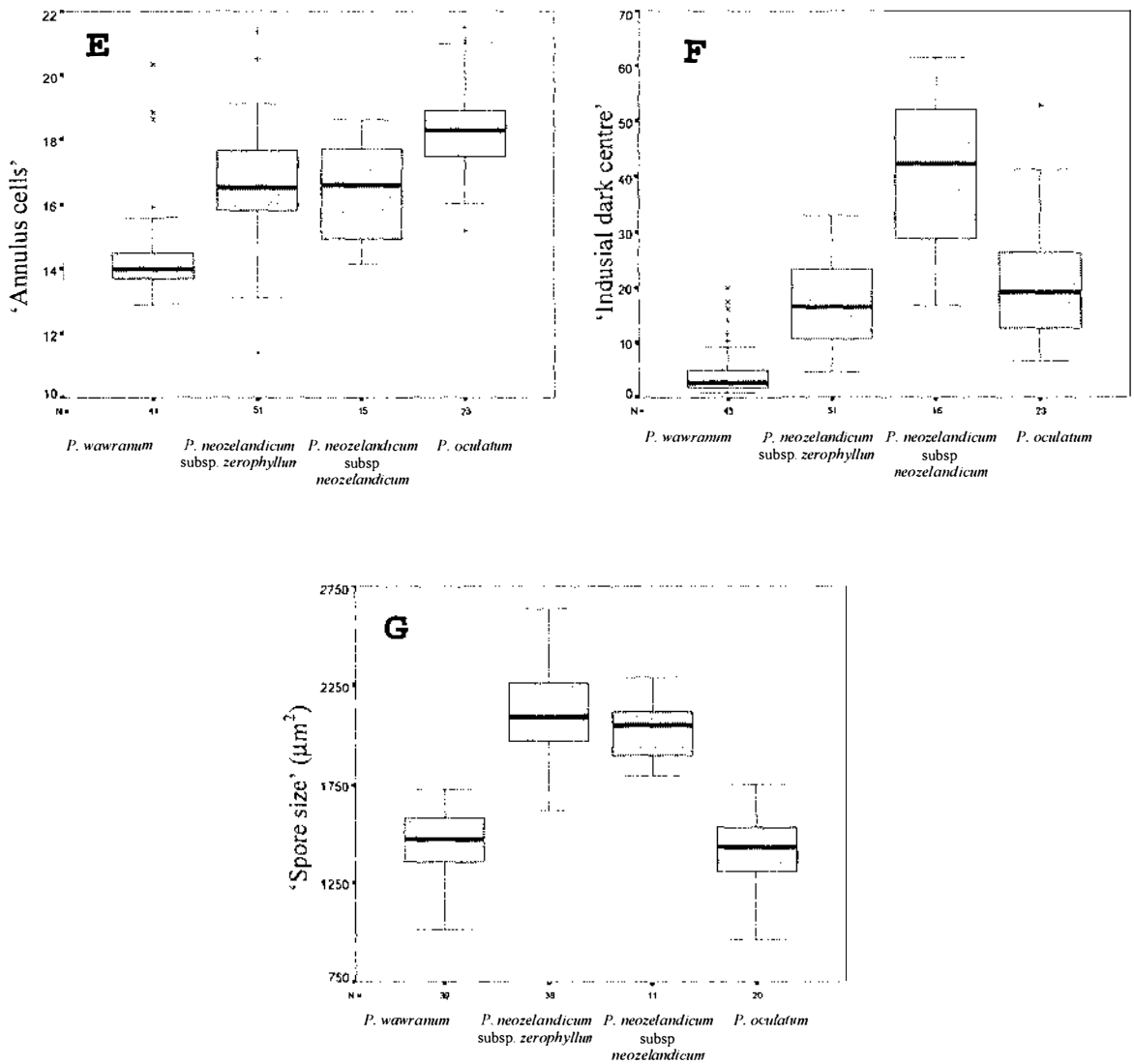


Figure 4.13 (cont.) *Polystichum neozelandicum* Complex Morphological Box-plots. Box-plot summaries of variation in morphological characters which show (at least some) differentiation between *Polystichum wawranum*, *P. neozelandicum* subsp. *zerophyllum*, *P. neozelandicum* subsp. *neozelandicum*, and *P. oculatum*. 'N=' denotes the number of samples analysed; '+' & '×' denote outliers. Characters are defined in Table 4.2, and details of samples analysed can be found in Appendix 2.1.1.

(E) 'Annulus cells.'
(F) 'Indusial dark centre.'
(G) 'Spore size.'

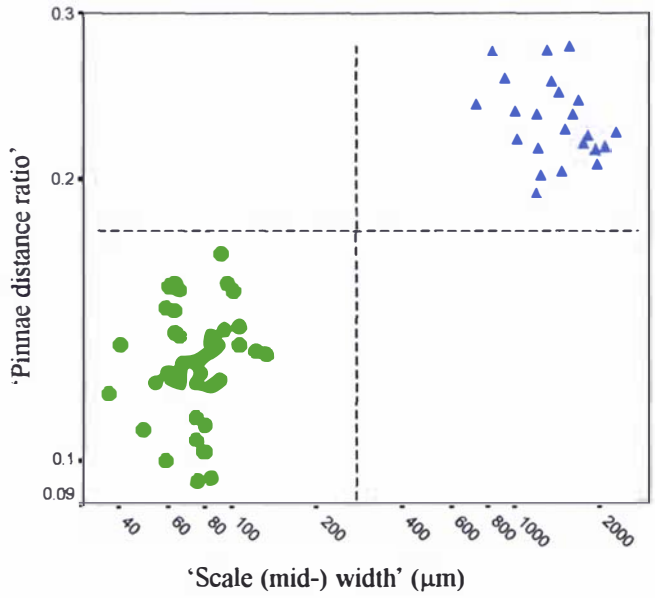


Figure 4.14. *Polystichum wawranum* and *P. oculatum* Scatter-plot.
Scatter-plot of 'scale (mid-) width' against 'pinnae distance ratio' for *P. wawranum* (●) and *P. oculatum* (▲). Note that the axes are logarithms. Dashed lines indicate partitioning.

(Figure 4.14), but all combinations of the characters 'scale (mid-) width,' 'pinnae distance ratio,' 'pinnae width ratio,' 'annulus cells,' and 'indusial dark centre' show some degree of concordant partitioning.

The character combination of 'scale (mid-) width' and 'pinnae distance ratio' also concordantly partitions *P. neozelandicum* from *P. oculatum*, and *P. neozelandicum* from *P. wawranum* (Figure 4.15). The split {*P. neozelandicum*}{complement} (and therefore also the partial splits of {*P. neozelandicum*}{*P. oculatum*} and {*P. neozelandicum*}{*P. wawranum*}) is concordantly partitioned by the character 'spore size' in combination with either 'scale (mid-) width' or 'pinnae distance ratio' (the partitioning afforded by 'scale (mid-) width' and 'spore size' is illustrated in Figure 4.16).

In most of the cases discussed above, the concordance between characters is not absolute. Nevertheless, the variation in the morphological characters discussed in this section provides support for the recognition of three lineages, and is largely congruent with the lineages recognised from analysis of the AFLP data (see Section 4.3.3). While

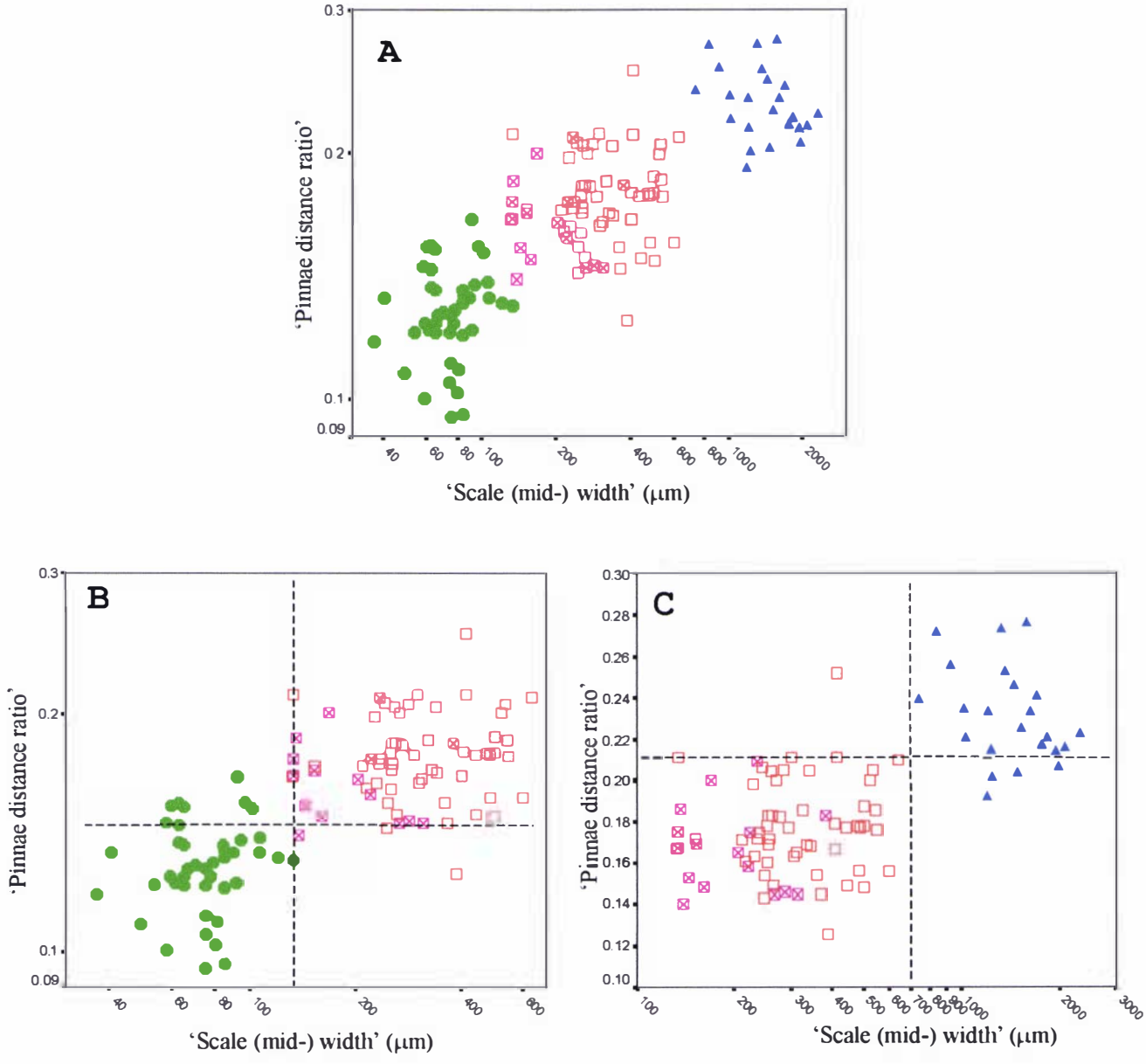


Figure 4.15. *Polystichum neozelandicum* Complex Scatter-plot I.
Scatter-plots of 'scale (mid-) width' against 'pinnae distance ratio' for *P. wawranum* (●), *P. neozelandicum* subsp. *neozelandicum* (⊠), *P. neozelandicum* subsp. *zerophyllum* (□), and *P. oculatum* (▲). Note that some of the axes are logarithms.
(A) All four taxa.
(B) Illustration of (almost) concordant-partitioning between *P. wawranum* and *P. neozelandicum* (both subspecies).
(C) Illustration of (almost) concordant partitioning between *P. neozelandicum* (both subspecies) and *P. oculatum*.
Dashed lines indicate partitioning.

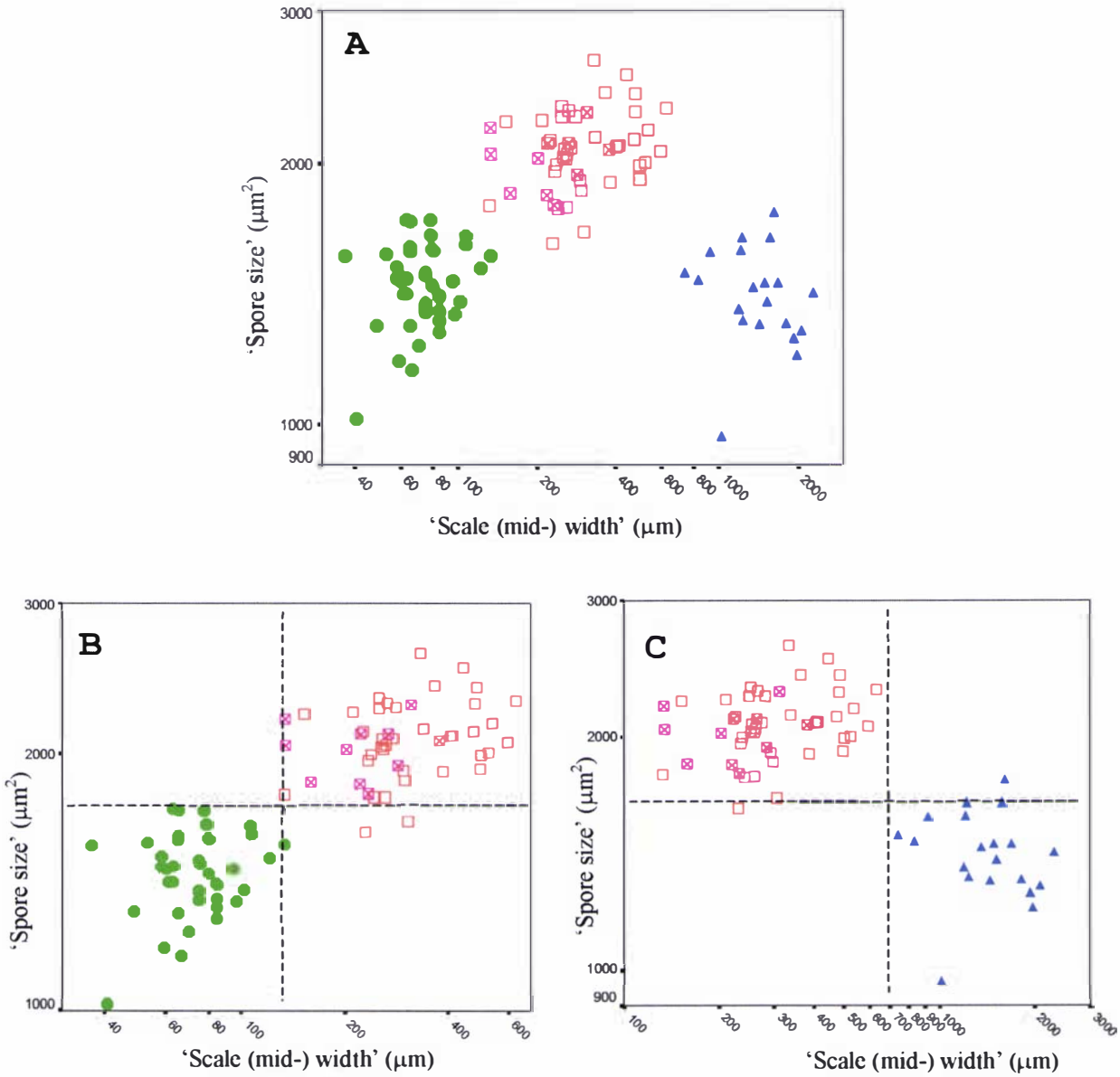


Figure 4.16. *Polystichum neozelandicum* Complex Scatter-plot II.
Scatter-plots of 'scale (mid-) width' against 'spore size' for *P. wawranum* (●), *P. neozelandicum* subsp. *neozelandicum* (⊠), *P. neozelandicum* subsp. *zerophyllum* (□), and *P. oculatum* (▲). Note that the axes are logarithms.
(A) All four taxa.
(B) Illustration of (almost) concordant-partitioning between *P. wawranum* and *P. neozelandicum* (both subspecies).
(C) Illustration of (almost) concordant partitioning between *P. neozelandicum* (both subspecies) and *P. oculatum*.
Dashed lines indicate partitioning.

the AFLP data (see Section 4.3.3) indicates the presence of two octoploid lineages, labelled as *P. neozelandicum* subsp. *neozelandicum* and *P. neozelandicum* and *zerophyllum*, there is little congruent morphological variation. Other than the dark centre of the indusia of *P. neozelandicum* subsp. *neozelandicum* being generally larger than that found in *P. neozelandicum* subsp. *zerophyllum* (see Figure 4.13f), these two taxa are essentially morphologically indistinguishable (although they do exhibit quite different morphological extremes). No pair of morphological characters, at least amongst those investigated, concordantly partition *P. neozelandicum* subsp. *neozelandicum* from *P. neozelandicum* subsp. *zerophyllum*.

4.3.3 AFLP Analysis.

Whilst three lineages might be recognisable from the morphological (and cytological) analyses, AFLP analysis provides strong support for the delimitation of four lineages. Perrie et al. (2000; manuscript provided in Appendix One) have already reported evidence from AFLP characters for the strong concordant partitioning of samples from the *P. neozelandicum* complex (as '*P. richardii*') into two groups (Appendix Four provides a nexus file of their data as 'AFLP-PerrieEtal2000.nex'). The sample set of Perrie et al. (2000) is detailed in Appendix 3.2.1. Their 'wide-scaled species' and 'narrow-scaled species' are equivalent to *P. oculatum*, and an amalgamation of *P. wawranum* and *P. neozelandicum*, respectively. The focus of Perrie et al. (2000) was on the separation of *P. oculatum* from the remainder of the complex. The distinction between *P. wawranum* and *P. neozelandicum*, and that between the subspecies of *P. neozelandicum*, was not fully appreciated at the time. Consequently *P. wawranum* and *P. neozelandicum* subsp. *neozelandicum* were under-represented in their sample set, with the inclusion of only two and one samples, respectively. However, Perrie et al. (2000 p.104) did note that "sympatric variation ... observed across several morphological characters in the narrow-scaled species [suggests] that it too may be a complex [of more than one species]." An analysis based on a broader sample set is described below.

4.3.3.1 Sampling.

The genetic relationships between seven samples of *P. wawranum*, six of *P. oculatum*, seven of *P. neozelandicum* subsp. *zerophyllum* and two of *P. neozelandicum* subsp. *neozelandicum* were investigated. Geographic origins of these samples are

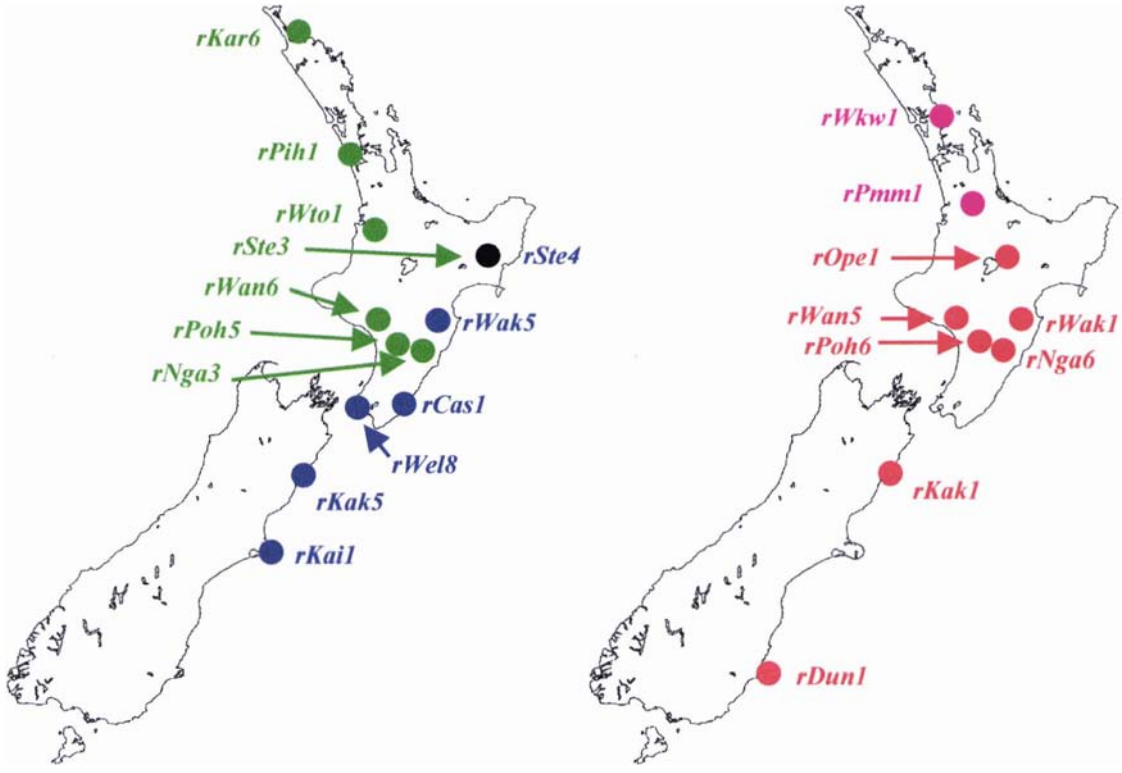


Figure 4.17. Maps of *Polystichum neozelandicum* Complex AFLP Samples.
Geographic origins of samples included in AFLP analysis of the *P. neozelandicum* complex. Samples are coloured as follows: *P. wawranum*, *P. neozelandicum* subsp. *neozelandicum*, *P. neozelandicum* subsp. *zerophyllum*, and *P. oculatum*. The black dot indicates the 'locally-sympatric' site from where *rSte3* and *rSte4* were sourced, representing *P. wawranum* and *P. oculatum* respectively. Further details of samples are given in Appendix 2.1.1

shown in Figure 4.17. Included were individuals from two locally sympatric sites (see Table 4.1 for further details) between *P. wawranum* and *P. neozelandicum* subsp. *zerophyllum* (*rPoh5* & *rPoh6*, and *rNga3* & *rNga6*), and one locally sympatric site between each of *P. neozelandicum* subsp. *zerophyllum* and *P. oculatum* (*rWak1* & *rWak5*) and *P. wawranum* and *P. oculatum* (*rSte3* & *rSte4*).

4.3.3.2 Results.

The methods outlined in Section 3.4.4 were used to produce AFLP profiles using the primer combinations E-ATA & M-CTG, E-AAT & M-CAG, and E-ATA & M-CAG. These generated 53, 132, and 126 scorable polymorphic bands, respectively. From these 311 characters, 243 different splits were recorded, of which 22 were parsimony-

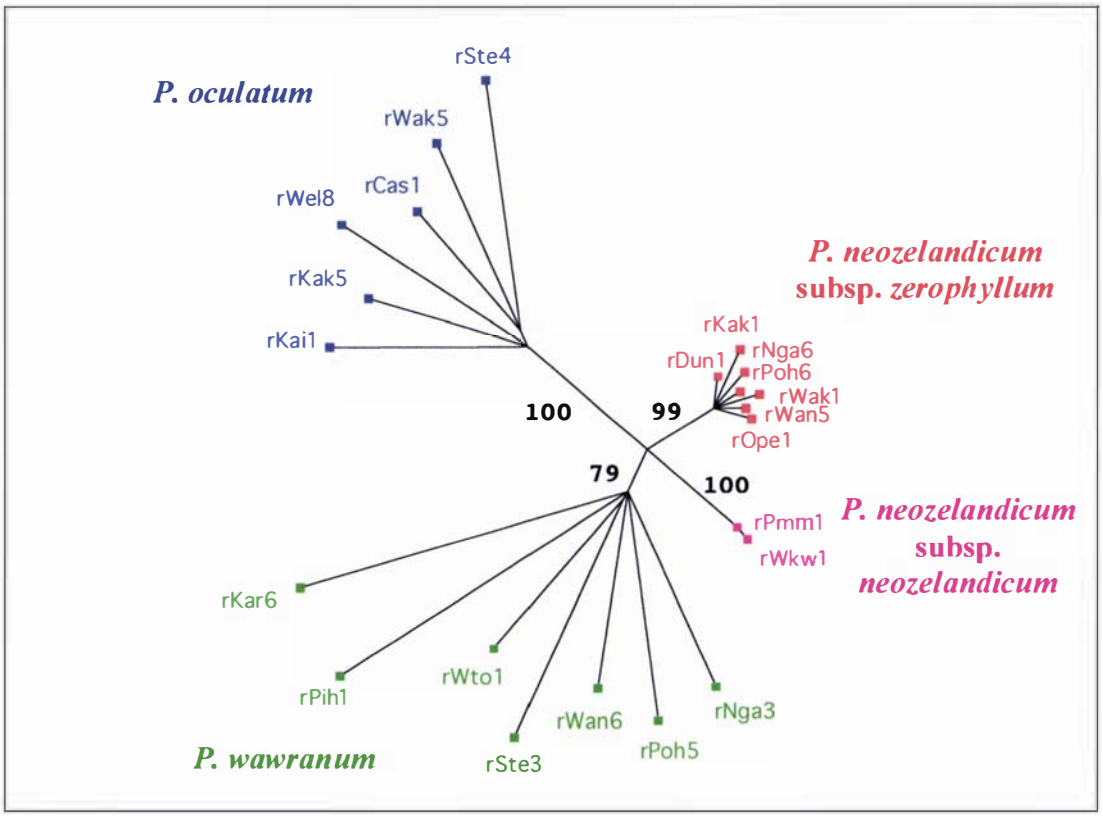


Figure 4.18. Parsimony Splits-Graph of the AFLP Data.
Bootstrap support (1000 replicates) for the major internal edges is shown. Internal edges recovered within *Polystichum oculatum* and *P. wawranum* were recovered with 19% and 11% bootstrap support, respectively. Collection details of samples are given in Appendix Four.

uninformative. Of the parsimony-informative splits, 24 occurred at more than one character, and these are given in Appendix 3.2.2. The remaining 197 parsimony-informative splits were recorded at only one character each. Nexus and spectrum files of this data are provided in Appendix Four ('AFLP-NeoCom.nex' & 'AFLP-NeoCom.spe').

Results of bootstrapping analysis (1000 replicates) under parsimony-based split-decomposition, parsimony and neighbour-joining are shown in Figures 4.18, 4.19 and 4.20, respectively. The 30 highest supported (parsimony-informative) splits under a distance-spectral analysis are detailed in Appendix 3.2.3, and graphed in Figure 4.21.

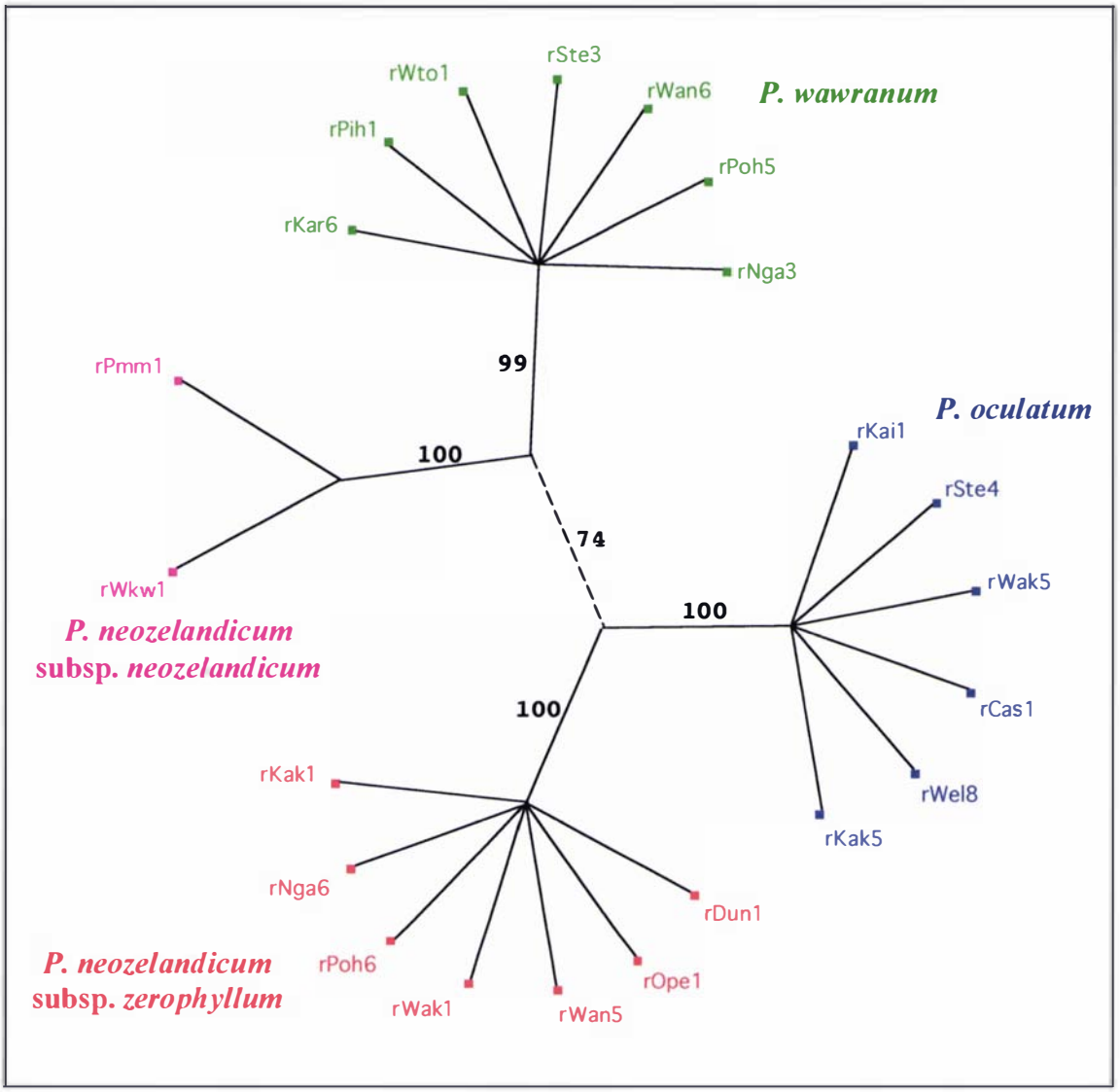


Figure 4.19. Bootstrap Analysis Under Parsimony of the AFLP Data.
Internal edges recovered with greater than 80% bootstrap support (1000 replicates) are indicated. The edge corresponding to the split {*Polystichum wawranum* & *P. neozelandicum* subsp. *neozelandicum*}{*P. neozelandicum* subsp. *zerophyllum* & *P. oculatum*} is indicated by the dashed line, and its bootstrap support is also given. Collection details of samples are given in Appendix Four.

The sample set is clearly resolved into four major groups, corresponding to *P. wawranum*, *P. oculatum*, *P. neozelandicum* subsp. *neozelandicum*, and *P. neozelandicum* subsp. *zerophyllum*. These groups are all subtended by internal edges with high bootstrap support (all 99% or 100% under split-decomposition, parsimony and neighbour-joining, except for *P. wawranum* under split-decomposition where the bootstrap support is 79%; see below). Further, their respective splits (ie. {*P. wawranum*}{complement}, {*P. oculatum*}{complement}, {*P. neozelandicum* subsp.

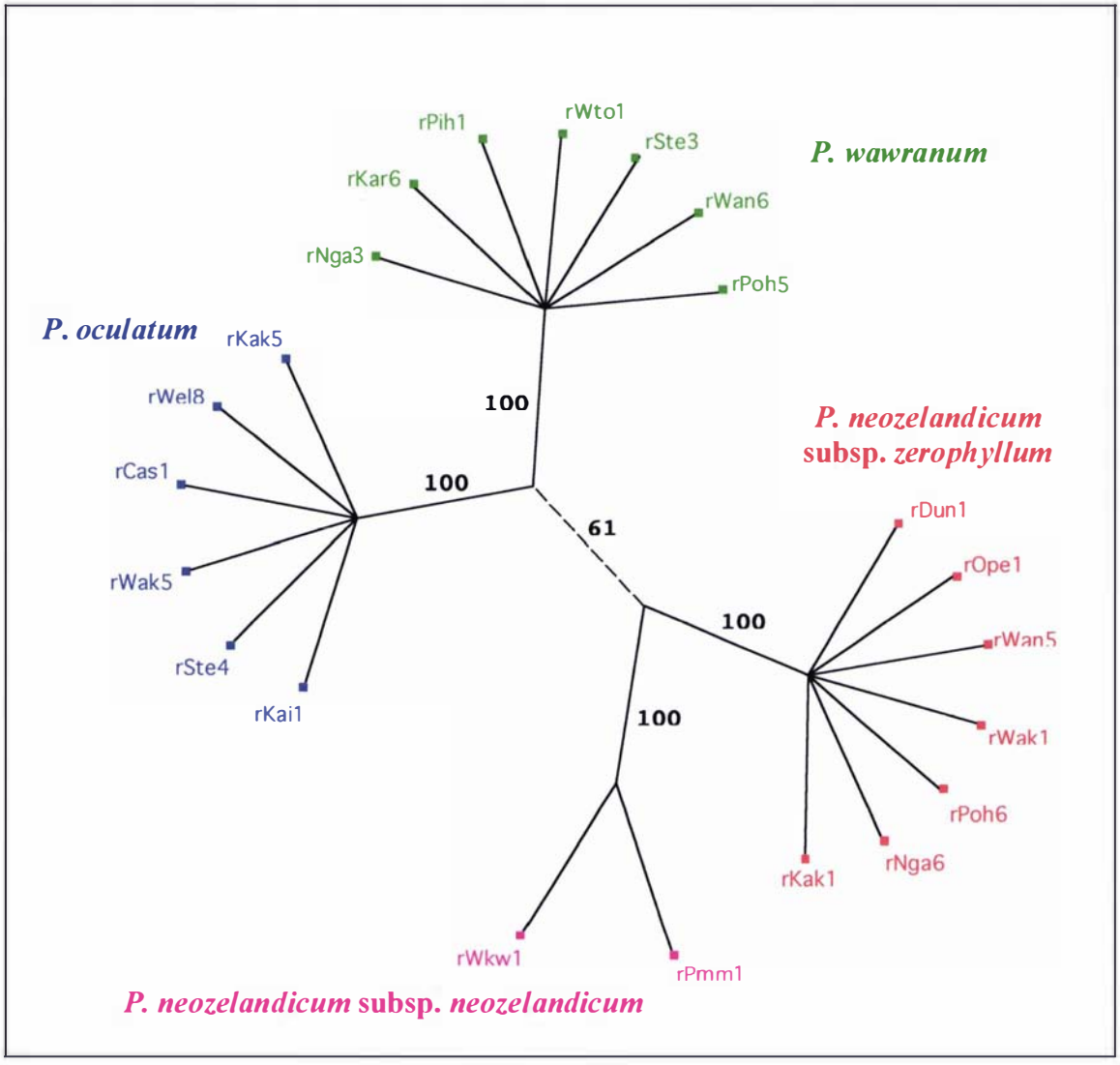


Figure 4.20. Bootstrap Analysis Under Neighbour-Joining of the AFLP Data.
Internal edges recovered with greater than 80% bootstrap support (1000 replicates) are indicated. The edge corresponding to the split {*Polystichum neozelandicum* subsp. *neozelandicum* & *P. neozelandicum* subsp. *zerophyllum*}{*P. wawranum* & *P. oculatum*} is indicated by the dashed line, and its bootstrap support is also given. Collection details of samples are given in Appendix Four.

neozelandicum}{complement}, and {*P. neozelandicum* subsp. *zerophyllum*} {complement}) are the four most supported splits in the distance spectral-analysis.

The samples of *P. wawranum* are recovered as a group with only 79% BS under split-decomposition. This appears to be because the most northerly *P. wawranum* samples, *rKar6* and *rPih1*, group preferentially with *P. oculatum*, whereas the remaining *P. wawranum* group either with *P. neozelandicum* (subsp. *neozelandicum*) or are

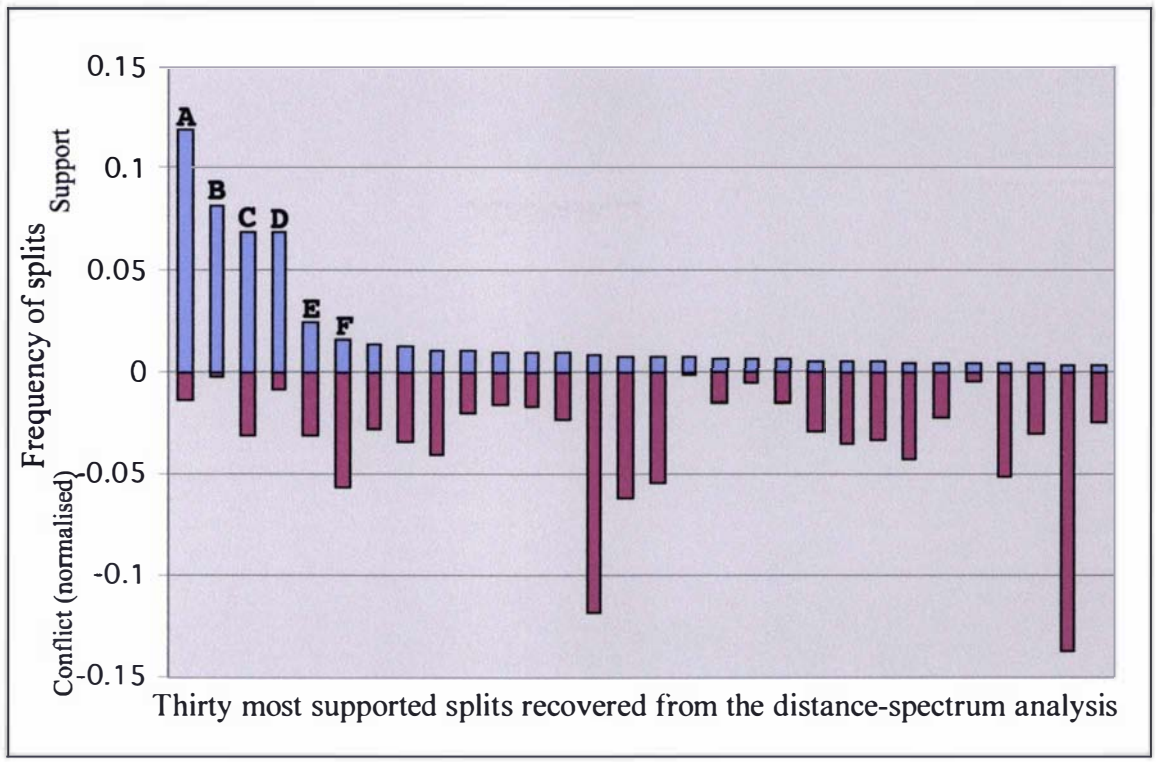


Figure 4.21. Distance-Spectral Analysis of the AFLP Data.
Plot of support for, and conflict against, the 30 most supported (parsimony-informative) splits recovered from the distance-spectrum analysis. Support (in blue) for each split is plotted on the positive y-axis; normalised conflict (in red) is plotted on the negative y-axis. Label (A) corresponds to the split {*Polystichum oculatum*}{complement}, (B) {*P. neozelandicum* subsp. *neozelandicum*}{complement}, (C) {*P. wawranum*} {complement}, and (D) {*P. neozelandicum* subsp. *zerophyllum*}{complement}. Label (E) denotes the split {*P. neozelandicum* subsp. *neozelandicum* & *P. neozelandicum* subsp. *zerophyllum*}{*P. wawranum* & *P. oculatum*}, which is recovered under neighbour-joining, and is consistent with a single allopolyploid origin of *P. neozelandicum*. Label (F) indicates the split {*P. wawranum* & *P. neozelandicum* subsp. *neozelandicum*}{*P. neozelandicum* subsp. *zerophyllum* & *P. oculatum*}, which is recovered under parsimony, and might be indicative of multiple origins of *P. neozelandicum*. Neither of these latter two splits are recovered under split-decomposition.

unresolved. This may indicate that the genomes of *rKar6* and *rPih1* are less representative of the *P. wawranum* genome that contributed to the allopolyploid *P. neozelandicum*. When *rKar6* and *rPih1* are excluded from the split-decomposition analysis, the remaining *P. wawranum* samples are supported as a group with 99% BS. Further, when *P. neozelandicum* is excluded, all *P. wawranum* samples are separated from all *P. oculatum* samples by an internal edge with 100% BS support.

That the groups labelled *P. wawranum*, *P. oculatum*, *P. neozelandicum* subsp. *neozelandicum* and *P. neozelandicum* subsp. *zerophyllum* are so well supported in the

data analyses indicates the presence of strong concordant partitioning in the AFLP data set. This, in turn, must reflect retrospective assortative-fertilising between these geographically widespread groups of samples, such that they could be termed (retrospectively) separate evolutionary lineages.

4.3.3.3 Comparison to Perrie et al. (2000).

The AFLP results presented above are entirely congruent with the earlier work reported in Perrie et al. (2000). Their splits-graph (Perrie et al. 2000 fig.7.) is also a four-way polytomy between *P. oculatum* (samples 15-22; 100% B.S.), *P. wawranum* (samples 1 & 7; 99% B.S.), *P. neozelandicum* subsp. *zerophyllum* (samples 3-6 & 8-14; 99% B.S.), and *P. neozelandicum* subsp. *neozelandicum* (sample 2; an external edge with 100% B.S.).

Many of the samples studied by Perrie et al. (2000) are the same as those reported here, providing a link between the sample sets. For instance, in *P. neozelandicum* subsp. *zerophyllum*, the samples *rWak1*, *rKak1*, and *rDun1* were analysed in both data sets. In the analysis of Perrie et al. (2000; see figures 1 & 7b), samples from Taranaki, the Cook Strait region, the central eastern coast of the South Island, and the Chatham Islands group closely with the aforementioned samples. This provides molecular corroboration for the presence of *P. neozelandicum* subsp. *zerophyllum* in these regions that were not included in the sample set reported here.

4.3.3.4 Subsequent Analysis of Polystichum neozelandicum *subsp.* neozelandicum.

Only two samples of *P. neozelandicum* subsp. *neozelandicum* were included in the analysis detailed in Sections 4.3.3.1 and 4.3.3.2, namely *rWkw1* and *rPmm1* from Warkworth and near Gordonton (in the northern Waikato), respectively. However, subsequent AFLP analysis of additional samples (*rKar9*, *rMan1*, *rCor3*, from Karikari Peninsula, near Whangarei, and Whangapoua in the Coromandel, respectively) found them to be virtually identical to *rWkw1* and *rPmm1*, but quite distinct from those of *P. neozelandicum* subsp. *zerophyllum* (see Figure 4.22). This finding provides molecular corroboration that the *P. neozelandicum* subsp. *neozelandicum* lineage is widely distributed in northern New Zealand.

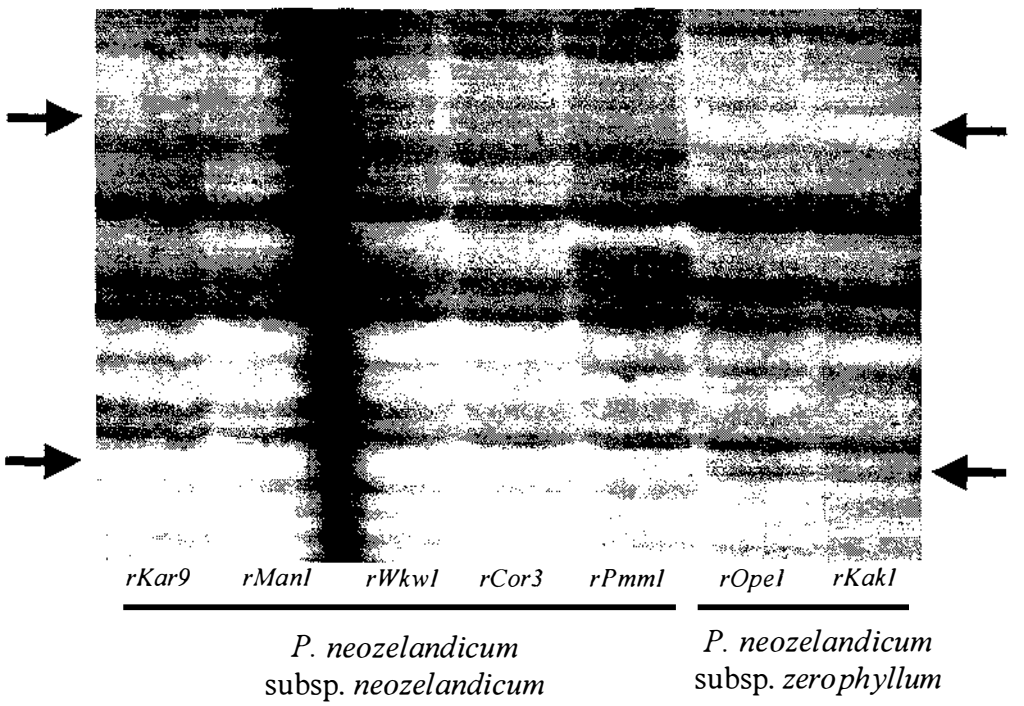


Figure 4.22. AFLP of the *Polystichum neozelandicum* Subspecies.
Portion of an AFLP gel (E-ATA M-CTG, 5% polyacrylamide, profiles between c.530-570 base-pairs pictured). Differences in the banding profiles between the samples of *P. neozelandicum* subsp. *neozelandicum* and *P. neozelandicum* subsp. *zerophyllum* are indicated by arrows. Details of the samples can be found in Appendix 2.1.1.

4.3.4 Allopolyploidy of *Polystichum neozelandicum.*

The cytological analyses reported earlier indicate that the *P. neozelandicum* subsp. *neozelandicum* and *P. neozelandicum* subsp. *zerophyllum* lineages are both octoploid, which is consistent with their larger spore size. Several aspects of the morphological variation exhibited by these octoploid plants suggest that they have been derived from an allopolyploid hybridisation event (or events) between the tetraploid *P. wawranum* and *P. oculatum* lineages (or at least between ancestors of the extant individuals of these lineages). Firstly, *P. neozelandicum* is generally morphologically intermediate between *P. wawranum* and *P. oculatum*, particularly in the 'scale (mid-) width' and 'pinnae distance ratio' characters (see Figure 4.15a). Further, *P. neozelandicum* exhibits few morphological character states not found in one or both of the putative parents. While the larger spore size of *P. neozelandicum* is an exception, this 'novel' character state is entirely compatible with the hypothesis of a polyploid origin (Barrington et al. 1986).

Table 4.4. Allopolyploid AFLP Banding Patterns.
Table of (A) 'Expected' and (B) 'Unexpected' banding patterns under a hypothesis of allopolyploid origins for *Polystichum neozelandicum* with a dominant marker system such as AFLP. 'Expected' patterns (exact as in (A), plus those one step away) predominate in the AFLP data set, being recorded at 28 characters, compared to 'Unexpected' patterns (exact as in (B), plus those one step away) which were recorded at 11 characters.

(A) 'Expected' patterns.

P. wawranum	*P. neozelandicum* subsp. *neozelandicum*	*P. neozelandicum* subsp. *zerophyllum*	*P. oculatum*
Fixed for band presence	Fixed for band presence	Fixed for band presence	Fixed for band absence
Fixed for band absence	Fixed for band presence	Fixed for band presence	Fixed for band presence

(B) 'Unexpected' patterns

P. wawranum	*P. neozelandicum* subsp. *neozelandicum*	*P. neozelandicum* subsp. *zerophyllum*	*P. oculatum*
Fixed for band presence	Fixed for band absence	Fixed for band absence	Fixed for band absence
Fixed for band absence	Fixed for band absence	Fixed for band absence	Fixed for band presence

Aspects of the AFLP data also support the hypothesis of an allopolyploid origin for *P. neozelandicum*. In the analyses of these data both octoploid lineages fall outside the tetraploid lineages, rather than inside one or other of them as would be expected from an autopolyploid event. Also, there is a predominance of patterns where *P. neozelandicum* shares the 'band-presence' allele, rather than the 'band-absence' allele, with one of the tetraploid lineages (see Table 4.4). This 'additive pattern' is to be expected in a hybrid scenario with a dominant marker system (Liu et al. 1998, Ayres & Strong 2001, Congiu et al. 2001). It would also be expected that the hybrid(s) be recovered on an internal node. This is clearly not the case here, and probably reflects a combination of an absence of samples adequately representative of the progenitor genotypes in the data set, synapomorphy on the hybrid lineage(s), and homoplasy (see Hedren et al 2001). Pertinent to the latter two points is the finding in some studies of rapid genomic change in new polyploids (Song et al. 1995, Soltis & Soltis 1999).

More generally, the octoploid lineages exhibit little intra-lineage genetic variation, as assayed by AFLP, relative to that found in either *P. wawranum* or *P. oculatum* (Table 4.5). This is consistent with the genetic 'bottlenecking' effect expected from a polyploid event, where the genetic variation inherent in one or two individuals (for

Table 4.5. Lineage Genetic Variation.
Table of intra- and inter-lineage genetic variation as assayed by AFLP. Given are minimum, median, and maximum Hamming distances, with 'n' indicates the number of pairwise distance comparisons made.

min, **median**, max n	*P. wawranum*	*P. neozelandicum* subsp. *neozelandicum*	*P. neozelandicum* subsp. *zerophyllum*	*P. oculatum*
P. wawranum	0.208, **0.304**, 0.362 21	0.305, **0.330**, 0.412 14	0.324, **0.362**, 0.449 49	0.442, **0.497**, 0.567 42
P. neozelandicum subsp. *neozelandicum*		**0.032** 1	0.189, **0.210**, 0.224 14	0.356, **0.407**, 0.426 12
P. neozelandicum subsp. *zerophyllum*			0.048, **0.071**, 0.093 21	0.323, **0.380**, 0.413 42
P. oculatum				0.192, **0.244**, 0.266 15

autopolyploidy and allopolyploidy, respectively) constitutes the founding stock of subsequent derivative polyploid individuals.

Although both exhibit little intra-lineage genetic variation, *P. neozelandicum* subsp. *neozelandicum* and *P. neozelandicum* subsp. *zerophyllum* are nevertheless quite distinct evolutionary lineages. However, whether this distinctiveness indicates two independent allopolyploid origins, or a single allopolyploid origin with subsequent divergence, is unclear. Soltis and Soltis (1993 p.243; also see Soltis & Soltis 1999, 2000) have claimed that "recurrent formation of polyploid species is the rule, rather than the exception," but Vogel et al. (1999) have questioned some of the evidence on which this hypothesis is based. In the case of the two lineages of *P. neozelandicum*, literal interpretation of their relationship, as inferred from the AFLP data, is ambiguous.

The split {*P. wawranum* & *P. neozelandicum* subsp. *neozelandicum*}{*P. neozelandicum* subsp. *zerophyllum* & *P. oculatum*} is potentially indicative of multiple allopolyploid origins, and is recovered with 74% bootstrap support under parsimony. However, the incompatible split {*P. neozelandicum* subsp. *neozelandicum* & *P. neozelandicum* subsp. *zerophyllum*}{*P. wawranum* & *P. oculatum*}, which is consistent with a single allopolyploid origin, is recovered under neighbour-joining, with 61% bootstrap support. In the distance-spectral analysis, the latter split is recovered with more support (and less

overall conflict) than the former (see Appendix 3.2.3). The simultaneous support for both of these incompatible splits is reflected in that neither are recovered under split-decomposition (not even under refined split-decomposition; see Huson 1998). The four-way polytomy of the splits-graph is evidently a fair depiction of this ambiguity.

Studies of hybrid formation have inferred multiple origins when both parental types of the uniparentally-inherited chloroplast have been found in hybrid individuals (eg. Anttila et al. 2000; see Soltis & Soltis 1993 p.247). The chloroplasts of *P. wawranum* and *P. oculatum* are differentiated by an apparently fixed five base-pair size difference in the *rps4-trnS* spacer region (see Section 6.2.2). However, both lineages of *P. neozelandicum* share the *P. wawranum* haplotype, rendering this avenue of investigation uninformative in this case.

The pattern of genetic 'bottlenecking' in *P. neozelandicum* is possibly suggestive of independent origins for subsp. *neozelandicum* and subsp. *zerophyllum*. Both appear to contain little genetic variation, but are nevertheless quite different to one another. A single origin hypothesis would require substantial diversification in the polyploid lineage to produce the observed differences between subsp. *neozelandicum* and subsp. *zerophyllum*, followed by a subsequent bottlenecking event (or events) in which much of this diversification was then lost with only two, quite different lineages surviving.

4.4 Taxonomic Delimitation.

From analysis of morphological and AFLP character state variation, the null hypothesis that 'a single evolutionary lineage' is present in the *P. neozelandicum* complex (=*P. richardii sensu* Allan 1961, Brownsey 1988, Brownsey & Smith-Dodsworth 1989, 2000) can be strongly rejected. Concordant partitioning by characters in the AFLP data set leads to the delimitation of four separate evolutionary lineages. Morphological variation is congruent with these lineages (although only three lineages could be recognised by morphology alone). Inter-lineage hybridisation suggests that at least three of these lineages are at least partially sexually-outcrossing. Consequently, the

concordant partitioning in the morphological and AFLP data must be due to assortative fertilisation, at least retrospectively.

The lineages labelled above as *P. wawranum*, *P. neozelandicum* subsp. *zerophyllum* and *P. oculatum* are broadly sympatric with each other over large geographic areas. Numerous local instances of sympatry are known between *P. wawranum* and *P. neozelandicum* subsp. *zerophyllum*, and between *P. neozelandicum* subsp. *zerophyllum* and *P. oculatum.* Such sympatry implies that the assortative fertilisation that has led to the retrospective separation of these lineages is not simply due to geographic isolation, but is likely to involve SMRS differentiation (see Chapter Two). Hence, not only are these lineages distinct retrospectively, but they can also be inferred to be prospectively separate, and therefore could be recognised as three separate species: *P. wawranum*, *P. neozelandicum*, and *P. oculatum.*

Taxonomic delimitation of the lineage labelled *P. neozelandicum* subsp. *neozelandicum* is less straightforward. Its broad sympatry with *P. wawranum* could be used to infer prospective evidence of their evolutionary independence, such that they should be regarded as separate species. However, *P. neozelandicum* subsp. *neozelandicum* is allopatric with respect to both *P. neozelandicum* subsp. *zerophyllum* and *P. oculatum.* Morphologically, *P. neozelandicum* subsp. *neozelandicum* is virtually indistinguishable from *P. neozelandicum* subsp. *zerophyllum*, both are octoploid, and some analyses of the AFLP data suggest that they are each other's closest relatives. Consequently, the lineage *P. neozelandicum* subsp. *neozelandicum* is best accommodated within the species *P. neozelandicum* (rather than within *P. oculatum*). It must be emphasised that *P. neozelandicum*, as defined here, is a taxonomic species first and foremost, in that it constitutes two retrospectively-distinct, evolutionary lineages, which may even have polyphyletic origins (ie. if they did arise from separate allopolyploid events. See Hedren et al. 2001 for a discussion of taxonomic issues in situations of multiple polyploid origins).

Further study may show *P. neozelandicum* subsp. *neozelandicum* and *P. neozelandicum* subsp. *zerophyllum* to be sympatric in the Bay of Plenty and/or lower Waikato regions (or these could be areas of genuine absence). Even if extensive introgression were to be found between them in any areas of sympatry, recognition of two subspecies is probably

still justifiable on the basis of their distinctiveness over the majority of the respective ranges (ie. two largely parapatric subspecies with a hybrid zone in the region of overlap). Alternatively, if their distinctiveness is retained in sympatry, this might be taken as indirect evidence of prospective evolutionary independence, such that they could be recognised as separate species.

4.5 Discussion.

4.5.1 The Relationship of *Polystichum wawranum* and *P. oculatum.*

Although the tetraploid lineages here recognised as *P. wawranum* and *P. oculatum* have been included in the same taxonomic species for almost one hundred years, they are in fact very different from one another. However, the intermediate morphology of the two octoploid lineages of *P. neozelandicum* blurs the otherwise discontinuous nature of the morphological variation between *P. wawranum* and *P. oculatum*. If it were not for the existence of *P. neozelandicum*, seemingly an allopolyploid derivative (or derivatives) of *P. wawranum* and *P. oculatum*, there is little doubt that the latter two would have been delimited long ago as separate species on morphological grounds.

There is even an absence of any strong evidence that the two tetraploid species are closely related. For instance, in the study of genetic relationships amongst south-west Pacific *Polystichum* (see Chapter Six), a sister-group relationship between *P. wawranum* and *P. oculatum* is not resolved with chloroplast *rps4-trnS* spacer sequence, or with AFLP data when both lineages of *P. neozelandicum* are excluded (see Sections 6.2.2 and 6.3.2). There is no morphological evidence for the grouping of *P. wawranum* and *P. oculatum*; they are not diagnosed by any morphological characters, let alone synapomorphies. The same applies to the *P. neozelandicum* complex as a whole (ie. *P. wawranum*, *P. neozelandicum* and *P. oculatum*). Brownsey & Smith-Dodsworth (1989 p.131) stated that this complex (as *P. richardii*) is "always recognisable by the indusia with black centres, and scales with fringed bases." However, the black centre of the indusia of many plants of *P. wawranum* is no bigger than that of *P. vestitum*. Further, the character-state of scales with marginal projections is also shared with some plants of *P. vestitum* from the Chatham Islands (see Chapter Five). Consequently, any hypothesis of a close relationship between *P. wawranum* and *P. oculatum* would appear to rest on

little more than their historical taxonomic association, itself due in large part to the illusion of morphological continuity conjured by their allopolyploid derivative(s). Barrington (1990 p.314) has already pointed out that "the scope of *Polystichum* species involved in secondary interactions is not limited by phylogenetic proximity," at least as inferred from morphology.

4.5.2 Allopolyploid Origin of *Polystichum neozelandicum.*

P. neozelandicum, as characterised here, represents the first demonstrated example of allopolyploidy in the New Zealand fern flora, although Brownsey (1977b) and Brownsey and de Lange (1997) have hypothesised that several of the New Zealand species of *Asplenium* L. may have allopolyploid origins. Elsewhere in *Polystichum*, allopolyploidy has previously been documented in Europe (Sleep & Reichstein 1967), North America (Wagner 1979, Soltis et al. 1991) and Central America (Barrington 1990). The geographic extension of this phenomenon in the genus into the Pacific region suggests that further study may reveal allopolyploidy in *Polystichum* to be as widespread as the genus itself.

The allopolyploid lineage here recognised as *P. neozelandicum* subsp. *zerophyllum* has a wider geographic distribution than either of its putative parents. Yet, samples of *P. neozelandicum* subsp. *zerophyllum* encompassing this range were found to have considerably less genetic variation (as assayed by AFLP) compared to samples from throughout the distributions of either *P. wawranum* or *P. oculatum*. Although not quantifiable as an absolute age, this finding nevertheless implies that range expansion of this allopolyploid lineage has been relatively rapid.

4.5.3 Ecology.

Little is known to ecologically differentiate the taxa recognised here, except for the broad differences in distribution, and that *P. wawranum* and *P. neozelandicum* subsp. *zerophyllum* range to higher altitudes than the others. All reach the coast, and are principally plants of sloping substrates and high-light habitats.

4.5.4 Conservation.

P. wawranum and *P. neozelandicum* subsp. *zerophyllum* are both widespread and often common. They are frequently protected within Department of Conservation lands, and

neither would appear to be threatened at the national level. *P. oculatum* is thought to be represented by large populations on Banks Peninsula, and around the Cook Strait region. However, despite occurring north to almost the East Cape, it is seemingly uncommon in the northern part of its range, where most recorded populations are small and unprotected (ie. frequently from roadside cuttings).

P. neozelandicum subsp. *neozelandicum* is widespread in the northern third of the North Island, and locally common in some places (eg. the Coromandel Peninsula). However, further investigation is warranted as most populations recorded during this study comprised few individuals (≤ c.20, and often < 10), and it is not currently known to be well-represented within the Department of Conservation estate.

Chapter Five

Polystichum vestitum (G. Forst.) C. Presl on the Chatham Islands.

5.1 Introduction.

The Chatham Islands are situated some 850 km to the east of Christchurch, and are home to a number of unique animals and plants. Many plant genera with species common throughout the three main islands of New Zealand are represented on the Chatham Islands by local endemics; eg. *Coprosma*, *Hebe*, *Astelia*, *Myrsine*, *Pseudopanax*, *Melicytus*, *Dracophyllum*, *Olearia* (Crisp et al. 2000). Indeed, about 10% of the flora is endemic (Dawson 1991).

Ferns and fern allies constitute a higher percentage of the Chatham Islands' flora (19%) than of New Zealand as a whole (8%; Dawson 1991), but only one described fern species, *Asplenium chathamense* Brownsey, is considered endemic (Brownsey 1985a). However, in several recent publications a form of *Polystichum* on the Chatham Islands has been informally regarded as a distinct, probably endemic species (Brownsey et al. 1985, Given & Williams 1985, Brownsey 1988, Brownsey & Smith-Dodsworth 1989, Large & Braggins 1991, Cameron et al. 1995, de Lange et al. 1999a, b, Crisp et al. 2000).

The earliest record in the literature of this unusual Chatham Islands' form of *Polystichum* is perhaps that of Crookes (1963 p.236), who described a "very vigorous, unusual form" with "very long fronds and a trunk two feet high," allying it with the diminutive, alpine *P. cystostegia* (Hook.) J.B. Armstr. However, Brownsey and Smith-Dodsworth (1989 p.133; and similarly, Brownsey 1988 p.26) indicated these Chatham Islands' plants were related to *P. vestitum* (G. Forst.) C. Presl, from which they could be distinguished by their "wider" fronds, and their rachis scales that were "uniformly pale brown and ... fringed at their bases." de Lange et al. (1999b p.24) stated that while some Chatham Island populations "are readily distinguished from *P. vestitum* with

regard to their ciliate scales, and larger, bright green fronds, the presence of intermediates trending towards *P. vestitum s.s.* has tended to cloud the issue [of their distinctiveness]." They further stated that field work undertaken by their lead author had "found that the majority of *Polystichum* populations [on the Chatham Islands] could not be readily separated into either taxon."

Despite never being formally described, the Chatham Islands' form was accepted as a distinct species (as *Polystichum* "Chathams") by Cameron et al. (1995) and listed as 'endangered' in their list of "Threatened and Local" New Zealand plants. In the subsequent revision of this list, de Lange et al. (1999a) moved the Chatham Islands' form (as *P.* aff. *vestitum*, AK230427-8, Chatham Islands) to their newly created sub-list of 'Taxonomically Indeterminate Taxa,' because of 'doubt regarding taxonomic status,' and re-categorised its conservation risk to the safer 'vulnerable.'

Brownsey (1988) has also noted that some plants he ascribed to *P. vestitum* from the Subantarctic Islands, especially the Snares, resemble the Chatham Islands' form (see also Brownsey et al. 1985). Indeed, understanding of the extensive morphological variation inherent in the taxon recognised as *P. vestitum* was perhaps well summarised by Allan (1961 p.88) who described it as "an ill-resolved aggregate of forms."

P. vestitum, even in the wider sense, is endemic to the New Zealand botanical region (which includes Macquarie Island). Despite even recent assertions otherwise (eg. Orchard 1993), it does not occur in Tasmania (Jones 1998). *P. silvaticum* (Colenso) Diels, another New Zealand endemic, appears closely allied to *P. vestitum* which it superficially resembles, but the former can be distinguished by its "more finely divided fronds, its pinna midribs with a slight wing, and the absence of indusia" and in "being ecologically distinct, growing only in damp, shaded forest" (Brownsey 1988 p.26).

Samples were obtained from plants of *P. vestitum s.l.* (including the 'divergent' forms from the Chatham Islands, and those from the Subantarctic region; hereafter simply *P. vestitum*) and *P. silvaticum*. Morphological and genetic (using AFLP DNA-fingerprinting) analyses were conducted to test: 1) whether *P. vestitum* and *P. silvaticum* constitute separate evolutionary lineages; and 2) whether the 'divergent'

morphological forms of *P. vestitum* from the Chatham Islands ('divergent Chathams' plants) constitute a separate evolutionary lineage.

This study confirms that *P. vestitum* and *P. silvaticum* should be recognised as separate species. They can be retrospectively identified as separate evolutionary lineages. Further, that they occur together over a broad area provides indirect evidence that they fertilise assortatively in sympatry, and are thus also prospectively separate.

It is also confirmed that the 'curious' forms of *Polystichum* noted previously in the literature from the Chatham Islands (and the Subantarctic Islands) are best allied with *P. vestitum*. While these forms exhibit morphological variation not known from the New Zealand 'mainland' (ie. the North and South Islands), analyses of morphological data and AFLP data (Section 5.3) do not provide strong evidence for the retrospective recognition of the 'divergent Chathams' plants as a separate evolutionary lineage. It is considered that the null hypothesis that 'a single evolutionary lineage is present' cannot be conclusively rejected. Consequently, *P. vestitum* is retained as a single, albeit morphologically variable, taxonomic species (Section 5.4). An intraspecific (or intra-lineage) taxonomy of *P. vestitum* (at the varietal level) is discussed, but its formal implementation is not recommended. Implications for the conservation of *P. vestitum* on the Chatham Islands are discussed in Section 5.5. A revised description of *P. vestitum* is presented below (Section 5.2).

5.2 Taxonomy.

5.2.1 Taxonomic Description.

To illustrate the morphological variation inherent in *P. vestitum*, representative fronds, and scales from the mid-rachis, are presented in Figures 5.1 and 5.2, respectively. Analysis of these and other morphological characters is discussed more fully in Section 5.3.1. Note that the ranges for quantitative characters given in the following description are based on 5th and 95th percentiles. Figure 5.3 shows distribution maps for *P. vestitum* and *P. silvaticum*, and is based on specimens obtained for this study (Appendices 2.1.2 & 2.1.3) together with the collections of AK, WELT, and CHR (Appendices 2.2.2 & 2.2.3).

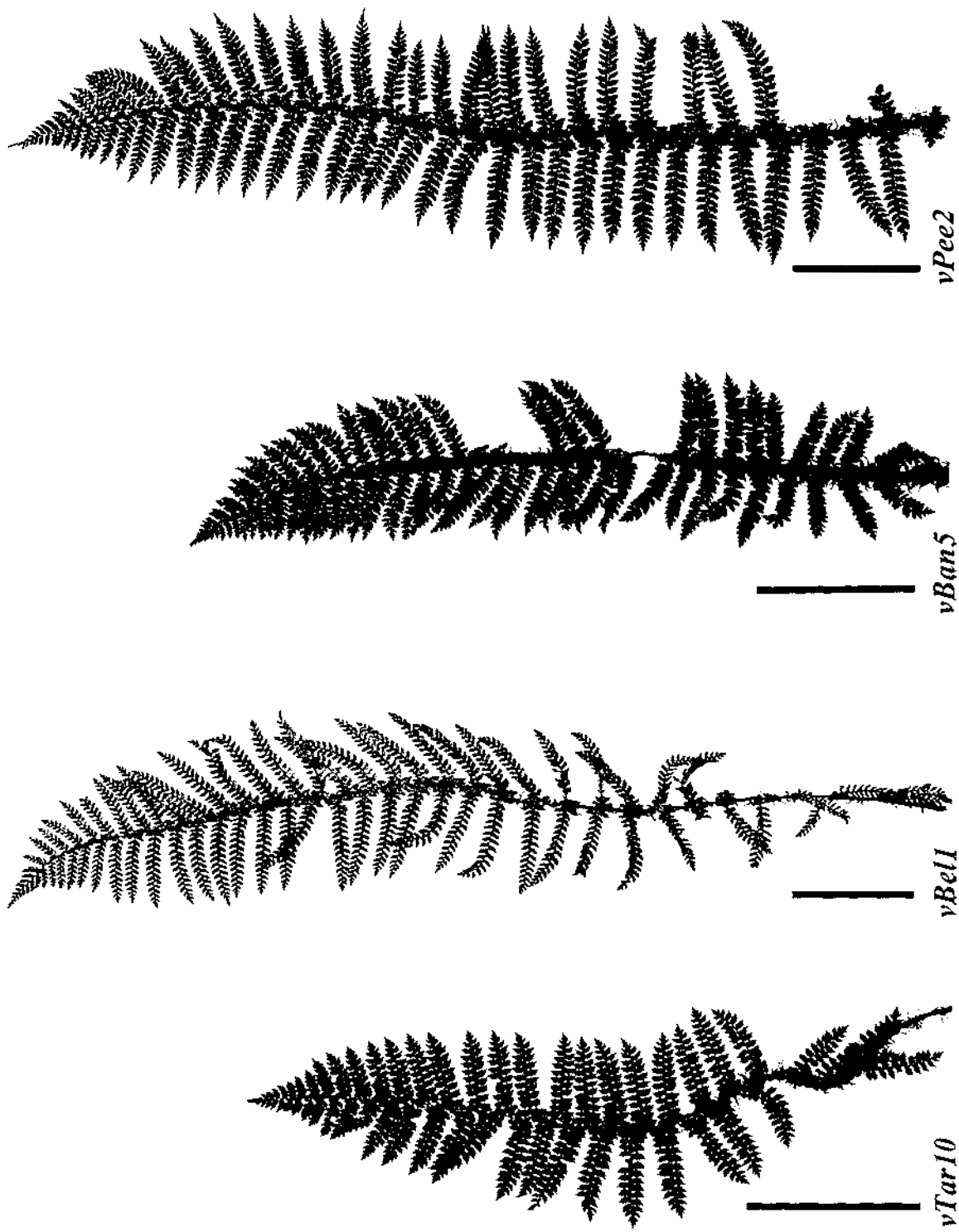


Figure 5.1. Fronds of *Polystichum vestitum*.
Representative fronds from 'mainland' plants of *P. vestitum* (morphological categories defined in Section 5.3.1). Sample details are given in Appendix 2.1.2. Fronds at different scales; scale bars indicate 10 cm. Note that some pinnae have been removed for molecular and/or spore analyses.

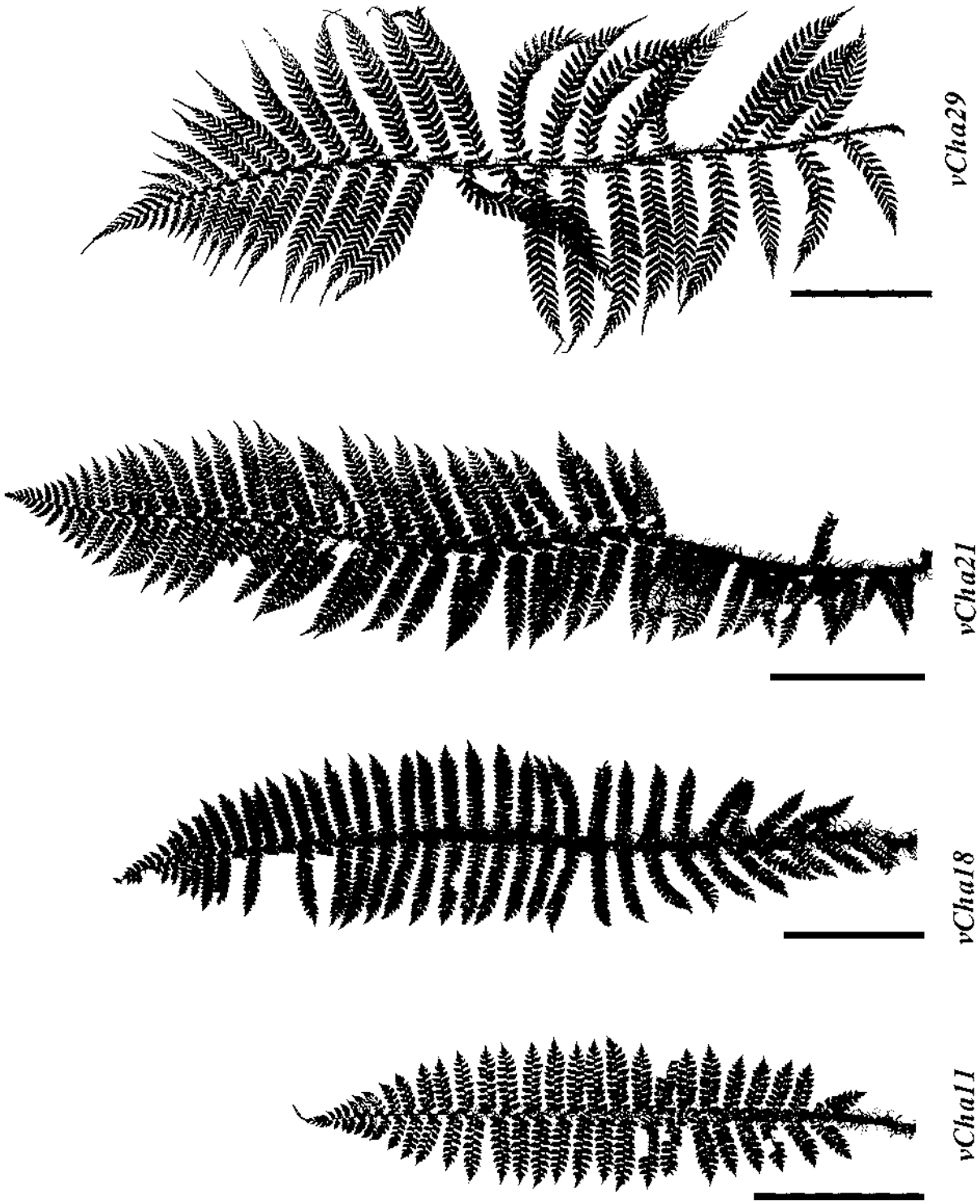


Figure 5.1 (continued). Fronds of *Polystichum vestitum*.
Representative fronds from 'mainland-like Chathams' (*vCha11* & *vCha18*) and 'intermediate Chathams' (*vCha21* & *vCha29*) plants of *P. vestitum* (morphological categories defined in Section 5.3.1). Sample details are given in Appendix 2.1.2. Fronds at different scales; scale bars indicate 10 cm. Note that some pinnae have been removed for molecular and/or spore analyses.

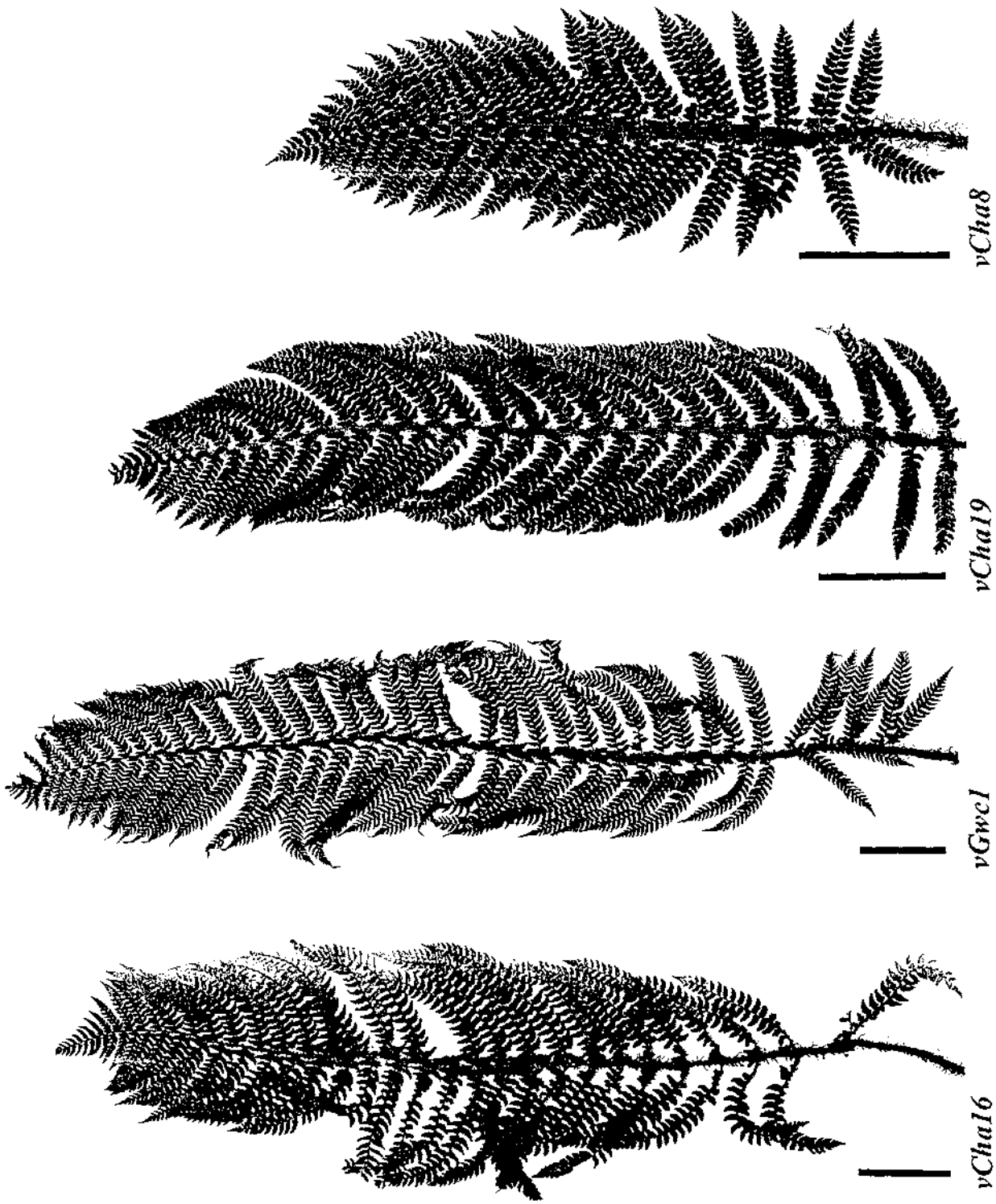


Figure 5.1 (continued). Fronds of *Polystichum vestitum*.
Representative fronds from 'divergent Chathams' plants of *P. vestitum* (morphological categories defined in Section 5.3.1). Sample details are given in Appendix 2.1.2. Fronds at different scales; scale bars indicate 10 cm. Note that some pinnae have been removed for molecular and/or spore analyses.

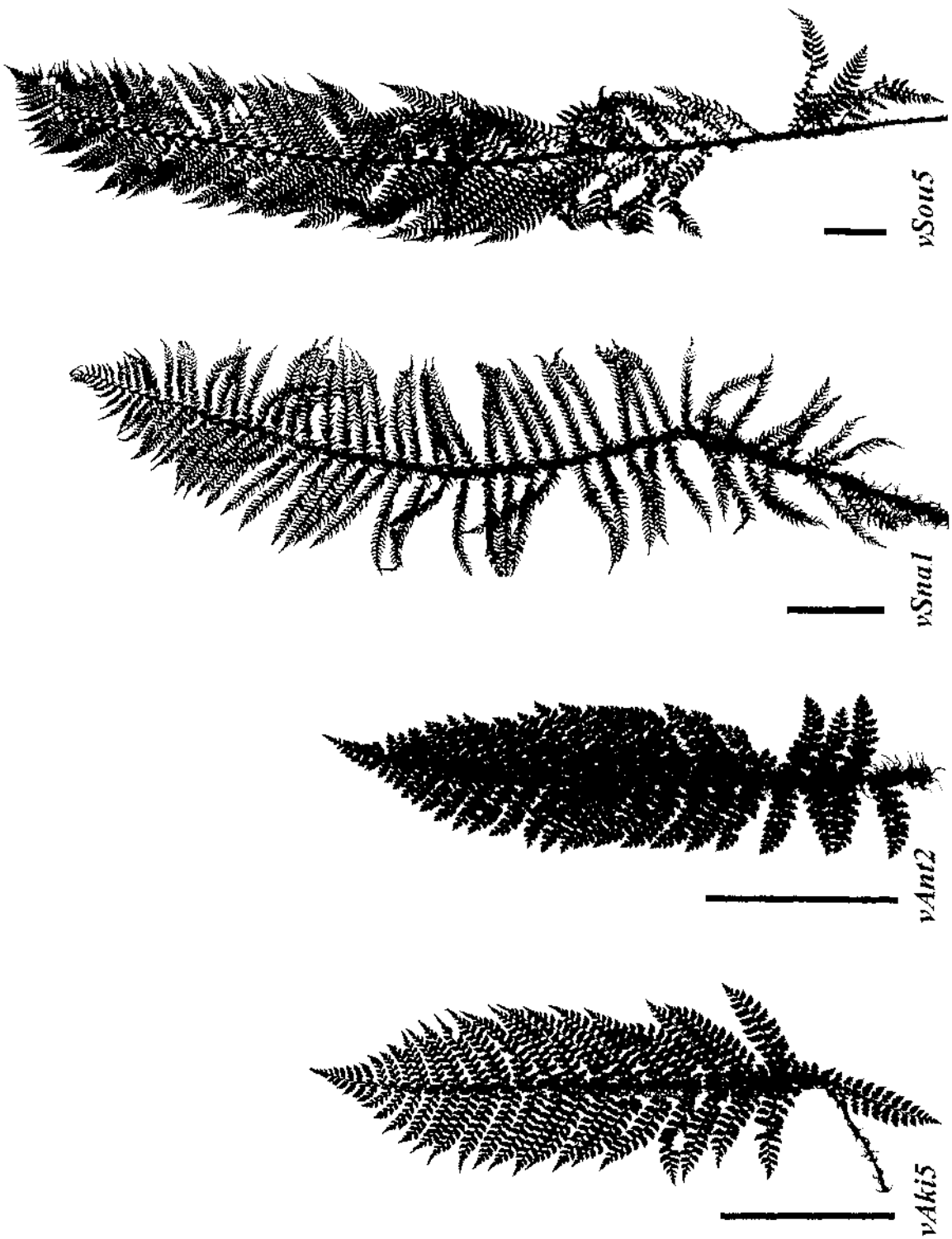


Figure 5.1 (continued). Fronds of *Polystichum vestitum.*
Representative fronds from 'mainland-like southern' (*vAki5 & vAnt2*) and 'divergent southern' (*vSna1 & vSou5*) plants of *P. vestitum* (morphological categories defined in Section 5.3.1). Sample details are given in Appendix 2.1.2. Fronds at different scales; scale bars indicate 10 cm. Note that some pinnae have been removed for molecular and/or spore analyses.

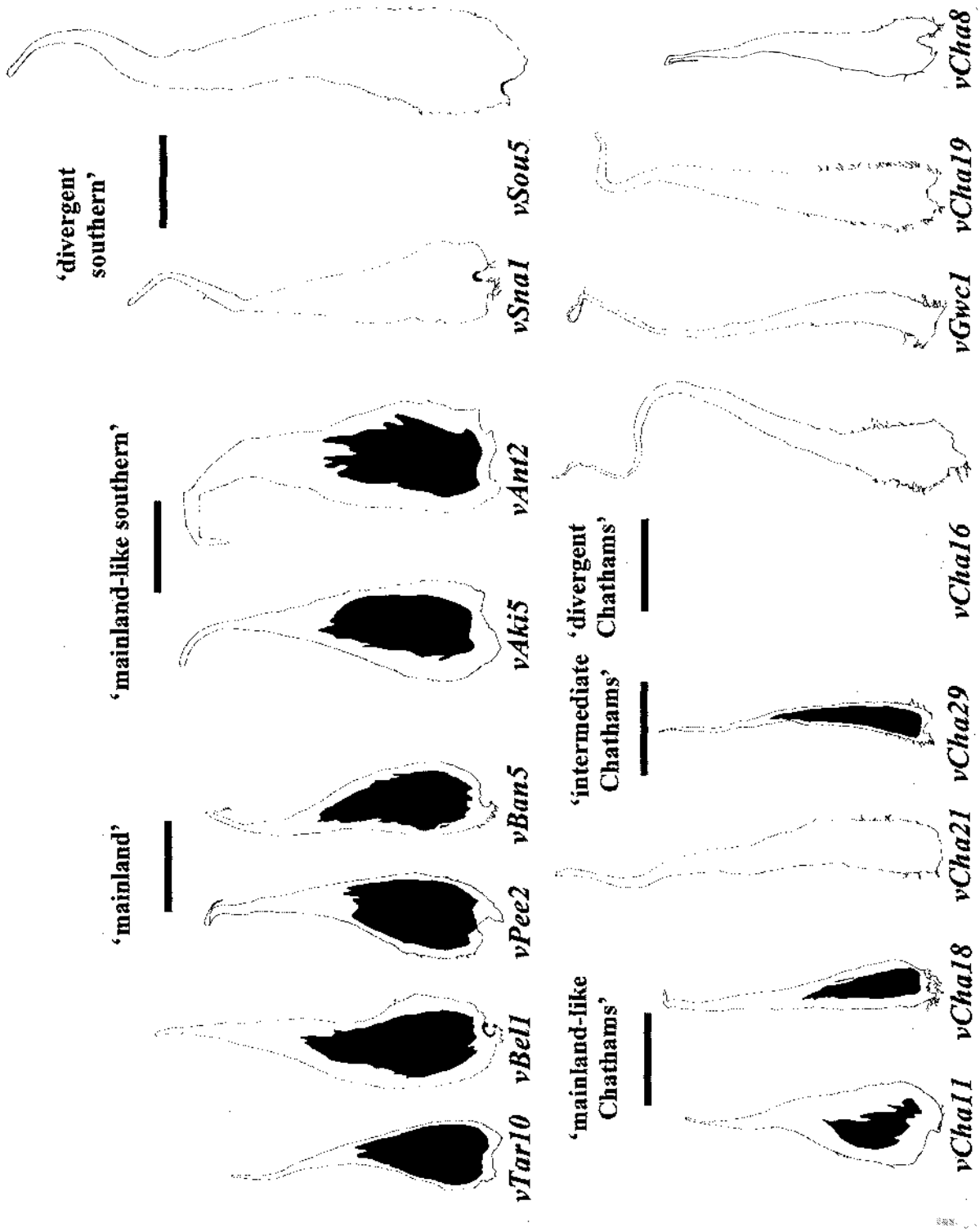


Figure 5.2. *Polystichum vestitum* Rachis Scales.
Representative scales from the mid-rachis of the different morphological categories (as defined in Section 5.3.1) of *Polystichum vestitum*. Collection details of samples are given in Appendix 2.1.2. Scale bars indicate 2000 μm.

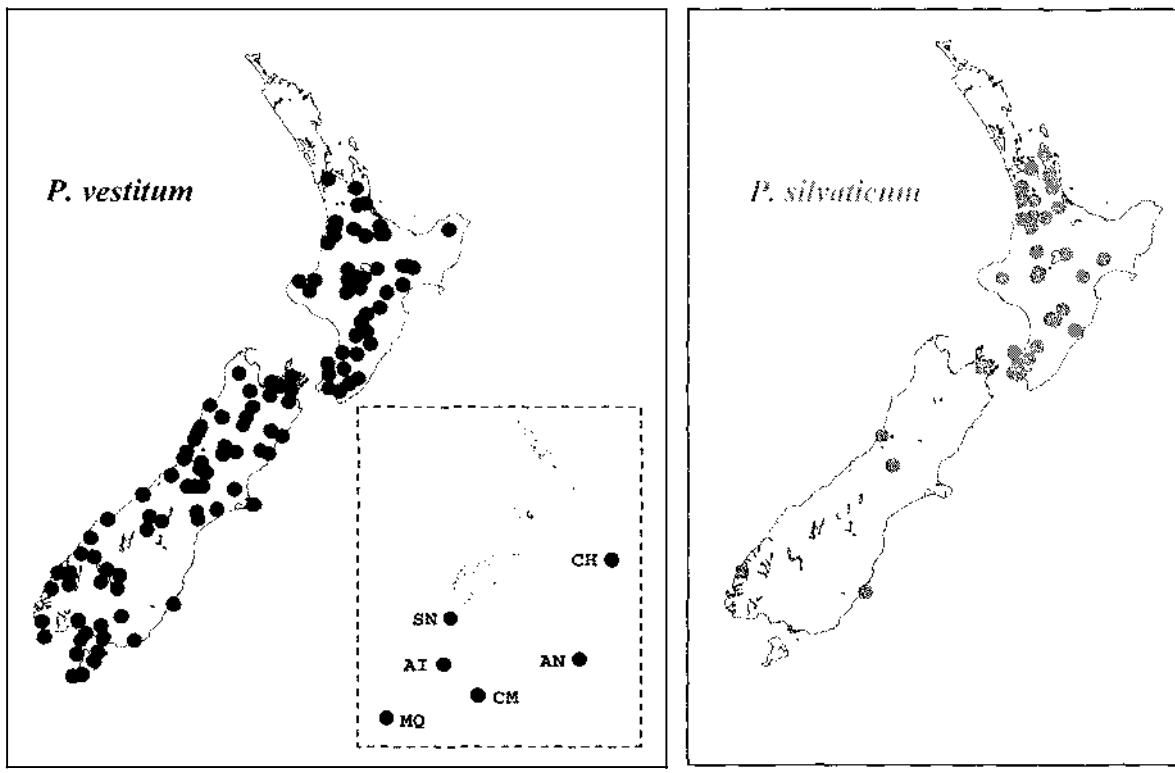


Figure 5.3. Distribution maps of *Polystichum vestitum* and *P. silvaticum.*
P. vestitum map compiled using collections made for this study (Appendix 2.1.2) and those of the herbaria AK, WELT and CHR! (Appendix 2.2.2). *P. silvaticum* map compiled using collections made for this study (Appendix 2.1.3) and those of the herbaria AK and WELT! (Appendix 2.2.3). Inserts: CH = Chatham Islands; SN = Snares Islands; AI = Auckland Islands; AN = Antipodes Islands; CM = Campbell Island; MQ = Macquarie Island.

Polystichum vestitum (G. Forst.) C. Presl, Tent. Pterid. 83 (1836)

Polypodium vestitum G. Forst., Fl. Ins. Austr. 82 (1786); *Aspidium vestitum* (G. Forst.) Sw., J. Bot. (Schrader) 1800 (2): 37 (1801); *Aspidium aculeatum* (L.) Sw. var. *vestitum* (G. Forst.) Hook. ex Hook. f., Handb. New Zealand Fl. 375 (1864); *Polystichum aculeatum* (L.) Schott var. *vestitum* (G. Forst.) Domin, Biblioth. Bot. 20, 85:55 (1913). Type: "Insulae Oceani Pacifici, Messrs Forster", no date recorded; lectotype (here designated) in BM (see Figure 5.4).

Polystichum venustum Hombr., Voy. Pôle Sud, Bot. t. 5 m-n (1844); *Aspidium venustum* (Hombr.) Hook. f., Fl. Antarct. 1: 106 (1844). Type: Auckland Islands, "Iles Auckland-Sud de la Novelle Zélande," M. Hombron, 1838-1840; lectotype (here designated) in P (specimen with rachis folded over itself; see Figure 5.5).

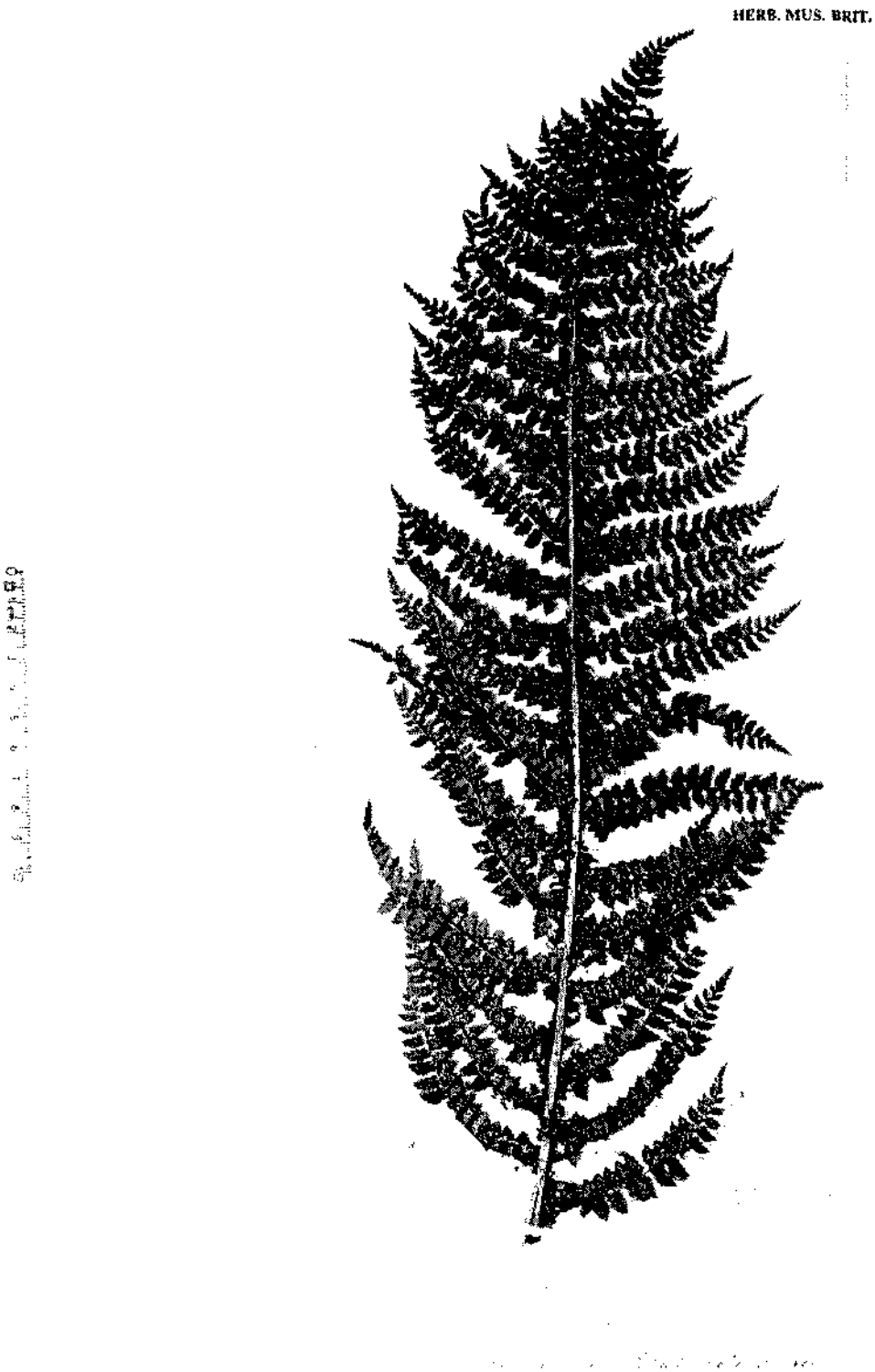


Figure 5.4. Lectotype of *Polypodium vestitum* G. Forst.
In BM. Label reads "Insulae Oceani Pacifici, Messrs Forster." The scale bar represents 10 cm.

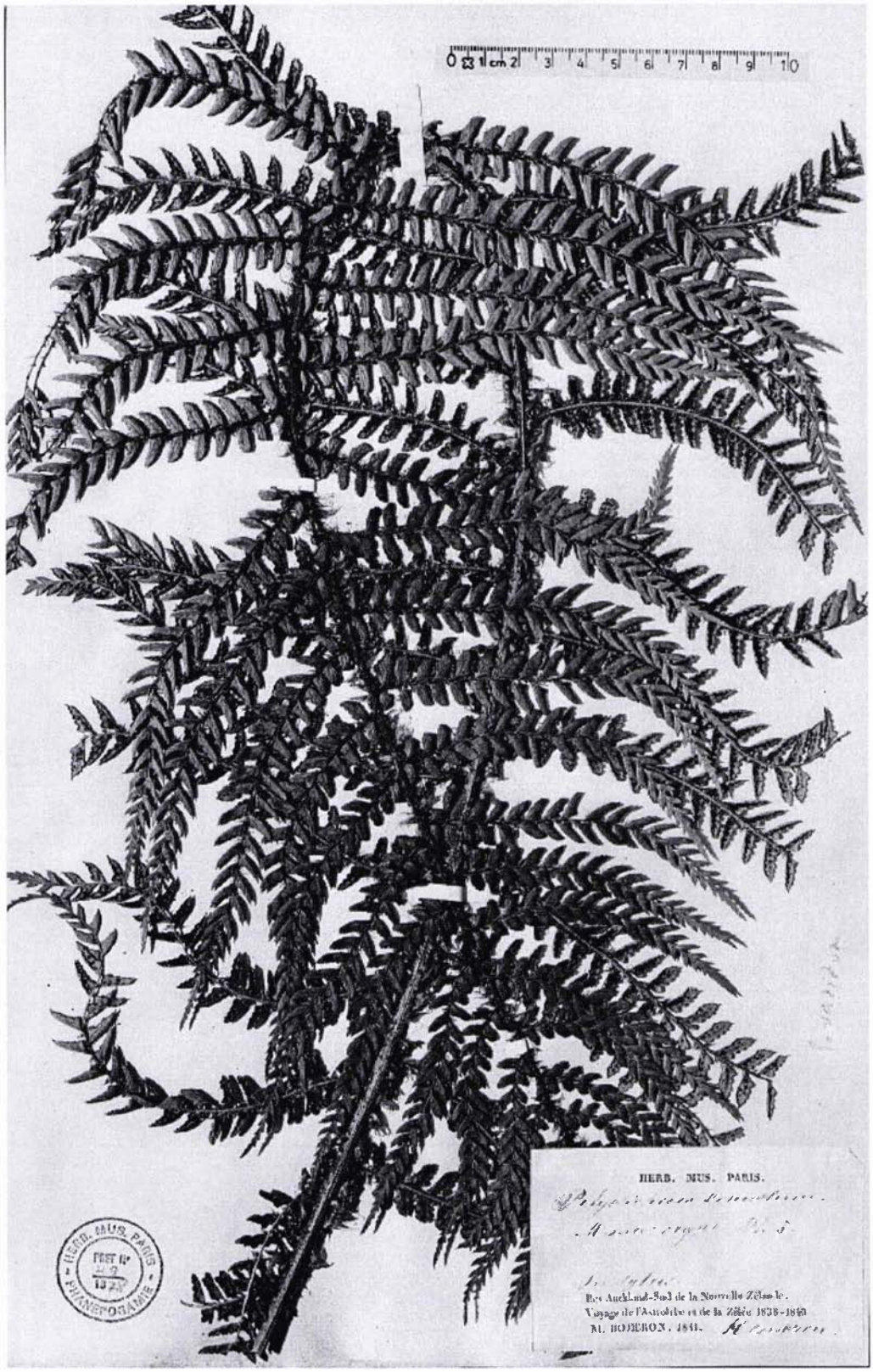


Figure 5.5. Lectotype of *Polystichum venustum* Hombr.
In P. Label reads "*Polystichum venustum.* Monoc. crypt. pl. 5. In sylvis."
Iles Auckland-Sud de la Novelle Zélande. Voyage de l'Astrolabe et de la Zélée 1838-40. M. Hombron. 1841. Hombron."

Aspidium pulcherrimum Colenso, Tasmanian J. Nat. Sci. 2: 167 (1845). Type: "Waikare Lake" [Lake Waikaremoana], W. Colenso, Dec. 1841; lectotype (here designated) WELT P3201 (see Figure 5.6).

Aspidium waikarense Colenso, Tasmanian J. Nat. Sci. 2: 168 (1845). Type: "near Waikare Lake" [Lake Waikaremoana], W. Colenso, Dec. 1841; lectotype (here designated) WELT P3200 (see Figure 5.7).

Aspidium perelegans Colenso, Trans. New Zealand Inst. 29: 416 (1897); *Polystichum perelegans* (Colenso) C. Chr., Index Fil. 87 (1905), 586 (1906); *Polystichum aculeatum* (L.) Schott var. *perelegans* (Colenso) Domin, Biblioth. Bot. 20, 85: 56 (1913). Type: forests south-west from Dannevirke, W. Colenso, 1896; lectotype (here designated) WELT P2577 (see Figure 5.8)

Aspidium aculeatum auct. non Sw. (1802): F. Muell., Veg. Chatham Isl. 70 (1864)

Aspidium proliferum auctt. non R. Br. (1810): A. Rich., Essai Fl. New Zealand 69 (1832); A. Cunn., Companion Bot. Mag. 2: 367 (1837); Raoul, Choix Pl. New Zealand 38 (1846)

DESCRIPTION: **Rhizome** erect, sometimes forming a trunk up to 50 cm tall. **Stipe** 90-410 mm long. Stipe and rachis densely clothed with scales of diverse form. Larger rachis **scales** usually ovate-lanceolate (those from the mid-rachis 340-1620 μm at their mid-length), becoming lanceolate-acicular in some plants from the Chatham Islands (those from the mid-rachis 180-780 μm at their mid-length); usually bicolourous, with an obvious dark brown centre surrounded on all sides by pale brown margins, but dark centre sometimes much reduced, particularly in some plants from the Chatham Islands, islands around Stewart Island, and the Subantarctic Islands, such that the rachis scales are uniformly pale brown; apex usually long and tapering; usually without marginal projections except for plants from the Chatham Islands, in which they may be very well developed; usually underlain by pale bristle-like scales. **Lamina** 230-1080 × 90-320 mm; narrowly-elliptic to narrowly-oblong; bipinnate; usually dark green above, paler below. **Primary pinnae** in 21-54 pairs, oblong. **Secondary pinnae** all stalked except those towards the apex of primary pinnae; with sharply pointed apex and prominent marginal teeth and/or crenulations. **Sori** round. Indusia peltate, ± flat, ± round; margins entire although sometimes undulate and/or scalloped; central dark area usually insignificant (< 10% surface area, and usually < 5%). Annulus cells of sporangia 12-17, but most commonly 13-14. Spore exine 36-44 × 26-32 μm; length-width product 980-1400 μm^2 (31 individuals, 16 populations).

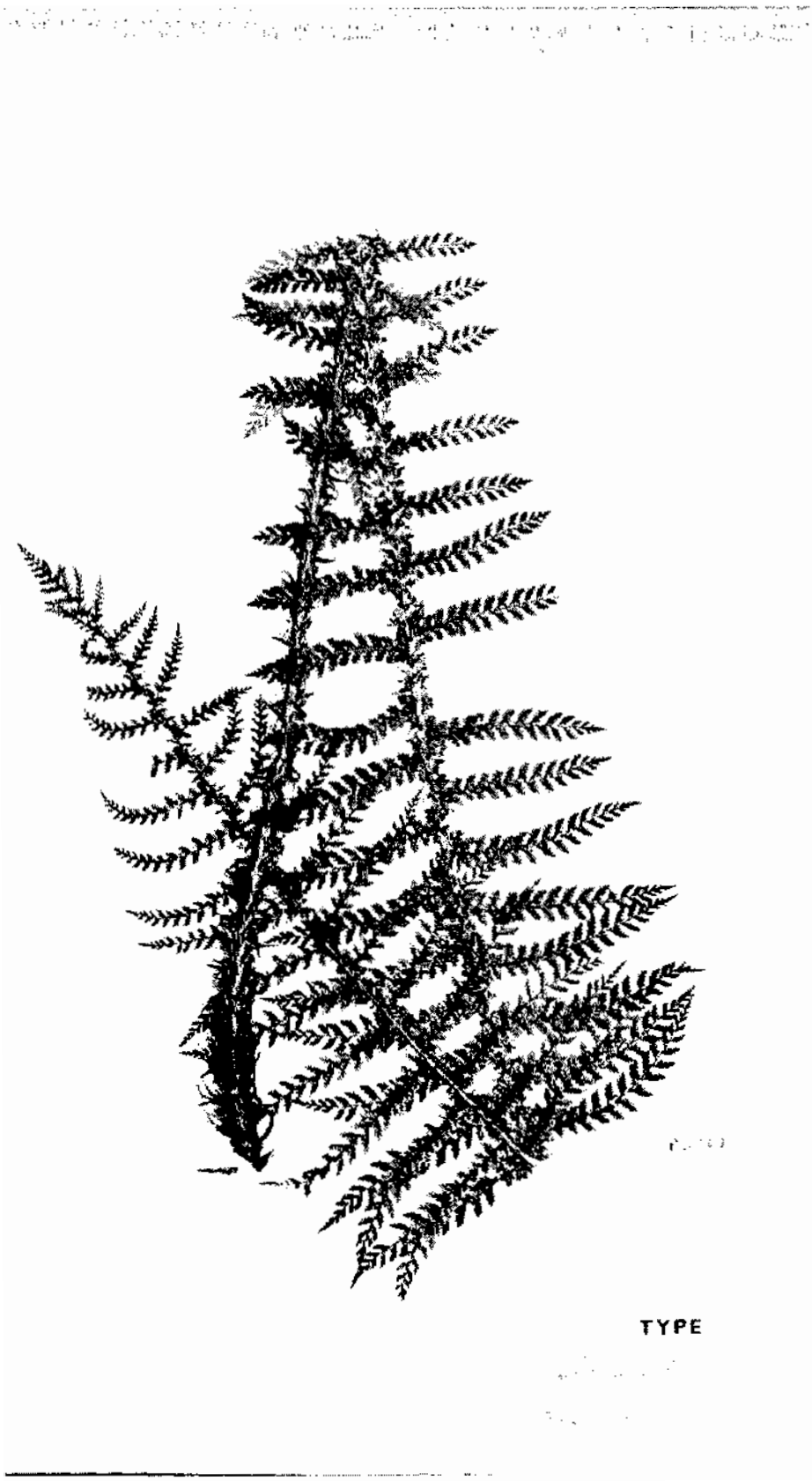


Figure 5.6. Lectotype of *Aspidium pulcherrimum* Colenso.
WELT P3201. Label reads "*Aspidium pulcherrimum*, n. sp., W. Colenso. Damp woods, mountains nr. [near] Waikare Lake, Dec., 1841." The scale bar represents 31 cm.

Figure 5.7. Lectotype of *Aspidium waikarense* Colenso.
WELT P3200. The larger specimen is designated as the lectotype. Label reads "*Aspidium waikarense*, n.sp., W. Colenso. Mountains, woods, near Waikare Lake, Dec., 1841." The scale bar represents 30 cm.

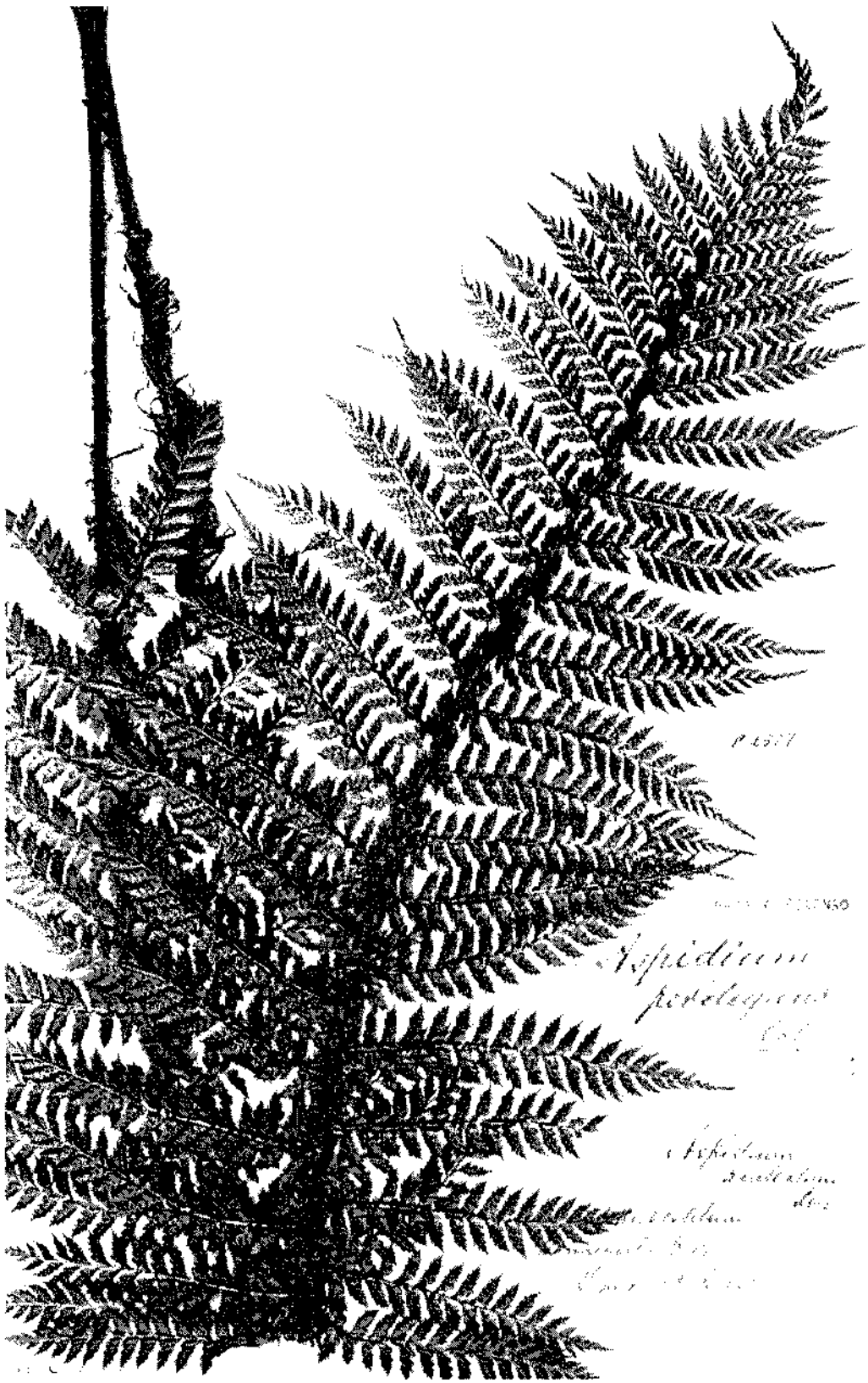


Figure 5.8. Lectotype of *Aspidium perelegans* Colenso.
WELT P2577. Labels read "*Aspidium perelegans*" in Colenso's handwriting, and "*Aspidium aculeatum* Sw. var. *vestitum.* Dannevirke, HB. Type of *A. perelegans* Col." in Cheeseman's handwriting. Approximately ×0.5 life-size.

CHROMOSOME NUMBER: tetraploid; n = 82, Brownlie (1954).

HABITAT & DISTRIBUTION: Endemic; North Island, from Auckland southwards; South Island, throughout; Stewart Island; Chatham Islands; Snares Islands; Auckland Islands; Campbell Islands; Antipodes Islands; Macquarie Island (see Figure 5.3). Only in montane regions in the north, but progressively extending to lower altitudes to the south; in the South Island ranging from almost coastal to subalpine regions. Primarily in exposed habitats, such as forest margins and grasslands, in the north, but extending into forest in colder, wetter regions.

NOTES: There are four specimens in P labelled by Hombron as *Polystichum venustum* and as being collected from the Auckland Islands. These constitute two pairs with distinct morphologies. The larger pair of specimens have relatively long primary pinnae, whereas those of the smaller pair of specimens are shorter. Table 5 in Hombron (1844) implies that these represent mature and juvenile forms, respectively. One of the larger specimens is consequently chosen as the lectotype of *P. venustum* Hombr.

5.2.2 Relationship of the Chatham Islands' form to *Polystichum vestitum.*

Several lines of evidence support the contention that the plants from the Chatham Islands with divergent morphology are allied to *P. vestitum* (cf. Brownsey 1988, Brownsey & Smith-Dodsworth 1989, de Lange et al. 1999a, b) rather than *P. cystostegia* (cf. Crookes 1963): 1) there is an apparent continuum in morphological form between these 'divergent' and 'mainland-like' (ie. plants resembling those from the North and South Islands) plants of *P. vestitum* on the Chatham Islands (see Sections 5.3.1 & 5.3.3; also de Lange et al. 1999b p.24), whereas *P. cystostegia* is no longer recognised as occurring on the Chathams (Brownsey & Smith-Dodsworth 1989, de Lange et al. 1999b); 2) the indusia of all Chatham Islands plants investigated are flat like *P. vestitum* rather than markedly convex as in *P. cystostegia*; 3) sequences from the chloroplast *rps4-trnS* spacer region of Chatham Islands plants are identical to those of mainland *P. vestitum*, but differ by a one base-pair deletion from those of *P. cystostegia* (see Section 6.2); and 4) AFLP fingerprinting indicates that of all the species investigated from the south-west Pacific the Chatham Islands plants are allied most closely to *P. vestitum* (and *P. silvaticum*; see Section 6.3).

5.2.3 Hybrids.

Plants with intermediate morphology and abnormally-developed spores, such that they are believed to be hybrids, have been recorded between *P. vestitum* and almost all of the other species of New Zealand *Polystichum*. Hybrids (eg. *rXvKaw2*, *rXvKak2*, *rXvPee2*; details of putative hybrids collected for this study are given in Appendix 2.1.4) between *P. vestitum* and *P. neozelandicum* subsp. *zerophyllum* are often common where the two grow together, particularly in ecologically disturbed areas. WELT P12512-12513 from the Chatham Islands is thought to be a hybrid between a plant of *P. vestitum* with 'divergent Chathams' morphology and *P. neozelandicum* subsp. *zerophyllum* (Brownsey 2001b, the latter parent listed as '*P. richardii*'). *cXvTar1* and *rXvBan9* are thought to be hybrids between *P. vestitum* and *P. cystostegia*, and *P. oculatum*, respectively. *P. vestitum* is also thought to hybridise with *P. silvaticum*, as perhaps best evidenced by the specimen *sXvAka1*. In general, this plant is morphologically intermediate, but it has abnormally developed indusia (which are reduced to scale-like paraphyses, probably reflecting the indusiate and exindusiate nature of its respective putative parents), and a mixture of normal- and abnormal-looking spores. Further, AFLP fingerprinting indicates that *sXvAka1* is also genetically intermediate between *P. silvaticum* and *P. vestitum* (Appendix 3.3.1).

While no direct analyses have been carried out, the involvement of *P. vestitum* in hybridisation with other lineages suggests that sexual-outcrossing must be at least a component of its breeding system. This inference can also be extended to 'divergent Chathams' plants, given the indication (see above) that they can hybridise with *P. neozelandicum*.

5.3 Lineage Delimitation.

5.3.1 Morphological (and Geographic) Category Delimitation.

Samples of *P. vestitum* were collected (or supplied) from throughout the New Zealand region. Those included in the morphological analysis are mapped in Figure 5.9, with further details given in Appendix 2.1.2. Field-work as part of this study on Chatham Island (the larger island of the Chatham Islands group) revealed an extensive range of morphological variation, more so than that found on the North and South Islands of

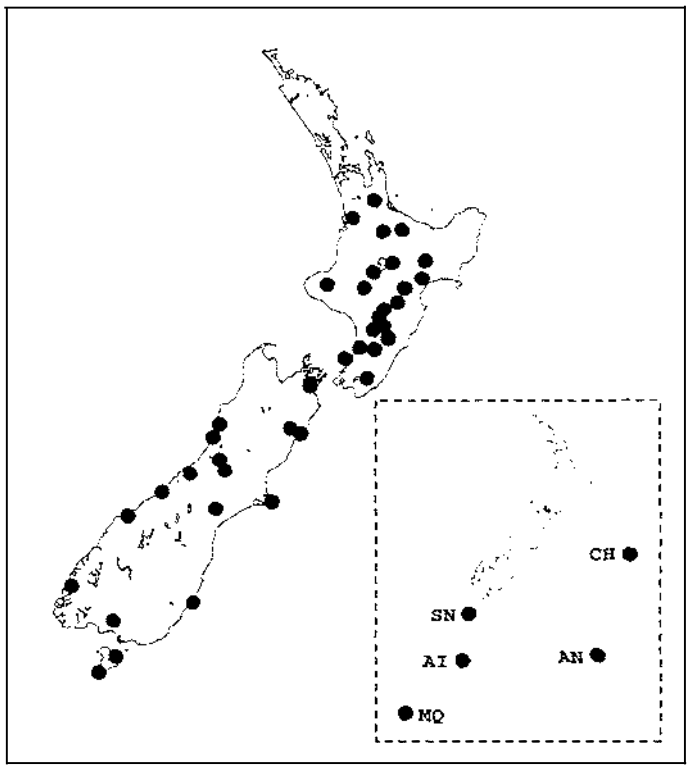


Figure 5.9. Map of *Polystichum vestitum* Morphological Samples.
Distribution map of *Polystichum vestitum* samples included in the morphological analyses. Further sample details can be found in Appendix 2.1.2. Inserts: CH = Chatham Islands; SN = Snares Islands; AI = Auckland Islands; AN = Antipodes Islands; MQ = Macquarie Island.

New Zealand. For comparison, plants collected from the Chatham Islands were assigned to three categories: those that looked like mainland *P. vestitum* (ie. like those from the North and South Islands), those that were intermediate, and those that looked distinctly different from mainland *P. vestitum* (ie. those with 'divergent' morphology).

This assignment was performed principally according to a superficial consideration of variation in the size and colouration of rachis scales, and the relative width of the frond (which is a reflection of primary pinnae length). Collection sites on Chatham Island are mapped in Figure 5.10. Samples from islands around Stewart Island (no samples from Stewart Island itself were included in the analysis) and from the Subantarctic were assigned to two categories of 'southern' plants, based on whether they resembled mainland *P. vestitum* or appeared morphologically divergent. This produced six morphological (and geographic) categories of *P. vestitum* plants: 'mainland,' 'mainland-like Chathams,' 'intermediate Chathams,' 'divergent Chathams,' 'mainland-like

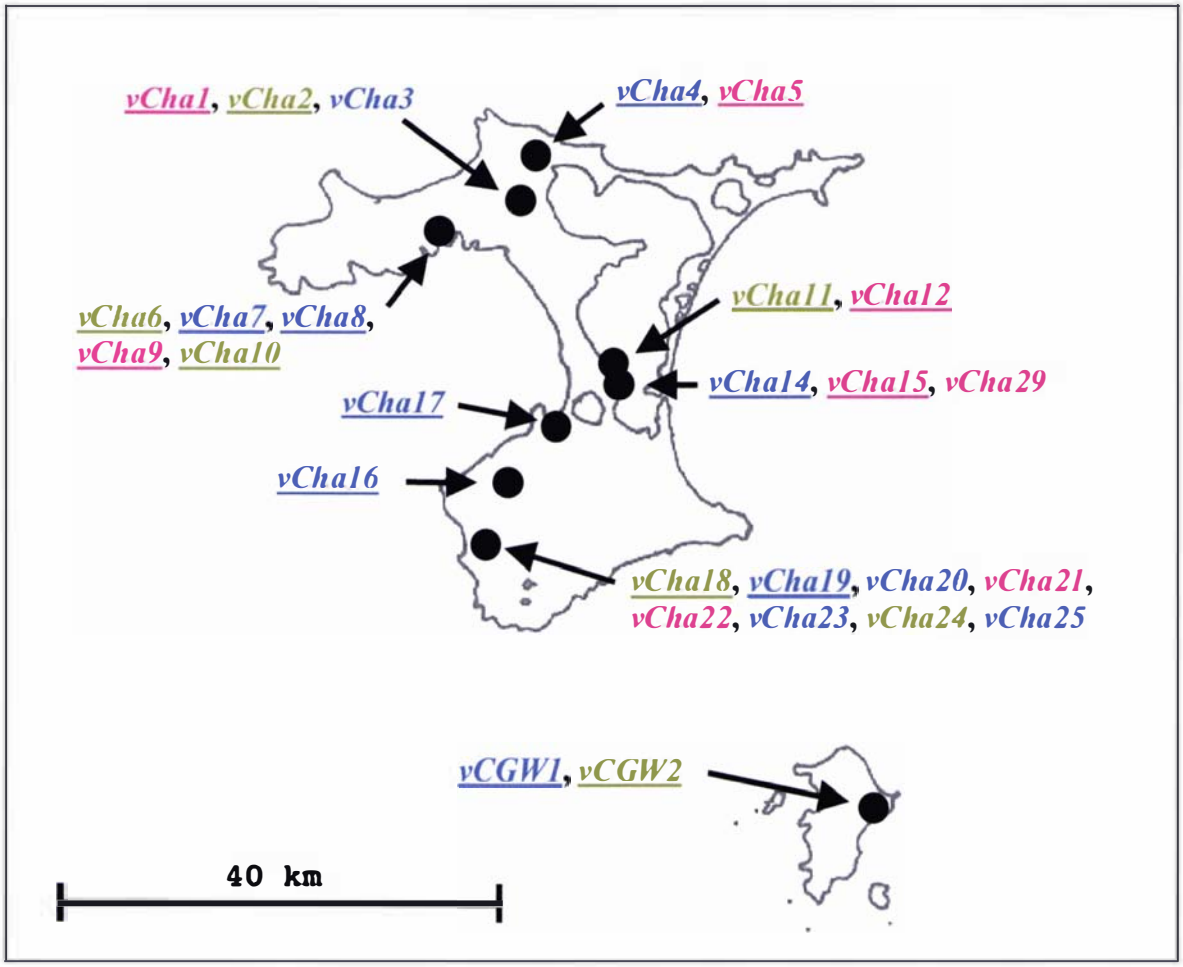


Figure 5.10. Map of Collection Sites on the Chatham Islands.
Indicated are all samples from the Chatham Islands included in the morphological analyses. Underlined samples were included in the 'Chatham Islands' AFLP sample set. Morphological categories are as follows: '**mainland-like Chathams**,' '**intermediate Chathams**,' and '**divergent Chathams**.' Further details of these samples can be found in the Appendix 2.1.2.

southern,' and 'divergent southern.' The assignment of individual samples to these categories is indicated in Appendix 2.1.2.

5.3.2 Ecological Observations.

No ecological differences between 'mainland-like Chathams' and 'divergent Chathams' plants were noted during the field-work on Chatham Island. However, this investigation was largely confined to the northern and central parts of Chatham Island which have undergone substantial environmental modification since human settlement. If ecological differentiation exists, it may be more apparent in the southern region of Chatham Island and parts of Pitt Island, which retain larger tracts of original forest.

Table 5.1. Definitions of Morphological Characters Used in Chapter Five.

Character Name	Character Definition
'Lamina shape ratio'	Maximum lamina width divided by rachis length.
'Lamina (dis-) width ratio'	Width of the lamina at the distal quartile length of the rachis, divided by the maximum lamina width.
'Pinnae length ratio'	Length of the longest primary pinnae, divided by the length of the rachis.
'Pinnae shape ratio'	Maximum width of the longest primary pinnae, divided by its length.
'Scale (max-) width'	Average maximum width of five scales from the mid-rachis (μm).
'Scale (mid-) width'	Average mid-length width of fives scales from the mid-rachis (μm).
'Scale (max-) shape ratio'	Average of the length divided by maximum width of five scales from the mid-rachis.
'Scale (mid-) shape ratio'	Average of the length divided by the mid-length width of five scales from the mid-rachis.
'Scale colour area'	Average of a quantitative estimate of the dark central area of five scales from the mid-rachis: length × mid-length width of the dark coloured area divided by length × mid-length width of the scale.

5.3.3 Morphological Analysis.

Several morphological characters show at least some differentiation between 'divergent Chathams' and 'mainland' (plus 'mainland-like Chathams') *P. vestitum* plants. These characters are defined in Table 5.1. Box-plot summaries of variation in three of these morphological characters, 'pinnae shape ratio,' 'scale (mid-) shape ratio,' and 'scale colour area,' across the six morphological categories of *P. vestitum* recognised in Section 5.3.1 are presented in Figure 5.11. The characters 'lamina shape ratio,' 'lamina (dis-) width ratio,' and 'pinnae length ratio' show a similar pattern of variation to 'pinnae shape ratio.' These characters are unlikely to be independent, and as the latter character shows the most pronounced differentiation between 'divergent Chathams' and 'mainland' (plus 'mainland-like Chathams'), only it is presented. Similarly, the characters 'scale (max-) width,' 'scale (mid-) width,' and 'scale (mid-) shape ratio' show similar patterns of variation to 'scale (mid-) shape ratio.' Again, these are unlikely to be independent and given that the latter character shows the most pronounced differentiation between 'divergent Chathams' and 'mainland' (plus 'mainland-like Chathams'), only it is presented. Scales from the mid rachis were also qualitatively scored for the number and length of marginal projections ('scale marginal projections'), with this data presented in Table 5.2. Appendix Four provides a file of the morphological data collected for *P. vestitum* ('Morph-Ves').

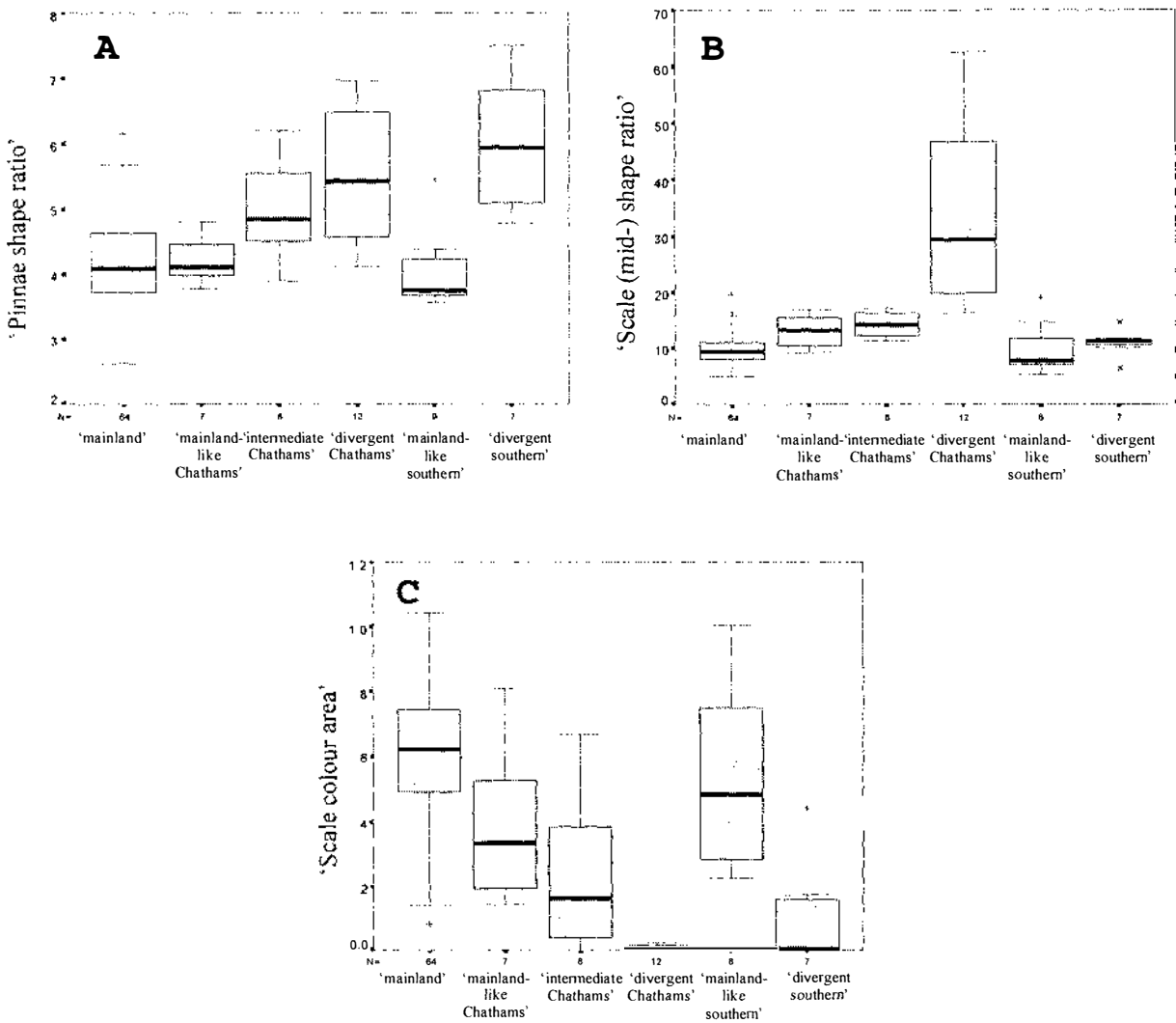


Figure 5.11. *Polystichum vestitum* Box-plots.
Box-plot summaries of variation in (representative) morphological characters showing (at least some) differentiation between the morphological categories described in the text. . 'N=' denotes the number of samples analysed; '+' & '×' denote outliers. Characters are defined in Table 5.1, and details of samples analysed can be found in Appendix 2.1.2.

(A) 'Pinnae shape ratio'
(B) 'Scale (mid-) shape ratio'
(C) 'Scale colour area'

Of the quantitative morphological characters investigated only 'scale (mid-) shape ratio' and 'scale colour area' differentiate 'divergent Chathams' individuals from the 'mainland' category (plus the 'mainland-like Chathams' category) without substantial overlap. It is therefore not surprising that this pair of quantitative morphological characters is the only combination to show any degree of concordant partitioning of the samples of the 'divergent Chathams' category from the other categories (Figure 5.12).

Table 5.2. Rachis Scale Marginal Projections.
The abundance of marginal projections on scales from the mid-rachis scored as 'none/few,' 'some,' or 'many' (eg. *vBel1*, *vCha8*, *vCha19*, respectively, in Figure 5.2), with their length scored as 'short,' 'medium,' or 'long' (eg. *vBel1*, *vCha8*, *vCha19*, respectively, in Figure 5.2). Note that these scores represent the 'average' from five randomly chosen scales from the mid-rachis. Results for each of the six superficial morphological categories are given.

'Mainland' *P. vestitum*

	None/few	Some	Many
Short	49	8	0
Medium	4	3	0
Long	0	0	0

'Mainland-like Chathams'

	None/few	Some	Many
Short	0	1	0
Medium	1	3	1
Long	0	1	0

'Intermediate Chathams'

	None/few	Some	Many
Short	2	0	0
Medium	0	3	2
Long	0	0	1

'Divergent Chathams'

	None/few	Some	Many
Short	0	0	0
Medium	0	1	4
Long	0	0	7

'Mainland-like southern'

	None/few	Some	Many
Short	7	1	0
Medium	1	0	0
Long	0	0	0

'Divergent southern'

	None/few	Some	Many
Short	4	1	0
Medium	1	1	0
Long	0	0	0

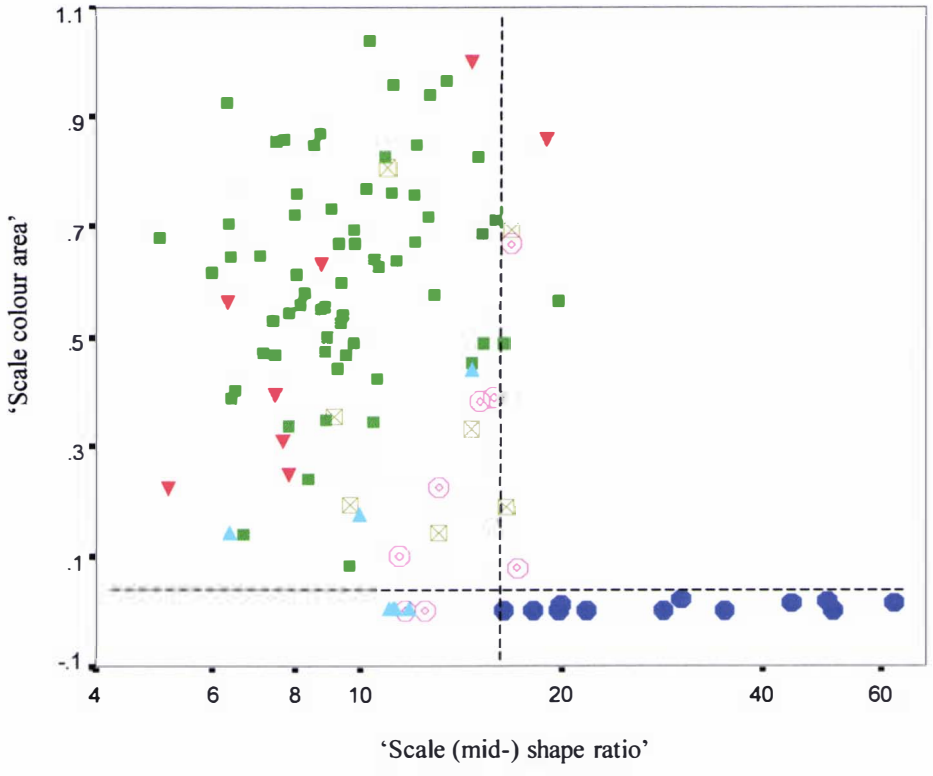


Figure 5.12. *Polystichum vestitum* Scatter-plot.
Scatter-plot of 'Scale (mid-) shape ratio' versus 'Scale colour area' for plants of *P. vestitum*. Samples are as follows: '**mainland**' (■), '**mainland-like Chathams**' (☒), '**intermediate Chathams**' (⊙), '**divergent Chathams**' (●), '**mainland-like southern**' (▼), and '**divergent southern**' (▲). Note that the 'Scale (mid-) shape ratio' axis is a logarithm, and that the minimum value for 'Scale colour area' is zero. The dashed lines indicate the 'almost' concordant-partitioning of the 'divergent Chathams' samples from the remainder.

However, several (but not all) 'divergent southern' and 'intermediate Chathams' samples have reduced 'scale colour area' like the 'divergent Chathams' plants whilst exhibiting the low 'scale (mid-) shape ratio' measurements characteristic of other plants of their own category and those from 'mainland' and 'mainland-like Chathams.' This means the concordant partitioning of the 'divergent Chathams' plants from the remaining samples is not 'absolute.'

'Divergent Chathams' plants (along with the 'divergent southern' plants) tend to have longer primary pinnae (and hence wider fronds) than those from the 'mainland' and 'mainland-like Chathams' (and 'mainland-like southern') categories. However, the respective ranges of the associated quantitative characters overlap significantly between

these categories such that they do not contribute to any concordant partitioning of the 'divergent Chathams' plants.

Consideration of Table 5.2 indicates that marginal projections of the rachis scales of 'mainland' plants are generally poorly developed, as are those from both 'mainland-like southern' and 'divergent southern' plants. The majority of 'mainland' samples were scored as having 'none/few' and 'short' marginal projections (61/64, or c.95%, were scored as 'none/few' or 'short'). In contrast, only 4/27 (c.15%) of the Chatham Islands ('mainland-like Chathams,' 'intermediate Chathams,' plus 'divergent Chathams') samples were scored as 'none/few' or 'short.' This indicates that marginal projections of the rachis scales of all Chatham Islands plants are generally better developed. The partitioning of the qualitative 'scale marginal projection' character between individuals with poorly developed projections and those with better developed projections is not concordant with the partitioning in the 'scale (mid-) shape ratio' and 'scale colour area' characters. Rather, it more or less partitions all Chatham Island individuals away from the rest, rather than just the 'divergent Chathams' samples.

However, marginal projections tend to be more developed in 'divergent Chathams' individuals than in those from the 'mainland-like Chathams' category. Of the 'divergent Chathams' samples, 11/12 (c.92%) were scored as having 'many' and/or 'long' marginal projections, compared to just 2/7 (c.29%) of 'mainland-like Chathams,' and 0/64 (0%) of 'mainland,' 0/9 (0%) of 'mainland-like southern,' and 0/7 (0%) of 'divergent southern' samples. Therefore, this partitioning in the 'scale marginal projection' character, between individuals with very well-developed marginal projections and those without, is more or less concordant with the partitioning in the 'scale (mid-) shape ratio' and 'scale colour area' characters.

Consequently, variation in the rachis scale characters of 'scale (mid-) shape ratio,' 'scale colour area,' and 'scale marginal projections' could be used to concordantly partition the 'divergent Chathams' individuals from those of the 'mainland' and 'mainland-like Chathams' categories. However, this concordance is of the 'almost' (rather than 'absolute') kind, and it is also bridged to some extent by the 'intermediate Chathams' samples. Further, all of these morphological characters pertain to the rachis scales, such that it might be doubted whether they are ontogenetically independent.

It should be noted that other characters seem to show, at least anecdotally, some correlation with those discussed above. For instance, the lamina of 'divergent Chathams' plants tend to be a lighter green (de Lange et al. 1999b p.24 described them as "bright green"), and there is a tendency for their primary pinnae to curl towards the apex of the frond (see fronds of *vCha16*, *vGwc1*, and *vCha19* in Figure 5.1), at least in large plants. These characters were not specifically investigated in this study because of the difficulty in quantifying them.

As indicated by Brownsey (1988), morphological variation that distinguishes the 'divergent Chathams' from 'mainland' plants also occurs in some plants from the Subantarctic Islands and islands around Stewart Island. 'Divergent southern' plants (eg. *vAki1* & *vAki4* from the Auckland Islands; *vSna1*, *vSna2*, & *vSna3* from the Snares Islands; and *vSou5* and *vSou7* from islands around Stewart Island) share with 'divergent Chathams plants' the tendency for wider fronds (with relatively longer primary pinnae) and reduced scale bicolouration. However, these characters do not concordantly partition these 'divergent southern' plants from 'mainland' (or 'mainland-like southern') *P. vestitum.*

There are also morphological differences between the 'divergent southern' and 'divergent Chathams' plants. The rachis scales of the former are never as narrow, and they also lack well-developed marginal projections. Other plants from the Subantarctic are much more similar to 'mainland' *P. vestitum* (ie. 'mainland-like southern;' eg. *vAki2*, *vAki3*, & *vAki5* from the Auckland Islands; *vAnt1*, *vAnt2*, & *vAnt3* from the Antipodes Islands; and *vMac1*, *vMac2* & *vMac4* from Macquarie Island).

Given that the morphological analyses do not provide strong evidence for the rejection of the null hypothesis that *P. vestitum* (encompassing the 'divergent Chathams' plants) is a single evolutionary lineage, genetic relationships were investigated using AFLP DNA-fingerprinting.

5.3.4 AFLP Analysis.

Three sets of *P. vestitum* samples were investigated with AFLP. The first set analysed relationships amongst *P. vestitum* from the North and South Islands (ie. 'mainland' *P.*

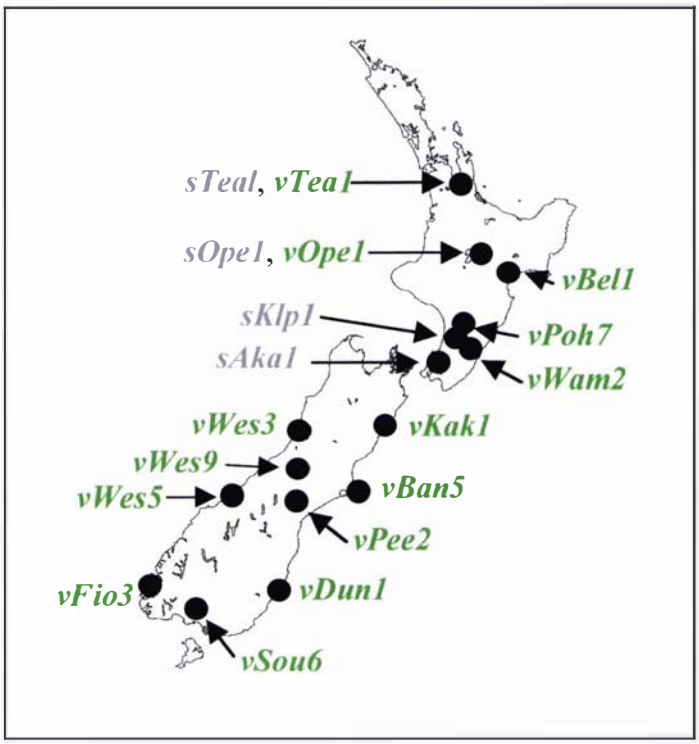


Figure 5.13. Map of 'North & South Island' AFLP Sample Set.
Geographic origins of samples included in the 'North & South Island' AFLP sample set; **'mainland' *Polystichum vestitum***, and *P. silvaticum*. Further details of these samples can be found in the Appendix 2.1.2.

vestitum), and of these to *P. silvaticum*. The second focused on relationships amongst Chatham Islands' samples, while the third was a 'combined' analysis, including samples of *P. vestitum* from the mainland, the Chatham Islands, the Subantarctic and Foveaux Strait, as well as *P. silvaticum*.

5.3.4.1 The 'North and South Island' Sample Set.

The 'North & South Island' sample set included 18 individuals, of which four were *P. silvaticum* and the remainder *P. vestitum* from throughout the North and South Islands. Geographic origins of these samples are mapped in Figure 5.13, and further details are given in Appendix 2.1.2. A sample of each species was included from two sites where they grow in close proximity (*sOpe1* & *vOpe1* from Opepe, Taupo; *sTea1* & *vTea1* from near the top of Mt. Te Aroha; less than 100 m distant at both sites).

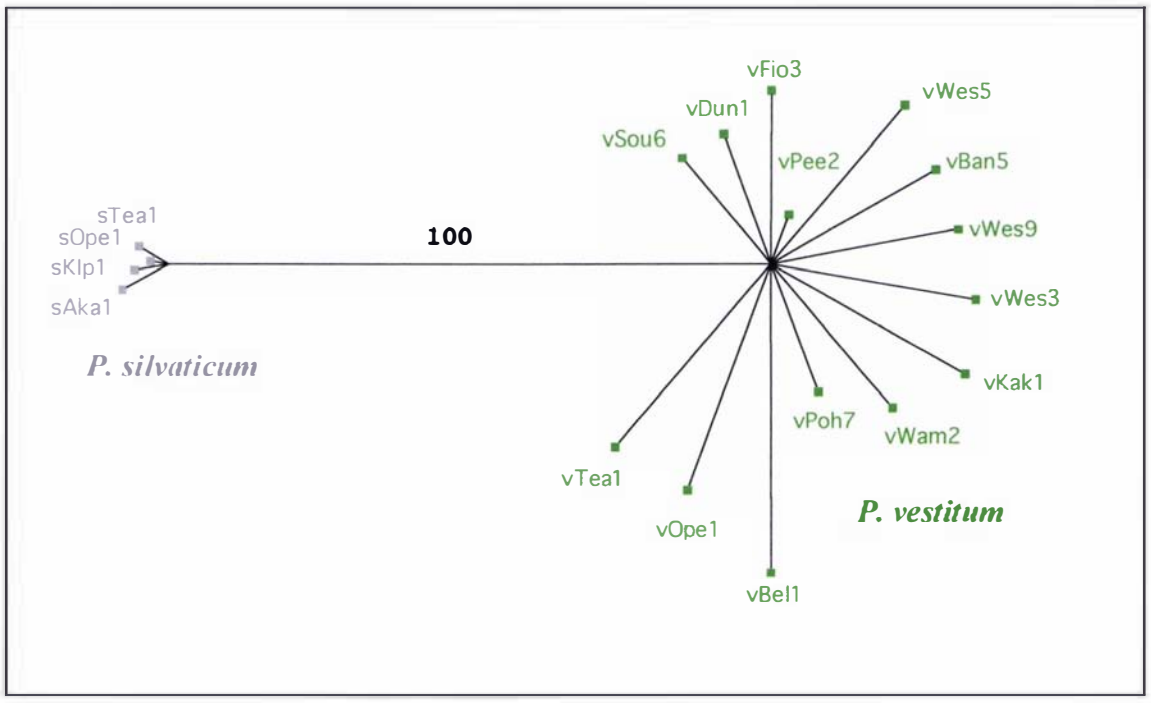


Figure 5.14. Parsimony Splits-Graph of the 'North & South Island' AFLP Data Set. The only internal edge recovered was that corresponding to the split {*Polystichum silvaticum*}{*P. vestitum*}, with bootstrap support of 100% (1000 replicates). Details of samples are given in Appendix 2.1.2.

The methods outlined in Section 3.4 were used to produce AFLP profiles using the primer combinations E-AGC & M-CTG and E-AAT & M-CTG, which generated 62 and 73 score-able polymorphic bands, respectively. A complete profile was not obtained for *vTar10* with the primer combination E-AGC & M-CTG, and it has consequently been excluded from the following analyses. (However, analyses of the *vTar10* profile obtained from the E-AAT & M-CTG primer combination found it to group with the other *P. vestitum* samples.) Analysis of these 135 characters with bootstrapping (1000 replicates) under parsimony-based split-decomposition, parsimony, and neighbour-joining found that the only well-supported split was that partitioning the *P. silvaticum* samples from the *P. vestitum* samples (ie. {*sTea1*, *sOpe1*, *sKlo1*, *sAka1*} {complement}), with the corresponding edge recovered with 100% bootstrap support under all analyses. The parsimony-based splits-graph is shown in Figure 5.14. The nexus file ('AFLP-Ves-NS.nex') is provided in Appendix Four.

5.3.4.2 The 'Chatham Islands' Sample Set.

The 'Chatham Islands' sample set included 20 samples, all except one of which (*vBan5* from Banks Peninsula) were from the Chatham Islands. The geographic origins of the Chatham Islands' samples are indicated in Figure 5.6, with further details presented in Appendix 2.1.2. The Chatham Islands' samples analysed included six, five, and eight samples assigned to the 'mainland-like Chathams,' 'intermediate Chathams,' and 'divergent Chathams' morphological categories, respectively.

The methods outlined in Section 3.4 were used to produce AFLP profiles using the primer combinations E-ATA & M-CAG, E-AAT & M-CTG, and E-AGC & M-CTG, which generated 42, 48, and 53 score-able polymorphic bands, respectively. These 143 characters were analysed with bootstrapping (1000 replicates) under parsimony-based split-decomposition, parsimony, and neighbour-joining. No internal edges were supported with 50%, or more, bootstrap support in any of the analyses. The nexus file ('AFLP-Ves-CI.nex') is provided in Appendix Four.

5.3.4.3 The 'Combined' Sample Set.

The 'Combined' sample set included 21 individuals, of which two were *P. silvaticum* (samples separated by more than 400 km), five were *P. vestitum* from the mainland, nine were from the Chatham Islands, and five were from the Subantarctic/Foveaux Strait region. The geographic origins of these samples are mapped in Figure 5.15, with further details given in Appendix 2.1.2. Of the samples from the Chatham Islands, four, one, and five samples had been assigned to the 'mainland-like Chathams,' 'intermediate Chathams,' and 'divergent Chathams' morphological categories, respectively. Of the samples from the Subantarctic/Foveaux Strait region, three (*vSou5*, *vSna1*, & *vAki1*) had been assigned to the 'divergent southern' category, with the remaining two (*vAki5* & *vAnt2*) assigned to the 'mainland-like southern' category.

The methods outlined in Section 3.4 were used to produce AFLP profiles using the primer combinations E-AAT & M-CTG, E-ATA M-CAG, E-AGC M-CTG, and E-AAT & M-CAG, which generated 89, 140, 57 and 96 score-able polymorphic bands, respectively. A complete profile was not obtained for *vAki1* with the primer combination E-AAT & M-CTG, and it has consequently been excluded from the following analyses. (However, analyses of the *vAki1* profile obtained from the other

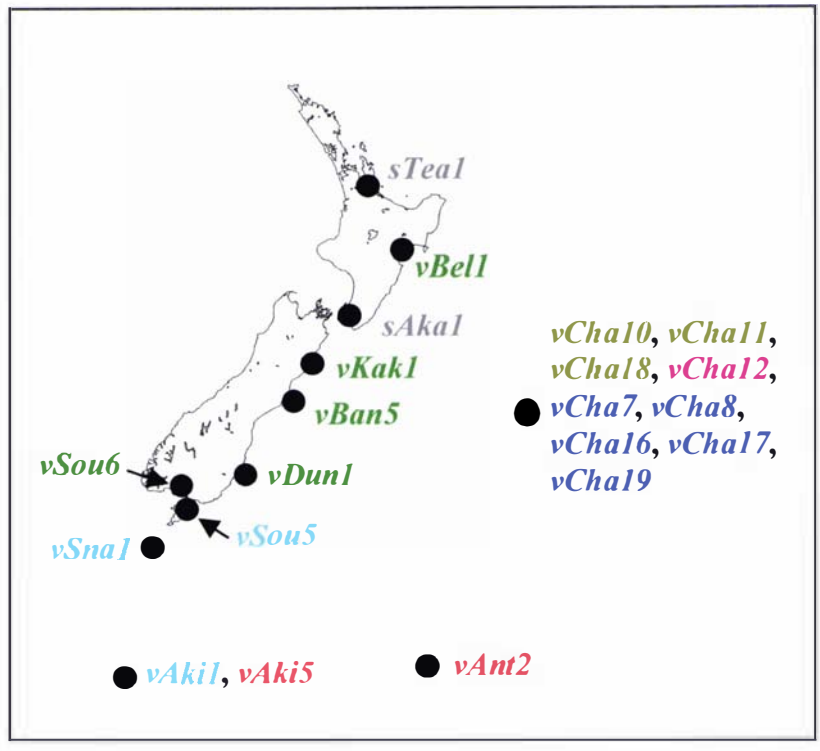


Figure 5.15. Map of 'Combined' AFLP Sample Set.
Geographic origins of samples included in the 'Combined' AFLP sample set. Samples are as follows: *Polystichum silvaticum*; and from *P. vestitum*; '**mainland**,' '**mainland-like Chathams**,' '**intermediate Chathams**,' '**divergent Chathams**,' '**mainland-like southern**,' and '**divergent southern**.' Further details of these samples can be found in the Appendix 2.1.2; 'Cha,' 'Sna,' 'Aki,' and 'Ant' denote samples from the Chatham, Snares, Auckland, and Antipodes Islands, respectively.

three primer combinations found it to group very strongly with the *vSou5* and *vSna1* samples.) Removal of the *vAki1* sample left a total of 377 polymorphic AFLP characters (89, 138, 56, & 94 from the above primer combinations, respectively), of which 332 were parsimony-informative. A total of 297 parsimony-informative splits are defined, of which 12 occurred at more than one character (Appendix 3.3.2). Nexus and spectrum files are provided in Appendix Four ('AFLP-Ves-CMB.nex' & 'AFLP-Ves-CMB.spe').

Results of boot-strapping analysis (1000 replicates) under parsimony-based split-decomposition, parsimony and neighbour-joining are shown in Figures 5.16, 5.17 and 5.18, respectively. The 30 highest supported (parsimony-informative) splits under a distance-spectral analysis are graphed in Figure 5.19, and detailed in Appendix 3.3.3.

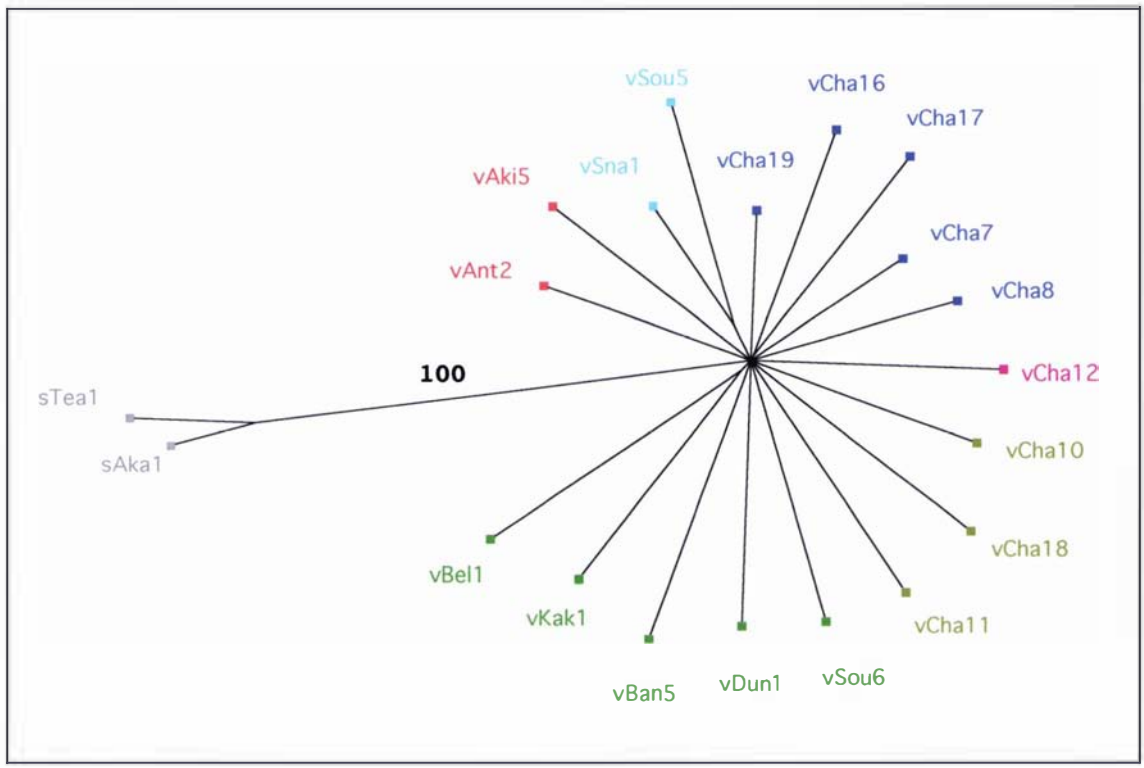


Figure 5.16. Parsimony Splits-Graph of the 'Combined' Data Set.
Samples are as follows: *Polystichum silvaticum*; and from *P. vestitum*; '**mainland**,' '**mainland-like Chathams**,' '**intermediate Chathams**,' '**divergent Chathams**,' '**mainland-like southern**,' and '**divergent southern**.' The only internal edges recovered (with bootstrap support; 1000 replicates) were those corresponding to the splits {*P. silvaticum*}{*P. vestitum*} (100% BS; shown above), {*vSou5*, *vSna1*}{complement} (66% BS), {*vBel1*, *vKak1*}{complement} (27% BS), and {*vCha16*, *vCha17*}{complement} (15% BS). Collection details of samples are given in Appendix 2.1.2. **Note** that although the '**divergent Chathams**' *appear* to group together in the same part of the graph, there is no edge in the graph separating them from the remainder of the samples. This apparent grouping is solely an artifact of the order in which the samples were analysed (see Morrison 1996 fig. 3).

There is very strong support from the analyses of this data for the concordant partitioning of the *P. silvaticum* samples from the remainder. Edges corresponding to the split {*P. silvaticum*}{complement} are recovered with 100% bootstrap support under parsimony-based split-decomposition, parsimony, and neighbour-joining. It is also the only split to occur at more than two characters in the data, and is by far the best supported split in the distance-spectral analysis.

In contrast to analyses of the 'Chatham Islands' AFLP sample set, there is some support in this data for the separation of the 'divergent Chathams' samples from the remainder

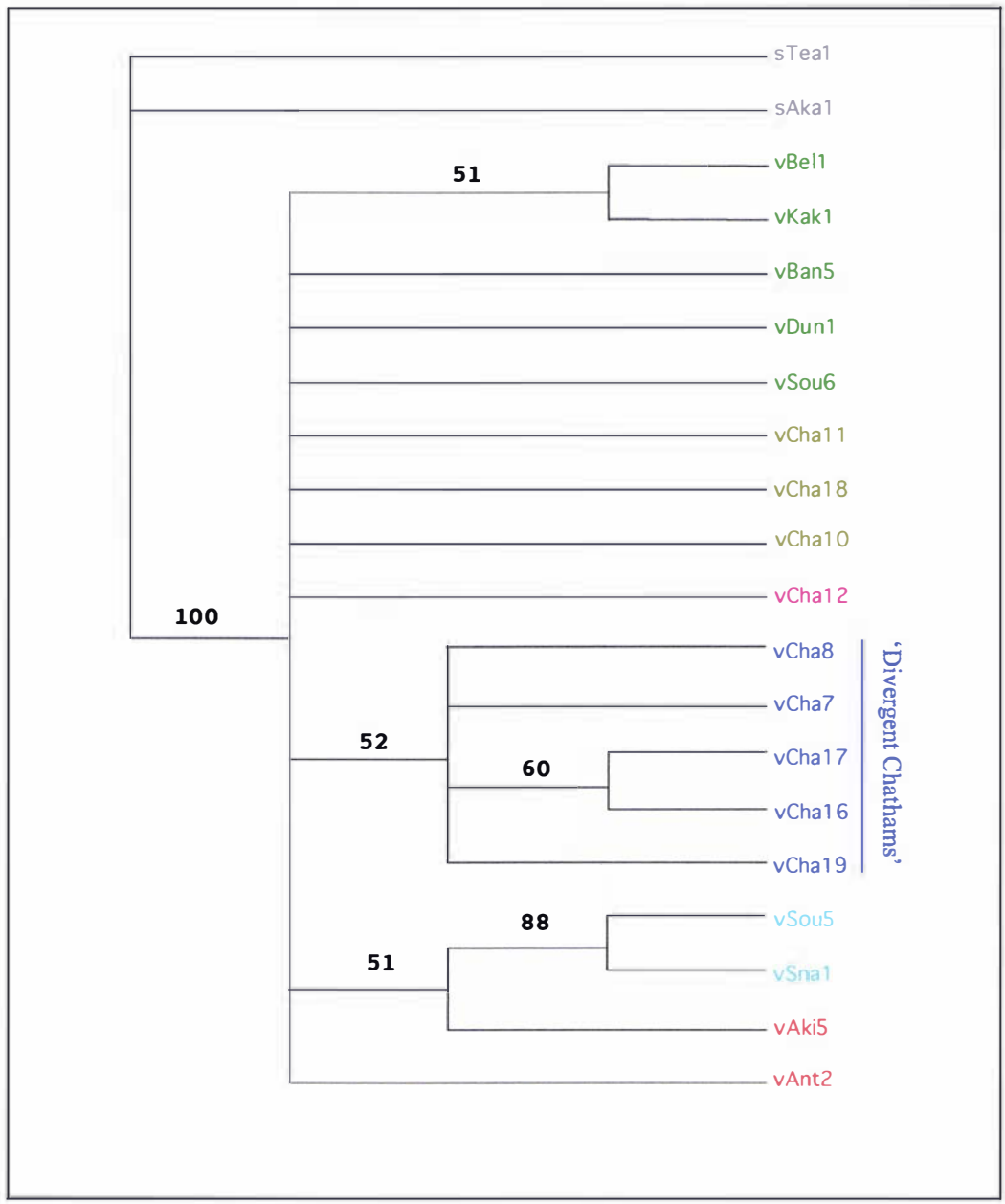


Figure 5.17. Bootstrap Analysis Under Parsimony of the 'Combined' AFLP Data.
Consensus tree of bootstrap analysis (1000 replicates) under parsimony of the 'Combined' AFLP data set. Internal edges recovered with greater than 50% bootstrap support are indicated. Samples are as follows: *Polystichum silvaticum*; and from *P. vestitum*; '**mainland**,' '**mainland-like Chathams**,' '**intermediate Chathams**,' '**divergent Chathams**,' '**mainland-like southern**,' and '**divergent southern**.' The consensus tree has been (arbitrarily) rooted on the edge between *P. silvaticum* and *P. vestitum*. Collection details of samples are given in Appendix 2.1.2.

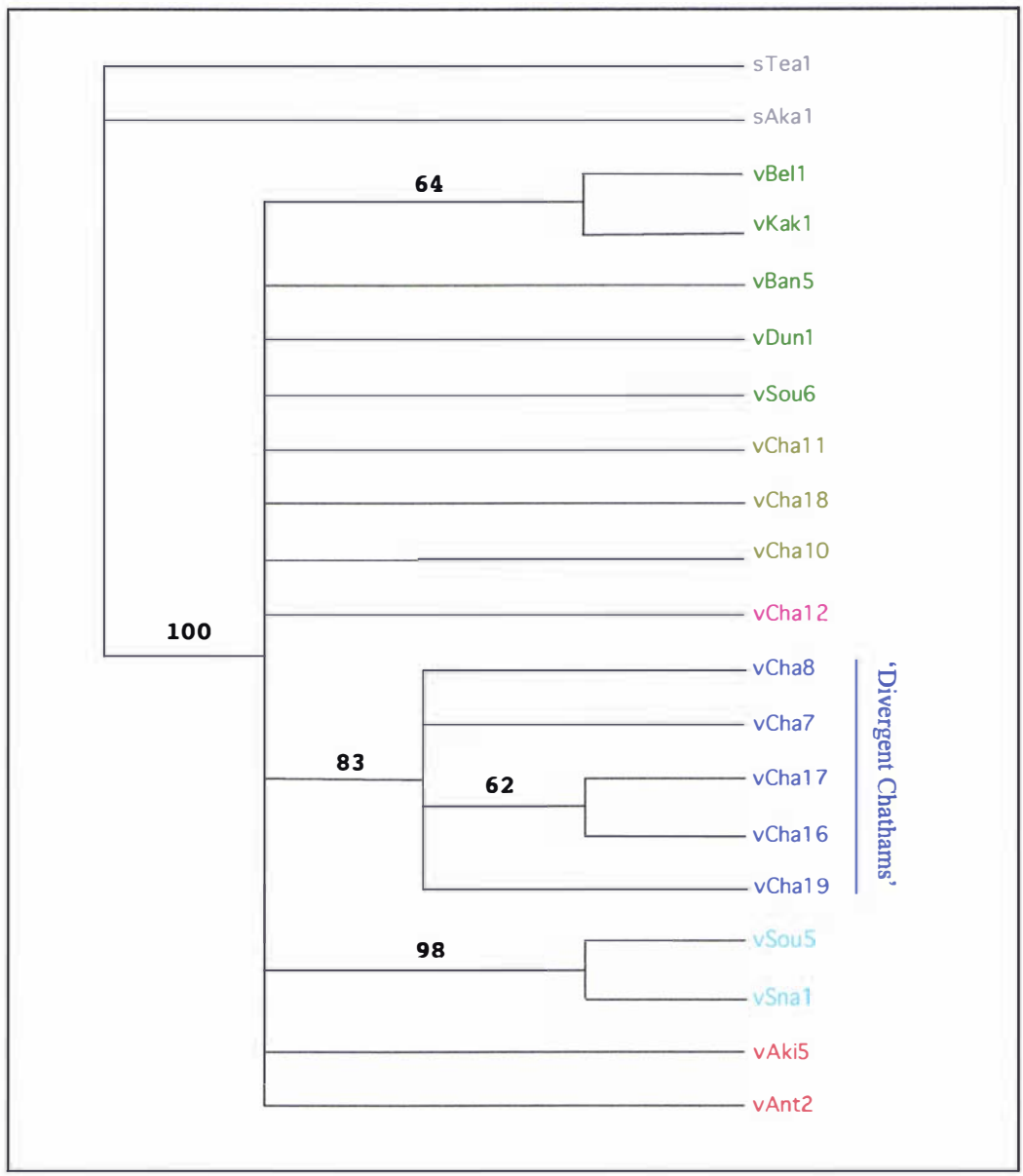


Figure 5.18. Bootstrap Analysis Under Neighbour-joining of the 'Combined' AFLP Data.

Consensus tree of bootstrap analysis (1000 replicates) under neighbour-joining of the 'Combined' AFLP data set. Internal edges recovered with greater than 50% bootstrap support are indicated. Samples are as follows: *Polystichum silvaticum*; and from *P. vestitum*; '**mainland**,' '**mainland-like Chathams**,' '**intermediate Chathams**,' '**divergent Chathams**,' '**mainland-like southern**,' and '**divergent southern**.' The consensus tree has been (arbitrarily) rooted on the edge between *P. silvaticum* and *P. vestitum.* Collection details of samples are given in Appendix 2.1.2.

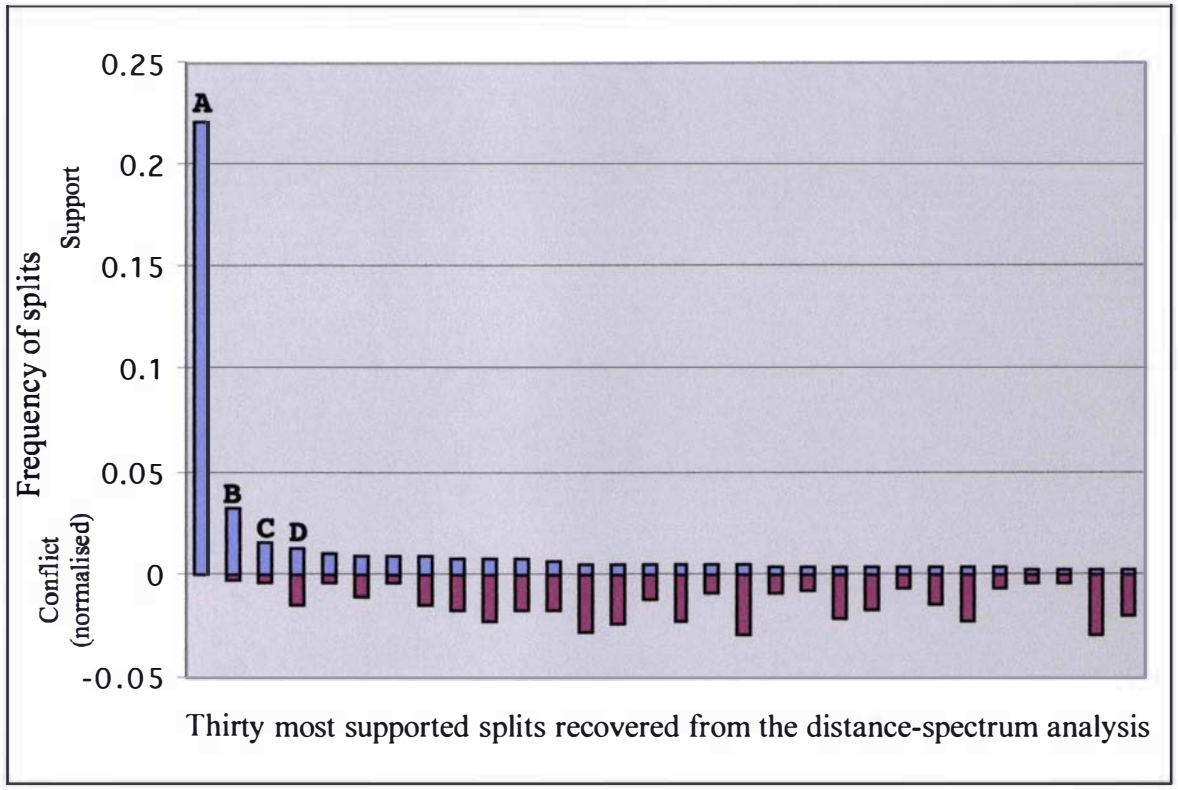


Figure 5.19. Distance-Spectral Analysis of the 'Combined' AFLP Data.
Plot of support for, and conflict against, the 30 most supported (parsimony-informative) splits recovered from the distance-spectrum analysis. Support (in blue) for each split is plotted on the positive y-axis; normalised conflict (in red) is plotted on the negative y-axis. Label (A) corresponds to the split {*Polystichum silvaticum*}{*P. vestitum*}, (B) {*vSou5*, *vSna1*}{complement}, (C) {'divergent Chathams'}{complement}, and (D) {*vBel1*, *vKak1*}{complement}.

of *P. vestitum*. The split {*vCha7*, *vCha8*, *vCha16*, *vCha17*, *vCha19*}{complement} is the third highest supported in the distance-spectral analysis, although its support is dwarfed by that for the {*P. silvaticum*}{*P. vestitum*} split. The edge corresponding to the split {*vCha7*, *vCha8*, *vCha16*, *vCha17*, *vCha19*}{complement} is recovered with 55% BS under parsimony and 83% BS under neighbour-joining, but not at all under parsimony-based split-decomposition. Of the 377 AFLP characters in this data set, none is diagnostic for this split, and only two are one step away, and another one two steps away.

Aside from *P. silvaticum*, the strongest signal in this data set was for the split partitioning the 'divergent southern' samples from the remainder (ie. {*vSou5*, *vSna1*}{complement}). This split was the second most supported in the distance-spectral analysis (although, again dwarfed by the support for the {*P. silvaticum*}{*P.*

vestitum} split), and the corresponding edges were recovered with 88%, 98%, and 66% BS under parsimony, neighbour-joining, and parsimony-based split-decomposition, respectively. There is no support for the 'divergent southern' plants being closely related to the 'divergent Chatham' plants. Bootstrap support for the split {'divergent Chathams' & 'divergent southern'}{complement} is less than 5% in all analyses. There is in fact weak evidence that they are not each other's closest relatives. The split {*vAki5*, *vSou5*, *vSna1*}{complement}, which is recovered with 51% BS under parsimony, suggests that the 'divergent southern' plants are most closely related to the 'mainland-like southern' *P. vestitum* plants.

Samples of 'mainland' *P. vestitum*, *vBel1* and *vKak1* from Napier and Kaikoura respectively, are also recovered together with similar support to that for the 'divergent Chathams' grouping (fourth highest split in the distance-spectral analysis, 27% BS under split-decomposition, 51% BS under parsimony, 64% BS under neighbour-joining). *vBel1* and *vKak1* do not exhibit morphological character states atypical of 'mainland' *P. vestitum*. (Note that the *vBel1* and *vKak1* samples are not recovered together in the full 'North & South Island' sample set, but are when it is trimmed to include only those samples also in the 'Combined' sample set.)

5.3.4.4 Interpretation of AFLP Results for Lineage Delimitation.

Analyses of the above data sets indicate that the samples of *P. silvaticum* are strongly concordantly partitioned from those of *P. vestitum* (ie. 100% BS in all analyses). Although the samples of *P. silvaticum* were less genetically variable compared to those of *P. vestitum*, the former nevertheless occurs over a wide geographic area (ie. *sTea1* and *sAka1* are over 400 km distant).

Relative to *P. vestitum* as a whole, the Chatham Islands' plants appear to be highly genetically variable (note the long external edges to the Chatham Islands' samples in Figure 5.16). They certainly do not represent a recent founder event (which would carry little genetic variation). This high level of genetic variation on the Chatham Islands might be explained by *in situ* accumulation over a long period of time. Alternatively, given that an edge subtending the Chatham Islands' samples as a whole is not recovered, the high genetic variation there might be explained by multiple dispersal

events from heterogeneous mainland sources. This would be consistent with the inferred high dispersability of *P. vestitum* (see Section 5.5.1).

Within the Chatham Islands, the 'divergent Chathams' samples also appear quite variable genetically, and there is some support in the AFLP data for their concordant partitioning from the remainder of *P. vestitum*. However, the partitioning of the 'divergent Chathams' samples is only observed in the 'Combined' data set, and not in the 'Chatham Island' data set.

This discrepancy between these data sets does not appear to be explained by the inclusion of a greater range of morphological forms in the latter. Reanalysis of the 'Chatham Islands' sample set using only those samples also included in the 'Combined' sample set does not recover any partitioning of the 'divergent Chatham' samples. However, the 'Combined' set includes data from one more primer combination (E-AAT & M-CAG) than the 'Chatham Islands' set. When the 94 characters from this primer combination are analysed by themselves, there is no recovery of separation of the 'divergent Chathams' samples. But, when they are removed from the entire 'Combined' data set, recovery of the 'divergent Chathams' separation is also lost (relevant nexus files provided in Appendix Four). Jack-knifing analysis (Swofford et al. 1996) indicates this is not simply the result of a reduction in character numbers, as 25% (= c.94/377) of the characters can be randomly removed without losing the recovery of the 'divergent Chathams' separation. It would seem that recovery of the 'divergent Chathams' separation in the full 'Combined' data set only results when the patterns in the E-AAT M-CAG data are analysed together with those from the remainder of the data (ie. the other three primer combinations).

In any case, the partitioning of the 'divergent Chathams' plants recovered from the 'Combined' data set is relatively weak when compared to that recovered between *P. silvaticum* and *P. vestitum*, or between *P. oculatum* and *P. wawranum* (Chapter Four). This weak partitioning suggests that the 'divergent Chathams' plants are *not* a lineage long separated from the remainder of *P. vestitum.*

Further, other groups (ie. the 'divergent southern' samples, and the 'mainland' samples *vBell & vKak1*) in the 'Combined' data set are recovered with similar support to that

for the 'divergent Chathams' samples. Although the 'divergent Chathams' and 'divergent southern' plants show some morphological similarities, the AFLP data does not support them as being closely related, at least on a 'genomic' level.

5.3.5 Summary of Lineage Delimitation.

P. silvaticum is clearly partitioned from *P. vestitum* by both molecular and morphological evidence (the former is exindusiate and possesses a slight wing on the pinna midribs; Brownsey 1988). Further both groups are widespread, and are broadly sympatric over a large area. Consequently, it is reasonable to infer that, in a retrospective context, they have been fertilising assortatively such that they represent separate evolutionary lineages.

The partitioning recovered within *P. vestitum* does not match that recovered with comparable methodology between *P. vestitum* and *P. silvaticum*, or within the *P. neozelandicum* complex (Chapter Four). Nevertheless, the 'divergent Chathams' plants are concordantly partitioned, albeit relatively weakly, in *both* the morphological and (some of the) AFLP analyses. It should also be remembered that the 'divergent Chathams' samples are broadly sympatric with the other Chatham Islands' samples from which they are partitioned.

That both the 'divergent Chathams' samples and the remainder of the *P. vestitum* samples are genetically variable, yet only weakly partitioned is not easily reconciled. (Note that *P. wawranum* and *P. oculatum* are both genetically variable and strongly partitioned; Chapter Four.) 'Divergent Chathams' and the remainder of *P. vestitum* do not appear to be long separated evolutionary lineages, as lineage-sorting would have been expected to result in their strong concordant partitioning. Neither does it seem that one is the recent derivative of the other, as both contain high levels of genetic variation.

This paradox of high, reciprocal genetic variation and weak partitioning is suggestive that genes responsible for the 'divergent Chathams' morphology may have introgressed into quite disparate genetic backgrounds. AFLP characters linked to this morphology may be responsible for the congruent (but weak) partitioning in the AFLP data. Such a scenario, with distinct morphologies 'floating' upon discordant genetic backgrounds, is

not consistent with the recognition of the 'divergent Chathams' plants as a separate lineage from the remainder of *P. vestitum.*

To summarise, the 'divergent Chathams' plants are only (relatively) weakly partitioned from the remainder of *P. vestitum,* and there is the paradox of high genetic variation in both. It is consequently suggested that the present morphological and molecular data are insufficient to reject the null hypothesis that 'a single evolutionary lineage is present' in *P. vestitum.* The 'divergent Chathams' plants are therefore not considered a separate lineage.

Concordant partitioning of groups beside 'divergent Chathams' was recovered in the AFLP analysis. However, sampling of these groups (eg. 'divergent southern') is inadequate to infer that they represent separate lineages. *P. vestitum* should thus be regarded as a single evolutionary lineage (unless additional data can demonstrate otherwise).

5.4 Taxonomic Delimitation.

5.4.1 *Polystichum silvaticum.*

There is good evidence that *P. silvaticum* and *P. vestitum* are, at least retrospectively, separate evolutionary lineages. They are broadly sympatric over a large geographic area, and often grow in relatively local proximity. The evidence presented in Section 5.2.2 indicates that both are at least partially sexually outcrossing. Given that they have remained separate in sympatry, it could be inferred that their SMRSs are differentiated, such they are also prospectively evolutionary separate (see Chapter Two). Thus, the data presented here is entirely consistent with their current taxonomic status as separate species.

5.4.2 *Polystichum vestitum.*

The delimitation of *P. vestitum* as a single evolutionary lineage is consistent with the current taxonomic recognition of this taxon as one species with no subspecies. *P. vestitum* is thus recognised as a single morphologically variable taxonomic species.

5.4.3 Intraspecific Taxonomy of *Polystichum vestitum.*

The species taxon *P. vestitum*, as delimited above, encompasses plants that exhibit quite 'obvious' morphological variation. Notably, as described in Section 5.3.3, the morphology of some plants from the Chatham Islands, the Subantarctic and islands around Stewart Island differs considerably in some characters from those from the North and South Islands.

Although this morphological variation is 'obvious,' it is set against a background of genomic discordance (ie. it is not supported by partitioning in other characters; hence the recognition of only one lineage). Any one of these discordant characters could be emphasised taxonomically, and to choose one over the others would be subjective.

However, the subjective taxonomic emphasis of obvious morphological variation through the recognition of non-lineage taxa could still be beneficial. For instance, it could indicate that the morphological variation in question, although 'obvious,' is not associated with a separate lineage. It would also provide a formal label, thereby facilitating communication of ideas pertaining to this variation (ie. its spatial distribution).

If the species and subspecies taxonomic categories are reserved for groups demonstrated to be separate lineages, the varietal category might be appropriate for non-lineage groups. Within *P. vestitum*, possible 'obvious' morphological variation to recognise at the varietal level includes the width, colouration, and marginal projections of the rachis scales. However, variation in colouration and marginal projections is discordant, and the taxonomic emphasis of the above morphological characters would require the recognition of four varietal taxa. For instance:

var. *vestitum* : plants from the North, South, Stewart and Subantarctic Islands with wide, bicoloured rachis scales without developed marginal projections (and which resemble the type of *P. vestitum*).

var. α : plants from the Chatham Islands with marginal projections on the rachis scales, but which otherwise closely resemble 'mainland' *P. vestitum* (var. *vestitum*)

var. β : plants from the Chatham Islands with rachis scales which are narrower, have reduced bicolouration and well-developed marginal projections.

var. λ : plants from the Subantarctic and Foveaux Strait regions with reduced bicolouration of the rachis scales.

Alternatively, (some of) the 'obvious' morphological variation might be summarised into just two varietal taxa. For instance, variety {*vestitum* + α) versus variety βλ {β + λ}, or variety *vestitum* versus variety αβλ {α +β + λ}. The choice is arbitrary, and might be driven largely by what partitioning is considered most useful. The fewer the varietal partitions, the more 'usable' the resultant scheme might be in that it is simpler, but the less information it conveys. For instance, an amalgamation of the varieties α +β + λ would demonstrate that the individuals assigned to this taxon are different (according to some subjectively chosen partition) from var. *vestitum*, but not that they are 'different in being different.' That is, the plants of the 'mainland-like Chathams' (var. α), 'divergent Chathams' (var. β), and 'divergent southern' (var. λ) morphological categories are all different in some morphological way from 'mainland' *P. vestitum*, but these differences are not the same, and they themselves are not closely related.

The 'obvious' characters emphasised above are all continuous. Therefore, as described in Section 2.4.1.1, the assignment of some individuals (ie. those with intermediate states) may be difficult or impossible. For example, in the above scheme of four varieties would 'intermediate Chathams' plants be regarded as variety α or variety β? Varietal taxa based on continuous characters may serve more as abstract, 'typological' signposts in the description of the variation observed, rather than necessarily facilitating the practical categorisation of all individuals.

Formal recognition of varietal taxa within *P. vestitum* is not recommended at the present time. This is in part because the discussion of morphological variation within *P. vestitum* can be facilitated via reference to the character states themselves. Further, in light of the continuous nature of the variation, the utility of formally naming varietal taxa in *P. vestitum* remains to be established. Particular pertinent is the ecological context of this morphological variation (Section 5.5.2), and the approach adopted for conserving Chatham Islands' *P. vestitum* (Section 5.5.3).

5.5 Discussion

5.5.1 The Dispersability of *Polystichum vestitum.*

The extant distribution of *P. vestitum* on all of the island groups in the southern New Zealand botanical region (except for the Bounty Islands, which are devoid of vascular plants) indicates that it is highly dispersable. Its distribution includes Macquarie Island, which lies over 600 km (approximately) south-west of all other extant *P. vestitum* populations. Assuming the Macquarie Island plants are derived from New Zealand, this necessitates a long-distance dispersal event against the prevailing westerly-winds (Brownsey 2001a; see Lockhart et al. 2001).

This apparent high dispersability is consistent with the suggestion that the high genetic variation of Chatham Islands' *P. vestitum* may stem from multiple colonisation events from genetically heterogeneous sources.

5.5.2 The 'Divergent' Morphological Variation.

This study indicates that the 'divergent' morphological variation exhibited by some *P. vestitum* plants from the Chatham Islands is not associated with a separate evolutionary lineage. However, it is not known whether this variation is adaptive. It is also uncertain if this variation is heritable.

The 'divergent' morphology could represent phenotypic plasticity, where a given genotype produces different morphological forms depending on the environment it is exposed to. In this sense, the morphological differences may not be heritable (although the ability to produce them is). A 'common garden' experiment, where the different morphological forms of *P. vestitum* are grown under uniform conditions, could resolve this. However, 'divergent Chathams' and 'mainland-like Chathams' plants have been recorded in close proximity (eg. at Whangamoe, map reference NZMS260 CH1 348725, and Tukuatamatea NZMS260 CH2 385426), suggesting that the different morphology is not simply a product of growing in a different environment.

It is also not known whether the observed morphological variation is adaptive (ie. could rachis-scale marginal projections or scale colouration be adaptive?), an explanation

which of course should not be assumed *per se* (see Gibson 2000). However, in this case the 'divergent' morphology may at least be linked to adaptive, non-'obvious' variation. That plants with 'divergent' morphology occur in habitats that might be considered 'divergent' from those typical of mainland *P. vestitum* suggests that there may be an adaptive explanation. The (pre-human) Chatham Islands' environment of coastal forests and large populations of ground-nesting seabirds, with their consequent enrichment of edaphic conditions, would have been quite different to the upland and cooler-lowland habitats of mainland *P. vestitum.*

Reciprocal transplantation provides a test of whether phenotypic differences are adaptive. However, this approach may not be appropriate when some of the variation concerned is rare, because of the risk of population disturbance to the rarer form, and of inadvertent gene-flow which might not otherwise be present (McKay et al. 2001).

However, if the 'divergent Chathams' and 'mainland-like Chathams' morphological forms are differentially adaptive it would be expected that their respective presence would be correlated with different habitats. As noted above, these morphological forms have been found growing in close proximity, although these records were from ecologically disturbed areas. A field survey of the different morphological forms of *P. vestitum* on the Chatham Islands and the habitats in which they occur where the environment is reasonably representative of 'natural' (pre-human) conditions would provide an initial starting point in determining their respective 'adaptiveness' or otherwise. Similar data from the Subantarctic Islands and the islands around Stewart Island would also be informative.

It is also not known whether the morphological similarities (ie. reduced rachis scale colouration, and tendency for wider fronds) between the 'divergent Chathams' and 'divergent southern' plants are analogous or homologous. These two groups do not appear closely related at a genomic level. However, this does not necessarily preclude that variation may have introgressed from one region into the quite disparate genomic background of the other, especially given the inferred high dispersability of *P. vestitum* and if the said variation was adaptive.

5.5.3 Conservation.

Conservation effort might be prioritised on any number of biological attributes (see Crozier 1997, Soltis & Gitzendanner 1999, Pearman 2001), one of which could be evolutionary distinctiveness *per se.* For instance, if only two taxa could be prioritised from the tuatara (*Sphenodon*) and two *Hoplodactylus* geckos, maximum evolutionary diversity would be conserved if the tuatara and one of the geckos were chosen (rather than choosing the two geckos over the tuatara). In that the 'divergent Chathams' plants do not constitute a separate lineage (based on the present data), preferential effort for their conservation (over eg. other *P. vestitum*) could not be justified on this basis. In this context, why conserve the 'obvious' morphological variation over and above variation in any of the numerous discordant characters?

Of course, biodiversity in the sense of separate lineages (inter-lineage biodiversity) is not the only attribute worthy of conservation. Studies of widespread plant groups regarded as single lineages often demonstrate instances of local adaptation (eg. Harris et al. 2001, McKay et al. 2001). It would seem sensible to attempt to conserve such intra-lineage biodiversity as best as possible, in that individuals adapted to local conditions are more likely to survive *in situ* (Pearman 2001).

Although the present evidence does not suggest that 'divergent Chathams' plants are a separate lineage, they may well represent local adaptation of *P. vestitum* to the Chatham Islands' environment, and thus be prioritised for conservation on this basis. This of course is not known for certain, in that the observation of polymorphism does not in itself demonstrate that it is adaptive (Gibson 2000). Hence the importance of an initial testing of whether the 'divergent Chathams' and 'mainland-like Chathams' morphological forms are correlated with different ecological settings (see Section 5.5.2).

The traditional conservation-prioritisation of New Zealand plants is explicitly taxon based (eg. Cameron et al. 1995, de Lange et al. 1999a). That is, in a broad sense, taxa, whether they be 'species,' 'subspecies,' or 'varieties' are prioritised on an equal footing. As circumscribed in this study, the taxonomic categories of species and subspecies might be prioritised on the basis of inter-lineage biodiversity, in that they represent distinct lineages. But, as circumscribed in this study, the varietal category is primarily

one of utility. It is for non-lineage (or intra-lineage) taxa, which are based on the subjective emphasis of variation in one character over discordant variation in other characters. In this context, such varietal partitions could justifiably be based on 'obvious' variation, regardless of whether it is neutral or adaptive. However, as discussed above, variation considered adaptive might be prioritised for conservation more so than that believed to be neutral, resulting in the situation where some varieties might be more 'worthy' of conservation than others.

An alternative to this taxon-focused approach to conservation is to decouple conservation effort from taxonomy. Dimmick et al. (1999 p.659), for instance, distinguished between the 'units of biodiversity,' "which are the result of evolutionary processes," and the 'units of conservation,' which "may be defined arbitrarily for practical management reasons" (see also Pennock & Dimmick 1997). Such decoupling of conservation and taxonomy would have the advantage in that a conservation focus on 'management units,' rather than 'taxonomic units,' might better conserve natural processes (Crandall et al. 2000).

In this sense, if the natural processes (ie. the pre-human environment) are conserved as best as possible, then the natural patterns will (hopefully) take care of themselves. It might then be preferable to conserve Chatham Islands' *P. vestitum* as a 'management unit' within the context of ecological restoration on the Chatham Islands, rather than through the formal naming of varietal taxa.

In the context of natural processes, it should be pointed out that it is not known how long 'divergent Chathams' and 'mainland-like Chathams' plants have been sympatric on the Chatham Islands. They may have coexisted for a long time, possibly in ecologically distinct habitats. Alternatively, vegetation clearance by humans may have facilitated the recent colonisation of 'mainland' plants. Indeed, in perhaps the biggest concern from a conservation viewpoint, the present data cannot rule out the possibility that an evolutionary lineage was once present on the Chatham Islands, but whose distinctiveness has now been blurred via introgression. Such introgression, possibly human-induced (eg. Rieseberg & Gerber 1995, Levin et al. 1996, Rhymer & Simberloff 1996, Arnold 1997, Antilla et al. 1998, Patten & Campbell 2000), between two once separate lineages could be considered consistent with the apparently paradoxical

observations from this study of only weak partitioning between the 'divergent Chathams' plants and the remainder of *P. vestitum*, yet with high genetic variation in both groups.

Whether the occurrence of 'mainland-like Chathams' plants in the more disturbed, northern part of Chatham Island is 'natural' or 'weedy' might really only be assessed by its distribution in relatively undisturbed areas of the Chatham Islands. Indeed, does this form even exist on the Chatham Islands outside environmentally disturbed areas? Hence the importance of additional study of the ecological context of morphological variation in *P. vestitum* on the Chatham Islands (and the Subantarctic and Foveaux Strait regions). Such data will hopefully provide further insights into the 'naturalness' of the extant patterns of morphological and genetic variation in *P. vestitum* from the Chatham Islands, and of the consequent priorities for conservation.

5.5.4 Southern *Polystichum vestitum.*

The sampling of *P. vestitum* from the Subantarctic and Stewart Island regions is not sufficient in this study to adequately determine whether plants with the 'divergent southern' morphology represent a separate evolutionary lineage. Nevertheless, despite some morphological similarities to the 'divergent Chathams' plants, the AFLP data of this study suggests that these two groups are not closely related on a genomic level. Further, although these results can only be regarded as preliminary, it also appears that while the 'divergent southern' plants are weakly (to moderately) partitioned from the remainder of *P. vestitum*, they too are genetically highly variable. That the 'divergent southern' plants represent a southern parallel to the paradoxical situation of the 'divergent Chathams' plants cannot be discounted. Like the Chatham Islands, a greater understanding of the morphological variation of Subantarctic and Stewart Island *P. vestitum* would be facilitated by more comprehensive knowledge of the ecological context of this variation.

The name *Polystichum venustum* Hombr., here treated as synonymous with *P. vestitum*, is based on material from the Auckland Islands. It may be relevant to any subsequent partitioning from *P. vestitum s.s.* of a taxon that encompasses Subantarctic plants.

Chapter Six

Origins of the New Zealand *Polystichum* Species, as Inferred from Genetic Relationships.

6.1 Introduction.

The origins of the New Zealand flora have been the subject of considerable recent debate (Winkworth et al. 1999, McGlone et al. 2001, Pole 2001). A prevailing view (eg. Nelson 1975, Salmon 1980, Enting & Molloy 1982) had held that the extant New Zealand flora was largely derived from taxa inherited *in situ* from Gondwana following the separation of the New Zealand landmass c.85 million years ago (mya). This 'vicariant' view has been challenged by Pole (1994; see also Macphail 1997, Pole 2001). He argued on the basis of fossil evidence that most, if not all, of the extant flora was derived from lineages that had reached New Zealand only recently (Miocene at the earliest). This view necessitates long-distance dispersal, as New Zealand has been separated from the nearest large landmass, Australia, by some 2000 km of the Tasman Sea since c.60 mya (McLoughlin 2001, Pole 2001).

Many recent molecular studies support this contention of long-distance dispersal, with several extant groups of plants seemingly colonising the New Zealand landmass only subsequent to its separation from Gondwana (eg. *Nothofagus*, Martin & Dowd 1993, Stöckler 2001; *Hebe,* Wagstaff & Garnock-Jones 1998; *Sophora*, Hurr et al. 1999; *Carmichaelia*, Wagstaff et al. 1999; *Laurelia*, Renner et al. 2000; *Aciphylla & Anisotome*, Winkworth 2000; *Toronia*, Stöckler 2001). Some groups appear especially recent, particularly subalpine and alpine taxa (eg. *Myosotis*, Winkworth et al. 1999; *Ranunculus*, Lockhart et al. 2001). However, other studies suggest some taxa have had a long-history in New Zealand, consistent with a vicariant scenario (eg. *Agathis*, *Dacrydium*, and *Pseudowintera*, Stöckler 2001), and thereby implying that both 'processes' have shaped the New Zealand flora (Winkworth et al. 1999).

Brownsey (2001a) synthesised evidence from previous fossil (Mildenhall 1980, Collinson 1996, Skog 2001) and molecular (Hasebe et al. 1994, 1995) studies with the

extant distributions of New Zealand ferns. From this he suggested that the origins of most ferns in New Zealand were post-separation from Gondwana, again necessitating long-distance dispersal. Brownsey (2001a) also highlighted the large number (89 extant species) of New Zealand ferns shared with temperate Australia. Assuming that these taxa have not remained virtually unchanged since New Zealand and Australia separated c.85 mya, their shared presence can only be accounted for by subsequent dispersal between the two regions. However, a contrary view has been put forward by Frey et al. (1999). They found minimal genetic variation between New Zealand and South American populations of the moss species *Lopidium concinnum* (Hook.) Wils., and, after dismissing any possibility of dispersal, concluded that it must be a case of stenoevolution (ie. where genetic evolution was very slow).

In light of Brownsey's (2001a) hypothesis of frequent trans-Tasman dispersal in ferns, genetic relationships amongst all of the twelve species of *Polystichum* in the south-west Pacific (New Zealand, Australia, and Lord Howe Island) were investigated. Each of these species occurs on only one of the aforementioned land-masses (ie. no species are shared between New Zealand, Australia, or Lord Howe Island): New Zealand (six endemic species; see Chapter Seven), *P. cystostegia*, *P. neozelandicum*, *P. oculatum*, *P. silvaticum*, *P. vestitum*, and *P. wawranum*; Australia (four endemic species, Jones 1998), *P. proliferum* (R. Br.) C. Presl, *P. australiense* Tindale, *P. fallax* Tindale, and *P. formosum* Tindale; and Lord Howe Island (two endemic species, Orchard 1994), *P. moorei* H. Christ, and *P. whiteleggei* Watts.

The first part of this investigation was to test in *Polystichum* the null hypothesis of a single disjunction event (irrespective of whether it be via vicariance or dispersal) between New Zealand and elsewhere. The geographical proximity to New Zealand of Australia and Lord Howe Island suggests their species are the most likely candidates to be closely related to the New Zealand species. Therefore, are the New Zealand *Polystichum* species all most closely related to each other, or to different species in the south-west Pacific?

Of particular interest is the relationship of New Zealand's *P. cystostegia*, the only alpine *Polystichum* in the south-west Pacific region. Does molecular evidence group *P. cystostegia* with the other New Zealand *Polystichum*, supporting the hypothesis of

Table 6.1. *rbcL* Variation Amongst South-West Pacific *Polystichum*.
Sequence variation detected at the *rbcL* locus in a preliminary study of south-west Pacific *Polystichum*. Only eight polymorphic sites were found in an alignment of 1186 base-pairs.
*Alignment position relative to the 'standard' *Marchantia polymorpha* L. Genbank accession U87079.

			Alignment Position*							
Taxon	Genbank accession	Herbarium voucher	201	261	312	718	744	819	972	1005
P. proliferum	AF208393	*oPro1*	C	C	T	A	G	C	G	C
P. cystostegia	AF208392	*cTar1*	C	T	T	T	G	T	G	C
P. wawranum	AF208394	*rPoh1*	T	C	T	A	G	T	A	T
P. vestitum	AF208395	*vWam4*	C	C	C	A	A	T	G	C

Wardle (1963, 1978) that New Zealand's alpine flora evolved from species already resident in New Zealand. Or, alternatively, do the relationships of *P. cystostegia* lie outside those of the other New Zealand species (and maybe even outside those from the south-west Pacific), perhaps more in agreement with Raven's (1973) contention that New Zealand's alpine flora evolved from immigrant species already adapted to an alpine habitat.

The second part of this investigation was to infer the approximate timing of the disjunction events? That is, did testing of the null hypothesis of vicariance (with disjunctions of 85 mya and older) with the data indicate that it could be rejected, thereby suggesting an explanation of dispersal (with disjunctions more recent than 85 mya)?

The data used to test these hypotheses was the genetic relationships amongst the species of *Polystichum* in the south-west Pacific as inferred from DNA-sequencing and AFLP DNA-fingerprinting. Previous DNA-sequencing studies of ferns have investigated variation in the *rbcL* gene of the chloroplast. While *rbcL* has proved useful for studies of relationships between genera and families (eg. Hasebe et al. 1994, 1995, Wolf 1995, Wolf et al. 1999, Lewis 2001, Pryer et al. 2001b), there is usually insufficient variation to resolve relationships amongst species (eg. Hauk 1995). This was found to be the case in a preliminary study of south-west Pacific *Polystichum* (see Table 6.1). Similarly, D. Little and D. Barrington (University of Vermont, pers. com.) found *rbcL* unable to resolve all but a few relationships within the 'crown' group of *Polystichum s.s.* (Interpolation of the south-west Pacific sequences in the data set of Little & Barrington reveals that they fall within a polytomy at the base of the crown group.)

Few other sequence loci have been investigated for analysis in ferns. However, Ray Cranfill (University of California, Berkley, pers. com.) has used sequence variation in the non-coding spacer region between the *rps4* and *trnS* genes (along with the *rps4* gene itself) of the chloroplast to resolve relationships within the genus *Blechnum s.l.* (see also Pryer et al. 2001a). This spacer region was adopted for use in investigating relationships amongst south-west Pacific *Polystichum.*

Although a given gene tree may accurately portray the relationships between alleles, because it is essentially one character, it may not necessarily reflect the relationships between the lineages from which the alleles were sampled (Baum & Shaw 1995, Doyle 1995, Maddison 1995, Avise & Wollenberg 1997). This problem may be particularly acute in reticulating systems, and *Polystichum* is known to include allopolyploid species (see Barrington 1985; Chapter Four). Consequently, genetic relationships between south-west Pacific *Polystichum* were also investigated using AFLP DNA-fingerprinting (Vos et al. 1995, reviewed by Mueller & Wolfenbarger 1999), which assays variation in multiple independent characters simultaneously.

AFLP has been previously used to investigate relationships between species of *Solanum* (Kardolus et al. 1998), the ariod genus *Caladium* (Loh et al. 2000a), and bamboo (Loh et al. 2000b, Hodkinson et al. 2000). The study of relationships between bamboo species of the genus *Phyllostachys* by Hodkinson et al. (2000) found AFLP and DNA-sequencing of the nuclear ITS locus to give congruent results, although the former was far more informative. They went as far as saying that AFLP "could often be the method of choice for phylogenetic studies of closely related taxa for which DNA sequence data provide insufficient resolution" (Hodkinson et al. 2000 p.259).

6.2 Sequence Analysis of the Chloroplast *rps4-trnS* Spacer in South-Western Pacific *Polystichum.*

Sequence of the *rps4-trnS* spacer region was collected according to the methods outlined in Section 3.4. All *Polystichum* species from the south-west Pacific were sampled. Samples of the European *P. setiferum* (Forssk.) Woyn. and the Asian *P.*

Table 6.2. Samples Sequenced (*rps4-trnS* spacer) and/or Analysed by AFLP for Chapter Six.
For further collection details see Appendix 2.1.

Species	Sample	Approximate locality	Full sequence	Partial sequence	AFLP
P. wawranum	*rKar6*	Northland	√		√
	rSte3	Gisborne		√	√
	rWan6	Wanganui			√
P. neozelandicum subsp. *neozelandicum*	*rPmm1*	Waikato		√	√
P. neozelandicum subsp. *zerophyllum*	*rPoh2*	Manawatu	√		
	rWan5	Wanganui			√
P. oculatum	*rSte4*	Gisborne	√		√
	rWel8	Wellington			√
	rKai1	Banks Peninsula		√	√
P. cystostegia	*cTar1*	Mt. Taranaki	√		√
	cKak1	Kaikoura		√	√
P. vestitum	*vTea1*	Mt. Te Aroha			√
	vBel1	Napier		√	√
	vBan5	Banks Peninsula	√		√
	vFio3	Fiordland			√
	vCha17	Chatham Island			√
	vCha18	Chatham Island			√
	vCha19	Chatham Island	√		
	vSou5	Foveaux Strait		√	√
	vAki5	Auckland Island			√
P. silvaticum	*sTea1*	Mt Te Aroha		√	√
	sAka1	Wellington	√		√
P. proliferum	*aPro1*	Palmerston North (cultivated)		√	
	aPro2	Sydney (cultivated)	√		√
P. australiense	*aAus1*	Sydney (cultivated)	√		√
P. fallax	*aFal1*	Sydney (cultivated)	√		√
P. formosum	*aFor1*	Sydney (cultivated)	√		√
P. moorei	*hMoo1*	Lord Howe Island	√		√
P. whiteleggei	*hWhi1*	Lord Howe Island	√		√
P. mohrioides	*oMoh1*	DNA supplied by Steve Wagstaff	√		
P. multifidum	*oMul1*	DNA supplied by Steve Wagstaff	√		
P. lentum	*oLen1*	Auckland (cultivated)	√		√
P. setiferum	*oSet1*	Palmerston North (cultivated)	√		√

lentum (D. Don) T. Moore were included as putative outgroups. DNA samples from the southern South American species *P. mohrioides* (Bory) C. Presl and *P. multifidum* (Mett.) T. Moore were available from Steve Wagstaff (Landcare, Lincoln), so these species were also analysed (vouchers CHR514069 and CHR514067, respectively). Table 6.2 provides a list of all of the samples sequenced.

Three sequencing reactions were required to get the complete sequence of the spacer region. However, c.95% of the region (all but c.20 base-pairs at the *trnS* end) could be obtained with two sequencing reactions. Such partial sequences were obtained from duplicate samples of *P. wawranum*, *P. oculatum*, *P. neozelandicum* (sequences from

Table 6.3. Region of Ambiguous Alignment in the *rps4-trnS* Sequence.
Polystichum fallax (*aFal1*) and *P. formosum* (*aFor1*) share two base-pair substitutions (base positions highlighted grey) within an eleven base-pair stretch (in box) deleted in *P. silvaticum* (*sAka1*) and *P. australiense* (*aAus1*). This ambiguity was arbitrarily recoded as shown for the analysis in which indel events were included as characters (see the file 'SpacerGapsIn.nex' in Appendix Four). For taxon identity of samples see Table 6.2. Collection details are given in Appendix 2.1.

	Alignment Base Positions 369 ↓		381 ↓	Recoding of base positions 370 to 380.
rKar6	CG	ACTCCAGCCCG	TCT	AC
rPoh2	CG	ACTCCAGCCCG	TCT	AC
rSte4	CG	ACTCCAGCCCG	TCT	AC
vBan5	CG	ACTCCAGCCCG	-CT	AC
vCha19	CG	ACTCCAGCCCG	-CT	AC
sAka1	CG	----------	TCT	TC
cTar1	CG	ACTCCAGCCCG	TCT	AC
hMoo1	CG	ACTCCAGCCCG	TCT	AC
hWhi1	CG	ACTCCAGCCCG	TCT	AC
aAus1	CG	----------	TCT	TC
aPro2	CG	ACTCCAGCCCG	TCT	AC
aFal1	CG	ATTCCAGCCTG	TCT	AT
aFor1	CG	ATTCCAGCCTG	TCT	AT
oMoh1	CG	ACTCCAGCCCG	TCT	AC
oMul1	CG	ACTCCAGCCCG	TCT	AC
oLen1	CG	ACTCCAGCCCG	TCT	AC
oSet1	CG	ACTCCAGCCCG	TCT	AC

samples of subsp. *neozelandicum* and subsp *zerophyllum* identical), *P. cystostegia*, *P. silvaticum*, *P. vestitum* and *P. proliferum*. No instances of intraspecific variation were found.

For those seventeen sequences spanning the entire non-coding spacer region between the *rps4* stop codon and the *trnS* coding sequence, an alignment of 456 base-pairs (b.p.) was generated using ClustalX 1.8 (Thompson et al. 1997), with inferred insertion-deletion ('indel') events checked manually. This alignment is given in clustal format in the file 'SpacerFull.aln' (see Appendix Four).

One region of ambiguous alignment was found, where the *P. formosum* and *P. fallax* samples share two substitutions in an eleven b.p. stretch deleted in the *P. australiense* and *P. silvaticum* samples (see Table 6.3). The remaining unambiguous alignment of 445 base-pairs contains 15 parsimony-informative substitutions, 32 parsimony-uninformative substitutions, as well as one parsimony-informative indel event (a single base-pair deletion) and three parsimony-uninformative indel events (of one, four and five b.p.).

Two parsimony-based splits-graphs were created with the program Splitstree (Huson 1997, 1998); the first (Figure 6.1a) with all characters containing gaps excluded, and the second (Figure 6.1b) with indel events recoded as characters (the region of ambiguous alignment was recoded as shown in Table 6.3). The respective nexus files are 'SpacerNoGaps.nex' & 'SpacerGapsIn.nex' (see Appendix Four). Because the conflict in both data sets is only two-dimensional, all inferred mutation events can be represented by the splits-graphs.

6.2.1 Number of Trans-Tasman Disjunction Events.

As seen in Figure 6.1a, the New Zealand samples are all split from the remaining samples, such that they could be said to be monophyletic with respect to this chloroplast sequence if *P. lentum* and *P. setiferum* are indeed suitable outgroups. This {NZ samples}{non-NZ samples} split occurs at a single b.p. character (a C→T transition at alignment position 339). It is consistent with the hypothesis of an origin from a single trans-Tasman (between New Zealand and Australia/Lord Howe Island) disjunction event for the extant New Zealand species (or at least their chloroplasts).

However, as seen in Figure 6.1b, this {NZ samples}{non-NZ samples} split is conflicted by the split {*P. australiense* & *P. silvaticum*}{complement} which is coded by the eleven b.p. deletion at alignment positions 370-380 (see Table 6.3). This second split implies a minimum of two disjunction events between Australia and New Zealand.

Because the chloroplast molecule is non-recombining (Ennos et al. 1999), state transformation at one of these characters must have occurred twice independently. That is, one of these characters is homoplasious, such that it is not consistent with phylogenetic history. Several studies have suggested that parallel indel events are more likely than parallel substitution events (eg. Golenberg et al. 1993; see also Mes et al. 2000). However, the likelihood of substitution homoplasy cannot be completely dismissed, as it does occur elsewhere in this present data set. Namely the split {*P. proliferum* & *P. lentum*}{complement} which occurs at alignment position 436, conflicts with the split {*P. lentum* & *P. setiferum*}{complement} which is coded by

Figure 6.1a

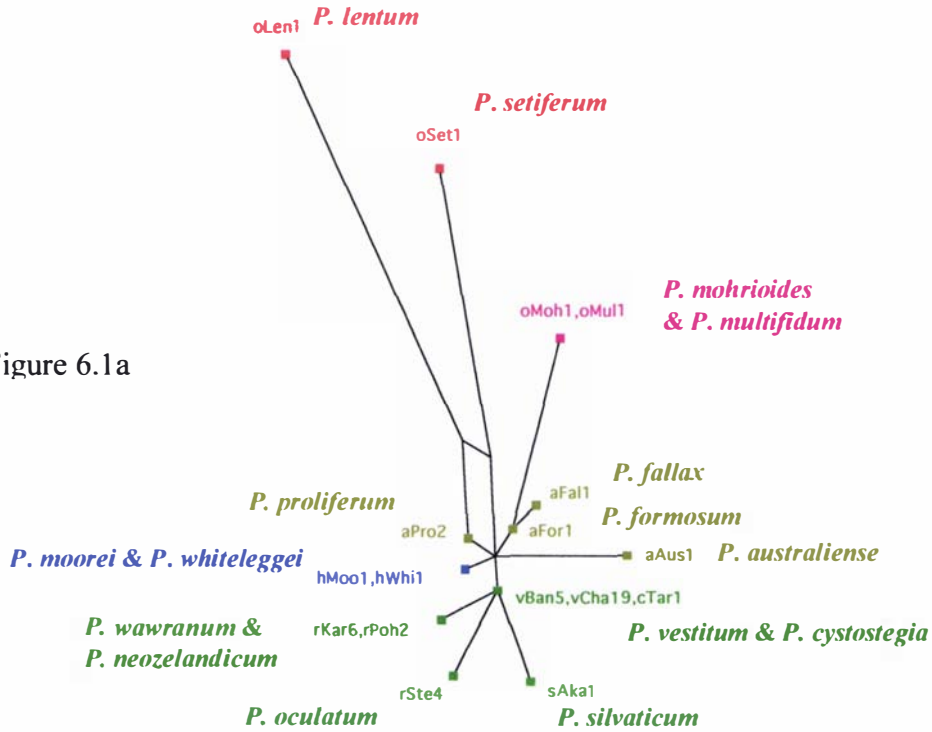


Figure 6.1b

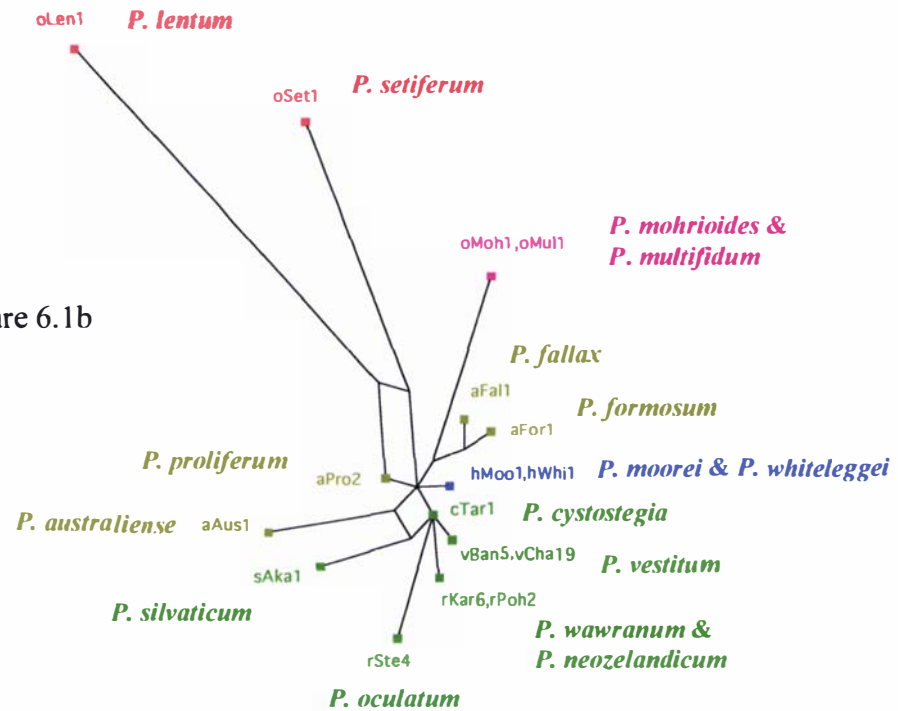


Figure 6.1. Parsimony-Based Splits-Graphs of the *rps4-trnS* Spacer Sequence Data.
6.1a) Characters with gaps excluded; ie. base substitution characters only.
6.1b) Characters with gaps recoded; ie. base substitutions and indel events included.
Samples are coloured: **New Zealand**, **Australia**, **Lord Howe Island**, **South America**, '**outgroups**.'
Collection details for samples are given in Appendix 2.1.

Figure 6.2a

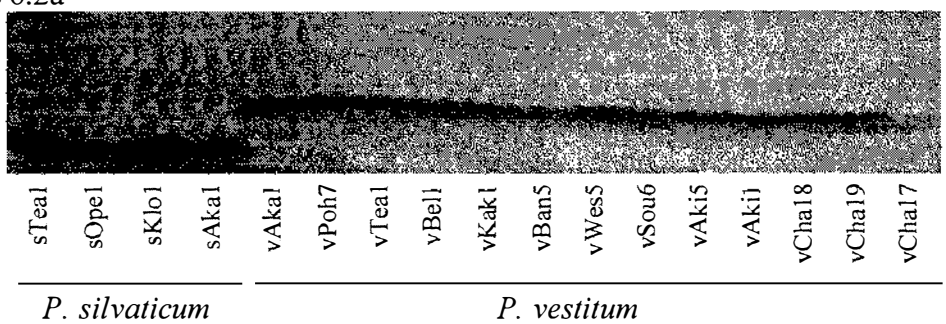


Figure 6.2b

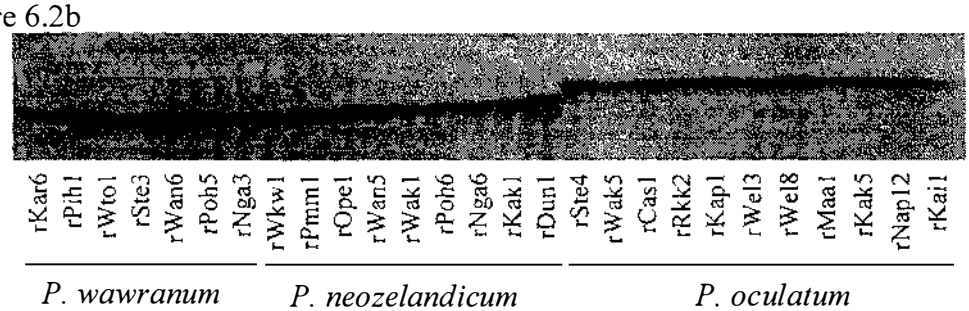


Figure 6.2. Indel Genotyping.
Genotyping of indel size differences in the *rps4-trnS* spacer sequence (on an 8% polyacrylamide gel). See Appendix 2.1 for details of the samples shown.
6.2a) The ten base size difference between *Polystichum silvaticum* and *P. vestitum*.
6.2b) The five base size difference between *P. oculatum* and the haplotype shared by *P. wawranum* and *P. neozelandicum* (*rWkw1* and *rPmm1* are from subsp. *neozelandicum*; the remaining *P. neozelandicum* samples are from subsp. *zerophyllum*).

three base substitutions (the conflict between these splits is responsible for the 'box' in Figure 6.1a).

6.2.2 Other Features of the Spacer Sequence Data.

Despite their close morphological similarity (Brownsey & Smith-Dodsworth 1989), the sequences of *P. silvaticum* and *P. vestitum* differ from one another at several sites. Relative to the inferred sequence of the common ancestor of the New Zealand species (represented in the extant *P. cystostegia*), the sequences of *P. silvaticum* and *P. vestitum* are characterised by three base-pair substitutions and one 11 base-pair deletion, and one single base-pair deletion, respectively. They consequently differ in size by ten base-pairs, a difference which, from genotyping a larger set of samples, is apparently fixed (Figure 6.2a).

The sequences of both subspecies of the allopolyploid *P. neozelandicum* are identical to that of *P. wawranum*, implying that the latter, rather than *P. oculatum*, was the chloroplast parent of *P. neozelandicum.* Relative to the inferred common ancestral New Zealand sequence, the sequences of *P. oculatum* and *P. wawranum* (plus *P. neozelandicum*) are characterised by three base-pair substitutions and one five base-pair insertion, and two base-pair substitutions, respectively. From genotyping a larger set of samples, this five base-pair difference in size is apparently fixed (Figure 6.2b).

That the sequence of *P. cystostegia* falls on the inferred ancestral node of the New Zealand species does not necessarily imply it was the ancestor of the other species. Given the low level of variation, this finding could simply reflect retention of the ancestral state in *P. cystostegia*.

The sequences for the two Lord Howe species are identical to one another, as are those for the two southern South American species. The southern South American species share a single b.p. synapomorphy (at alignment position 29) with the Australian *P. formosum* and *P. fallax*, suggesting that the former may have an Australasian origin.

6.3 AFLP Analysis of South-Western Pacific *Polystichum.*

Because they have only two character states (ie. band presence or absence at a particular band-size), a given AFLP character contains relatively little information. They are also prone to homoplasy (particularly in the independent acquisition of the band-absent state), a problem that increases in severity with increasing genetic distance between the taxa sampled (Mueller & Wolfenbarger 1999). However, the AFLP method has the great advantage that data can be collected from numerous, independent characters, and although the information of any given AFLP character may be poor, when analysed collectively they can provide extremely powerful insights into the relationships between lineages (Sections 3.4.4.5 & 3.4.4.7). As indicated by both Farris (1983 p.12-14) and Brower et al. (1996 p.433), as long as any homoplasy is random with respect to phylogenetic signal, it can still be possible to recover that signal from the data.

Lineage-sorting between divergent lineages is expected to create 'phylogenetic signal,' namely, concordant partitioning across multiple characters. In such a noisy data set as an AFLP analysis of different species, this concordance may not be 'absolute' in the form of multiple, congruent 'diagnostic' characters, but rather be 'almost' concordant. That is, a split may occur only a few times in the data, but there may be enough characters with splits only a few steps away for the former to still be recovered with strong support in bootstrap analyses using parsimony or distance criteria, or in a distance-spectrum analysis.

The AFLP sample set included three individuals of *P. wawranum*, two of *P. neozelandicum* (one of both subspecies), three of *P. oculatum*, eight of the morphologically-heterogeneous *P. vestitum* (including four 'mainland' plants, together with single plants from each of the following four morphological categories; 'mainland-like Chathams,' 'divergent Chathams,' 'mainland-like southern,' and 'divergent southern;' see Chapter Five), two of *P. silvaticum*, two of *P. cystostegia*, and one each of *P. moorei*, *P. whiteleggei*, *P. proliferum*, *P. australiense*, *P. formosum*, *P. fallax*, *P. lentum* and *P. setiferum*; the latter two as putative outgroups. Unfortunately, the supplied DNA samples of *P. mohrioides* and *P. multifidum* used to generate *rps4-trnS* spacer sequence were too degraded for AFLP profiling. Further details of the samples analysed are given in Table 6.2.

Parsimony and neighbour-joining were used to infer relationships between the samples based on AFLP character variation. These methods assume a hierarchical, or bifurcating, pattern of relationship, which should be the case assuming that these taxonomic species represent divergently related evolutionary lineages. Consequently, all edges recovered by these analyses are reported, even those with low bootstrap support (BS), although such poorly supported edges should be interpreted with caution. Edges recovered with greater than 50% BS are highlighted. (The high BS values recovered for some intraspecific relationships, principally within *P. wawranum* and *P. oculatum*, are likely to reflect limited intraspecific sampling. They are not apparent with more thorough sampling of these taxa; see Chapter Four.)

A distance-spectral analysis was also used to assess support for and conflict against particular splits. Because of Spectrum 2.0 (Charleston 1997, 1998) memory limitations,

the sample set had to be reduced to 22 individuals. The six individuals excluded for this analysis were *vTea1*, *vFio3*, *vCha18* (all *P. vestitum*), *sTea1* (*P. silvaticum*), *cTar1* (*P. cystostegia*) and *rPmm1* (*P. neozelandicum* subsp. *neozelandicum*). Reanalysis of this reduced sample set by neighbour-joining and parsimony gave results consistent with the full sample set (results not shown).

AFLP profiles were generated with the E-ATA M-CTG and E-AAT M-CAG primer combinations according to the methods detailed in Section 3.4. 230 polymorphic characters were scored. These identified 220 different splits, of which 10 were parsimony-uninformative. Of the 210 parsimony informative splits, one occurred at three characters, five occurred at two characters, and the remaining 204 were each coded by one character. The 'common' parsimony-informative splits (ie. those occurring more than once) are given in Appendix 3.4.1. Appendix Four provides the nexus and spectrum files ('AFLP-SWPac.nex' & 'AFLP-SWPac.spe,' respectively).

The neighbour-joining tree is shown in Figure 6.3. Two most parsimonious trees, differing only in the internal arrangement of the *P. oculatum* samples, of 946 steps were recovered (Consistency Index: 0.243, Rescaled Consistency Index: 0.122); one is shown in Figure 6.4. The distance spectrum is given in Figure 6.5.

6.3.1 Trans-Tasman Relationships.

The edge corresponding to the split {NZ samples}{non-NZ samples} was recovered under both parsimony and neighbour-joining, with moderate to high bootstrap support (72% BS under parsimony, 83% BS under neighbour-joining). There are no diagnostic characters for this split (ie. it is not identified by any character). The distance-spectral analysis indicates little relative support for splits conflicting with the {NZ samples} {non-NZ samples} split; such splits might necessitate inference of more than one disjunction event. While the {NZ samples}{non-NZ samples} split was recovered as the eleventh best-supported parsimony-informative split, the highest ranking split in conflict with it was only 58th (see Figure 6.5).

Such support for the split {NZ samples}{non-NZ samples} suggests that the genomes of the extant New Zealand species share a common ancestor to the exclusion of the non-

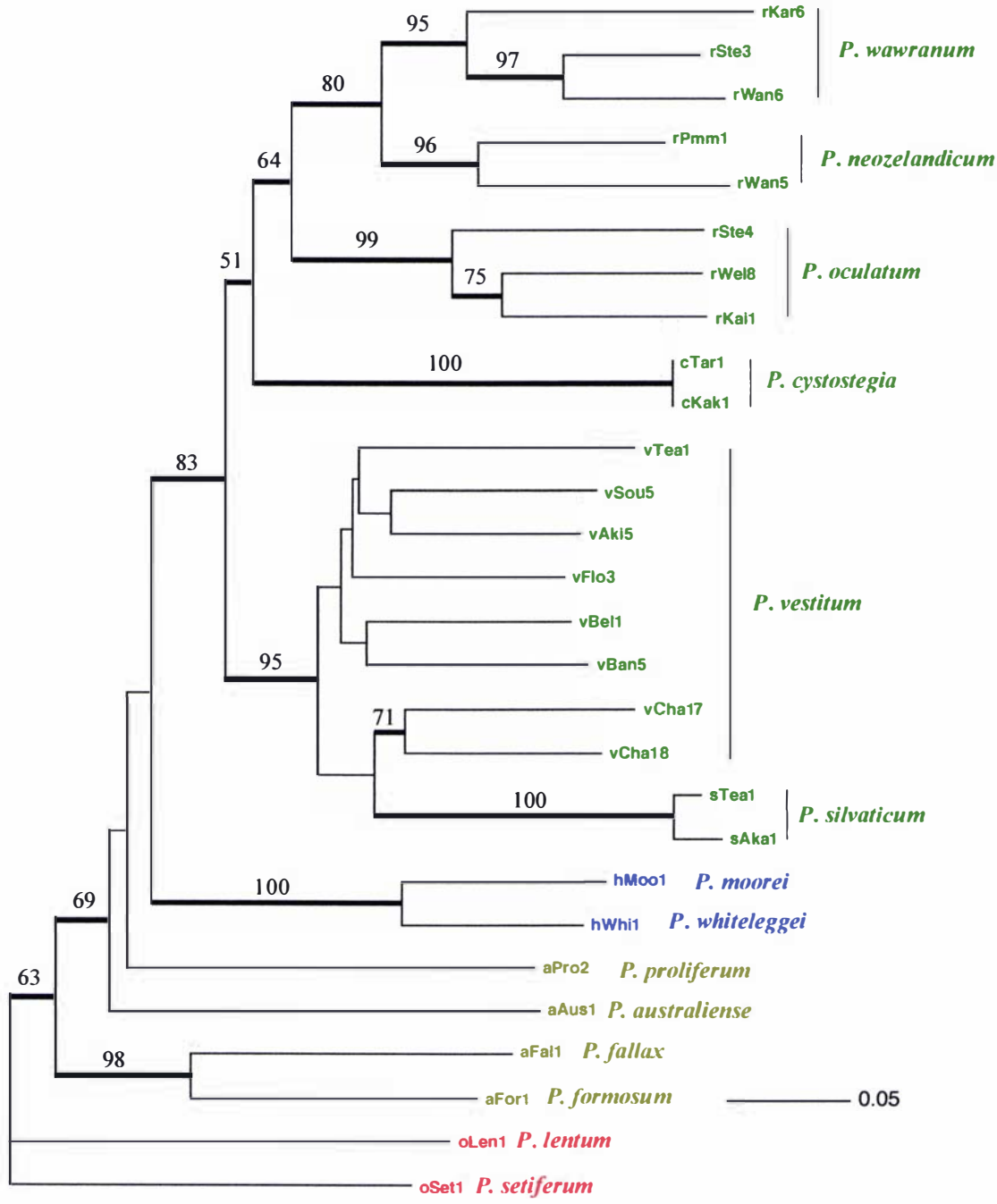


Figure 6.3. Neighbour-Joining Tree of the AFLP Data.
Edges recovered with more than 50% bootstrap support (1000 replicates) are highlighted. Samples are coloured: **New Zealand**, **Australia**, **Lord Howe Island**, '**outgroups**.' Collection details for samples are given in Appendix 2.1.

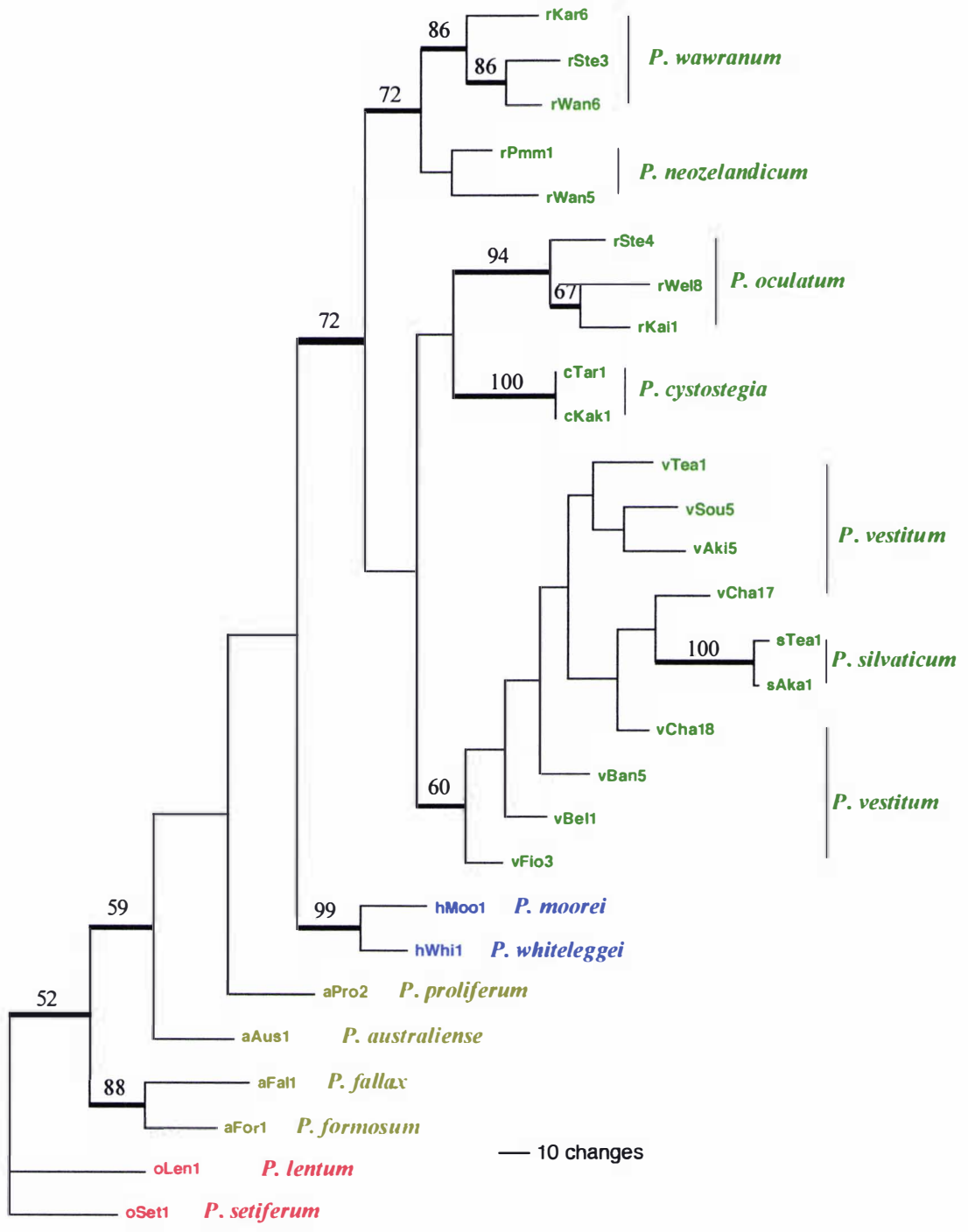


Figure 6.4. Parsimony Analysis of the AFLP Data.
One of two most parsimonious trees of the AFLP data. The other differs only in the internal arrangement of the *Polystichum oculatum* samples. Steps 946, CI: 0.243, RC: 0.122. Edges recovered with more than 50% bootstrap support (1000 replicates) are highlighted. Samples are coloured: **New Zealand**, **Australia**, **Lord Howe Island**, '**outgroups**.' Collection details for samples are given in Appendix 2.1.

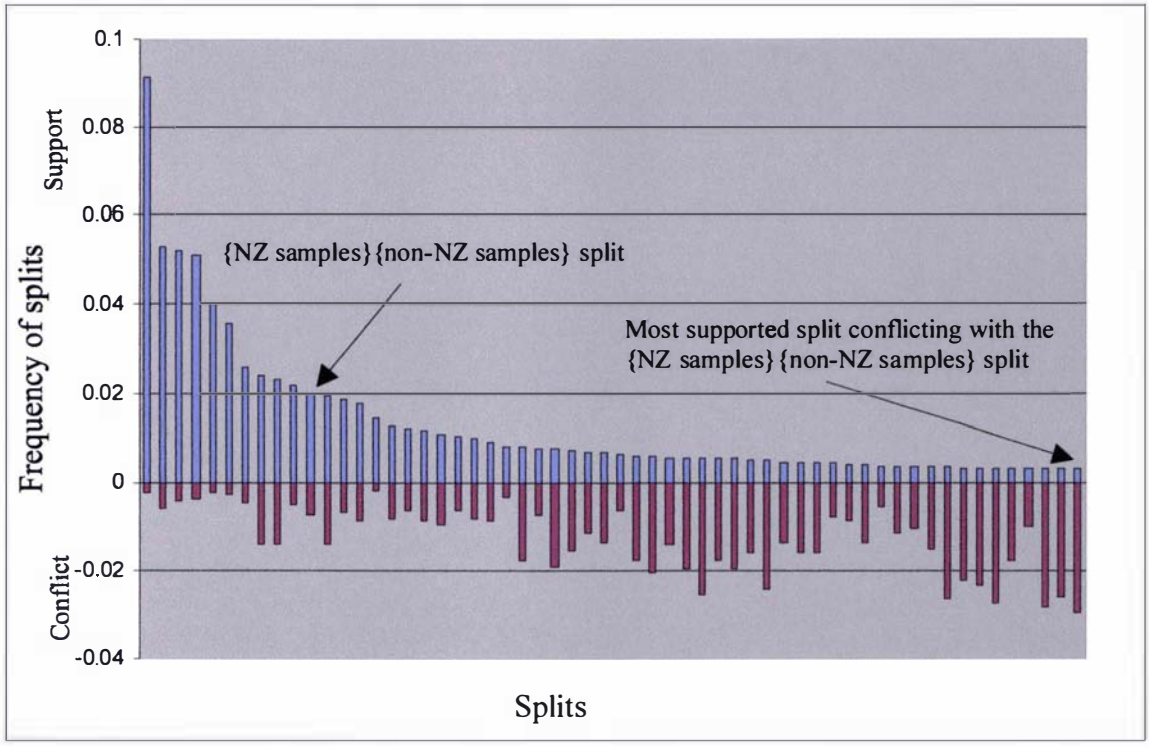


Figure 6.5. Distance-Spectral Analysis of the AFLP Data.
Plot of support for, and conflict against, the 58 most supported internal splits recovered from the distance-spectrum analysis. Support (in blue) for each split is plotted on the positive y-axis; normalised conflict (in red) is plotted on the negative y-axis. The {NZ samples}{non-NZ samples} split is indicated, as is the most supported split which conflicts with it.

New Zealand samples analysed; ie. the extant New Zealand species could be considered a monophyletic group. This is consistent with a single trans-Tasman disjunction event (ie. between New Zealand and Australia/Lord Howe Island).

6.3.2 Relationships Between the New Zealand Species.

The two samples of *P. silvaticum*, from localities over 400 km distant, were recovered together as a group with 100% BS under parsimony and neighbour-joining. Both methods nested *P. silvaticum* within *P. vestitum* (and more so, with the Chatham Island samples, a relationship not found in Chapter Four), although this nesting had less than 50% BS. Nevertheless, the group of *P. silvaticum* and *P. vestitum* was recovered with moderate to strong support under parsimony (60% BS) and neighbour-joining (95% BS). In the distance-spectral analysis, the relationship of *P. silvaticum* to *P. vestitum* was recovered with much higher support than that of *P. silvaticum* to *P. australiense*, which was indicated by the eleven b.p. deletion at alignment positions 370-380 of the

rps4-trnS spacer sequence data. Relative support for the former relationship was c.×36 than that for the latter.

Samples of *P. wawranum* were recovered as a well-supported group under parsimony and neighbour-joining, as were those of *P. oculatum*. The samples of *P. neozelandicum* were recovered as each others closest relative under both parsimony and neighbour-joining, although this edge had less than 50% BS under parsimony. *P. wawranum* and *P. neozelandicum* were recovered as sister groups with moderate to high support under both parsimony and neighbour-joining.

P. oculatum was recovered as the sister group to that of *P. wawranum* and *P. neozelandicum* under neighbour-joining (64% BS), but not under parsimony where the sister-group relationship of *P. oculatum* and *P. cystostegia* was recovered. However, this curious relationship (between a lowland and an alpine species) had less than 50% BS. Interestingly, when *P. neozelandicum* is excluded from the neighbour-joining analysis, *P. oculatum* no longer groups with *P. wawranum* but with *P. cystostegia* (57% BS), as happens in the parsimony analysis (69% BS with *P. neozelandicum* excluded). This suggests that the grouping of *P. oculatum* with *P. wawranum* and *P. neozelandicum* under neighbour-joining is an artifact, with the inclusion of the allopolyploid *P. neozelandicum* in the analysis apparently pulling together its two putative parents, which otherwise seem to be not closely related. The profiles of the two samples of *P. cystostegia*, one from Taranaki in the North Island and the other from Kaikoura in the South Island, were identical.

6.3.3 Relationships Between the non-New Zealand Species.

Inference of relationships amongst the non-New Zealand species should be limited in the absence of intraspecific sampling of these species. However, the samples of the two Lord Howe species, *P. moorei* and *P. whiteleggei*, were recovered as each other's closest relative with 99% BS under parsimony and 100% BS under neighbour-joining. A close relationship between the Australian *P. fallax* and *P. formosum* was also strongly supported, being recovered together with 88% BS under parsimony and 98% BS under neighbour-joining.

Interestingly, the Lord Howe species were recovered as the sister group to the New Zealand species under both parsimony and neighbour-joining, although this relationships received less than 50% BS under both methods.

6.4 Timing of Disjunction Events.

There is no suitable internal temporal calibration point within the sets of samples reported in Sections 6.2 and 6.3. Inclusion of more distantly related taxa, for which lineage divergences have been dated from fossils, is not possible because of indel-induced alignment difficulties in the *rps4-trnS* spacer region (eg. *Cyrtomium* and *Dryopteris* sequences; data not shown), and the expectation of homoplasy-induced randomness in AFLP profiles.

The *rbcL* sequences reported in Table 6.1 are, however, informative in the context of the large database of accessions from ferns already available from Genbank and that indel events do not occur at this locus. The average sequence divergence between the Australian *P. proliferum* and the three New Zealand species reported in Table 6.1 is c.0.3% (c.3 b.p.). The average (uncorrected) sequence divergence between these south-west Pacific *Polystichum* species and three *Dryopteris* sequences from Genbank (Accessions AF240653, DDU05622 & DAU05923) is c.3.75% (c.40 b.p.) for an alignment of 1154 b.p. (file 'rbcL-PolDry.aln' in Appendix Four).

Skog (2001) reports the earliest Dryopteridaceae fossils from the early Cretaceous, say 140 mya. If *Polystichum* and *Dryopteris* diverged at this time, with an extant *rbcL* sequence divergence of c.3.75%, then a sequence divergence of c.2.3% (c.26 b.p.) might be expected for lineages separating c.85 mya (ie. at the time New Zealand separated from Australia). However, the average *rbcL* sequence divergence between the Australian *P. proliferum* and three New Zealand species, at c.0.3%, is an order of magnitude less, suggesting that their divergence was much more recent than 85 mya.

While molecular dating can be fraught with difficulties, the approach employed here is conservative, simple and probably robust against all but the most extreme non-clock-like molecular evolution. It could be contended that the *Polystichum-Dryopteris* split

might be older than 140 mya. However, the Dryopteridaceae is a relatively modern fern family (Skog 2001), and although a diverse group, it still represents only a fraction of fern diversity, hence putting an upper bound on how old it really can be.

It is far more likely that the *Polystichum-Dryopteris* split is actually much younger than the conservative 140 mya calibrated above, especially as these genera constitute only a portion of the Dryopteridaceae as it is usually circumscribed. If so, the split between the Australian *P. proliferum* and the New Zealand *Polystichum* is even more recent than initially implied. Correcting for multiple substitution events would also push this divergence towards a more recent date. Hence, the *rbcL* sequence data implies that the divergence between the Australian *P. proliferum* and the New Zealand *Polystichum* was much younger than 85 mya, necessitating that their disjunction be explained by long-distance dispersal.

6.5 Discussion.

The principal objectives of the work reported in this chapter were to determine whether there was genetic evidence amongst extant *Polystichum* from the south-west Pacific for more than one trans-Tasman disjunction event, and to infer whether these were due to vicariance or dispersal. The recovery of the split {NZ samples}{non-NZ samples} would be consistent with one disjunction event. The recovery of splits conflicting the split {NZ samples}{non-NZ samples} would otherwise necessitate inference of multiple disjunction events.

Interpretation of the chloroplast *rps4-trnS* spacer sequence data, with an equal weighting of substitution and indel events, is ambiguous in resolving this objective, because of the equal support found for the conflicting splits {NZ samples}{non-NZ samples} and {*P. silvaticum* & *P. australiense*}{complement}. The former is consistent with one disjunction event, while the latter is consistent with two. Because the chloroplast genome is non-recombining, one of these splits must be homoplasious. Given that external evidence (morphology, Brownsey & Smith-Dodsworth 1989; AFLP, this study) indicates *P. silvaticum* is more closely related to *P. vestitum* than any other species, it is most likely that the homoplasious split is {*P. silvaticum* & *P.*

australiense}{complement}. Allowing such appeal to external knowledge, the *rps4-trnS* sequence data is then consistent with one trans-Tasman disjunction event.

Of course, this sequence data only really implies that the extant chloroplasts found in New Zealand *Polystichum* originated from one trans-Tasman disjunction event, and does not necessarily imply the same for the extant lineages themselves. For instance, it could be argued that one chloroplast haplotype may have introgressed into, and gone to fixation within, otherwise unrelated lineages. However, the AFLP analysis also recovers the split {NZ samples}{non-NZ samples}, and with strong support. Because AFLP characters come from throughout the genome, the AFLP results provide strong evidence that the extant lineages of New Zealand *Polystichum* have indeed originated from a single trans-Tasman disjunction event.

It should be noted that this 'single' event does not necessarily imply that the New Zealand *Polystichum* species originated from one spore. Rather, there could have been multiple spores but of such similar genomic composition that, when viewed retrospectively there is extant evidence of only the 'one' event. More correctly, the data presented here should be interpreted as providing no evidence of multiple disjunction events between *Polystichum* from New Zealand and the rest of the south-west Pacific. The low levels of variation between the *rbcL* sequence of Australian *P. proliferum* and those of several New Zealand species indicates that this disjunction event is almost certainly (much) younger than 85 mya, implicating long-distance dispersal rather than vicariance.

Relationships amongst the New Zealand *Polystichum* species are largely poorly supported, except for the close association of *P. silvaticum* with *P. vestitum*. Nevertheless, as a putatively monophyletic group, the New Zealand *Polystichum* species comprise an extensive *in situ* ecological radiation, ranging in habitat occupation from the coast to the alpine zone, and from the high-light conditions of forest margins to underneath dark, temperate-rainforest. The common ancestor of the New Zealand species was probably of lowland and/or montane habitat, as are all the extant non-New Zealand south-west Pacific species. Consequently, the occupation of an alpine habitat by *P. cystostegia* is consistent with Wardle's (1963, 1978) contention that New Zealand alpine plants evolved from lower altitude plants already resident in the country.

Chapter Seven

Summary, Conclusions, and Discussion.

7.1 Summary.

7.1.1 General Species Delimitation.

As discussed in Chapter Two, basing the taxonomic scheme on evolutionary history provides an objective framework, on which competing hypotheses of relationship and classification can be tested. In this context, a minimum requirement for entities assigned to the species category could be that they constitute separate evolutionary lineages. The delimitation of separate evolutionary lineages must then precede taxonomic delimitation.

Evolutionary lineages can be delimited from both prospective (looking to the future) and/or retrospective (looking to the past) perspectives. Prospectively, only pre-fertilisation barriers, and not post-fertilisation barriers, can engender assortative fertilisation and lineage separation. Retrospectively, character state variation can be used to infer that two groups of sexually-outcrossing organisms have been fertilising assortatively. More robust inferences of retrospective assortative fertilisation will result from the recovery of concordant partitioning across multiple, independent characters. Inferences based on single characters are likely to be misleading. In any case, lineage and taxonomic delimitation should always be viewed as a hypothesis, subject to corroboration or refutation by future evidence.

7.1.2 Species Delimitation in New Zealand *Polystichum*.

The morphological, cytological and molecular analyses documented in Chapter Four indicate that the morphologically variable *P. richardii* (*sensu* Brownsey 1988, Brownsey & Smith-Dodsworth 1989) is an allopolyploid complex of four evolutionary lineages. Cytological analysis demonstrated the presence of both tetraploid and octoploid plants. Morphological analysis indicated that the tetraploid plants could be concordantly partitioned into two quite discrete groups. Further, both of these tetraploid groups could be concordantly partitioned from the octoploid plants, with the latter being

morphologically intermediate between the former. AFLP DNA-fingerprinting analysis confirmed the partitioning of the two tetraploid groups from each other and from the octoploid plants, but indicated that the latter actually comprised two genetically-distinct, but morphologically very similar, groups.

Four lineages were consequently retrospectively recognised. The two tetraploid lineages and the southern octoploid lineage are sympatric over large areas, such that they could also be inferred to be prospectively separate (ie. with Specific Mate Recognition System, or SMRS, differentiation). They are consequently delimited as three separate species. The northern octoploid lineage is included as a subspecies with the southern octoploid lineage, with which it is allopatric.

There is morphological and molecular evidence that the two octoploid lineages are allopolyploid derivatives of the two tetraploid lineages; indeed, this is the best documented example of allopolyploidy in the New Zealand fern flora. However, whether the two octoploid lineages arose from the same allopolyploid event and have subsequently diverged, or have had independent origins is uncertain.

The name *P. richardii* is a later synonym of *P. neozelandicum*, which is reinstated for the taxonomic species encompassing both octoploid lineages. The new combination *P. neozelandicum* subsp. *zerophyllum* is proposed for the southern octoploid lineage. The new combination *P. wawranum* is adopted for one of the tetraploid lineages, while *P. oculatum* is reinstated for the other.

In contrast, as discussed in Chapter Five, similar methodology applied to the morphologically variable *P. vestitum* (*sensu* Brownsey and Smith-Dodsworth 1989), and in particular plants from the Chatham Islands, did not (strongly) indicate the presence of separate lineages. Morphological variation occurs on the Chatham Islands that is not known from the 'mainland' of New Zealand. However, the concordant partitioning it engenders is insufficient to (retrospectively) recognise a separate Chatham Islands' lineage, especially given that the ecological context of this variation is not well understood.

Nor does AFLP analysis provide strong support for the delimitation of the morphologically 'divergent Chathams' plants as a separate lineage, although the separation between *P. silvaticum* and *P. vestitum* is strongly recovered. The 'divergent Chathams' plants are recovered as a separate group in some, but not all, of the analyses, and then only weakly. Further, the 'divergent Chathams' plants, and indeed the Chatham Islands' plants in general, appear genetically very diverse. The high genetic diversity within Chatham Islands' plants of *P. vestitum* is consistent with its inferred high dispersability; *P. vestitum* occurs on all major southern island groups in the New Zealand Botanical Region. The genetic diversity within 'divergent Chathams' plants combined with their only weak partitioning, suggests that the 'divergent' morphology may have introgressed into heterogeneous genomic backgrounds. Such introgression would not be consistent with the recognition of these morphologically 'divergent' plants as a separate evolutionary lineage. Consequently, only a single lineage is delimited within *P. vestitum*, which is consistent with its present taxonomic delimitation as a single species (with no subspecies). The ecological context of the morphological variation found on the Chatham Islands requires further study.

There are plants from the Subantarctic and Foveaux Strait regions that also exhibit some of the 'divergent' morphological character states found on the Chatham Islands. Sampling of the former in this study was insufficient to adequately address whether they represent a separate lineage. However, the results presented here suggest that despite some morphological similarities, the 'divergent southern' and 'divergent Chathams' plants are not closely related at a genomic level.

Consequently, six native (all endemic) and three adventive species of *Polystichum* are recognised in New Zealand. A revised morphological key to these species is presented in Section 7.2.

7.1.3 Relationships of *Polystichum* within New Zealand, and within the SW Pacific.

As discussed in Chapter Six, from a genetic investigation of relationships the New Zealand species of *Polystichum* appear to be a monophyletic group with respect to the other *Polystichum* species from the south-west Pacific (Australia and Lord Howe Island). This is consistent with a single disjunction event across the Tasman Sea (between New Zealand and Australia/Lord Howe Island). Further, this disjunction

event appears to be much younger than 85 mya, implicating long-distance dispersal rather than vicariance. The New Zealand species comprise an extensive ecological radiation, ranging in habitat from the alpine zone to coastal areas, and from high-light open conditions to under dark, wet forest. The alpine *P. cystostegia* appears to have evolved from lowland relatives already resident within New Zealand, rather than representing an immigrant already adapted to the alpine zone.

Within New Zealand *Polystichum*, the relationships between the different species are largely unresolved, although *P. silvaticum* is unquestionably allied to *P. vestitum*. *P. oculatum* and *P. wawranum*, despite being the parents of the allopolyploid *P. neozelandicum*, may not be closely related to one another.

7.2 Revised Morphological Key to New Zealand *Polystichum*.

With the recognition of three species in the place of the taxon *P. richardii*, a revised morphological key to the species of *Polystichum* in New Zealand is necessary, and is presented below. The section dealing with the adventive species of *Polystichum* in New Zealand draws strongly from the keys of Brownsey (1988) and Brownsey and Smith-Dodsworth (1989).

1. Fronds bearing bulbils 2
   Fronds lacking bulbils 4
2. Fronds with numerous bulbils, borne along rachises at junctions with pinnae ..... ***P. setiferum***
   Fronds with one or a few bulbils only, borne near apices of rachises 3
3. Basal primary pinnae bearing stalked secondary pinnae ..... ***P. proliferum***
   Basal primary pinnae divided, sometimes to midribs, but never bearing more than one stalked secondary pinna ..... ***P. lentum***
4. Indusia markedly convex; alpine plants with stipe and rachis scales uniformly pale orange-brown ..... ***P. cystostegia***
   Indusia flat or absent; lowland or montane plants, or if from the alpine zone with bicolourous (pale brown margins surrounding dark brown centre) stipe and rachis scales 5
5. Rachis scales appearing hair-like to the naked eye (scales from the stipe-rachis junction < 130 μm at mid length); AND spore exine 40-48 × 29-36 μm ..... ***P. wawranum***
   Rachis scales obviously scale-like to the naked eye (scales from the stipe-rachis junction > 130 μm at mid length); OR if rachis scales appearing hair-like to the naked eye, then with spore exine 46-58 × 36-45 μm 6

6. Indusia absent ..... ***P. silvaticum***
   Indusia present 7
7. Indusia lacking an obvious dark centre; majority of the stipe scales bicolourous with pale brown margin completely encompassing dark brown centre (except for some plants from the Chatham Islands where the scales may be uniformly pale brown); rachis scales without marginal projections (except for some plants from the Chatham Islands) ..... ***P. vestitum***
   Indusia with an obvious dark centre; stipe scales concolourous (uniformly dark brown, or pale brown), or if bicolourous not with a pale brown margin completely encompassing a dark brown centre; rachis scales usually with marginal projections 8
8. Scales from the stipe-rachis junction > 750 μm (and usually > 1000 μm) wide at their mid-length, usually pentagonal; spores small (spore exine 36-48 × 27-36 μm) ..... ***P. oculatum***
   Scales from the stipe-rachis junction < 650 μm wide at their mid-length, generally acicular-lanceolate; spores large (spore exine 46-58 × 36-45 μm) ..... (***P. neozelandicum***) 9
9. Dark centre of indusia usually occupying > 30% of surface area, often much more so; from Kawhia and the Bay of Plenty northwards ..... ***P. neozelandicum*** subsp. ***neozelandicum***
   Dark centre of indusia usually occupying < 30% of surface area; from Taranaki, Taupo, and the Urewera Ranges southwards ..... ***P. neozelandicum*** subsp. ***zerophyllum***

7.3 Future Work.

The body of work presented in this thesis suggests a number of possible avenues for future investigation. Some of these are discussed below.

7.3.1 Molecular Markers.

The robust delimitation of lineages requires the ability to assay polymorphism in multiple, independent characters from throughout the genome. AFLP DNA fingerprinting is thought, for the most part, to meet this requirement. However, there may be a desire to go beyond the fingerprinting of the genome *per se*. For instance, sequence variation at particular AFLP loci may be characterised as SCAR (Sequence-Characterised Amplified Region) markers for further evaluation (eg. McLenachan et al. 2000; see also Lockhart & McLenachan 1997, Lockhart et al. 2001). Variation in the sequence itself amongst different taxa may be used to reconstruct the evolutionary relationships of the alleles concerned (eg. Lockhart et al. 2001).

Sequencing multiple loci in many individuals is very expensive, and a cheaper (in the long-run) alternative to this is to SCAR many AFLP loci. Primers could then be designed which encompass both the AFLP linkers and the internal sequence of the SCAR locus. These primers could then recapitulate the presence or absence of the original AFLP locus bands when amplified from restricted DNA, producing binary-SCAR-AFLP markers. This approach does not appear to exist in the literature, but it has several advantages. Such binary-SCAR-AFLP markers could be assayed singularly, such that their scoring would be demonstrably unambiguous. They could be assayed on agarose rather than polyacrylamide gels, and their robust amplification, involving a single locus rather than multiple loci, is more likely than for AFLP-PCR from poorer quality templates. They could be assayed in numerous samples (no technical limit to sample size). Of course, there is no guarantee that such markers, whether they be assayed for presence or absence, or sequenced in full, are genomically independent. This can really only be determined by crossing experiments, but in a general sense, the more chromosomes the organism of interest has, the more likely a given pair of markers will segregate independently.

SCAR-AFLP markers could complement other 'easily-assayable' marker systems, such as low-haplotype microsatellites and chloroplast 'indels,' to provide a large suite of characters for the delimitation of lineages amongst SO organisms. Of course, such markers are unlikely to be general in their application (ie. a given marker is likely to only work in the specific group in which it was designed). Consequently, the development of such a suite of markers is likely to only be cost-effective in groups that require detailed study, or are particularly contentious. Straight AFLP, as implemented in this thesis, with its assaying of multiple characters is likely to be a more cost efficient molecular method for the testing of lineage boundaries.

7.3.2 New Zealand *Polystichum*.

- The ecological context in which the morphological variation within *P. vestitum* is exhibited requires study.
- The geographic boundary between the two octoploid lineages of *P. neozelandicum* remains uncertain. *P. neozelandicum* appears to be rare, if not absent from the

Waikato between Hamilton (subsp. *neozelandicum*) and Taupo (subsp. *zerophyllum*). However, the situation within the Bay of Plenty is unknown.

- The size of spores in plants of *P. cystostegia* from Taranaki and Kaikoura was found to be comparable (if not bigger) to those of the octoploid *P. neozelandicum*, and much bigger than the tetraploids *P. wawranum*, *P. oculatum*, *P. silvaticum*, and *P. vestitum*. However, *P. cystostegia* is currently regarded as a tetraploid (Brownlie 1958, Dawson et al. 2000). Interestingly, the spore size of *P. cystostegia* (for plants from Nelson & Arthur's Pass) given by Large and Braggins (1991) is similar to that which they report for *P. silvaticum* and *P. vestitum*. This suggests that two ploidy levels may be present in *P. cystostegia*.
- The finding of a seemingly at least partially fertile (in that some of its spores appear normal), putative hybrid (*sXvAka1*) between *P. silvaticum* and *P. vestitum* from the Akatawara Ranges presents several exciting opportunities to investigate the reproductive and ecological barriers between these sympatric lineages. Preliminary genetic study suggests this plant may not be an F_1 hybrid, although more detailed study of the parental populations would be required to confirm this. While hybridisation in ferns is generally considered to be limited to the F_1 generation, back-crossing hybrid swarms are known from ecotonal regions between the North American diploids *P. munitum* and *P. imbricans* (Mayer & Mesler 1993, Mullenniex et al. 1999, Kentner & Mesler 2000).

Further, the abnormally developed indusia of *sXvAka1* may be deserving of further study. The reduction of the indusium to scale-like paraphyses in this putative cross between an exindusiate parent and an indusiate parent is suggestive that the peltate indusia of *Polystichum*, and indeed the 'dryopteroid' ferns in general, may be developmentally homologous to a scale (or group of scales).

7.3.3 New Zealand Ferns in General.

A few (known) taxa remain undescribed in the New Zealand fern flora (eg. from the genera *Tmesipteris*, *Lastreopsis*; also the polyploid series in *Blechnum fluviatile*). Evaluation of their taxonomic status may be amenable to similar methodology to that employed here in *Polystichum*, although several undescribed taxa are part of large, widespread complexes (eg. *Nephrolepis*, *Christella*) requiring worldwide revisions. In any case, genetic typing might be developed as a less intrusive method for

distinguishing the weedy *N. cordifolia* (L.) C. Presl from the morphologically similar, but undescribed native *Nephrolepis* taxon (which are otherwise best separated on their respective presence or absence of underground tubers).

Some of the larger fern genera in New Zealand provide excellent opportunities for testing biogeographic and evolutionary hypotheses, *Asplenium* in particular. For instance, do the several species and species-groups of *Asplenium* shared between New Zealand and Australia (Brownsey 2001a) represent ancient vicariance or recent dispersal? In each of the groups characterised by polyploidy, has chromosome doubling occurred once or several times?

7.3.4 The Specific Mate Recognition System (SMRS) in Plants.

Study of the functioning of the SMRS in plants would be extremely informative in the understanding of 'speciation.' This thesis has inferred the presence of SMRS differentiation between *P. silvaticum*, *P. vestitum*, *P. wawranum*, *P. oculatum*, and *P. neozelandicum*, but can it be detected directly? The interplay between SMRS differentiation and ecological differentiation would also be interesting to investigate in the ferns *Asplenium bulbiferum* G. Forst and *A. flaccidum* G. Forst. These taxa are widely sympatric, ecologically differentiated (terrestrial and epiphytic, respectively), and morphologically quite distinct. Their hybrid is one of, if not the, most commonly encountered in the New Zealand fern flora, and usually occurs only in their 'ecotone,' at the base of a tree trunk.

The hermaphroditic gametophyte of ferns may not make them the easiest plants in which to study SMRS differentiation. Other groups of New Zealand plants, like the charismatic, well-studied *Hebe*, and the dioecious *Coprosma* may be more amenable to this kind of study. Of particular interest is the prediction of SMRS conservation, or stability, amongst the individuals of even widespread lineages. If true, for instance, male gametophytes of plants from Kaitaia should engender similar levels of successful fertilisation to those from Invercargill in female gametophytes from the latter population, and vice versa, given that these plants all belong to the same lineage (or, at least evidence cannot be found to reject this assumption). This last caveat necessitates that such study should be carried out within an explicitly lineage-based framework.

References.

Allan HH. 1961. Flora of New Zealand, Volume I. Government Printer, Wellington.

Anttila CK, Daehler CC, Rank NE, & Strong DR. 1998. Greater male fitness of a rare invader (*Spartina alterniflora*, Poaceae) threatens a common native (*Spartina foliosa*) with hybridisation. *American Journal of Botany* 85: 1597-1601.

Anttila CK, King RA, Ferris C, Ayres DR, & Strong DR. 2000. Reciprocal hybrid formation of *Spartina* in San Franciso Bay. *Molecular Ecology* 9: 765-770.

Arnold ML. 1997. Natural Hybridisation and Evolution. Oxford University Press, New York.

Avise JC. 1994. Molecular Markers, Natural History, and Evolution. Chapman & Hall, London.

Avise JC, & Ball RM. 1990. Principles of genealogical concordance in species concepts and biological taxonomy. *In* Futuyma D, & Antonovics J (eds.), p.45-67. Oxford Surveys In Evolutionary Biology, Vol. 7. Oxford University Press.

Avise JC, & Johns GC. 1999. Proposal for a standardised temporal scheme of biological classification for extant species. *Proceedings of the National Academy of Sciences USA* 96: 7358-7363.

Avise JC, & Wollenberg K. 1997. Phylogenetics and the origin of species. *Proceedings of the National Academy of Sciences USA* 94: 7748-7755.

Ayres DR, & Strong DR. 2001. Origin and genetic diversity of *Spartina anglica* (Poaceae) using nuclear DNA markers. *American Journal of Botany* 88: 1863-1867.

Bandelt HJ, & Dress AWM. 1992. Split decomposition: a new and useful approach to phylogenetic analysis of distance data. *Molecular Phylogenetics & Evolution* 1: 242-252.

Barraclough TG, & Vogler AP. 2000. Detecting the geographical pattern of speciation from species-level phylogenies. *American Naturalist* 155: 419-434.

Barrington DS. 1985. The present evolutionary and taxonomic status of the fern genus *Polystichum*: The 1984 Botanical Society of America Pteridophyte section symposium. *American Fern Journal* 75: 22-28.

Barrington DS. 1989. New species and combinations in tropical American *Polystichum* (Dryopteridaceae). *Annals of the Missouri Botanical Gardens* 76: 365-373.

Barrington DS. 1990. Hybridisation and allopolyploidy in Central American *Polystichum*: Cytological and isozyme documentation. *Annals of the Missouri Botanical Gardens* 77: 297-305.

Barrington DS. 1993. Ecological and historical factors in fern biogeography. *Journal of Biogeography* 20: 275-280.

Barrington DS, Paris CA, & Ranker TA. 1986. Systematic inferences from spore and stomate size in the ferns. *American Fern Journal* 76: 149-159.

Bartholomew B, Eaton LC, & Raven PH. 1973. *Clarkia rubicunda*: a model of plant evolution in semiarid regions. *Evolution* 27: 505-517.

Barton NH, & Hewitt GM. 1985. Analysis of hybrid zones. *Annual Review of Ecology & Systematics* 16: 113-148.

Baum DA. 1992. Phylogenetic species concepts. *Trends in Ecology & Evolution* 7: 1-2.

Baum DA, & Donoghue MJ. 1995. Choosing among alternative "phylogenetic" species concepts. *Systematic Botany* 20: 560-573.

Baum DA, & Shaw KL. 1995. Genealogical perspectives on the species problem. *In* Hoch PC, & Stephenson AG (eds.), p.289-303. Experimental and Molecular Approaches to Plant Biosystematics. Missouri Botanical Gardens, St. Louis.

Bodénès C, Labbé T, Pradère S, & Kremer A. 1997. General vs. local differentiation between two closely related white oak species. *Molecular Ecology* 6: 713-724.

Brower AVZ. 1999. Delimitation of phylogenetic species with DNA sequences: a critique of Davis and Nixon's population aggregation analysis. *Systematic Biology* 48: 199-213.

Brower AVZ, DeSalle R, & Vogler A. 1996. Gene trees, species trees, and systematics: a cladistic perspective. *Annual Review of Ecology & Systematics* 27: 423-450.

Brownlie G. 1954. Introductory note to cyto-taxonomic studies of New Zealand ferns. *Transactions of the Royal Society of New Zealand* 82: 665-666.

Brownlie G. 1958. Chromosome numbers in New Zealand ferns. *Transactions of the Royal Society of New Zealand* 85: 213-216.

Brownsey PJ. 1977a. A taxonomic revision of the New Zealand species of *Asplenium*. *New Zealand Journal of Botany* 15: 39-86.

Brownsey PJ. 1977b. *Asplenium* hybrids in the New Zealand flora. *New Zealand Journal of Botany* 15: 601-637.

Brownsey PJ. 1981. A biosystematic study of a wild population of *Leptopteris* hybrids in New Zealand. *New Zealand Journal of Botany* 19: 343-352.

Brownsey PJ. 1985a. *Asplenium chathamense* – a new fern species from the Chatham Islands, New Zealand. *New Zealand Journal of Botany* 23: 135-140.

Brownsey PJ. 1985b. Biosystematics of the cryptogamic flora of New Zealand: Pteridophytes. *New Zealand Journal of Botany* 23: 681-685.

Brownsey PJ. 1988. Pteridophyta. *In* Webb CJ, Sykes WR, & Garnock-Jones PJ (eds.), p.2-37. Flora of New Zealand, Volume IV. Botany Division, DSIR, Christchurch.

Brownsey PJ. 2001a. New Zealand's pteridophyte flora – plants of ancient lineage but recent arrival? *Brittonia* 53: 284-303.

Brownsey PJ. 2001b. Recent pteridophytes. *In* Gordon D (ed.). The New Zealand Inventory of Biodiversity: A Species 2000 Symposium Review. (In press.)

Brownsey PJ, & de Lange PJ. 1997. *Asplenium cimmeriorum*, a new fern species from New Zealand. *New Zealand Journal of Botany* 35: 283-292.

Brownsey PJ, & Smith-Dodsworth JC. 1989. New Zealand Ferns and Allied Plants. David Bateman Ltd., Auckland.

Brownsey PJ, & Smith-Dodsworth JC. 2000. New Zealand Ferns and Allied Plants. 2nd edition. David Bateman Ltd., Auckland.

Brownsey PJ, Given DR, & Lovis JD. 1985. A revised classification of New Zealand pteridophytes with a synonymic checklist of species. *New Zealand Journal of Botany* 23: 431-489.

Brummitt RK, & Powell CE. 1992. Authors of Plant Names. Kew, Royal Botanic Gardens.

Bush GL. 1969. Sympatric host race formation and speciation in frugivorous flies of the genus *Rhagoletis* (Diptera: Tephritidae). *Evolution* 23: 237-251.

Butlin RK. 1987a. Species, speciation, and reinforcement. *The American Naturalist* 130: 461-464.

Butlin RK. 1987b. Speciation by reinforcement. *Trends in Ecology & Evolution* 2: 8-13.

Cain ML, Milligan BG, & Strand AE. 2000. Long-distance seed dispersal in plant populations. *American Journal of Botany* 87: 1217-1227.

Cameron EK, de Lange PJ, Given DR, Johnson PN, & Ogle CC. 1995. New Zealand Botanical Society threatened and local plant lists (1995 revision). *New Zealand Botanical Society Newsletter* 39: 15-28.

Carney SE, Cruzan MB, & Arnold ML. 1994. Reproductive interaction between hybridising irises: analyses of pollen tube growth and fertilisation success. *American Journal of Botany* 81: 1169-1175.

Carney SE, Hodges SA, & Arnold ML. 1996. Effects of pollen-tube growth and ovule position on hybridisation in the Louisiana irises. *Evolution* 50: 1871-1878.

Charleston MA. 1997. Spectrum 2.0. http://taxonomy.zoology.gla.ac.uk/~mac/spectrum/spectrum.html

Charleston MA. 1998. Spectrum: spectral analysis of phylogenetic data. *Bioinformatics* 14: 98-99.

Charleston MA, & Page RDM. 1999. Spectral analysis – a brief introduction. *In* Hollingsworth PM, Bateman RM, & Gornall RJ (eds.), p.255-267. Molecular systematics and plant evolution. Taylor & Francis, London.

Charleston MA, Hendy MD, & Penny D. 1994. The effects of sequence length, tree topology, and number of taxa on the performance of phylogenetic methods. *Journal of Computational Biology* 1: 133-151.

Chase MW, & Hills HH. 1991. Silica gel: an ideal material for field preservation of leaf samples for DNA studies. *Taxon* 40: 215.

Chen FC, & Li WH. 2001. Genomic divergences between humans and other hominoids and the effective population size of the common ancestor of humans and chimpanzees. *American Journal of Human Genetics* 68: 444-456.

Christensen C. 1905. Index Filicum. Hagerup, Hafniae.

Claridge MF, Dawah HA, & Wilson MR (eds.). 1997. Species. The Units of Biodiversity. Chapman & Hall, London.

Clark JS, Fastie C, Hurtt G, Jackson ST, Johnson C, King GA, Lewis M, Lynch J, Pacala S, Prentice C, Schupp EW, Webb T, & Wyckoff P. 1998. Reid's paradox of rapid plant migration. *BioScience* 48: 13-24.

Clarkson BD. 1991. Ecology and conservation of heart-leaved kohuhu (*Pittosporum obcordatum* s.s.) in the East Coast Regional Conservancy. DSIR Land Resources Contract Report 91/57, Part 1. DSIR Land Resources, Lower Hutt.

Colenso WJ. 1897. A description of some new indigenous New Zealand forest ferns. *Transactions and Proceedings of the New Zealand Institute* 29: 414-421.

Collinson ME. 1996. "What use are fossil ferns?" – 20 years on: with a review of the fossil history of extant pteridophyte families and genera. *In* Camus JM, Gibby M, & Johns RJ (eds.), p.349-394. Pteridology in Perspective. Royal Botanic Gardens, Kew.

Congiu L, Dupanloup I, Patarnello T, Fontana F, Rossi R, Arlati G, & Zane L. 2001. Identification of interspecific hybrids by amplified fragment length polymorphism: the case of sturgeon. *Molecular Ecology* 10: 2355-2359.

Cooley JR, Simon C, Marshall DC, Slon K, & Ehrhardt C. 2001. Allochronic speciation, secondary contact, and reproductive character displacement in periodical cicadas (Hemiptera: *Magicicada* spp.): genetic, morphological, and behavioural evidence. *Molecular Ecology* 10: 661-671.

Coyne JA, & Orr HA. 1989. Patterns of speciation in *Drosophila*. *Evolution* 43: 362-381.

Coyne JA, Orr HA, & Futuyma DJ. 1988. Do we need a new species concept? *Systematic Zoology* 37: 190-200.

Cracraft J. 1983. Species concepts and speciation analysis. *Current Ornithology* 1: 159-187.

Crandall KA, Bininda-Emonds ORF, Mace GM, & Wayne RK. 2000. Considering evolutionary processes in conservation biology. *Trends in Ecology & Evolution* 15: 290-295.

Crisp P, Miskelly C, & Sawyer J. 2000. Endemic plants of the Chatham Islands. Department of Conservation, Wellington.

Cronquist A. 1978. Once again, what is a species? *In* Romberger JA (ed.), p.3-22. [2] Biosystematics in Agriculture. Allanheld, Osmun & Co. Publishers, Montclair.

Crookes ME. 1963. New Zealand Ferns, 6th edition. Incorporating illustrations and original work of Dobbie HB. Whitcombe & Tombs, Christchurch.

Crozier RH. 1997. Preserving the information content of species: genetic diversity, phylogeny, and conservation worth. *Annual Review of Ecology and Systematics* 28: 243-268.

Daigobo S. 1972. Taxonomical studies on the fern genus *Polystichum* in Japan, Ryukyu, and Taiwan. *Scientific Report of the Tokyo Bunrika Daigaku, Section B* 15: 57-80.

Darwin C. 1868. The Variation of Animals and Plants Under Domestication. John Murray, London.

Darwin C. 1900. The Effects of Cross and Self Fertilisation in the Vegetable Kingdom. John Murray, London.

Davis JI, & Manos PS. 1991. Isozyme variation and species delimitation in the *Puccinellia nuttalliana* complex (Poaceae): an application of the phylogenetic species concept. *Systematic Botany* 16: 431-445.

Davis JI, & Nixon KC. 1992. Populations, genetic variation, and the delimitation of phylogenetic species. *Systematic Biology* 41: 421-435.

Dawson JW. 1991. New Zealand botany with a difference – the Chatham Islands. *Tuatara* 31: 23-42.

Dawson MI, Brownsey PJ, & Lovis JD. 2000. Index of chromosome numbers of indigenous New Zealand pteridophytes. *New Zealand Journal of Botany* 38: 25-46.

de Lange PJ, Heenan PB, Given DR, Norton DA, Ogle CC, Johnson PN, & Cameron EK. 1999a. Threatened and uncommon plants of New Zealand. *New Zealand Journal of Botany* 37: 603-628.

de Lange PJ, Sawyer JWD, & Ansell R. 1999b. Checklist of indigenous vascular plant species recorded from Chatham Islands. Department of Conservation, Wellington.

de Queiroz K. 1998. The general lineage concept of species, species criteria, and the process of speciation. *In* Howard DJ, & Berlocher SH (eds.), p.57-75. Endless Forms: Species and Speciation. Oxford University Press, New York.

de Queiroz K, & Donoghue MJ. 1990. Phylogenetic systematics and species revisited. *Cladistics* 6: 83-90.

DeSalle R, & Vogler AP. 1994. Phylogenetic analysis on the edge: the application of cladistic techniques at the population level. *In* Golding B (eds.), p.154-174. Non-Neutral Evolution. Chapman & Hall, New York.

Diels L. 1899. Polypodiaceae. *In* Engler A, & Prantl K, p.139-339. Die Naturlichen Pflanzenfamilien. Edition 1, volume 1(4). W. Engelmann, Leipzig.

Dimmick WW, Ghedotti MJ, Grose MJ, Maglia AM, Meihhardt DJ, & Pennock DS. 1999. The importance of systematic biology in defining units of conservation. *Conservation Biology* 13: 653-660.

Dobzhansky T. 1941. Genetics and the Origin of Species. 2nd edition. Columbia University Press, New York.

Dobzhansky T. 1970. Genetics of the Evolutionary Process. Columbia University Press, New York.

Dobzhansky T. 1976. Organismic and molecular aspects of species formation. *In* Ayala FJ (ed.), p.95-105. Molecular Evolution. Sinauer Associates, Sunderland, Massachusetts.

Donoghue MJ. 1985. A critique of the biological species concept and recommendations for a phylogenetic alternative. *The Bryologist* 88: 172-181.

Donoghue MJ, & Cantino PD. 1988. Paraphyly, ancestors, and the goals of taxonomy: a botanical defence of cladism. *The Botanical Review* 54: 107-128.

Doyle JJ. 1995. The irrelevance of allele tree topologies for species delimitation, and a non-topological alternative. *Systematic Botany* 20: 574-588.

Doyle JJ, & Doyle JL. 1990. Isolation of plant DNA from fresh tissue. *Focus* 12: 13-15.

Ehrlich PR, & Raven PH. 1969. Differentiation of populations. *Science* 165: 1228-1232.

Emms SK, Hodges SA, & Arnold ML. 1996. Pollen-tube competition, siring success, and consistent asymmetric hybridisation in Louisiana irises. *Evolution* 50: 2201-2206.

Ennos RA, Sinclair WT, Hu XS, & Langdon A. 1999. Using organelle markers to elucidate the history, ecology and evolution of plant populations. *In* Hollingsworth PM, Bateman RM, & Gornall RJ (eds.), p.1-19. Molecular Systematics and Plant Evolution. Taylor & Francis, London.

Enting B, & Molloy L. 1982. The Ancient Islands: New Zealand's Natural Environments. Port Nicholson Press, Wellington.

Farr ER, Leussink JA, & Staflue FA. 1979. Index nominum genericorum (plantarum). Volume III. *Regnum Vegetabile* 102: 1277-1896.

Farris JS. 1983. Distance data in phylogenetic analysis. *In* Funk VA, & Brooks DR (eds.), p.3-23. Advances In Cladistics. The New York Botanical Garden, New York.

Feder JL. 1998. The apple maggot fly, *Rhagoletis pomonella*: flies in the face of conventional wisdom about speciation? *In* Howard DJ, & Berlocher SH (eds.), p.130-144. Endless Forms: Species and Speciation. Oxford University Press, New York.

Frey W, Stech M, & Meissner K. 1999. Chloroplast DNA-relationship in palaeoaustral *Lopidium concinnum* (Hypopterygiaceae, Musci). An example of stenoevolution in mosses. Studies in austral temperate rain forest bryophytes 2. *Plant Systematics & Evolution* 218: 67-75.

Gibco BRL: AFLP Analysis System I. AFLP Starter Primer Kit. Instruction Manual. http://www.invitrogen.com/Content/Tech-Online/molecular_biology/manuals_pps/10544013.pdf

Gibson G. 2000. *Hox* genes and the cellared wine principle. *Current Biology* 10: 452-455.

Given DR, & Williams PA. 1985. Conservation of Chatham Island flora and vegetation. (Reprinted with amendments.) Botany Division, DSIR, Christchurch.

Givnish TJ, & Sytsma KJ. 1997. Homoplasy in molecular vs. morphological data: the likelihood of correct phylogenetic inference. *In* Givnish TJ, & Sytsma KJ (eds.), p.55-101. Molecular Evolution and Adaptive Radiation. Cambridge University Press, Cambridge.

Goldstein PZ, & DeSalle R. 2000. Phylogenetic species, nested hierarchies, and character fixation. *Cladistics* 16: 364-384.

Goldstein PZ, DeSalle R, Amato G, & Vogler AP. 2000. Conservation genetics at the species boundary. *Conservation Biology* 14: 120-131.

Golenberg EM, Clegg MT, Durbin ML, Doebley J, & Ma DP. 1993. Evolution of a noncoding region of the chloroplast genome. *Molecular Phylogenetics & Evolution* 2: 52-64.

Goulding JH. 1978. Annotated checklist of type specimens of New Zealand plants in the Auckland Institute and Museum herbarium. *Records of the Auckland Institute and Museum* 15: 37-44.

Grady JM, & Quattro JM. 1999. Using character concordance to define taxonomic and conservation units. *Conservation Biology* 13: 1004-1007.

Gray DA, & Cade WH. 2000. Sexual selection and speciation in field crickets. *Proceedings of the National Academy of Sciences USA* 97: 14449-14454.

Harper AA, & Lambert DM. 1983. The population genetics of reinforcing selection. *Genetica* 62: 15-23.

Harris W, Beever RE, & Smallfield B. 2001. Variation in response to cold damage by populations of *Cordyline australis* and of some other species of *Cordyline* (Lomandraceae). *New Zealand Journal of Botany* 39: 147-159.

Harrison RG. 1998. Linking evolutionary pattern and process. *In* Howard DJ, & Berlocher SH (eds.), p.19-31. Endless Forms: Species and Speciation. Oxford University Press, New York.

Hasebe M, Omori T, Nakazawa M, Sano T, Kato M, Iwatsuki K. 1994. *rbcL* gene sequences provide evidence for the evolutionary lineages of leptosporangiate ferns. *Proceedings of the National Academy of Sciences USA* 91: 5370-5374.

Hasebe M, Wolf PG, Pryer KM, Ueda K, Ito M, Sano R, Gastony GJ, Yokoyama J, Manhart JR, Murakami N, Crane EH, Haufler CH, & Hauk WD. 1995. Fern phylogeny based on *rbcL* nucleotide sequences. *American Fern Journal* 85: 134-181.

Haufler CH. 1996. Species concepts and speciation in pteridophytes. *In* Camus JM, Gibby M, & Johns RJ. (eds.), p.291-305. Pteridology in Perspective. Royal Botanic Gardens, Kew.

Hauk WD. 1995. A molecular assessment of relationships among cryptic species of *Botrychium* subgenus *Botrychium* (Ophioglossaceae). *American Fern Journal* 85: 375-394.

Hedren M, Fay MF, & Chase MW. 2001. Amplified fragment length polymorphisms (AFLP) reveal details of polyploid evolution in *Dactylorhiza* (Orchidaceae). *American Journal of Botany* 88: 1868-1880.

Henderson NR, & Lambert DM. 1982. No significant deviation from random mating of world-wide populations of *Drosophila melanogaster*. *Nature* 300: 437-440.

Hennig W. 1966. Phylogenetic Systematics. University of Illinois Press, Urbana.

Hey J. 2001. The mind of the species problem. *Trends in Ecology & Evolution* 16: 326-329.

Hodkinson TR, Renvoize SA, Chonghaile GN, Stapleton CMA, & Chase MW. 2000. A comparison of ITS nuclear rDNA sequence data and AFLP markers for phylogenetic studies in *Phyllostachys* (Bambusoideae, Poaceae). *Journal of Plant Research* 113: 259-269.

Hombon JB. 1844. Atlas, pl. 5, in Dumount D'Urville J. Voyage au Pôle Sud et dans l'Oceanie sur les corvettes l'Astrolabe et la Zelee. Paris.

Hooker JD. 1855. Flora Novae Zelandiae. Part II. Flowerless plants. Reeve, London.

Hooker WJ. 1863. Species Filicum, Volume 4. W. Pamplin, London.

Holmgren PK, Holmgren NH, & Barnett LC. 1990. Index Herbariorum. Part I: The Herbaria of the World. 8th ed. *Regnum Vegetabile 120.*

Howard DJ, Reece M, Gregory PG, Chu J, & Cain ML. 1998. The evolution of barriers to fertilisation between closely related organisms. *In* Howard DJ, & Berlocher SH (eds.), p.279-290. Endless Forms: Species and Speciation. Oxford University Press, New York.

Hudson RR. 1990. Gene genealogies and the coalescent process. *In* Futuyma D, & Antonovics J (eds.), p.1-44. Oxford Surveys In Evolutionary Biology, Vol. 7. Oxford University Press.

Hull DL. 1997. The ideal species concept - and why we can't get it. *In* Claridge MF, Dawah HA, & Wilson MR (eds.), p.357-380. Species: The Units of Biodiversity. Chapman & Hall, London.

Hull DL. 1999. On the plurality of species: questioning the party line. *In* Wilson RA (ed.), p.23-48. Species: New Interdisciplinary Essays. The MIT Press, Cambridge, Massachusetts.

Hurr KA, Lockhart PJ, Heenan PB, & Penny D. 1999. Evidence for the recent dispersal of *Sophora* (Leguminosae) around the Southern Oceans: molecular data. *Journal of Biogeography* 26: 565-577.

Husband BC, & Schemske DW. 2000. Ecological mechanisms of reproductive isolation between diploid and tetraploid *Chamerion angustifolium. Journal of Ecology* 88: 689-701.

Huson DH. 1997. Splitstree 2.4. ftp://ftp.uni-bielefeld.de/pub/math/splits/splitstree2/

Huson DH. 1998. SplitsTree: analysing and visualising evolutionary data. *Bioinformatics* 14: 68-73.

Johannesson K. 2001. Parallel speciation: a key to sympatric divergence. *Trends in Ecology & Evolution* 16: 148-153.

Jones DL. 1998. Dryopteridaceae. *In* Orchard AE (executive ed.), & McCarthy PM (volume ed.), p.393-418. Flora of Australia, Volume 48, Ferns, Gymnosperms and Allied Groups. CSIRO Publishing, Collingwood.

Kardolus JP, van Eck HJ, & van den Berg RG. 1998. The potential of AFLPs in biosystematics: a first application in *Solanum* taxonomy (Solanaceae). *Plant Systematics & Evolution* 210: 87-103.

Keller MJ, & Gerhardt HC. 2000. Polyploidy alters advertisement call structure in gray treefrogs. *Proceedings of the Royal Society of London, Series B* 268: 341-345.

Kentner EK, & Mesler MR. 2000. Evidence for natural selection in a fern hybrid zone. *American Journal of Botany* 87: 1168-1174.

Kirkpatrick M. 2001. Reinforcement during ecological speciation. *Proceedings of the Royal Society of London, Series B* 268: 1259-1263.

Klips RA. 1999. Pollen competition as a reproductive isolating mechanism between two sympatric *Hibiscus* species (Malvaceae). *American Journal of Botany* 86: 269-272.

Koopman WJM, Zevenbergen MJ, & van den Berg RG. 2001. Species relationships in *Lactuca* s.l. (Lactuceae, Asteraceae) inferred from AFLP fingerprints. *American Journal of Botany* 88: 1881-1887.

Kramer KU. 1990. Dryopteridaceae. *In* Kramer KU, & Green PS (eds.), p.101-143. The Families and Genera of Vascular Plants. Volume 1, Pteridophytes and Gymnosperms. Sringer-Verlag, Berlin.

Lambert DM, & Paterson HEH. 1982. Morphological resemblance and its relationship to genetic distance measures. *Evolutionary Theory* 5: 291-300.

Lambert DM, & Paterson HEH. 1984. On 'bridging the gap between race and species': the Isolation Concept and an alternative. *Proceedings of the Linnean Society of New South Wales* 107: 501-514.

Lambert DM, Kingett PD, & Slooten E. 1982. Intersexual selection: the problem and a discussion of the evidence. *Evolutionary Theory* 6: 67-78.

Lambert DM, Centner MR, & Paterson HEH. 1984. Simulation of the conditions necessary for the evolution of species by reinforcement. *South African Journal of Science* 80: 308-311

Lambert DM, Michaux B, & White CS. 1987. Are species self-defining? *Systematic Zoology* 36: 196-205.

Lan R, & Reeves PR. 2000. Intraspecies variation in bacterial genomes: the need for a species genome concept. *Trends in Microbiology* 8: 396-401.

Langton M. 2001. Spectronet Version 1.0. Massey University, Palmerston North.

Large MF, & Braggins JE. 1991. Spore Atlas of New Zealand Ferns & Fern Allies. SIR Publishing, Wellington.

Lento GM, Hickson RE, Chambers GK, & Penny D. 1995. Use of spectral analysis to test hypotheses on the origin of pinipeds. *Molecular Biology & Evolution* 12: 28-52.

Levin DA. 1983. Polyploidy and novelty in flowering plants. *The American Naturalist* 122: 1-25.

Levin DA. 1993. Local speciation in plants: the rule not the exception. *Systematic Botany* 18: 197-208.

Levin DA. 2000. The origin, expansion, and demise of plant species. Oxford University Press, New York.

Levin DA, Francisco-Ortega J, & Jansen RK. 1996. Hybridisation and the extinction of rare plant species. *Conservation Biology* 10: 10-16.

Lewin B. 1994. Genes V. Oxford University Press, Oxford.

Lewis H. 1961. Experimental sympatric populations of *Clarkia*. *The American Naturalist* 95: 155-168.

Lewis R. 2001. Molecular Studies of the New Zealand Tree Ferns. Unpublished MSc thesis, Massey University, Palmerston North.

Liou LW, & Price TD. 1994. Speciation by reinforcement of premating isolation. *Evolution* 48: 1451-1459.

Liu Z, Nichols A, Li P, & Dunham RA. 1998. Inheritance and usefulness of AFLP markers in channel catfish (*Ictalurus punctatus*), blue catfish (*I. furcatus*), and their F1, F2, and backcross hybrids. *Molecular & General Genetics* 258: 260-268.

Lloyd RM. 1974. Mating systems and genetic load in pioneer and non-pioneer Hawaiian Pteridophyta. *Botanical Journal of the Linnean Society* 69: 23-35.

Lockhart PJ, & McLenachan PA. 1997. Isolating polymorphic plant DNA fragments identified using AFLP technology without using acrylamide gels: markers for evolutionary studies. *Focus* 19: 70-71.

Lockhart PJ, Penny D, & Meyer A. 1995. Testing the phylogeny of swordtail fishes using split decomposition and spectral analysis. *Journal of Molecular Evolution* 41: 666-674.

Lockhart PJ, Steel MA, Barbrook AC, Huson DH, Charleston MA, & Howe CJ. 1998. A covariotide model explains apparent phylogenetic structure of oxygenic photosynthetic lineages. *Molecular Biology & Evolution* 15: 1183-1188.

Lockhart PJ, Howe CJ, Barbrook AC, Larkum AWD, & Penny D. 1999. Spectral analysis, systematic bias, and the evolution of chloroplasts. *Molecular Biology & Evolution* 16: 573-576.

Lockhart PJ, McLenachan PA, Havell D, Glenny D, Huson D, & Jensen U. 2001. Phylogeny, radiation and transoceanic dispersal of New Zealand alpine buttercups: molecular evidence under split decomposition. *Annals of the Missouri Botanical Gardens* 88: 458-477.

Loh JP, Kiew R, Hay A, Kee A, Gan LH, & Gan YY. 2000a. Intergenic and interspecific relationships in Araceae tribe Caladieae and development of molecular markers using Amplified Fragment Length Polymorphism (AFLP). *Annals of Botany* 85: 371-378.

Loh JP, Kiew R, Set O, Gan LH, & Gan YY. 2000b. A study of genetic variation and relationships within the bamboo subtribe Bambusinae using Amplified Fragment Length Polymorphism. *Annals of Botany* 85: 607-612.

Lovis JD. 1977. Evolutionary patterns and processes in ferns. *In* Preston RD, & Woolhouse HW (eds.), p.229-415. Advances in botanical research, vol. 4. Academic Press, London.

Luckow M. 1995. Species concepts: assumptions, methods, and applications. *Systematic Botany* 20: 589-605.

Macphail MK. 1997. Comment on M. Pole (1994): 'The New Zealand flora – entirely long-distance dispersal? *Journal of Biogeography* 24: 113-117.

Maddison W. 1995. Phylogenetic histories within and among species. *In* Hoch PC, & Stephenson AG (eds.), p.273-287. Experimental and Molecular Approaches to Plant Biosystematics. Missouri Botanical Gardens, St. Louis.

Maheswaran M, Subudhi PK, Nandi S, Xu JC, Parco A, Yang DC, & Huang N. 1997. Polymorphism, distribution, and segregation of AFLP markers in a doubled haploid rice population. *Theoretical & Applied Genetics* 94: 39-45.

Mallet J. 1995. A species definition for the modern synthesis. *Trends in Ecology & Evolution* 10: 294-299.

Martin PG, & Dowd JM. 1993. Using sequences of *rbcL* to study phylogeny and biogeography of *Nothofagus* species. *Australian Systematic Botany* 6: 441-447.

Martinsen GD, Whitham TG, Turek RJ, & Keim P. 2001. Hybrid populations selectively filter gene introgression between species. *Evolution* 55: 1325-1335.

Masters JC, & Spencer HG. 1989. Why we need a new genetic species concept. *Systematic Zoology* 38: 270-279.

Maughan PJ, Saghai Maroof MA, Buss GR, & Huestis GM. 1996. Amplified Fragment Length Polymorphism (AFLP) in soybean: species diversity, inheritance, and near-isogenic line analysis. *Theoretical & Applied Genetics* 93: 392-401.

Mayden RL. 1997. A hierarchy of species concepts; the denouement in the sage of the species problem. *In* Claridge MF, Dawah HA, & Wilson MR (eds.), p.381-424. Species. The Units of Biodiversity. Chapman & Hall, London.

Mayer MS, & Mesler MR. 1993. Morphometric evidence of hybrid swarms in mixed populations of *Polystichum munitum* and *P. imbricans* (Dryopteridaceae). *Systematic Botany* 18: 248-260.

Mayr E. 1942. Systematics and the Origin of Species. Columbia University Press, New York.

Mayr E. 1976. Evolution and the Diversity of Life. Harvard University Press, Cambridge, Massachusetts.

Mayr E. 1982. The Growth of Biological Thought. Harvard University Press, Cambridge, Massachusetts.

McGlone MS, Duncan RP, & Heenan PB. 2001. Endemism, species selection and the origin and distribution of the vascular plant flora of New Zealand. *Journal of Biogeography* 28: 199-216.

McKay JK, Bishop JC, Lin JZ, Richards JH, Sala A, & Mitchell-Olds T. 2001. Local adaptation across a climatic gradient despite small effective population size in the rare sapphire rockcress. *Proceedings of the Royal Society of London, Series B* 268: 1715-1721.

McLenachan PA, Stöckler K, Winkworth RC, McBreen K, Zauner S, & Lockhart PJ. 2000. Markers derived from amplified fragment length polymorphism gels for plant ecology and evolution studies. *Molecular Ecology* 9: 1899-1903.

McLoughlin S. 2001. The breakup history of Gondwana and its impact on pre-Cenozoic floristic provincialism. *Australian Journal of Botany* 49: 271-300.

Meier R, & Willmann R. 2000. The Hennigian species concept. *In* Wheeler QD, & Meier R (eds.), p.30-43. Species concepts and phylogenetic theory. Columbia University Press, New York.

Mes THM, Kuperus P, Kirschner J, Stepanek J, Oosterveld P, Storcova H, & den Nijs JCM. 2000. Hairpins involving both inverted and direct repeats are associated with homoplasious indels in non-coding chloroplast DNA of *Taraxacum* (Lactuceae: Asteraceae). *Genome* 43: 634-641.

Mildenhall DC. 1980. New Zealand late Cretaceous and Cenozoic plant biogeography: a contribution. *Palaeogeography, Palaeoclimatology, Paleoecology* 31: 197-233.

Mishler BD, & Brandon RN. 1987. Individuality, pluralism, and the phylogenetic species concept. *Biology & Philosophy* 2: 397-414.

Mishler BD, & Donoghue MJ. 1982. Species concepts: a case for pluralism. *Systematic Zoology* 31: 491-503.

Mishler BD, & Theriot EC. 2000a. The phylogenetic species concept (sensu Mishler and Theriot): monophyly, apomorphy, and phylogenetic species concepts. *In* Wheeler QD, & Meier R (eds.), p.44-54. Species concepts and phylogenetic theory. Columbia University Press, New York.

Mishler BD, & Theriot EC. 2000b. A critique from the Mishler and Theriot phylogenetic species concept perspective: monophyly, apomorphy, and phylogenetic species concepts. *In* Wheeler QD, & Meier R (eds.), p.119-132. Species concepts and phylogenetic theory. Columbia University Press, New York.

Mishler BD, & Theriot EC. 2000c. A defence of the phylogenetic species concept (sensu Mishler and Theriot): monophyly, apomorphy, and phylogenetic species concepts. *In* Wheeler QD, & Meier R (eds.), p.179-184. Species concepts and phylogenetic theory. Columbia University Press, New York.

Morrison DA. 1996. Phylogenetic tree-building. *International Journal of Parasitology* 26: 589-617.

Mueller UG, & Wolfenbarger LL. 1999. AFLP genotyping and fingerprinting. *Trends in Ecology & Evolution* 14: 389-394.

Muir G, Fleming CC, & Schlötterer C. 2000. Species status of hybridising oaks. *Nature* 405: 1016.

Mullenniex A, Hardig TM, & Mesler MR. 1999. Molecular confirmation of hybrid swarms in the fern genus *Polystichum* (Dryopteridaceae). *Systematic Botany* 23: 421-426.

Nelson G. 1975. Reviews: Biogeography, the vicariance paradigm, and continental drift. *Systematic Zoology* 24: 490-504.

Nichols R. 2001. Gene trees and species trees are not the same. *Trends in Ecology & Evolution* 16: 358-364.

Nilsson NO, Hansen M, Panagopoulos AH, Tuvesson S, Edlde M, Christiansson M, Rading IM, Riessler M, & Kraft T. 1999. QTL analysis of *Cercospora* leaf spot resistance in sugar beat. *Plant Breeding* 118: 327-334.

Nixon KC, & Wheeler QD. 1990. An amplification of the phylogenetic species concept. *Cladistics* 6: 211-223.

Noor, MAF. 1999. Reinforcement and other consequences of sympatry. *Heredity* 83: 503-508.

Nordenskiöld H. 1971. Hybridisation experiments in the genus *Luzula*. *Hereditas* 68: 47-59.

O'Hanlon PC, & Peakall R. 2000. A simple method for the detection of size homoplasy among amplified fragment length polymorphism fragments. *Molecular Ecology* 9: 815-816.

O'Hara RJ. 1993. Systematic generalisation, historical fate, and the species problem. *Systematic Biology* 42: 231-246.

Orchard AE. 1993. Dryopteridaceae. *In* George AS, Orchard AE, & Hewson HJ (executive ed.), & Hewson HJ, & Thompson HS (scientific ed.), p.559. Flora of Australia, Volume 50, Oceanic Islands 2. CSIRO Publishing, Collingwood.

Orchard AE. 1994. Dryopteridaceae. *In* Orchard AE (executive ed.), & Wilson AJG (volume ed.), p.603-604. Flora of Australia, Volume 49, Oceanic Islands 1. Australian Government Publishing Service.

Palmer JD. 1992. Mitochondrial DNA in plant systematics: applications and limitations. *In* Soltis PS, Soltis DE, & Doyle JJ (eds.), p.36-49. Molecular Systematics of Plants. Chapman & Hall, New York.

Palumbi SR. 1998. Species formation and the evolution of gamete recognition loci. *In* Howard DJ, & Berlocher SH (eds.), p.271-278. Endless Forms: Species and Speciation. Oxford University Press, New York.

Palumbi SR. 1999. All mates are not created equal: Fertility differences depend on gamete recognition polymorphisms in sea urchins. *Proceedings of the National Academy of Sciences USA* 96: 12632-12637.

Panhuis TM, Butlin R, Zuk M, & Tregenza T. 2001. Sexual selection and speciation. *Trends in Ecology & Evolution* 16:364-371.

Paterson HEH. 1978. More evidence against speciation by reinforcement. *South African Journal of Science* 74: 369-371.

Paterson HEH. 1981. The continuing search for the unknown and unknowable: a critique of contemporary ideas on speciation. *South African Journal of Science* 77: 113-119.

Paterson HEH. 1982a. Perspective on speciation by reinforcement. *South African Journal of Science* 78: 53-57.

Paterson HEH. 1982b. Darwin and the origin of species. *South African Journal of Science* 78: 272-275.

Paterson HEH. 1985. The recognition concept of species. *In* Vrba ES (ed.), p.21-29. Species and Speciation. Transvaal Museum Monograph No. 4. Transvaal Museum.

Paterson HEH. 1986. Environment and species. *South African Journal of Science* 82: 62-65.

Paterson HEH. 1988. On defining species in terms of sterility: problems and alternatives. *Pacific Science* 42: 65-71.

Paterson HEH. 1989. A view of species. *In* Goodwin B, Sibatani A, & Webster G (eds.), p.77-88. Dynamic Structure in Biology. Edinburgh University Press, Edinburgh.

Paterson HEH. 1993. The term "isolating mechanisms" as a canaliser of evolutionary thought. *In* Patterson HEH: edited by McEvery SF, p.1-10. Evolution and the Recognition Concept of Species: Collected Writings. Chapter One. The John Hopkins University Press, Maryland.

Paterson HEH, & Macnamara M. 1984. The recognition concept of species: Macnamara interviews Paterson. *South African Journal of Science* 80: 312-318.

Patten MA, & Campbell KF. 2000. Typological thinking and the conservation of subspecies: the case of the San Clemente Island loggerhead shrike. *Diversity & Distributions* 6: 177-188.

Pearman PB. 2001. Conservation value of independently evolving units: sacred cow or testable hypothesis? *Conservation Biology* 15: 780-783.

Peck JH, Peck CJ, & Farrar DR. 1990. Influences of life history attributes on formation of local and distant fern populations. *American Fern Journal* 80: 126-142.

Pennock DS, & Dimmick WW. 1997. Critique of the evolutionary significant unit as a definition for "distinct population segments" under the U.S. endangered species act. *Conservation Biology* 11: 611-619.

Penny D, Hendy MD, & Steel MA. 1992. Progress with methods for constructing evolutionary trees. *Trends in Ecology & Evolution* 7: 73-79.

Penny D, Hendy MD, Lockhart PJ, & Steel MA. 1996. Corrected parsimony, minimum evolution, and hadamard conjugations. *Systematic Biology* 45: 596-606.

Perrie LR, Lockhart PJ, Brownsey PJ, & Large MF. 2000. Morphological and molecular concordance for the recognition of two species in the New Zealand *Polystichum richardii* (Hook.) J. Smith complex. *Plant Systematics & Evolution* 224: 97-107.

Piepho HP & Koch G. 2000. Codominant analysis of banding data from a dominant marker system by normal mixtures. *Genetics* 155: 1459-1468.

Pole M. 1994. The New Zealand flora – entirely long-distance dispersal? *Journal of Biogeography* 21: 625-635.

Pole MS. 2001. Can long-distance dispersal be inferred from the New Zealand plant fossil record. *Australian Journal of Botany* 49: 357-366.

Price CSC, Kim CH, Posluszny J, & Coyne JA. 2000. Mechanisms of conspecific sperm precedence in *Drosophila*. *Evolution* 54: 2028-2037.

Promega. 1998. Silver Sequence. DNA Sequencing System. Technical Manual. http://www.promega.com/tbs/Tm023/tm023.pdf

Pryer KM, Smith AR, & Skog JE. 1995. Phylogenetic relationships of extant ferns based on evidence from morphology and *rbcL* sequences. *American Fern Journal* 85: 205-282.

Pryer KM, Schneider H, Smith AR, Cranfill R, Wolf PG, Hunt JS, & Sipes SD. 2001a. Horsetails and ferns are a monophyletic group and the closest living relatives to seed plants. *Nature* 409: 618-622.

Pryer KM, Smith AR, Hunt JS, & Dubuisson JV. 2001b. *rbcL* data reveal two monophyletic groups of filmy ferns (Filicopsida: Hymenophyllaceae). *American Journal of Botany* 88: 1118-1130.

Ramsey J, & Schemske DW. 1998. Pathways, mechanisms, and rates of polyploid formation in flowering plants. *Annual Review of Ecology & Systematics* 29: 467-501.

Raven PH. 1973. Evolution of subalpine and alpine plant groups in New Zealand. *New Zealand Journal of Botany* 11: 177-200.

Raven PH, & Raven TE. 1976. The Genus *Epilobium* (Onagraceae) in Australasia: a systematic and evolutionary study. Bascands Ltd., Christchurch.

Renner SS, Foreman DB, & Murrary D. 2000. Timing transantarctic disjunctions in the Atherospermataceae (Laurales): Evidence from coding and noncoding chloroplast sequences. *Systematic Botany*: 49: 579-591.

Rhymer JM, & Simberloff D. 1996. Extinction by hybridisation and introgression. *Annual Review of Ecology & Systematics* 27: 83-109.

Ridley M. 1986. Evolution and classification: the reformation of cladism. Longman, London.

Ridley M. 1996. Evolution. 2nd edition. Blackwell Science, Cambridge, Massachusetts.

Rieseberg LH, & Brouillet L. 1994. Are many plant species paraphyletic? *Taxon* 43: 21-32.

Rieseberg LH, & Burke JM. 2001. The biological reality of species: gene flow, selection, and collective evolution. *Taxon* 50: 47-67.

Rieseberg LH, & Gerber D. 1995. Hybridisation in the Catalina Island mountain mahogany (*Cercocarpus traskiae*): RAPD evidence. *Conservation Biology* 9: 199-203.

Rieseberg LH, Desrochers AM, & Young SJ. 1995. Interspecific pollen competition as a reproductive barrier between sympatric species of *Helianthus* (Asteraceae). *American Journal of Botany* 85: 515-519.

Ritchie MG. 2001. Chronic speciation in periodical cicadas. *Trends in Ecology & Evolution* 16: 59-61.

Roth AW. 1799. Tentamen Florae Germanicae. Volume 3. Leipzig.

Roux JP. 2000. The genus *Polystichum* (Dryopteridaceae) in Africa. *Bulletin of Natural History of the Museum of London (Botany)* 30: 33-79.

Salmon TJ. 1980. The Native Trees of New Zealand. Reed, Wellington.

Schemske DW. 2000. Understanding the origin of species. *Evolution* 54: 1069-1073.

Schluter D. 2001. Ecology and the origin of species. *Trends in Ecology & Evolution* 16: 372-380.

Schwarzbach AE, Donovan LA, & Rieseberg LH. 2001. Transgressive character expression in a hybrid sunflower species. *American Journal of Botany* 88: 270-277.

Shaw KL. 1998. Species and the diversity of natural groups. *In* Howard DJ, & Berlocher SH (eds.), p.44-56. Endless Forms: Species and Speciation. Oxford University Press, New York.

Simon C, Tang J, Dalwadi S, Staley G, Deniega J, & Unnasch TR. 2000. Genetic evidence for assortative mating between 13-year cicadas and sympatric "17-year cicadas with 13-year life cycles" provides support for allochronic speciation. *Evolution* 54: 1326-1336.

Simpson GG. 1961. Principles of Animal Taxonomy. Columbia University Press, New York.

Sites JW, & Crandall KA. 1997. Testing species boundaries in biodiversity studies. *Conservation Biology* 11: 1289-1297.

Skog JE. 2001. Biogeography of Mesozoic leptosporangiate ferns related to extant ferns. *Brittonia* 53: 236-269.

Sleep A, & Reichstein T. 1967. Der farnbastard *Polystichum meyeri* hybr. nov. = *Polystichum braunii* (Spencer) Fée × *P. lonchitis* (L.) Roth und sine cytologie. *Bauhinia* 3: 299-309, 363-374.

Smith AR. 1995. Non-molecular phylogenetic hypotheses for ferns. *American Fern Journal* 85: 104-122.

Sneath PHA, & Sokal RR. 1973. Numerical Taxonomy. The Principles and Practice of Numerical Classification. Freeman, San Francisco.

Soltis DE, & Soltis PS. 1993. Molecular data and the dynamic nature of polyploidy. *Critical Reviews in Plant Sciences* 12: 243-273.

Soltis DE, & Soltis PS. 1999. Polyploidy: recurrent formation and genome evolution. *Trends in Ecology & Evolution* 14: 348-352.

Soltis PS, & Gitzendanner MA. 1999. Molecular systematics and the conservation of rare species. *Conservation Biology* 13: 471-483.

Soltis PS, & Soltis DE. 2000. The role of genetic and genomic attributes in the success of polyploids. *Proceedings of the National Academy of Sciences USA* 97: 7051-7057.

Soltis PS, Soltis DE, & Wolf PG. 1991. Allozymic and chloroplast DNA analyses of polyploidy in *Polystichum* (Dryopteridaceae). I. The origins of *P. californicum* and *P. scopulinum*. *Systematic Botany* 16: 245-256.

Song K, Lu P, Tang K, & Osborn TC. 1995. Rapid genome change in synthetic polyploids of *Brassica* and its implications for polyploid evolution. *Proceedings of the National Academy of Sciences USA* 92: 7719-7723.

Spencer HG, McArdle BH, & Lambert DM. 1986. A theoretical investigation of speciation by reinforcement. *The American Naturalist* 128: 241-262.

Spencer HG, Lambert DM, & McArdle BH. 1987. Reinforcement, species, and speciation: a reply to Butlin. *The American Naturalist* 130: 958-962.

SPSS. 2000. SPSS for windows, release 10.1.0.

Stevenson DW, & Loconte H. 1996. Ordinal and familial relationships of pteridophyte genera. *In* Camus JM, Gibby M, & Johns RJ. (eds.), p.435-467. Pteridology in Perspective. Royal Botanic Gardens, Kew.

Stöckler K. 2001. Origins and Evolution of the New Zealand Forest Flora: a Molecular Phylogenetic Approach. Unpublished PhD thesis, Massey University, Palmerston North.

Stuessy TF. 1990. Plant Taxonomy. Columbia University Press, New York.

Swanson WJ, & Vacquier VD. 2002. The rapid evolution of reproductive proteins. *Nature Reviews Genetics* 3: 137-144.

Swofford DL. 2001. PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4. Sinauer Associates, Sunderland, Massachusetts.

Swofford DL, Olsen GJ, Waddell PJ, & Hillis DM. 1996. Phylogenetic inference. *In* Hillis DM, Moritz C, & Mable BK (eds.), p.407-514. Molecular Systematics, 2nd edition. Sinauer Associates, Sunderland, Massachusetts.

Templeton AR. 1981. Mechanisms of speciation – a population genetic approach. *Annual Review of Ecology & Systematics* 12: 23-48.

Templeton AR. 1989. The meaning of species and speciation: a genetic perspective. *In* Otte D, & Endler JA (eds.), p.3-27. Speciation and its Consequences. Sinauer Associates, Sunderland, Massachusetts.

Templeton AR. 1994. The role of molecular genetics in speciation studies. *In* Schierwater B, Streit B, Wagner GP, & DeSalle R (eds.), p.261-273. Molecular Ecology and Evolution: Approaches and Applications. Birkhäuser Verlag Basel.

Templeton AR. 1998a. Nested clade analyses of phylogeographic data: testing hypotheses about gene flow and population history. *Molecular Ecology* 7: 381-397.

Templeton AR. 1998b. Species and speciation. *In* Howard DJ, & Berlocher SH (eds.), p.32-43. Endless Forms: Species and Speciation. Oxford University Press, New York.

Templeton AR. 2001. Using phylogeographic analyses of gene trees to test species status and processes. *Molecular Ecology* 10: 779-791.

Templeton AR, Routman E, & Phillips CA. 1995. Separating population structure from population history: a cladistic analysis of the geographical distribution of mitochondrial DNA haplotypes in the tiger salamander, *Ambystoma tigrinum*. *Genetics* 140: 767-782.

Templeton AR, Maskas SD, & Cruzan MB. 2000. Gene trees: a powerful tool for exploring the evolutionary biology of species and speciation. *Plant Species Biology* 15: 211-222.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, & Higgins DG. 1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* 24: 4876-4882.

Turelli M, Barton NH, & Coyne JA. 2001. Theory and speciation. *Trends in Ecology & Evolution* 16: 330-343.

Van Valen L. 1976. Ecological species, multispecies, and oaks. *Taxon* 25: 233-239.

Via S. 2001. Sympatric speciation in animals: the ugly duckling grows up. *Trends in Ecology & Evolution* 16: 381-390.

Vogel JC, Russell SJ, Rumsey FJ, Barett JA, & Gibby M. 1998. On hybrid formation in the rock fern *Asplenium* x *alternifolium* (Aspleniaceae, Pteridophyta). *Botanica Acta* 111: 241-246.

Vogel JC, Barrett JA, Rumsey FJ, & Gibby M. 1999. Identifying multiple origins in polyploid homosporous pteridophytes. *In.* Hollingsworth PM, Bateman RM, & Gornall RJ (eds.), p.101-117. Molecular Systematics and Plant Evolution. Taylor & Francis, London.

Vos P, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, & Zabeau M. 1995. AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Research* 23: 4407-4414.

Vrana P, & Wheeler W. 1992. Individual organisms as terminal entities: laying the species problem to rest. *Cladistics* 8: 67-72.

Vrba ES. 1995. Species as habitat-specific, complex systems. *In* Lambert DM, & Spencer HG (eds.), p.3-44. Speciation and the Recognition Concept. John Hopkins University Press, Baltimore.

Waddell PJ, Penny D, Hendy MD, & Arnold G. 1994. Variance-covariance matrices for evolutionary trees using Hadamard transforms. *Molecular Biology & Evolution* 11: 630-642.

Wagner DH. 1979. Systematics of *Polystichum* in western North America north of Mexico. *Pteridologia* 1: 1-61.

Wagstaff SJ, & Garnock-Jones PJ. 1998. Evolution and biogeography of the *Hebe* complex (Scrophulariaceae) inferred from ITS sequences. *New Zealand Journal of Botany* 36: 425-437.

Wagstaff SJ, Heenan PB, & Sanderson MJ. 1999. Classification, origins, and patterns of diversification in New Zealand Carmichaelinae (Fabaceae). *American Journal of Botany* 86: 1346-1356.

Wardle P. 1963. Evolution and distribution of the New Zealand flora, as affected by Quaternary climates. *New Zealand Journal of Botany* 1: 3-17.

Wardle P. 1978. Origin of the New Zealand mountain flora, with special reference to trans-Tasman relationships. *New Zealand Journal of Botany* 16: 535-550.

Webb CJ, & Edgar E. 1999. Spelling New Zealand in the specific and infraspecific epithets of vascular plants. *New Zealand Journal of Botany* 37: 71-77.

Wheeler MJ, Franklin-Tong VE, & Franklin FCH. 2001. The molecular and genetic basis of pollen-pistil interactions. *New Phytologist* 151: 565-584.

Wheeler QD, & Meier R. 2000. Species Concepts and Phylogenetic Theory. A Debate. Columbia University Press, New York.

Wheeler QD, & Nixon KC. 1990. Another way of looking at the species problem: a reply to de Queiroz and Donoghue. *Cladistics* 6: 77-81.

Wheeler QD, & Platnick NI. 2000a. The phylogenetic species concept (sensu Wheeler and Platnick). *In* Wheeler QD, & Meier R (eds.), p.55-69. Species concepts and phylogenetic theory. Columbia University Press, New York.

Wheeler QD, & Platnick NI. 2000b. A critique from the Wheeler and Platnick phylogenetic species concept perspective: problems with alternative concepts of species. *In* Wheeler QD, & Meier R (eds.), p.133-145. Species concepts and phylogenetic theory. Columbia University Press, New York.

Wheeler QD, & Platnick NI. 2000c. A defence of the phylogenetic species concept perspective (sensu Wheeler and Platnick). *In* Wheeler QD, & Meier R (eds.), p.185-197. Species concepts and phylogenetic theory. Columbia University Press, New York.

White CS, Michaux B, & Lambert DM. 1990. Species and neo-Darwinism. *Systematic Zoology* 39: 399-413.

Wiley EO, & Mayden RL. 2000. The evolutionary species concept. *In* Wheeler QD, & Meier R (eds.), p.70-89. Species concepts and phylogenetic theory. Columbia University Press, New York.

Wiley EO. 1978. The evolutionary species concept reconsidered. *Systematic Zoology* 27: 17-26.

Williams GC. 1966. Adaptation and Natural Selection. Princeton University Press, Princeton, New Jersey.

Wilson RA (ed.). 1999. Species: New Interdisciplinary Essays. The MIT Press, Cambridge, Massachusetts.

Winkworth RC. 2000. Evolution of the New Zealand Alpine Flora: Origins, Diversification and Disperal. Unpublished PhD thesis, Massey University, Palmerston North.

Winkworth RC, Robertson AW, Ehrendorfer F, & Lockhart PJ. 1999. The importance of dispersal and recent speciation in the flora of New Zealand. *Journal of Biogeography* 26: 1323-1325.

Wolf PG. 1995. Phylogenetic analyses of *rbcL* and nuclear ribosomal RNA gene sequences in Dennstaedtiaceae. *American Fern Journal* 85: 306-327.

Wolf PG, Sipes SD, White MR, Martines ML, Pryer KL, Smith AR, & Ueda K. 1999. Phylogenetic relationships of the enigmatic fern families Hymenophyllopsidaceae and Lophosoriaceae: evidence from *rbcL* nucleotide sequences. *Plant Systematics & Evolution* 219: 263-270.

Wolf PG, Campbell DR, Waser NK, Sipes SD, Toler TR, & Archibald JK. 2001. Tests of pre- and post-pollination barriers to hybridisation between sympatric species of *Ipomopsis* (Polemoniaceae). *American Journal of Botany* 88: 213-219.

Wolfe KH, Li WH, & Sharpe PM. 1987. Rates of nucleotide substitution vary greatly among plant mitochondria, chloroplast and nuclear DNAs. *Proceedings of the National Academy of Sciences USA* 84: 9054-9058.

Wright S. 1931. Evolution in Mendelian populations. *Genetics* 16: 97-159.

Yatskievych G. 1996. A revision of the fern genus *Phanerophlebia* (Dryopteridaceae). *Annals of the Missouri Botanical Gardens* 83: 168-199.

Yatskievych G, Stein DB, & Gastony GJ. 1988. Chloroplast DNA evolution and systematics of *Phanerophlebia* (Dryopteridaceae) and related fern genera. *Proceedings of the National Academy of Sciences USA* 85: 2589-2593.

Zink RM, & McKitrick MC. 1995. The debate over species concepts and its implications for ornithology. *The Auk* 112: 701-719.

Appendix Two: Sample Details.

Appendix 2.1: Samples Collected for this Study.

Sample Codes.

Samples collected during the course of this study have each been given a unique code of the format '*tXxxN*.'
't' denotes the taxon: '*c*' = *Polystichum cystostegia*, '*r*' = *P. neozelandicum* complex (cf. '*P. richardii*'), '*s*' = *P. silvaticum*, and '*v*' = *P. vestitum*. '*a*' indicates an Australian species, '*h*' a species from Lord Howe Island, and '*o*' an 'outgroup' species.
'Xxx' is a locality code.
'N' is the numbered sample from a given locality for a specific taxon.

The tables in Appendix 2.1 have the general format:

[1] Sample: the sample code.
[2] Taxon: taxon abbreviations (where necessary) are given under each appendix.
[3] M: '1' = inclusion in morphological analyses, '0' = not included, but mapped.
[4] Locality: brief locality details; full collection details can be found in Appendix Four.
[5] Collector: 'LP' = Leon Perrie, 'LS' = Lara Shepherd, 'ML' = Mark Large.
[6] Date.
[7] NZMS 260: grid reference from the NZMS 260 map series.
[8] Lat.: latitude south.
[9] Long.: longitude east, except longitude west where indicated '*'.

Appendix 2.1.1: Samples from the *Polystichum neozelandicum* Complex.

Taxon abbreviations: 'neoneo' = *Polystichum neozelandicum* subsp. *neozelandicum*, 'neozer' = *P. neozelandicum* subsp. *zerophyllum*, 'ocu' = *P. oculatum*, 'waw' = *P. wawranum*.

Sample[1]	Taxon[2]	M[3]	Locality[4]	Collector[5]	Date[6]	NZMS 260[7]	Lat.[8]	Long.[9]
rAral	waw	1	Northland, Aranga Beach	LP & LS	Jan 2000	O07 625036	35 46	173 34
rAuc1	neoneo	1	Auckland, near Waiwera	LP	Jan 1999	R10 637168	36 32	174 42
rAuc2	waw	1	Auckland, near Waiwera	LP	Jan 1999	R10 637168	36 32	174 42
rAwh1	neoneo	1	Awhitu Peninsula, J. Renall Rd.	Merilyn Merret	16 May 1997	Q12 552472	37 10	174 38
rBan4	ocu	1	Banks Peninsula, Summit Rd.	LP & ML	Dec 1998	N36 08-20-	43 4-	172 5-
rBdc3	ocu	1	East Cape, Anaura Bay	Bruce Clarkson	1990<?<1997	Z16 74-16-	38 14	178 18
rBel2	neozer	0	Napier, Bellbird Bush	LP & LS	Jun 2000	V19 394246	39 07	176 49
rBel4	waw	0	Napier, Bellbird Bush	LP & LS	Jun 2000	V19 394246	39 07	176 49
rCar1	ocu	1	Wairarapa, near Carswell	LP & ML	1997	T26 54-265	40 56	175 54
rCar2	ocu	1	Wairarapa, near Carswell	LP & ML	1997	T26 54-265	40 56	175 54
rCar4	neozer	0	Wairarapa, near Carswell	LP & ML	1997	T26 54-265	40 56	175 54
rCar5	neozer	1	Wairarapa, near Whareama	LP & ML	1997	T26 65-23-	40 57	176 02
rCas1	ocu	1	Wairarapa, near Castlepoint	LP & ML	1997	U26 823327	49 52	176 14
rCas2	neozer	1	Wairarapa, near Castlepoint	LP & ML	1997	U26 823327	49 52	176 14
rCha2	neozer	1	Chatham Island, Nikau Bush	LP	Feb 1999	CH1 442764	43 36	*176 36
rCha3	neozer	1	Chatham Island, Plumtree Bush	LP	Feb 1999	CH1 516597	43 55	*176 30
rCol1	neozer	1	Ruahine Ranges, Lake Colenso area	LP & LS	2000	U21 80-66-	39 4-	176 08

rCol2	waw	1	Ruahine Ranges, Lake Colenso area	LP & LS	2000	U21 80-66-	39 4-	176 08
rCol8	neozer	1	Ruahine Ranges, Lake Colenso area	LP & LS	2000	U21 74-64-	39 41	176 05
rCoo2	waw	1	Wairarapa, near Coonoor	LP & LS	2000	U24 8--7--	40 2-	176 0-
rCoo3	neozer	1	Wairarapa, near Coonoor	LP & LS	2000	U24 8--7--	40 2-	176 0-
rCoo5	neozer	1	Wairarapa, near Coonoor	LP & LS	2000	U24 8--7--	40 2-	176 0-
rCor2	neoneo	1	Coromandel Peninsula, Tairua	LP, Richard Perrie & Judith Perrie	1998	T11 636667	36 58	175 51
rCor3	neoneo	1	Coromandel Peninsula, Whangapoua	LP & John Armstrong	Jan 2001	T10 434960	36 43	175 36
rCor4	neoneo	1	Coromandel Peninsula, Whangapoua	LP & John Armstrong	Jan 2001	T10 434960	36 43	175 36
rDre1	waw	0	Rangitikei, north of Rangiwahia	LP & Trish McLenachan	16 May 2000	T22 577471	39 50	175 53
rDre2	neozer	0	Rangitikei, north of Rangiwahia	LP & Trish McLenachan	16 May 2000	T22 577471	39 50	175 53
rDun1	neozer	1	Dunedin, Flagstaff Creek area	LP	13 Dec 1998	144 14-83-	45 50	170 29
rDun2	neozer	1	Dunedin, Flagstaff Creek area	LP	13 Dec 1998	144 152823	45 51	170 29
rGer2	neozer	1	Canterbury, near Geraldine	LP & ML	Dec 1998	J38 6--7--	44 0-	171 1-
rGer3	neozer	1	Canterbury, near Geraldine	LP & ML	Dec 1998	J38 6--7--	44 0-	171 1-
rHun1	waw	1	Auckland, Hunua Ranges	LP & LS	Dec 1998	S12 964577	37 04	175 05
rJsd1	neoneo	1	Coromandel Peninsula, Golden Bay	John Smith-Dodsworth	20 May 1997	T10 434960	36 44	175 28
rKai1	ocu	1	Banks Peninsula, Kaituna Reserve	LP & ML	Dec 1998	M36 848181	43 45	172 41
rKai2	ocu	1	Banks Peninsula, Kaituna Reserve	LP & ML	Dec 1998	M36 848181	43 45	172 41
rKai3	neozer	1	Banks Peninsula, Kaituna Reserve	LP & ML	Dec 1998	M36 848181	43 45	172 41
rKak1	neozer	1	Kaikoura, Puhi Puhi Reserve	LP	Feb 1999	P31 71-82-	42 16	173 44
rKak3	neozer	1	Kaikoura, Hapuku Reserve	LP	Feb 1999	O31 69-76-	42 19	173 43
rKak5	ocu	1	Kaikoura, vehicle track to Mt. Fyffe	LP	Feb 1999	O31 57-73-	42 21	173 34
rKap1	ocu	1	Wellington, Kapiti Island	LP	1999	R26 727376	40 51	174 56
rKap2	neozer	1	Wellington, Kapiti Island	LP	1999	R26 727376	40 51	174 56
rKap5	ocu	1	Wellington, Kapiti Island	LP	1999	R26 723380	40 51	174 55
rKap6	neozer	1	Wellington, Kapiti Island	LP	1999	R26 723380	40 51	174 55
rKar6	waw	1	Karikari Peninsula, Whangatupere Bay	LP	Jan 1999	O03 513075	34 50	173 26
rKar9	neoneo	1	Karikari Peninsula, Whatuwhiwhi	LP	Jan 2000	O03 463027	34 53	173 21
rKar10	neoneo	1	Karikari Peninsula, Maitai Bay	LP	Jan 2000	O03 499079	34 50	173 25
rKar11	waw	1	Karikari Peninsula, Whangatupere Bay	LP & LS	Jan 2000	O03 513075	34 50	173 26
rKar12	waw	1	Karikari Peninsula, Whangatupere Bay	LP & LS	Jan 2000	O03 513075	34 50	173 26
rKaw1	neozer	1	Kaweka Ranges, Makahu Road track	LP & LS	1999	U20 07-15-	39 13	176 27
rKaw7	neozer	1	Hawkes Bay, near Puketitiri	LP & LS	1999	V20 188053	39 18	176 34
rKee1	neozer	1	Palmerston North, Keebles Bush	LP	29 May 1997	T24 305864	40 24	175 36
rKwh1	waw	1	Kawhia, south of Makomako	LP & LS	Dec 1999	R15 73-50-	38 02	174 51
rKwh3	waw	1	Kawhia, south of Makomako	LP & LS	Dec 1999	R15 73-50-	38 02	174 51
rMaa1	ocu	1	Marlborough, near Rarangi	LP	Dec 1998	P28 982784	41 23	174 03
rMaa2	ocu	1	Marlborough, near Rarangi	LP	Dec 1998	P28 982784	41 23	174 03
rMaa4	neozer	1	Marlborough, near Rarangi	LP	Dec 1998	P28 982784	41 23	174 03
rMah1	waw	1	Hawkes Bay, Mahia Peninsula Reserve	LP & LS	4 Jun 2000	Y20 312195	39 08	177 52
rMah3	waw	1	Hawkes Bay, Mahia Peninsula Reserve	LP & LS	4 Jun 2000	Y20 312195	39 08	177 52
rMah5	waw	1	Hawkes Bay, Morere Reserve	LP & LS	3 Jun 2000	X19 253355	38 59	177 48
rMan1	neoneo	1	Northland, Mt. Mania	LP	Jan 2000	Q07 475966	35 50	174 31
rMat1	waw	1	Waikato, near Matamata	LP & LS	Apr 2000	T14 635812	37 44	175 53
rNap2	ocu	1	Canterbury, Napenape Reserve	LP & ML	Dec 1998	N33 30-07-	42 57	173 15
rNap3	neozer	1	Canterbury, Napenape Reserve	LP & ML	Dec 1998	N33 30-07-	42 57	173 15
rNap4	neozer	1	Canterbury, Napenape Reserve	LP & ML	Dec 1998	N33 30-07-	42 57	173 15
rNap7	neozer	1	Canterbury, Napenape Reserve	LP & ML	Dec 1998	N33 30-07-	42 57	173 15
rNap10	ocu	1	Canterbury, Napenape Reserve	LP & ML	Dec 1998	N33 30-07-	42 57	173 15
rNap12	ocu	1	Canterbury, Napenape Reserve	LP & ML	Dec 1998	N33 30-07-	42 57	173 15

rNap17	ocu	1	Canterbury, Napenape Reserve	LP & ML	Dec 1998	N33 30-07-	42 57	173 15
rNel1	neozer	1	Nelson, Whangamoa Hill	Nelson Fern Society	Jun 1997	O27 46-98-	41 13	173 26
rNel4	neozer	1	Nelson, Mt. Richmond Park	Eileen Heatherbell	Aug 1997	N28 28-79-	41 23	173 13
rNga1	waw	1	Wairarapa, Ngapaeruru Reserve	LP & David Havell	Dec 1999	U24 835992	40 17	176 13
rNga2	neozer	1	Wairarapa, Ngapaeruru Reserve	LP & David Havell	Dec 1999	U24 835992	40 17	176 13
rNga3	waw	1	Wairarapa, Ngapaeruru Reserve	LP & LS	2000	U24 835992	40 17	176 13
rNga6	neozer	1	Wairarapa, Ngapaeruru Reserve	LP & LS	2000	U24 835992	40 17	176 13
rNor1	neozer	1	Wairarapa, near Norsewood	LP & LS	1999	U23 848228	40 04	176 13
rOpe1	neozer	1	Taupo, Opepe Reserve	LP & LS	Apr 2000	U18 898657	38 47	176 13
rOpo2	waw	1	Northland, Opononi	LP & LS	Jan 2000	O06 49-33-	35 30	173 25
rOta1	waw	1	Wellington, Otaki Forks	LP & LS	Nov 2000	S26 987345	40 53	175 14
rPar1	neozer	1	Wellington, Paraparaumu	LP	1998	R26 804313	40 54	175 01
rPar2	neozer	1	Wellington, Paraparaumu	LP	1998	R26 804313	40 54	175 01
rPah1	waw	1	Wairarapa, Makuri Valley	LP & David Havell	Spring 1999	T25 627689	40 33	175 59
rPee1	neozer	1	Canterbury, Peel Forest	LP & ML	Dec 1998	K37 706006	43 53	171 16
rPee4	neozer	1	Canterbury, Peel Forest	LP & ML	Dec 1998	K37 706006	43 53	171 16
rPih1	waw	1	Auckland, Piha	LP & LS	Jan 2000	Q11 405728	36 56	174 27
rPih3	waw	1	Auckland, Piha	LP & LS	Jan 2000	Q11 405728	36 56	174 27
rPmm1	neoneo	1	Waikato, near Gordonton	LP & Tony Dugdale	3 Apr 1997	S14 197989	37 35	175 22
rPmm2	neoneo	1	Waikato, near Gordonton	LP & Tony Dugdale	3 Apr 1997	S14 197990	37 35	175 22
rPng1	waw	0	Wairarapa, Pongaroa Reserve	LP & LS	2001	U25 808690	40 33	176 12
rPng2	neozer	0	Wairarapa, Pongaroa Reserve	LP & LS	2001	U25 808690	40 33	176 12
rPoh1	waw	1	Manawatu, Totara Reserve	LP & ML	19 Mar 1997	T23 532167	40 08	175 51
rPoh2	neozer	1	Manawatu, Totara Reserve	LP & ML	19 Mar 1997	T23 532167	40 08	175 51
rPoh5	waw	1	Manawatu, Totara Reserve	LP & LS	1999	T23 525152	40 09	175 50
rPoh6	neozer	1	Manawatu, Totara Reserve	LP & LS	1999	T23 525152	40 09	175 50
rPuk1	waw	1	Northland, Puketi Forest	LP & Merilyn Merret	1999	P05 720579	35 17	173 40
rPuk4	waw	1	Northland, Puketi Forest	LP & LS	Jan 2000	P05 720579	35 17	173 40
rRag3	waw	1	Waikato, Raglan, Mt. Karioi	LP & LS	Dec 1999	R14 69-74-	37 50	174 48
rRim1	neozer	1	Wellington, Rimutaka Ranges	LP & ML	1999	S26 95-10-	41 06	175 12
rRim2	neozer	1	Wellington, Rimutaka Ranges	LP & ML	1999	S26 95-10-	41 06	175 12
rRkk1	neozer	1	Wairarapa, near Ruakokoputuna	LP & Dave Havell	2000	S27 10-85-	41 20	175 2-
rRkk2	ocu	1	Wairarapa, near Ruakokoputuna	LP & Dave Havell	2000	S27 10-85-	41 20	175 2-
rRot2	waw	1	Rotorua, Lake Okataina	LP & LS	Apr 2000	V16 110391	38 06	176 26
rRot3	waw	1	Rotorua, Lake Okataina	LP & LS	Apr 2000	V16 110391	38 06	176 26
rSte1	waw	1	Gisborne, near Pehiri	LP & Dave King	9 Jun 1997	X18 194743	38 38	177 42
rSte3	waw	1	Gisborne, near Pehiri	LP & Dave King	9 Jun 1997	X18 194743	38 38	177 42
rSte4	ocu	1	Gisborne, near Pehiri	LP & Dave King	9 Jun 1997	X18 194743	38 38	177 42
rTar1	neozer	1	Taranaki, Urenui	LP & Merilyn Merret	1997	Q19 308454	38 59	174 24
rTar3	neozer	1	Taranaki, Opunake	Chrissy Ryan	1999	P20 841938	39 28	173 52
rTar5	neozer	1	Taranaki, Opunake	LP & LS	2000	P20 841938	39 28	173 52
rTar7	neozer	1	Taranaki, near Okato	LP & LS	2000	P19 855289	39 09	173 52
rTea1	waw	1	Waikato, Mt. Te Aroha	LP & LS	Jan 2000	T13 514054	37 32	175 44
rTea3	waw	1	Waikato, Mt. Te Aroha	LP & LS	Jan 2000	T13 514054	37 32	175 44
rTok1	waw	1	Waikato, near Tokoroa	LP & LS	Apr 2000	T16 56-29-	38 13	175 49
rTur2	waw	1	Wanganui, McPhersons Bush	LS	1999	S22 096378	39 57	175 20
rTur6	waw	0	Wanganui, Sutherlands Bush	LP & LS	12 May 2001	S22 117423	39 54	175 21
rTur7	neozer	0	Wanganui, Sutherlands Bush	LP & LS	12 May 2001	S22 117423	39 54	175 21
rUre1	waw	1	Ureweras, Hopuruahine catchment	LP & Richard Perrie	1998	W18 64-74-	38 40	177 04
rUre2	waw	1	Ureweras, Hopuruahine catchment	LP & Richard Perrie	1998	W18 64-74-	38 40	177 04
rUre3	waw	1	Ureweras, Hopuruahine catchment	LP & Richard Perrie	Apr 2001	W18 643747	38 40	177 04

rVin4	neozer	1	Rangitikei, near Mangaweka	LP & ML	13 May 1997	T22 502514	39 49	175 48
rVin5	neozer	1	Rangitikei, near Mangaweka	LP & ML	13 May 1997	T22 502514	39 49	175 48
rWae2	neoneo	1	Waikato, W. of Waerenga	LP	1999	S13 036270	37 21	175 10
rWai1	neoneo	1	Waikato, E. of Waerenga	LP & Richard Perrie	28 Mar 1997	S13 148217	37 23	175 18
rWak1	neozer	1	Hawkes Bay, Wakarara	LP & LS	1999	U22 897528	39 47	176 16
rWak2	neozer	1	Hawkes Bay, Wakarara	LP & LS	1999	U22 897528	39 47	176 16
rWak4	ocu	1	Hawkes Bay, Wakarara	LP & LS	1999	U22 897528	39 47	176 16
rWak5	ocu	1	Hawkes Bay, Wakarara	LP & LS	1999	U22 897528	39 47	176 16
rWan1	neozer	1	Wanganui, Virginia Lake	LS	1999	R22 837416	39 55	175 02
rWan5	neozer	1	Wanganui, south of Pungarehu	LP & LS	1999	S22 94-51-	39 50	175 09
rWan6	waw	1	Wanagnui, north of Pungarehu	LP & LS	1999	S22 94-54-	39 48	175 09
rWan7	waw	1	Wanagnui, north of Pungarehu	LP & LS	1999	S22 94-54-	39 48	175 09
rWel3	ocu	1	Wellington, near Seatoun	LP & Richard Winkworth	30 Jun 1997	R27 637885	41 18	174 50
rWel5	ocu	1	Wellington, Moa Point	LP & Richard Winkworth	30 Jun 1997	R27 615835	41 20	174 49
rWel8	ocu	1	Wellington, Makara	LP & LS	Sep 2000	R27 536887	41 18	174 43
rWel9	ocu	0	Wellington, Makara	LP & LS	Sep 2000	R27 538885	41 18	174 43
rWel10	neozer	0	Wellington, Makara	LP & LS	Sep 2000	R27 538885	41 18	174 43
rWen3	waw	1	Auckland, near Waiwera	LP & LS	Dec 1999	R10 639166	36 33	174 43
rWhi1	neozer	1	Hawkes Bay, north of Napier	LP	12 Jun 1997	V20 445059	39 17	176 52
rWhi3	neozer	1	Hawkes Bay, north of Napier	LP	12 Jun 1997	V20 445059	39 17	176 52
rWkw1	neoneo	1	Northland, near Warkworth	LP & LS	Dec 1999	R09 685260	36 28	174 46
rWkw2	neoneo	1	Northland, near Warkworth	LP & LS	Dec 1999	R09 685260	36 28	174 46
rWil1	neozer	1	Hawkes Bay, near Willowflat	LP & LS	Jun 2000	W19 56-32-	39 03	177 00
rWto1	waw	1	Waikato, near Waitomo	LP & LS	Dec 1999	S16 948248	38 16	175 06
rWto3	waw	1	Waikato, near Waitomo	LP & LS	Dec 1999	S16 948248	38 16	175 06
rWwp2	waw	1	Wairarapa, Waewaepa Ranges	LP & LS	2000	U24 71-82-	40 26	176 05

Appendix 2.1.2: *Polystichum vestitum* Samples.

'Taxon' abbreviations (see Section 5.3.1 for further details): 'mainland' = mainland (North and South Island) *Polystichum vestitum*, 'm.l. Cha.' = 'mainland-like Chathams,' 'int. Cha.' = 'intermediate Chathams,' 'div. Cha.' = 'divergent Chathams,' 'm.l. sou.' = 'mainland-like southern,' and 'div. sou.' = 'divergent southern.'

Sample[1]	'Taxon'[2]	M[3]	Locality[4]	Collector[5]	Date[6]	NZMS 260[7]	Lat.[8]	Long.[9]
vAki1	div. sou.	1	Auckland Islands, Enderby Island	Brian Rance	19 Dec 1998	77183		
vAki2	m.l. sou.	1	Auckland Islands, Auckland Island	Brian Rance	20 Dec 1998	932843		
vAki3	m.l. sou.	1	Auckland Islands, Auckland Island	Brian Rance	20 Dec 1998	828823		
vAki4	div. sou.	1	Auckland Islands, Auckland Island	Brian Rance	22 Dec 1998	75141		
vAki5	m.l. sou.	1	Auckland Islands, Auckland Island	Brian Rance	23 Dec 1998	38157		
vAnt1	m.l. sou.	1	Antipodes Island	Sheryl Hamilton & Alan Wiltshire	15 Feb 1999	115-081		
vAnt2	m.l. sou.	1	Antipodes Island	Sheryl Hamilton & Alan Wiltshire	18 Feb 1999	097-075		
vAnt3	m.l. sou.	1	Antipodes Island	Sheryl Hamilton & Alan Wiltshire	18 Feb 1999	077-056		
vBan2	mainland	1	Banks Peninsula, Kaituna Reserve	LP & ML	Dec 1998	M36 848181	43 45	172 41
vBan3	mainland	1	Banks Peninsula, near Montgomery Reserve	LP & ML	Dec 1998	N36 99-18-	43 45	172 52
vBan4	mainland	1	Banks Peninsula, Summit Rd.	LP & ML	Dec 1998	N36 08-20-	43 45	172 52
vBan5	mainland	1	Banks Peninsula, near Hinewai Reserve	LP & ML	Dec 1998	N36 12-10-	43 49	173 01
vBel1	mainland	1	Hawkes Bay, Bellbird Bush	LP & LS	Jun 2000	V19 393243	39 08	176 49
vCen1	mainland	1	Central Plateau, Lake Rotopounamu	LP	1998	T19 466387	39 02	175 44
vCgw1	div. Cha.	1	Chatham Islands, Pitt Island	Geoff Walls	20 May 1999	CH2 754185	44 17	*176 11
vCgw2	m.l. Cha.	1	Chatham Islands, Pitt Island	Geoff Walls	20 May 1999	CH2 754185	44 17	*176 11
vCha1	int. Cha.	1	Chathams Islands, Chatham Island	LP	Feb 1999	CHI 439764	43 46	*176 35
vCha2	m.l. Cha.	1	Chathams Islands, Chatham Island	LP	Feb 1999	CHI 439764	43 46	*176 35
vCha3	div. Cha.	1	Chathams Islands, Chatham Island	LP	Feb 1999	CHI 448762	43 46	*176 34
vCha4	div. Cha.	1	Chathams Islands, Chatham Island	LP	Feb 1999	CHI 446804	43 44	*176 34
vCha5	int. Cha.	1	Chathams Islands, Chatham Island	LP	Feb 1999	CHI 446804	43 44	*176 34
vCha6	m.l. Cha.	1	Chathams Islands, Chatham Island	LP & Geoff Walls	Feb 1999	CHI 348725	43 48	*176 42
vCha7	div. Cha.	1	Chathams Islands, Chatham Island	LP & Geoff Walls	Feb 1999	CHI 348725	43 48	*176 42
vCha8	div. Cha.	1	Chathams Islands, Chatham Island	LP & Geoff Walls	Feb 1999	CHI 348725	43 48	*176 42
vCha9	int. Cha.	1	Chathams Islands, Chatham Island	LP & Geoff Walls	Feb 1999	CHI 348725	43 48	*176 42
vCha10	m.l. Cha.	1	Chathams Islands, Chatham Island	LP & Geoff Walls	Feb 1999	CHI 348725	43 48	*176 42
vCha11	m.l. Cha.	1	Chathams Islands, Chatham Island	LP	Feb 1999	CHI 516597	43 55	*176 30
vCha12	int. Cha.	1	Chathams Islands, Chatham Island	LP	Feb 1999	CHI 516597	43 55	*176 30
vCha14	div. Cha.	1	Chathams Islands, Chatham Island	LP	Feb 1999	CHI 522578	43 56	*176 29
vCha15	int. Cha.	1	Chathams Islands, Chatham Island	LP	Feb 1999	CHI 522578	43 56	*176 29
vCha16	div. Cha.	1	Chathams Islands, Chatham Island	LP	Feb 1999	CHI 423523	43 59	*176 36
vCha17	div. Cha.	1	Chathams Islands, Chatham Island	LP	Feb 1999	CHI 453551	43 57	*176 33
vCha18	m.l. Cha.	1	Chathams Islands, Chatham Island	LP	Feb 1999	CH2 385426	44 04	*176 39
vCha19	div. Cha.	1	Chathams Islands, Chatham Island	LP	Feb 1999	CH2 385426	44 04	*176 39
vCha20	div. Cha.	1	Chathams Islands, Chatham Island	LP	Feb 1999	CH2 385425	44 04	*176 39
vCha21	int. Cha.	1	Chathams Islands, Chatham Island	LP	Feb 1999	CH2 385425	44 04	*176 39
vCha22	int. Cha.	1	Chathams Islands, Chatham Island	LP	Feb 1999	CH2 385425	44 04	*176 39
vCha23	div. Cha.	1	Chathams Islands, Chatham Island	LP	Feb 1999	CH2 385425	44 04	*176 39
vCha24	m.l. Cha.	1	Chathams Islands, Chatham Island	LP	Feb 1999	CH2 385425	44 04	*176 39

vCha25	div. Cha.	1	Chathams Islands, Chatham Island	LP	Feb 1999	CH2 385425	44 04	*176 39
vCha29	int. Cha.	1	Chathams Islands, Chatham Island	LP	Feb 1999	CH1 522578	43 56	*176 29
vDan1	mainland	1	Hawkes Bay, near Ongaonga	LP & LS	1999	U22 902327	39 58	176 17
vDre3	mainland	1	Rangitikei, north of Rangiwahia	LP & Trish McLenachan	16 May 2000	T22 577471	39 50	175 53
vDre6	mainland	1	Rangitikei, north of Rangiwahia	LP & Trish McLenachan	16 May 2000	T22 577471	39 50	175 53
vDun1	mainland	1	Dunedin, Flagstaff Creek area	LP	Dec 1998	144 153820	45 51	170 29
vDun2	mainland	1	Dunedin, Flagstaff Creek area	LP	Dec 1998	144 14-83-	45 50	170 29
vFio3	mainland	0	Fiordland, Broughton Arm	Roland Foster & Gina Williams	21 Feb 2000	B44 36-99-	45 34	166 56
vHil4	mainland	1	Ruahine Ranges, near Ikawetea	LP & Richard Winkworth	1998	U21 900752	39 35	176 16
vInv1	mainland	1	Southland, near Invercargill	Llyod Esler	11 Oct 1997	E47 48-08-	46 27	168 17
vIro1	mainland	1	Ruahines Ranges, Lake Colenso area	LP & LS	1999	U21 74-64-	39 41	176 05
vKaa1	mainland	1	Rangitikei, south of Rangiwahia	LP & LS	21 Apr 1999	T22 575409	39 54	175 54
vKak1	mainland	1	Kaikoura, Blue Duck Reserve	LP	Feb 1999	P31 745843	42 15	173 47
vKak2	mainland	1	Kaikoura, Puhi Puhi Reserve	LP	Feb 1999	P31 71-82-	42 16	173 44
vKak6	mainland	1	Kaikoura, Mt. Fyffe	LP	Feb 1999	031 60-77-	42 19	173 36
vKap1	mainland	1	Wellington, Kapiti Island	LP	1999	R26 717378	40 51	174 55
vKaw3	mainland	1	Kaweka Ranges, Makahu Road track	LP & LS	1999	U20 07-15-	39 13	176 27
vKaw6	mainland	1	Kaweka Ranges, Makahu Road track	LP & LS	1999	U20 083168	39 12	176 27
vKin1	mainland	1	Ruahines Ranges, Kinvig Track	LP	1998	T23 68-29-	40 00	176 02
vKiw1	mainland	1	Wairarapa, east of Dannevirke	LP, LS & Dave Havell	1999	U23 83-05-	40 13	176 13
vKlo1	mainland	1	Arthurs Pass, near Bealey	LP & ML	Dec 1998	K34 948995	43 00	171 35
vKlo2	mainland	1	Arthurs Pass, near Bealey	LP & ML	Dec 1998	K34 948995	43 00	171 35
vKmw1	mainland	1	Kaimanawas, Hinemaiaia Catchment	LP	Dec 1999	U19 828411	39 00	176 09
vKmw2	mainland	1	Kaimanawas, Hinemaiaia Catchment	LP	Dec 1999	U19 828411	39 00	176 09
vKmw3	mainland	1	Kaimanawas, Hinemaiaia Catchment	LP	Dec 1999	U19 829404	39 00	176 09
vKaa4	mainland	1	Rangitikei, south of Rangiwahia	LP & LS	19 Dec 1999	T22 575409	39 54	175 54
vMac1	m.l. sou.	1	Macquarie Island	W. Misiak	20 Jan 1999			
vMac2	m.l. sou.	1	Macquarie Island	W. Misiak	18 Jan 1999			
vMac4	m.l. sou.	1	Macquarie Island	W. Misiak	20 Jan 1999			
vOpe1	mainland	1	Taupo, Opepe Reserve	LP & LS	Apr 2000	U18 898657	38 47	176 13
vOta1	mainland	1	Wellington, Otaki Forks	LP & LS	Nov 2000	S26 991346	40 53	175 15
vPee2	mainland	1	Canterbury, Peel Forest	LP & ML	Dec 1998	J37 69-99-	43 54	171 15
vPee5	mainland	1	Canterbury, Peel Forest	LP & ML	Dec 1998	K37 706006	43 53	171 16
vPir3	mainland	1	Waikato, Mt. Pirongia	LP & LS	Dec 1999	S15 96-56-	37 59	175 07
vPoh7	mainland	1	Manawatu, Totara Reserve	LP & LS	1999	T23 525152	40 09	175 50
vRan1	mainland	1	Ruahines Ranges, Rangiwahia track	LP & LS	21 Apr 1999	T22 676411	39 54	176 00
vRkk1	mainland	1	Wairarapa, Ruakokoputuna Station	LP & Dave Havell	2000	S28 ------	41 2-	175 2-
vRkk2	mainland	1	Wairarapa, near Ruakokoputuna	LP & Dave Havell	2000	S27 10-85-	41 20	175 2-
vRob1	mainland	1	Marlborough, Mt. Robertson	LP	Dec 1998	P27 955838	41 21	174 01
vRot2	mainland	1	Rotorua, Whakarewarewa forest	LP & LS	Apr 2000	U16 955314	38 10	176 15
vRot3	mainland	1	Rotorua, Lake Okataina	LP & LS	Apr 2000	V16 110391	38 06	176 26
vSna1	div. sou.	1	Snares Islands	JC Stahl	13 Mar 2000			
vSna2	div. sou.	1	Snares Islands	JC Stahl	13 Mar 2000			
vSna3	div. sou.	1	Snares Islands	JC Stahl	13 Mar 2000			
vSou5	div. sou.	1	Foveaux Strait, Bench Island	Carol J. West	15 Oct 1999	E48 47-56-	46 54	168 14
vSou6	mainland	1	Southland, south of Mossburn	Dave Havell	2000	E44 32-78-	45 50	168 09
vSou7	div. sou.	1	Foveaux Strait, Hebe Island	Carol J. West	Feb 2000	D50 378189	47 13	167 38
vTar1	mainland	1	Taranaki, North Egmont	LP & LS	26 Oct 1998	P20 045143	39 16	174 06
vTar10	mainland	1	Taranaki, Fanthams Peak track	LP & LS	Mar 2000	P20 031097	39 16	174 05

vTea1	mainland	1	Waikato, Mt. Te Aroha	LP & LS	Apr 2000	T13 526039	37 32	175 45
vTok2	mainland	1	Waikato, near Tokoroa	LP & LS	Apr 2000	T16 58-25-	38 14	175 50
vTon1	mainland	1	Central Plateau, near Turoa	LP	1999	S20 255255	39 20	175 30
vTon2	mainland	1	Central Plateau, near Turoa	LP	1999	S20 255255	39 20	175 30
vTon3	mainland	1	Central Plateau, near Turoa	LP	1999	S20 255255	39 20	175 30
vTru2	mainland	1	Tararua Ranges, Herepai	LP	1998	S25 25-54-	40 42	175 32
vUre1	mainland	1	Ureweras, Hopuruahine catchment	LP & Richard Perrie	1998	W18 64-74-	38 40	177 04
vUre2	mainland	1	Ureweras, Hopuruahine catchment	LP & Richard Perrie	1998	W18 64-74-	38 40	177 04
vUre3	mainland	1	Ureweras, Hopuruahine catchment	LP	9 Apr 2001	W18 643747	38 40	177 04
vWam2	mainland	1	Wairarapa, near Ekatahuna	LP & ML	1997	T25 341522	40 43	175 39
vWam3	mainland	1	Wairarapa, near Ekatahuna	LP & ML	1997	T25 341522	40 43	175 39
vWes2	mainland	1	West Coast, near Punakaiki	LP & ML	Feb 1999	K30 72-98-	42 06	171 20
vWes3	mainland	1	West Coast, north of Greymouth	LP & ML	Feb 1999	J31 66-71-	42 21	171 15
vWes4	mainland	1	West Coast, south of Harihari	LP & ML	Feb 1999	134 015704	43 15	170 26
vWes5	mainland	1	West Coast, Lake Matheson	LP & ML	Feb 1999	H35 647474	43 27	169 57
vWes6	mainland	1	West Coast, near Haast Pass	LP & ML	Feb 1999	G38 184715	44 06	169 21
vWes7	mainland	1	West Coast, near Haast	LP & ML	Feb 1999	F37 93-99-	43 51	169 04
vWes8	mainland	1	Arthurs Pass, near Jacksons	LP & ML	Feb 1999	K33 89-28-	42 45	171 32
vWes9	mainland	1	Arthurs Pass, Otira Valley	LP & ML	Feb 1999	K33 919168	42 51	171 33
vWwp1	mainland	1	Wairarapa, Waewaepa Ranges	LP & LS	2000	U24 71-82-	40 26	176 05

Appendix 2.1.3: *Polystichum cystostegia* and *P. silvaticum* Samples.

Sample[1]	Species	Locality[4]	Collector[5]	Date[6]	NZMS 260[7]	Lat.[8]	Long.[9]
cKak1	*P. cystostegia*	Kaikoura, Mt. Fyffe	LP	Feb 1999	O31 60-77-	42 03	173 35
cTar1	*P. cystostegia*	Taranaki, south-eastern Mt. Taranaki	LP & LS	Dec 1998	P20 01-09-	39 12	174 03
sAka1	*P. silvaticum*	Wellington, Akatarawa Ranges	LP & ML	1999	R26 877254	40 58	175 07
sKlp1	*P. silvaticum*	Manawatu, near Palmerston North	LP & LS	2000	T24 305780	40 28	175 36
sOpe1	*P. silvaticum*	Taupo, Opepe Reserve	LP & LS	Apr 2000	U18 898657	38 47	176 13
sTea1	*P. silvaticum*	Waikato, Mt. Te Aroha	LP & LS	Apr 2000	T13 527039	37 32	175 44

Appendix 2.1.4: Hybrid Specimens.

Abbreviations used to denote putative parents: 'cys' = *Polystichum cystostegia*, 'neozer' = *P. neozelandicum* subsp. *zerophyllum*, 'ocu' = *P. oculatum*, 'sil' = *P. silvaticum*, 'ves' = *P. vestitum*, and 'waw' = *P. wawranum*.

Sample[1]	Cross[2]	Locality[4]	Collector[5]	Date[6]	NZMS 260[7]	Lat.[8]	Long.[9]
rXvKak2	neozer X ves	Kaikoura, Puhi Puhi Reserve	LP	Feb 1999	O31 695784	42 18	173 43
rXvKaw2	neozer X ves	Kaweka Ranges, Makahu Road track	LP & LS	1999	U20 07-15-	39 13	176 27
rXvPee2	neozer X ves	Canterbury, Peel Forest	LP & ML	Dec 1998	K37 706006	43 53	171 16
rXvBan9	ves X ocu	Banks Peninsula, Summit Rd.	LP & ML	Dec 1998	N36 08-20-	43 4-	172 5-
rXvNap1	ves X ?ocu	Canterbury, Napenape Reserve	LP & ML	Dec 1998	N33 30-07-	42 57	173 15
rXvNap2	ves X ?ocu	Canterbury, Napenape Reserve	LP & ML	Dec 1998	N33 30-07-	42 57	173 15
rWeb1	neozer X waw	Wairarapa, north of Coonoor	LP & Dave Havell	1999	U24 733817	40 26	176 06
rNap13	? (neozer X ocu)	Canterbury, Napenape Reserve	LP & ML	Dec 1998	N33 30-07-	42 57	173 15
rNap15	? (neozer X ocu)	Canterbury, Napenape Reserve	LP & ML	Dec 1998	N33 30-07-	42 57	173 15
rNap16	? (neozer X ocu)	Canterbury, Napenape Reserve	LP & ML	Dec 1998	N33 30-07-	42 57	173 15
rNap18	? (neozer X ocu)	Canterbury, Napenape Reserve	LP & ML	Dec 1998	N33 30-07-	42 57	173 15
cXvTar1	cys X ves	Taranaki, south-eastern Mt. Taranaki	LP & LS	Dec 1998	P20 02-09-	39 19	174 04
sXvAka1	sil X ves	Wellington, Akatarawa Ranges	LP & LS	1999	R26 877254	40 58	175 07

Appendix 2.1.5: Non-New Zealand *Polystichum* Samples.

Sample	Species	Origin	Locality	Collector
aAus1	*P. australiense*	Australia	Cultivated Sydney, Mt. Druitt	Elizabeth Brown
aFal1	*P. fallax*	Australia	Cultivated Sydney, Mt. Druitt	Elizabeth Brown
aFor1	*P. formosum*	Australia	Cultivated Sydney, Mt. Druitt	Elizabeth Brown
aPro1	*P. proliferum*	Australia	Cultivated Palmerston North	LP
aPro2	*P. proliferum*	Australia	Cultivated Sydney, Mt. Druitt	Elizabeth Brown
hMoo1	*P. moorei*	Lord Howe Island	Lord Howe Island	Elizabeth Brown
hWhi1	*P. whiteleggei*	Lord Howe Island	Lord Howe Island	Elizabeth Brown
oLen1	*P. lentum*	Asia	Cultivated Auckland	ML
oMoh1	*P. mohrioides*	South America	Chile, Los Lagos, Rio Futalefu	P. Wardle & S. Wagstaff
oMul1	*P. multifidum*	South America	Chile, Los Lagos, Puente Ventisquero	P. Wardle & S. Wagstaff
oSet1	*P. setiferum*	Europe	Cultivated Palmerston North	LP

Appendix 2.2: Herbarium Collections.

The following are lists of herbarium specimens examined, redesignated, and/or mapped.

Appendix 2.2.1: *Polystichum neozelandicum* complex.

* indicates samples not mapped.

Polystichum neozelandicum subsp. *neozelandicum*

AK: *NORTHLAND*: **114800**, Kawau Island, HB Mathews, 1920; **121784**, Whangarei, RC Cooper, 1965; **122318**, Great Barrier Island, Frater, 1965; **123167**, Whatuwhiwhi, R Cooper, 1965; **131602**, Whangaruru Harbour, AE Orchard, 1972; **138290**, North Cape, ?, ?; **150244**, Motukawanui Island, DJ Court, 1979; **171179**, Great Barrier Island, AE Wright, 1984; **182074**, Great Barrier Island, DJ Court, 1967; **189744**, Hokianga, AE Wright, 1989; **203112**, Kaipara, LJ Forester, 1990; **205252**, Kaipara, AE Wright, 1991; **207808** Cape Reinga, BS Parris, 1977; **207892**, Poor Knights Islands, BS Parris, ?; **213663**, Whangaroa, H Carse, 1921; *WAIKATO*: **11030***, Auckland, T Kirk, ?; **14072***, Thames, J Adams, ?; **31513**, Tairua, P Hynes, 1953; **92926**, Kawhia, P Hynes, 1963; **114295**, Tamaki, ?, 1932; **114787**, Tamaki, ?, ?; **114788**, Tamaki, ?, ?; **114801**, Tamaki, HB Matthews, 1919; **114804***, Tamaki, E Craig, ?; **121310**, Waerenga, DVG Woods, 1962; **122364**, Orere Point, RC Cooper, 1965; **126432**, Auckland, AE Wright, 1970; **129129**, Thames, P Hynes, 1971; **132073**, Pakatoa, AE Wright, 1973; **138284***, Coromandel, TF Cheeseman, ?; **138289***, ?Muriwai, TF Cheeseman, 1919; **141328**, Waitemata, AE Wright, 1977; **144428**, Tamaki, P Hynes, 1952; **164591**, Tamaki, RO Gardner, 1983; **170386**, Kawhia, PJ de Lange, 1985; **211799***, Tairua, IL Barton, 1956; **213302**, Waihou, MR Woodhead, 1940; **214097**, Auckland, AE Wright, 1970.

WELT: *NORTHLAND*: **P001590**, Waipu, WRI Oliver, 1952; **P008151**, Hen & Chickens Islands, WRI Oliver, 1924; **P008995***, North Auckland, ?, 1889; *WAIKATO*: **P001604**, Mt. Maunganui, WRI Oliver, 1920; **P008144***, Auckland, ?, ?; **P008157***, Auckland, D Petrie, 1921; **P008159***, Auckland, ?, ?; **P008168**, Waitakare, H Dobbie, 1924; **P008171***, Coromandel Peninsula, TF Cheeseman, ?; **P008172**, Muriwai, ?, ?; **P011925***, Auckland, ?, ?; **P015819***, Auckland, S Mossman, 1850.

Polystichum neozelandicum subsp. *zerophyllum*

AK: *WAIKATO*: **137011**, Taupo, A Leahy, 1975; *HAWKES BAY*: **138281**, Dannevirke, W Colenso, ?; **214099**, Dannevirke, W Colenso, ?; **214100**, Dannevirke, W Colenso, ?; **214101**, Dannevirke, W Colenso, ?; *TARANAKI/RANGITIKEI*: **113572**, Pohangina, P Hynes, 1967; *NELSON/MARLBOROUGH*: **138282**, Queen Charlotte Sound, JM McMahon, ?; **143085**, Farewell Spit, AE Wright, 1977; **151470**, Nelson, AE Wright, 1979; *CANTERBURY*: **179702**, Mt. Somers, EM Chapman, 1973; *OTAGO*: **114797***, Dunedin, D Petrie, ?; **114798***, Dunedin, D Petrie, ?; **114799***, Dunedin, D Petrie, ?; *SOUTHLAND*: **92017**, Stewart Island, E Hynes, ?; **163491**, Stewart Island, MW Crookes, 1960; *CHATHAMS*: **170658**, Chatham Island, AM Ringer, 1980;

CHR: *NELSON/MARLBOROUGH*: **179030** (lower-most specimen), Nelson, P Wardle, 1965; **188511**, Pohara, WR Sykes, 1969; **219955**, Nelson, BH Macmillan, 1971; **245268**, Kekerengu, AP Druce, 1971; **278035**, NW Nelson, AP Druce, 1974; **290351**, Kenepuru, JH McMahon, ?; **356629**, Nelson, DR Given, 1974; **385979**, Clarence River, BPJ Molloy, 1976; **387976***, Marlborough, BPJ Molloy, 1975; *CANTERBURY*: **201616**, Rikaia River, BPJ Molloy, 1970; **202859**, Kaituna, DR Given, 1970; **211427**, Kimberley, BH Macmillan, 1970; **212856**, Peel Forest, BPJ Molloy, 1973; **218323**, Waimakariri Gorge, DR Given, 1971; **258558**, Mt. Somers, EM Chapman, 1973; **510191**, Geraldine, PJ Bellingham, 1996; *OTAGO*: **431232**, Lake Roxburgh, KJM Dickinson, 1986; *SOUTHLAND*: **355391**, Stewart Island, HD Wilson, 1978; **368809**, Stewart Island, HD Wilson, 1980; *CHATHAMS*: **397620**, Pitt Island, GN Park, 1980; **403369**, Chatham Island, DR Given, 1982; *UNCERTAIN*: **226120***, Grey River, BH Macmillan, 1972; **274535***, Mt. Drew, D Kelley, 1975; **428879***, Devils Gorge, KJM Dickinson, 1986.

WELT: *TARANAKI/RANGITIKEI*: **P008152**, Paritutu, D Petrie, 1912; **P015536**, Wanganui, AE King, 1988; **P015535***, Wanganui, AE King, 1988; **P015537**, Wanganui, AE King, 1988; **P015726***,

Wanganui, AE King, 1988; **P016078**, Wanganui, AE King, 1989; **P016310**, Wanganui, AE King, 1989; **P017747**, Wanganui, AE King, 1991; **P017793**, Wanganui, AE King, 1989; **P017929**, Marton, AE King, 1991; *HAWKES BAY*: **P001611**, Waikaremoana, WRI Oliver, 1946; **P002581**, Dannevirke, W Colenso, 1887; **P002582**, Dannevirke, W Colenso, 1887; **P002586***, ?, W Colenso, ?; **P002587***, ?, W Colenso, ?; **P002588**, Dannevirke, W Colenso, 1887; **P002589**, Dannevirke, W Colenso, ?; **P002590**, Dannevirke, W Colenso, ?; **P002591**, Dannevirke, W Colenso, ?; **P002592**, Dannevirke, W Colenso, ?; **P002593**, Dannevirke, W Colenso, ?; **P002594**, Dannevirke, W Colenso, ?; **P002595**, Dannevirke, W Colenso, ?; **P003385**, Waikaremoana, WRI Oliver, 1946; **P008131**, Petane, A Hamilton, 1882; **P008132**, Petane, A Hamilton, 1883; **P008133***, Wellington, A Hamilton, 1905; **P008138**, Petane, A Hamilton, 1882; **P008139**, Petane, A Hamilton, 1883; **P008170**, Dannevirke, W Colenso, ?; **P013870**, Napier, AE King, 1988; **P016003**, Puketitiri, F Pitt, 1988; **P016028**, Akito, F Pitt, 1988; *WELLINGTON*: **P001608**, Kapiti Island, WRI Oliver, 1935; **P001609**, Kapiti Island, WRI Oliver, 1935; **P008123**, Wadestown, A Hamilton, 1905; **P008176**, Tinakori, H Kirk, 1875; *NELSON/MARLBOROUGH*: **P001599**, D'Urville Island, WRI Oliver, 1943; **P008160** (two upper specimens), Kenepuru, JH McMahon, ?; **P009677**, Kenepuru, JH Mahon, ?; **P009678**, Kenepuru, ?, 1919; P09682, Kenepuru, JH McMahon, 1926; **P009683***, Marlborough, ?, 1928; **P009688**, Kenepuru, JH McMahon, ?; **P009690**, Kenepuru, JH McMahon, 1920; **P009691**, Kenepuru, JH McMahon, 1918; **P009692**, Mahakipawa, ?, 1922; **P010987**, Maud Island, CC Ogle, 1980; **P017521**, Nelson, PJ Brownsey, 1991; *WESTLAND*: **P001649**, Lake Adelaide, ?, ?; *OTAGO*: **P001589**, The Remarkables, ?, 1853; **P008154**, Dunedin, D Petrie, ?; *SOUTHLAND*: **P005897***, Stewart Island, EA Willa, 1960; *CHATHAMS*: **P012508**, Chatham Island, J Smith-Dodsworth, 1986; **P012509**, Chatham Island, J Smith-Dodsworth, 1986; **P012515**, Chatham Island, J Smith-Dodsworth, 1986; **P012518**, Chatham Island, J Smith-Dodsworth, 1986; *UNCERTAIN*: **P001593***, Castle Rock, WRI Oliver, 1953; **P009039***, South Island, ?, 1909; **P009684***, South Island, ?, 1918; **P011926***, South Island, ?, ?.

Polystichum oculatum

AK:*WELLINGTON*: **143478**, Wellington, EP Turner, ?; **230891**, Wellington, PJ de Lange, 1996; *NELSON/MARLBOROUGH*: **114752**, Nelson, E Craig, ?; **138279**, Nelson, TF Cheeseman, 1881; **174653**, Chetwoode Islands, AE Wright, 1984; *CANTERBURY*: **114805**, Timaru, E Craig, ?.

CHR: *NELSON/MARLBOROUGH*: **147547**, Waihopai Valley, DR Given, 1963; **179030** (right-most specimen), Nelson, P Wardle, 1965; **186533**, Stephens Island, BH Macmillan, 1968; **217646**, Stephens Island, BH Macmillan, 1971; **219735**, Awatere Valley, EM Chapman, 1972; **322908**, Rarangi, DR Given, 1978; **323035**, Avon River, DR Given, 1977; **323358**, Forsyth Island, AJ Healy, 1938; **388014**, Marlborough, B Molloy, 1975; **512429**, Leatham, DR Given, 1990; *CANTERBURY*: **202999**, Kaituna, BH Macmillan, 1970; **325363**, Lyttelton, EM Chapman, ?; **483364**, Napenape, DR Given, 1974; **483369**, Banks Peninsula, DR Given, 1974; **497755**, Banks Peninsula, HD Wilson, 1986; **498245**, Banks Peninsula, DR Given, 1975.

WELT: *HAWKES BAY*: **P008140**, Napier, A Hamilton, 1882; **P008180**B, Petane, A Hamilton, ?; *WELLINGTON*: **P001594**, Paraparaumu, ?, 1954; **P001606**, Seatoun, WRI Oliver, 1921; **P006490**, Porirua, A Lush, 1950; **P006544**, Stephens Island, WRI Oliver, 1922; **P008162**, Orongorongo Ranges, Butcher, ?; **P008178**, Wellington, N Adams, 1973; **P008746**, Wellington, H Roberts, 1973; **P009093***, Wellington, A Stock, 1863; **P009527**, Wellington, CC Ogle, 1974; **P009616**, Kapiti Island, AS Wilkinson, ?; **P017969**, Cape Palliser, PJ Brownsey, 1991; **P018657**, Cape Turakirae, K Baxter, 1984; *NELSON/MARLBOROUGH*: **P008125**, Trio Islands, WRI Oliver, 1922; **P008128**, Stephens Island, WRI Oliver, 1922; **P008158**, Awatere Valley, D Petrie, 1922; **P008160** (lower specimen), Kenepuru, JH McMahon, ?; **P009677**, Queen Charlotte Sound, JH McMahon, ?; **P009680** (two left-most specimens), Long Island, JH McMahon, 1922; **P009685**, Picton, ?, 1919; **P009686**, Picton, JH McMahon, 1928; **P009687**, Picton, ?, 1933; **P017859**, Kaikoura, AM Buchanan, 1989; *CANTERBURY*: **P008122***, Banks Peninsula, WRI Oliver, 1945.

Polystichum wawranum

AK: *NORTHLAND*: **23288**, Hen & Chickens Islands, AWB Powell, 1945; **24413**, Kapowairua, RC Cooper, 1949; **50806**, Spirits Bay, P Hynes, 1957; **103813**, Hen & Chickens Islands, P Hynes, 1964; **114792** Hen & Chickens Islands, LB Moore, 1934; **114803**, Ahipara, HB Matthews, 1920 **118317**, Little Barrier Island, EM Smith, 1897; **119618**, Cuvier Island, BS Parris, 1968; **123200**, Hen & Chickens Islands, BS Parris, 1968; **126184**, Whale Bay, RC Cooper, 1965; **127614**, Little Barrier Island, F

Shakespeare, c.1900; **141837**, Herekino, JK Bartlett, 1977; **142706**, Hen & Chickens Islands, AE Wright 1977; **149398**, Motumuka Island, AE Wright, 1978; **149578**, Nukutaunga Island, AE Wright, 1979; **149653**, Motukawaiti Island, AE Wright, 1979; **150158**, Rodney, RO Gardner, 1979; **153563**, Arid Island, AE Wright, 1981; **155146**, Motukawanui Island, KP Olsen, 1976; **155312**, Poor Knights Islands, AE Wright, 1980; **156983**, Motuarohia Island, RE Beever, 1980; **159990**, Hen & Chickens Islands, AE Wright, 1982; **159991**, Hen & Chickens Islands, AE Wright, 1982; **160828**, Great Barrier Island, AE Wright, 1983; **163494**, Whangarei, MW Crookes, 1964; **166444**, Tangihua Forest, PJ Bellingham, 1984; **166586**, Puketi, PJ Bellingham, 1984; **167907**, Herekino, PJ Bellingham, 1984; **168159**, Hokianga, PJ Bellingham, 1984; **169513**, Poor Knights Islands, AE Wright, 1984; **177478**, Hokianga, AE Wright, 1986; **183143**, Three Kings Islands, AE Wright, 1989; **213838**, Little Barrier Island, AJ Dakin, 1973; **214095**, Hen & Chickens Islands, TF Cheeseman, 1884; *WAIKATO*: **27636**, Waitemata, K Wood, 1949; **29643**, Waitakere, ED Hatch, 1951; **71060**, Moeatoa, P Hynes, 1961; **71063**, Waitomo, P Hynes, 1961; 11029*, Waitakere, T Kirk, ?; **114789**, Maungatautari, JE Attwood, 1932; **114790**, Maungatautari, JE Attwood, 1932; **114791**, Maungatautari, JE Attwood, 1932; **114792**, Maungatautari, JE Attwood, 1932; **114793**, Maungatautari, JE Attwood, 1932; **114794**, Maungatautari, JE Attwood, 1932; ; **114806**, Waitakere, E Craig, ?; **122453**, Waitakere, RC Cooper, 1965; **126459**, Whale Island, P Hynes, 1970; **128843**, Red Mercury Island, P Hynes, 1971; **129152**, Auckland, AE Wright, 1971; **133363**, Slipper Island, AE Wright, 1973; **133372**, Penguin Island, AE Wright, 1973; **133395**, Rabbit Island, AE Wright, 1973; **138280**, Waitakere, TF Cheeseman, 1883; **138283**, Mt. Te Aroha, TF Cheeseman, 1906; **138285**, North Manakau Heads, TF Cheeseman, ?; **138286***, Auckland Harbour, TF Cheeseman, ?; **138287***, Waitakere, TF Cheeseman, ?; **138288***, Waitakere, TF Cheeseman, ?; **141328**, Waitemata, AE Wright, 1977; **142193**, Kaimai Range, JK Bartlett, 1977; **169901**, Waipa, J Smith-Dodsworth, 1985; **174038**, Whale Island, AE Wright, 1985; **174067**, Whale Island, RJ Lusk, 1986; **208375**, Hunua Ranges, IL Barton, 1969; **212026**, Hunua Ranges, AJ Dakin, 1972; **212027**, Hunua Ranges, AJ Dakin, 1972; **212028**, Hunua Ranges, AJ Dakin, 1972; *TARANAKI/RANGITIKEI*: **114802**, Tarata, HB Matthews, 1961; *UNCERTAIN*: **140536***, ? ("Goat Island"), E M Dickson, 1970; **140543***, ? ("Goat Island"), E M Dickson, 1970.

CHR: *UNCERTAIN*: **506135***; ?; MF Large, 1987.

WELT: *NORTHLAND*: **P001600**, Spirits Bay, WRI Oliver, 1916; **P001603**, Whangamumu, WRI Oliver, 1911; **P006486**, Poor Knights Islands, WRI Oliver, 1924; **P008150**, Great Barrier Island, WRI Oliver, 1929; **P016115**, Warawara, PJ Brownsey, 1990; *WAIKATO*: **P001591**, Te Whetumatarau, WRI Oliver, 1949; **P001592**, Te Whetumatarau, WRI Oliver, 1949; **P001596**, Port Waikato, WRI Oliver, 1941; **P001607**, North Manakau Heads, WRI Oliver, 1912; **P006516**, Cuvier Island, RR Forster, 1943; **P008124**, Cuvier Island, ?, 1919; **P008134***, Auckland, ?A Hamilton, ?; **P008135***, Auckland, ?A Hamilton, ?; **P008137**, Tarawera, A Hamilton, 1883; **P008141**, Raglan, D Petrie, ?; **P008153**, Raglan, D Petrie, ?; **P008156**, Whakatane, D Petrie, ?; **P008165**, Raglan, D Petrie, ?; P00166, Raglan, 1915; **P008167**, Waitakere, H Dobbie, 1924; **P008173***, Waitakere, TF Cheeseman, ?; *TARANAKI/RANGITIKEI*: **P008161**, Tarata, HB Matthews, ?; **P017776**, Wanganui, AE King, 1989; **P018027**, Wanganui, AE King, 1989; *HAWKES BAY*: **P001610**, Waikaremoana, WRI Oliver, 1946; **P003383**, Waikaremoana, WRI Oliver, 1946; **P003386**, Waikaremoana, WRI Oliver, 1946; **P003390**, Waikaremoana, WRI Oliver, 1946; **P008143**, Mohaka, A Hamilton, 1881; **P008145**, Petane, A Hamilton, 1882; **P008180**A, Petane, A Hamilton, ?; **P010734**, Gisborne, F Pitt, 1980; **P010737**, Tokomaru Bay, F Pitt, 1980; **P010746**, Gentle Annie, F Pitt, 1980; *UNCERTAIN*: **P001595***, North Island, ?, 1954; **P002584***, ?, W Colenso, ?; **P002585***, ?, W Colenso, ?; **P008149***, Tamahua, WRI Oliver, 1929.

Appendix 2.2.2: *Polystichum vestitum.*

Herbaria Specimens of *Polystichum vestitum* used to Construct Distribution Map. '!' indicates collections where individual specimens examined; other collections have been mapped from database locality data only.

AK: *WAIKATO*: **14076,** Thames, J Adams, ?; **14077,** Thames, J Adams, ?; **50096,** Ohakune, RC Cooper, 1957; **114753,** Ohakune, JE Attwood, 1932; **114754,** Maungatautari, JE Attwood, 1932; **114761,** Waihohonu, JE Attwood, ?; **114762,** Waimarino, HB Matthews, 1918; **114763,** Waimarino, HB Matthews, 1918; **114786,** Waimarino, HB Matthews, 1918; **119130,** Rotorua, DVG Woods, 1959; **126933,** Rotorua, RC Cooper, 1966; **138081,** Kakahi, E Phillips Turner, ?; **139722,** Opepe, TF Cheeseman, 1903; **143477,** Erua , E Phillips Turner, ?; **158784,** Waitomo, RO Gardner, 1981; **181162,** Rotorua, DJ Court, 1968; **214111,** Opepe, TF Cheeseman, 1903; **223143,** Ketetahi, AE Wright, 1975; **223145,** Auckland, AE Wright, 1969; **223147,** Waitomo , AE Wright, 1971; **223149,** Martha Hill, AE Wright, ?; **223150,** Whirinaki , AE Wright, 1979; **233155,** Te Ranga, DC Slaven, 1997; **237937,** Mt. Pirongia , PJ de Lange, 1999; *TARANAKI/RANGITIKEI*: **22214,** Mt Egmont, BEG Molesworth, 1939; **113573,** Rangiwahia, P Hynes, 1967; **114758,** Tarata, HB Matthews, 1916; *HAWKES BAY*: **114755,** Maungapohatu, LM Cranwell, 1932; **114756,** Maungapohatu, L B Moore, 1930; **122541,** Waikaremoana, P Hynes, 1970; **139718,** Dannevirke, W Colenso, ?; **221785,** Dannevirke, W Colenso, ?; **223144,** Lake Waikaremoana, AE Wright, 1972; *WELLINGTON*: **145555,** Kapiti Island, AS Wilkinson, ?; **159256,** Kapiti Island, AE Wright, 1982; *NELSON/MARLBOROUGH*: **70297,** Mt. Arthur Range, P Hynes, 1961; **105405,** Lake Rotoiti, P Hynes, 1965; **139724,** Nelson, TF Cheeseman, 1878; **139773,** Queen Charlotte Sound, JH MacMahon, ?; **213571,** Kenepuru, JH McMahon, ?; **214112,** Nelson, TF Cheeseman, 1878; **214113,** Queen Charlotte Sound, JH MacMahon, ?; **215155,** Queen Charlotte Sound, JH MacMahon, ?; *CANTERBURY*: **71380,** Akaroa, P Hynes, 1962; **141782,** Lewis Pass, AE Orchard, 1977; **179831,** Mt. Somers, EM Chapman, 1973; *WESTLAND*: **144557,** Arthur's Pass, P Hynes, 1956; **172030,** Barrytown, RO Gardner, 1985; **211800,** Mt. Kowhitirangi, AL Rockell, 1958; **223146,** Franz Josef , T Payne, 1970; *SOUTHLAND*: **92016,** Stewart Island, P Hynes, 1963; **114759,** Stewart Island, JE Attwood, 1940; **114760,** Stewart Island, JE Attwood, 1940; **119132,** Lake Manapouri, DVG Woods, 1959; **251685,** Queenstown, ME Sexton, 1959; *CHATHAMS*: **139619,** Chatham Islands, T Kirk, ?; **139620,** Chatham Islands, Seddon, ?; **139726,** Chatham Islands, Seddon, ?; **150075,** Chatham Island, AM Ringer, 1979; **174224,** Chatham Islands, RO Gardner, 1985; **174318,** Chatham Islands, RO Gardner, 1985; **174331,** Chatham Islands, RO Gardner, 1985; **214114,** Chatham Islands, T Kirk, ?; *SUBANTARCTIC*: **26540,** Antipodes Island, EG Turbott, 1950; **139727,** Auckland Islands, HR Field, 1907; **139728,** Auckland Islands, BC Aston, 1909; **139729,** Auckland Islands, JS Tennant, ?; **139730,** Macquarie Island, H Hamilton, ?; **209150,** Auckland Islands, MG Easton, 1944; **209151,** Auckland Islands , MG Easton, 1944; **209152,** Auckland Islands, MG Easton, 1944; **209153,** Auckland Islands, EG Turbott, 1944; **209154,** Auckland Islands, EG Turbott, 1944; **214115,** Auckland Islands, HR Field, 1907; **232373,** Antipodes Group, AJD Tennyson, 1995.

CHR!: *NELSON/MARLBOROUGH*: **185111,** Kaikoura, WR Sykes, 1968; **189365,** Parnassus, I Robins, 1969; **274518,** Rongo Valley, D Kelly, 1975; **311871,** Taylor Pass, AP Druce, 1976; *CANTERBURY*: **163026,** Lake Pukaki, R Mason, 1970; **169025,** Hinds, R Mason, 1965; **202864,** Kaituna, DR Given, 1970; **211440,** Geraldine, BH Macmillan, 1970; **226107,** Ashley, BH Macmillan, 1972; **234134,** Ashley, BH Macmillan, 1972; **257180,** Crawford Range, BH Macmillan, 1973; **302162,** Waiau River, BH Macmillan, 1975; **305318,** Lake Ohau, BH Macmillan, 1976; **305773,** Lewis Pass, AE Orchard, 1977; **460744,** Peel Forest, RP Buxton, 1989; **472259,** Organ Range, EH Woods, 1991; **479957,** Upper Hope Valley, DR Given, 1978; **495136,** Lake Forsyth, DR Given, 1980; *WESTLAND*: **254164,** Mueller Valley, HD Wilson, 1970; *OTAGO*: **363963,** Dunedin, PN Johnson, 1981; **428876,** Pamahaka River, KJM Dickinson, 1986; *SOUTHLAND*: **151331,** Centre Island, GI Collett, 1964; **176376,** Codfish Island, IM Ritchie, 1966; **189004,** Waikawa, PK Dorizac, 1968; **233187,** Eyre Peak, DR Given, 1972; **238067,** Riverton, R Mason, 1973; **253018,** Solander Island, PN Johnson, 1973; **360611,** Big South Cape Island, MA Ritchie, 1969; *CHATHAMS*: **187382,** Chatham Island, MA Ritchie, 1968; **397619,** Chatham Island, GN Park, 1980; *SUBANTARCTIC*: **49832,** Campbell Island, JH Sorenson, 1943; **52029,** Auckland Islands, MG Easton, 1945; **88843,** Auckland Islands, NT Moar, 1954; **88844,** Auckland Islands, NT Moar, 1954; **117937,** Campbell Island, EJ Godley, 1961; **134137,** Auckland Islands, EJ Godley, 1963;

134167, Auckland Islands, EJ Godley, 1966; **148631,** Snares Island, ?, 1947; **218458,** Campbell Island, C Meurk, 1971; **280086,** Antipodes Islands, EJ Godley, 1969; **303748,** Campbell Island, DR Given, 1976; **343054,** Antipodes Islands, G Llano, 1967; **398098,** Antipodes Islands, RH Taylor, 1978.

WELT: *WAIKATO*: **P001632,** Mt. Ruapehu, WRB Oliver, 1954; **P001633,** Mt. Ruapehu, WRB Oliver, 1954; **P001638,** Ngongotaha Mt., WRB Oliver, 1920; **P001639,** Mt. Tauhara, WRB Oliver, 1917; **P008040,** Tarawera, A Hamilton, 1881; **P008049,** Ohakune, D Petrie, ?; **P008050,** Te Whaiti, D Petrie, ?; **P008054,** Ohakune, D Petrie, ?; **P008056,** Waimarino, D Petrie, 1921; **P008075,** Mt. Tauhara, M Sutherland, 1934; **P008097,** Waimarino, D Petrie, 1921; **P012098,** Owhango, PG Edwards, 1983; *TARANAKI/RANGITIKEI*: **P006492,** Mt. Taranaki, DR McQueen, 1947; **P008057,** Tarata, HB Matthews, ?; **P009644,** Mt.Taranaki, JH McMahon, ?; **P017960,** Hunterville, AE King, 1991; *HAWKES BAY*: **P002572,** Dannevirke, ?, ?; **P002578,** Dannevirke, ?, ?; **P006559,** Ngamoko, WRB Oliver, 1946; **P006565,** Lake Waikaremoana, WRB Oliver, 1946; **P008080,** Mt. Hikurangi, WRB Oliver, 1926; **P008109,** Puketiritiri, A Hamilton, 1882; *WELLINGTON*: **P001631,** Wellington, WRB Oliver, 1949; **P001637,** Kapiti Island, WRB Oliver, 1935; **P001640,** Mt Matthews, ?, ?; **P001644,** Tauherenikau Valley, WRB Oliver, 1942; **P001648,** Kapiti Island, WRB Oliver, 1935; **P006540,** Tauherenikau Valley, DR McQueen, 1946; **P007894,** Tinakori Hills, HB Kirk, 1875; **P008077,** Kapiti Island, WRB Oliver, 1935; **P008082,** Mt. Matthews, EM Heine, 1930; **P008084,** Mt. Holdsworth, EM Heine, 1930; **P008089,** Mt. Hector, WRB Oliver, 1932; **P008093,** Kapiti Island, EM Heine, 1931; **P008108,** Horokiwi, A Hamilton, 1877; **P009545,** Pukerua Bay, CC Ogle, 1975; **P009625,** Kapiti Island, Wilkinson, AS, ?; **P010143,** Mt. Holdsworth, BL Enting, 1972; **P010145,** Mt. Holdsworth, BL Enting, 1972; **P013498,** Plimmerton, CC Ogle, 1984; *NELSON/MARLBOROUGH*: **P005148,** Mt. Franklin, ?, ?; **P007902,** Kenepuru Valley, JH McMahon, 1923; **P007904,** Pelorus Valley, JH McMahon, ?; **P007905,** Kenepuru Valley, JH McMahon, 1923; **P007906,** Mt. Stokes, JH McMahon, 1923; **P008044,** Mt. Stokes, JH McMahon, 1923; **P008045,** Kenepuru Valley, JH McMahon, 1923; **P008047,** Kenepuru Valley, JH McMahon, 1923; **P008058/A,** Kenepuru Valley, JH McMahon, 1923; **P008063,** Mt. Stokes, JH McMahon, ?; **P008079,** Mt. Arthur, EM Heine, 1933; **P008096,** Kenepuru Valley, JH McMahon, 1923; **P008107,** Mt. Arthur, A McKay, 1879; **P008111,** Picton, ?, ?; **P009640,** Picton, JH McMahon, 1939; **P009641,** Queen Charlotte Sound, JH McMahon, 1932; **P009642,** Kenepuru, JH McMahon, 1929; **P009643,** Kenepuru, JH McMahon, 1926; **P009645,** Kenepuru Valley, JH McMahon, 1923; **P009646,** Kenepuru Valley, ?, 1922; **P009647,** Kenepuru Valley, JH McMahon, 1924; **P009648,** Kenepuru Valley, ?, 1920; **P009649,** Kenepuru Valley, ?, 1921; **P009650,** Kenepuru Valley, ?, 1917; **P009651,** Kenepuru Valley, JH McMahon, 1923; **P009652,** Kenepuru Valley, ?, 1918; **P009653,** Kenepuru Valley, ?, 1919; **P009654,** Kenepuru, ?, ?; **P009655,** Mt. Stokes, JH McMahon, 1927; **P009656,** Mt. Stokes, JH McMahon, ?; **P009657,** Mt. Stokes Range, JH McMahon, 1923; **P009661,** Maruia, ?, 1931; **P009662,** Maruia, JH McMahon, 1931; **P009664,** Endeavour Inlet, JH McMahon, 1927; **P009666,** Mahakipawa Creek, JH McMahon, 1922; **P009667,** Wairau Valley, ?, ?; **P009668,** Wairau Valley, ?, ?; **P009670,** Queen Charlotte Sound, ?, ?; **P009671,** Kenepuru, ?, ?; **P009672,** Kenepuru Valley, ?, 1921; **P009674,** Grants Pass, ?, ?; **P011180,** Lake Wapiti, GB Stevenson, 1981; **P016399,** Mt. Stokes, PJ Brownsey, 1992; **P017540,** Nelson, PJ Brownsey, 1991; **P018659,** Mt. Richmond Forest, K Baxter, 1983; *CANTERBURY*: **P001641,** Mt Torlesse, WRB Oliver, 1910; **P006499,** Mt. Peel, WRB Oliver, ?; **P008041,** Oxford, ?, ?; **P008059,** Poplars Range, WG Morrison, ?; **P008091,** Mt. Torlesse, ?, 1909; **P008092,** Banks Peninsula, WRB Oliver, 1910; **P008106,** Wilberforce Valley, J Buchanan, ?; **P009735,** Banks Peninsula, N Adams, 1978; *WESTLAND*: **P001642,** Cleddau River, WRB Oliver, 1944; **P007295,** Whitcombe River, DR McQueen, 1947; **P008076,** Haast, M Sutherland, 1936; **P008085,** Arthurs Pass, WRB Oliver, 1927; **P008086,** Upper Hollyford, WRB Oliver, 1944; **P008101,** Westport, PG Morgan, 1912; **P009665,** Mt. Barron, JW Brame, 1923; **P011940,** Hokitika, ?, ?; **P018660,** Arthurs Pass, K Baxter, 1983; *OTAGO*: **P008090,** Mt. Cargill, ?, 1920; **P008102,** Dunedin, A Hamilton, ?; **P008103,** Dunedin, A Hamilton, ?; **P008104,** Dunedin, A Hamilton, ?; **P010131,** Lake Wakatipu, BL Enting, 1973; **P017650,** Tahakopa, PJ Brownsey, 1990; **P018661,** Duffers Saddle, K Baxter, 1984; *SOUTHLAND*: **P001629,** Caswell Sound, WRB Oliver, 1949; **P001630,** Caswell Sound, WRB Oliver, 1949; **P003543,** Caswell Sound, WRB Oliver, 1949; **P004827,** Stewart Island, LC Hudson, 1972; **P005895,** Stewart Island, EA Willa, 1960; **P007306,** Caswell Sound, WRB Oliver, 1949; **P008074,** Clifden, RL Oliver, 1941; **P008118,** Puysegur Point, ?, ?; **P008120,** Stewart Island, LC Hudson, 1972; **P009026,** Puysegur Point, T Kirk, ?; **P009207,** Murchison Range, F Newcombe, 1951; **P010132,** Homer Tunnel, BL Enting, 1972; *SUBANTARTIC*: **P001627,** Snares Islands, F Newcombe, 1947; **P001634,** Auckland Islands, RL Oliver, 1944; **P001635,** Antipodes Islands, WRB Oliver, 1927; **P001636,** Campbell Island, WRB Oliver, 1927; **P003468,** Snares Islands, ?, 1947; **P003470,** Snares Islands, ?, 1947; **P003480,** Campbell Island, WRB Oliver, 1927; **P004847,** Campbell Island, ?, ?; **P004854,** Campbell Island, Rathouis, 1873; **P004856,** Antipodes Islands, T Kirk, ?; **P005170,** Antipodes Islands, T Kirk, ?; **P008051,** Campbell Island, RL

Oliver, 1944; **P008053,** Auckland Islands, L Cockayne, 1903; **P008061,** Auckland Islands, BC Aston, 1909; **P008062,** Campbell Island, WR Chambers, 1907; **P008065,** Campbell Island, JH Sorensen, 1947; **P008066,** Campbell Island, JH Sorensen, 1947; **P008067,** Campbell Island, ?, 1947; **P008069,** Campbell Island, RA Falla, RA, 1951; **P008072,** Campbell Island, RL Oliver, ?; **P008081,** Auckland Islands, ?, ?; **P008119,** Macquarie Island, A Hamilton, ?; **P008121,** Macquarie Island, A Hamilton, 1894; **P009340,** Antipodes Islands, T Kirk, ?; **P010848,** Auckland Islands, MC Wassilieff, 1980; **P010851,** Auckland Islands, MC Wassilieff, 1980; **P011094,** Snares Islands, ASW Penniket, 1981; **P014124,** Snares Islands, GS Hardy, 1984; **P014125,** Snares Islands, GS Hardy, 1984; **P014126,** Snares Islands, GS Hardy, 1984.

Appendix 2.2.3: *Polystichum silvaticum.*

Herbaria Specimens of *Polystichum vestitum* used to Construct Distribution Map. '!' indicates collections where individual specimens examined; other collections have been mapped from database locality data only.

AK: *WAIKATO*: **11028**, Thames ?, ?; **14079**, Thames, J Adams, ?; **31514**, Mt Pirongia, P Hynes, 1953; **71193**, Wairoa River, P Hynes, 1961; **71194**, Wairoa River, P Hynes, 1961; **114773**, Hamilton, HB Matthews, 1920; **114774**, Ruapehu, JE Attwood, ?; **114775**, Ruapehu, JE Attwood, ?; **114776**, Ruapehu, JE Attwood, ?; **114780**, Hamilton, HB Matthews, 1920; **114781**, Hamilton, HB Matthews, 1920; **114782**, Ruapehu, JE Attwood, ?; **114783**, Horopito, JE Attwood, 1933; **114785**, Ruapehu, JE Attwood, ?; **114865**, Ruapehu, JE Attwood, 1932; **114866**, Ruapehu, JE Attwood, 1932; **114868**, Ruapehu, JE Attwood, 1932; **114869**, Maungatautari, JE Attwood, 1932; **114870**, Maungatautari, JE Attwood, 1932; **114871**, Ruapehu, JE Attwood, 1932; **114872**, Manganuiateao River, JE Attwood, 1932; **128175**, Mt Te Aroha, BS Parris, 1971; **128744**, Waitomo, AE Wright, 1971; **139622**, Coromandel, TF Cheeseman, 1882; **139623**, Thames, TF Cheeseman, ?; **139627**, Hauhungatahi, E Phillips Turner, ?; **144558**, Coromandel, P Hynes, 1953; **163515**, Pirongia, MC Gudex, 1947; **165472**, Priongia, PJ de Lange, 1984; **166037**, Coromandel Range, B Burns, 1983; **166054**, Coromandel Range, RO Gardner, 1983; **166055**, Coromandel Range, RO Gardner, 1983; **208376**, Hunua Range, IL Barton, 1969; **212728**, Mangakahu Valley, AE Wright, 1992; **213303**, Horopito, JE Attwood, 1933; **214103**, Manganui-a-teao River, JE Attwood, 1932; **22330**, Hunua Ranges, BEG Molesworth, 1944; *TARANAKI/RANGITIKEI*: **50687**, Mt Egmont, P Hynes, 1958; **139624**, Mt Egmont, TF Cheeseman, 1885; **214104**, Mt Egmont Ranges, TF Cheeseman, 1885; *HAWKES BAY*: **114764**, Woodville ?, ?; **223148**, Lake Waikaremoana, AE Wright, 1972; **233836**, Lake Waikaremoana, AE Wright, 1972; *WELLINGTON*: **200870**, Wellington, PJ de Lange, 1990; *NELSON/MARLBOROUGH*: **139625**, Queen Charlotte Sound, JH MacMahon, ?; **214105**, Queen Charlotte Sound, JH MacMahon, ?; **214106**, Queen Charlotte Sound, JH MacMahon, ?; **214107**, Queen Charlotte Sound, JH MacMahon, ?; **214108**, Queen Charlotte Sound, JH MacMahon, ?; *CANTERBURY*: **235063**, Craigieburn, EK Cameron , 1997; *SOUTHLAND*: **251686**, Doubtful Sound, ME Sexton, 1959.

WELT!: *WAIKATO*: **P007913**, Opuatia Creek, A Hamilton, 1905; **P015762**, Te Kauri, MJ Gilmour, 1987; *TARANAKI/RANGITIKEI*: **P007893**, Mt Taranaki, WRB Oliver, 1931; *HAWKES BAY*: **P001624**, Waikaremoana, WRB Oliver, 1946; **P006566**, Waikaremoana, WRB Oliver, 1946; **P007890**, Puketiritiri, A Hamilton, 1881; **P007891**, Porangahau, A Hamilton, 1883; *WELLINGTON*: **P001625**, Colonial Knob, WRB Oliver, 1947; **P006457**, Colonial Knob, WRB Oliver, 1947; **P007888**, Wainuiomata, ?, ?; **P017800**, Otari , PJ de Lange, 1993; **P020216**, Wellington, CK Jeffs, 1879; *NELSON/MARLBOROUGH*: **P009634**, Kenepuru Valley, ?, 1932; **P009635**, Kenepuru Valley, ?, 1918; **P009636**, Kenepuru, ?, 1918; **P009637**, Kenepuru, ?, 1931; *WESTLAND*: **P011924**, Hokitika, ?, ?; *OTAGO*: **P007892**, Mt Cargill, A Hamilton, ?.

Appendix Three: Appendices for Chapters Three, Four, Five, and Six.

Appendix 3.1: Appendices from Chapter Three.

Appendix 3.1.1

Details of primers used to amplify single locus regions investigated during this study.

Name	Locus	Source	Orientation	Sequence
RB27F	*rbcL* gene	novel	forward	5'-GTCACCACAAACAGAGACTAAAGC-3'
RB422F	*rbcL* gene	novel	forward	5'-GCTTGGAAGACCTTCGAATTC-3'
RB961F	*rbcL* gene	novel	forward	5'-GTATTGGCCAAAGCATTACGAATG-3'
RB579R	*rbcL* gene	novel	reverse	5'-GTGAAATCAAGTCCGCCGCG-3'
RB988R	*rbcL* gene	novel	reverse	5'-CCTCCAGTTTACCTACTACAG-3'
RB1381R	*rbcL* gene	novel	reverse	5'-CAAGCTTCACGAATAACTTCATTACC-3'
rps4F	*rps4* gene & spacer	Ray Cranfill	forward	5'-ATGTCCCGTTATCGAGGACCT-3'
rps4-2	*rps4* gene & spacer	novel	forward	5'-GTACCACTGCAATTACTC-3'
rps4-3	*rps4* spacer	novel	forward	5'-CAATAAGCAGATTACTTAG-3'
rpsSTOP	*rps4* spacer	novel	forward	5'-GTTAGTTGTTGAGTATTAC-3'
trnSr	*rps4* gene & spacer	Ray Cranfill	reverse	5'-TACCGAGGGTTCGAATC-3'

Appendix 3.1.2

Details of oligonucleotide sequences used in the AFLP analyses.

Adapter-Linker Oligonucleotides	
*Eco*RI adapter I	5'-CTCGTAGACTGCGTACC-3'
*Eco*RI adapter II	5'-AATTGGTACGCAGTCTAC-3'
*Mse*I adapter I	5'-GACGATGAGTCCTGAG-3'
*Mse*I adapter II	5'-TACTCAGGACTCAT-3'
Pre-amplification Primers	
*Eco*RI-PA	5'-GACTGCGTACCAATTCA-3'
*Mse*I-PA	5'-GATGAGTCCTGAGTAAC-3'
Selective-amplification Primers	
Eco-AAT	5'-GACTGCGTACCAATTCAAT-3'
Eco-ATA	5'-GACTGCGTACCAATTCATA-3'
Eco-AGC	5'-GACTGCGTACCAATTCAGC-3'
Mse-CAG	5'-GATGAGTCCTGAGTAACAG-3'
Mse-CTG	5'-GATGAGTCCTGAGTAACTG-3'

Appendix 3.2: Appendices from Chapter Four.

Appendix 3.2.1

Sample (see Appendix 2.1.1 for collection details) and taxonomic details of the *Polystichum* samples analysed by AFLP in Perrie et al. (2000).
* indicates samples from locally-sympatric sites discussed in Table 4.1.
† *rKap1* & *rKap6* were c.100 m distant.
‡ *rKak1* & *rKak5* were c.10 km distant

Sample Number in Perrie et al. (2000)	Sample	Designation of Perrie et al. (2000)	As Recognised in this Study
1	*rKar6*	'Narrow-scaled species'	*P. wawranum*
2	*rPmm1*	'Narrow-scaled species'	*P. neozelandicum* subsp. *neozelandicum*
3	*rTar3*	'Narrow-scaled species'	*P. neozelandicum* subsp. *zerophyllum*
4	*rCar5*	'Narrow-scaled species'	*P. neozelandicum* subsp. *zerophyllum*
5	*rDun1*	'Narrow-scaled species'	*P. neozelandicum* subsp. *zerophyllum*
6	*rCha3*	'Narrow-scaled species'	*P. neozelandicum* subsp. *zerophyllum*
7	*rSte3* *	'Narrow-scaled species'	*P. wawranum*
8	*rWak1* *	'Narrow-scaled species'	*P. neozelandicum* subsp. *zerophyllum*
9	*rKap6* †	'Narrow-scaled species'	*P. neozelandicum* subsp. *zerophyllum*
10	*rCas2* *	'Narrow-scaled species'	*P. neozelandicum* subsp. *zerophyllum*
11	*rMaa4* *	'Narrow-scaled species'	*P. neozelandicum* subsp. *zerophyllum*
12	*rKak1* ‡	'Narrow-scaled species'	*P. neozelandicum* subsp. *zerophyllum*
13	*rNap3* *	'Narrow-scaled species'	*P. neozelandicum* subsp. *zerophyllum*
14	*rKai3* *	'Narrow-scaled species'	*P. neozelandicum* subsp. *zerophyllum*
15	*rSte4* *	'Wide-scaled species'	*P. oculatum*
16	*rWak5* *	'Wide-scaled species'	*P. oculatum*
17	*rKap1* †	'Wide-scaled species'	*P. oculatum*
18	*rCas1* *	'Wide-scaled species'	*P. oculatum*
19	*rMaa1* *	'Wide-scaled species'	*P. oculatum*
20	*rKak5* ‡	'Wide-scaled species'	*P. oculatum*
21	*rNap12* *	'Wide-scaled species'	*P. oculatum*
22	*rKai1* *	'Wide-scaled species'	*P. oculatum*

Appendix 3.2.2

List of the parsimony-informative splits occurring more than once in the *Polystichum neozelandicum* complex AFLP data set described in Section 4.3.3.

Split	Interpretation	Frequency of occurrence	Number of incompatible characters
{*rSte4*, *rWak5*, *rCas1*, *rWel8*, *rKak5*, *rKai1*}{complement}	{*P. oculatum*} {complement}	15	113
{*rKar6*, *rPih1*, *rWto1*, *rSte3*, *rWan6*, *rPoh5*, *rNga3*}{complement}	{*P. wawranum*} {complement}	5	144
{*rKar6*, *rPih1*}{complement}		4	97
{*rKar6*, *rPoh5*, *rSte4*, *rWak5*, *rCas1*, *rWel8*, *rKak5*, *rKai1*}{complement}		3	196
{*rSte4*, *rWak5*, *rCas1*, *rKak5*, *rKai1*}{complement}		3	119
{*rKar6*, *rSte3*}{complement}		3	102
{*rKar6*, *rWto1*, *rSte3*, *rPoh5*, *rNga3*}{complement}		3	163
{*rSte4*, *rWak5*, *rWel8*}{complement}		3	103
{*rPih1*, *rWto1*, *rSte3*, *rWan6*, *rPoh5*, *rNga3*}{complement}		2	163
{*rWkw1*, *rPmm1*, *rSte4*, *rWak5*}{complement}		2	156
{*rSte4*, *rWak5*, *rCas1*}{complement}		2	92
{*rSte4*, *rWak5*, *rCas1*, *rWel8*, *rKak5*}{complement}		2	117
{*rSte4*, *rWak5*, *rWel8*, *rKak5*}{complement}		2	116
{*rPih1*, *rWto1*, *rWan6*, *rPoh5*, *rNga3*}{complement}		2	152
{*rWto1*, *rSte3*, *rWan6*, *rPoh5*, *rNga3*}{complement}		2	145
{*rKar6*, *rPih1*, *rWto1*}{complement}		2	139
{*rKar6*, *rPih1*, *rWto1*, *rSte3*, *rWan6*, *rPoh5*, *rNga3*, *rWkw1*, *rPmm1*}{complement}	{*P. wawranum* & *P. neozelandicum* subsp. *neozelandicum*} {*P. neozelandicum* subsp. *zerophyllum* & *P. oculatum*}	2	150
{*rWkw1*, *rPmm1*, *rOpe1*, *rWan5*, *rWak1*, *rPoh6*, *rNga6*, *rKak1*, *rDun1*}{complement}	{*P. neozelandicum* subsp. *neozelandicum* & *P. neozelandicum* subsp. *zerophyllum*}{*P. wawranum* & *P. oculatum*}	2	93
{*rKar6*, *rSte4*, *rWak5*, *rCas1*, *rWel8*, *rKak5*, *rKai1*}{complement}		2	170
{*rKar6*, *rNga3*, *rSte4*, *rWak5*, *rCas1*, *rWel8*, *rKak5*}{complement}		2	228
{*rPoh5*, *rNga3*}{complement}		2	75
{*rWkw1*, *rPmm1*}{complement}	{*P. neozelandicum* subsp. *neozelandicum*} {complement}	2	10
{*rSte4*, *rKak5*}{complement}		2	73
{*rPih1*, *rSte4*, *rWak5*, *rCas1*, *rWel8*, *rKak5*, *rKai1*}{complement}		2	166

Appendix 3.2.3

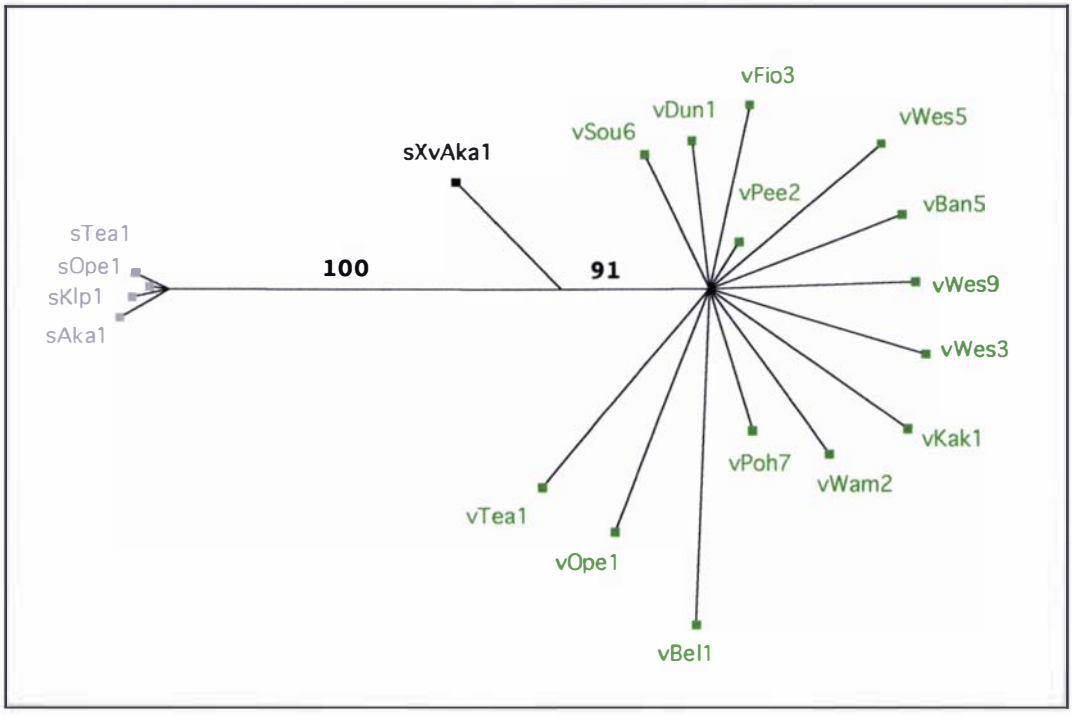
Support and conflict values calculated under a distance-spectral analysis for the 30 most supported (parsimony-informative) splits in the AFLP data set of the *Polystichum neozelandicum* complex described in Section 4.3.3.

Split	Interpretation	Calculated support	Calculated conflict (normalised)
{*rSte4*, *rWak5*, *rCas1*, *rWel8*, *rKak5*, *rKai1*}{complement}	{*P. oculatum*} {complement}	0.1188	0.0214 (0.0141)
{*rWkw1*, *rPmm1*}{complement}	{*P. neozelandicum* subsp. *neozelandicum*}{complement}	0.0824	0.0037 (0.0024)
{*rKar6*, *rPih1*, *rWto1*, *rSte3*, *rWan6*, *rPoh5*, *rNga3*}{complement}	{*P. wawranum*} {complement}	0.0695	0.0478 (0.0314)
{*rOpe1*, *rWan5*, *rWak1*, *rPoh6*, *rNga6*, *rKak1*, *rDun1*}{complement}	{*P. neozelandicum* subsp. *zerophyllum*}{complement}	0.0688	0.0131 (0.0086)
{*rWkw1*, *rPmm1*, *rOpe1*, *rWan5*, *rWak1*, *rPoh6*, *rNga6*, *rKak1*, *rDun1*}{complement}	{*P. neozelandicum* subsp. *neozelandicum* & *P. neozelandicum* subsp. *zerophyllum*} {*P. wawranum* & *P. oculatum*}	0.0247	0.0476 (0.0312)
{*rKar6*, *rPih1*, *rWto1*, *rSte3*, *rWan6*, *rPoh5*, *rNga3*, *rWkw1*, *rPmm1*} {complement}	{*P. wawranum* & *P. neozelandicum* subsp. *neozelandicum*} {*P. neozelandicum* subsp. *zerophyllum* & *P. oculatum*}	0.0161	0.0857 (0.0563)
{*rKar6*, *rPih1*}{complement}		0.0143	0.0426 (0.0280)
{*rWto1*, *rWan6*, *rPoh5*, *rNga3*}{complement}		0.0123	0.0524 (0.0344)
{*rWto1*, *rSte3*, *rWan6*, *rPoh5*, *rNga3*}{complement}		0.0105	0.0620 (0.0407)
{*rSte4*, *rWak5*, *rCas1*, *rWel8*}{complement}		0.0103	0.0307 (0.0202)
{*rSte4*, *rWak5*, *rCas1*, *rWel8*, *rKak5*}{complement}		0.0098	0.0243 (0.0160)
{*rWak5*, *rCas1*}{complement}		0.0096	0.0253 (0.0166)
{*rSte4*, *rWak5*, *rCas1*} {complement}		0.0091	0.0351 (0.0230)
{*rKar6*, *rPih1*, *rSte4*, *rWak5*, *rCas1*, *rWel8*, *rKak5*, *rKai1*}{complement}		0.0083	0.1799 (0.1181)
{*rKar6*, *rPih1*, *rSte3*}{complement}		0.0074	0.0944 (0.0620)
{*rPih1*, *rWto1*, *rSte3*, *rWan6*, *rPoh5*, *rNga3*}{complement}		0.0071	0.0822 (0.0540)
{*rWak1*, *rPoh6*}{complement}		0.0071	0.0010 (0.0007)
{*rSte4*, *rWel8*}{complement}		0.0064	0.0220 (0.0144)
{*rOpe1*, *rWan5*, *rWak1*, *rPoh6*, *rNga6*}{complement}		0.0063	0.0076 (0.0050)
{*rPoh5*, *rNga3*}{complement}		0.0059	0.0235 (0.0154)
{*rKak5*, *rKai1*}{complement}		0.0058	0.0438 (0.0289)
{*rWto1*, *rWan6*}{complement}		0.0055	0.0527 (0.0346)
{*rSte4*, *rWak5*, *rWel8*, *rKak5*}{complement}		0.0053	0.0507 (0.0333)
{*rWto1*, *rSte3*}{complement}		0.0047	0.0653 (0.0429)
{*rWto1*, *rNga3*}{complement}		0.0045	0.0348 (0.0228)
{*rWan5*, *rWak1*, *rPoh6*}{complement}		0.0042	0.0064 (0.0042)
{*rWto1*, *rSte3*, *rPoh5*, *rNga3*}{complement}		0.0042	0.0776 (0.0509)
{*rWan6*, *rPoh5*}{complement}		0.0041	0.0448 (0.0294)
{*rWkw1*, *rPmm1*, *rOpe1*, *rWan5*, *rWak1*, *rPoh6*, *rNga6*, *rKak1*, *rDun1*, *rKai1*}{complement}		0.0036	0.2083 (0.1367)
{*rSte4*, *rWak5*, *rWel8*}{complement}		0.0036	0.0368 (0.0242)

Appendix 3.3: Appendices from Chapter Five.

Appendix 3.3.1

Parsimony splits-graph illustrating the genetic intermediacy of the putative hybrid *sXvAka1* between *P. silvaticum* and *P. vestitum.* Collection details for samples are given in Appendices 2.1.2 and 2.1.4.



Appendix 3.3.2

List of the parsimony-informative splits occurring more than once in the 'Combined' AFLP data set of *Polystichum vestitum* and *P. silvaticum.*

Split	Interpretation	Frequency of occurrence	Number of incompatible characters
{*sTea1*, *sAka1*}{complement}	{*P. silvaticum*} {*P. vestitum*}	25	33
{*vBan5*, *vDun1*}{complement}		2	106
{*sTea1*, *sAka1*, *vCha17*}{complement}		2	158
{*vCha17*, *vCha16*}{complement}		2	86
{*vCha17*, *vCha18*}{complement}		2	112
{*sTea1*, *sAka1*, *vSou6*}{complement}		2	156
{*vBel1*, *vCha11*, *vCha18*}{complement}		2	164
{*sTea1*, *sAka1*, *vBan5*}{complement}		2	147
{*sTea1*, *sAka1*, *vSou5*, *vSna1*}{complement}		2	190
{*vBel1*, *vSou5*}{complement}		2	120
{*vBel1*, *vKak1*, *vBan5*, *vCha11*, *vCha12*, *vSou5*, *vAki5*} {complement}		2	254
{*sTea1*, *sAka1*, *vCha11*}{complement}		2	155

Appendix 3.3.3

Support and conflict values calculated under a distance-spectral analysis for the 30 most supported (parsimony-informative) splits for the 'Combined' AFLP data set of *Polystichum vestitum* and *P. silvaticum.*

Split	Interpretation	Calculated support	Calculated conflict (normalised)
{*sTea1*, *sAka1*}{complement}	{*P. silvaticum*}{*P. vestitum*}	0.2217	0 (0)
{*vSou5*, *vSna1*}{complement}	{'Divergent southern' samples} {complement}	0.0326	0.0083 (0.0035)
{*vCha8*, *vCha7*, *vCha16*, *vCha17*, *vCha19*}{complement}	{'Divergent Chathams' samples} {complement}	0.0150	0.0098 (0.0041)
{*vBel1*, *vKak1*}{complement}		0.0135	0.0361 (0.0151)
{*vCha17*, *vCha16*}{complement}		0.0104	0.0090 (0.0038)
{*vSou5*, *vSna1*, *vAki5*}{complement}		0.0095	0.0268 (0.0112)
{*vCha18*, *vCha10*}{complement}		0.0091	0.0098 (0.0041)
{*sTea1*, *sAka1*, *vBan5*}{complement}		0.0083	0.0342 (0.0143)
{*vAki5*, *vAnt2*}{complement}	{'Mainland-like southern' samples} {complement}	0.0080	0.0400 (0.0167)
{*vKak1*, *vSou6*}{complement}		0.0005	0.0546 (0.0229)
{*vSou5*, *vSna1*, *vAki5*, *vAnt2*}{complement}	{All 'southern' samples} {complement}	0.0072	0.0406 (0.0170)
{*vBel1*, *vCha11*}{complement}		0.0057	0.0418 (0.0175)
{*vBel1*, *vKak1*, *vSou6*}{complement}		0.0053	0.0672 (0.0281)
{*vBan5*, *vDun1*}{complement}		0.0052	0.0556 (0.0233)
{*vCha10*, *vCha12*}{complement}		0.0048	0.0288 (0.0120)
{*vBel1*, *vKak1*, *vCha11*}{complement}		0.0048	0.0531 (0.0222)
{*vCha18*, *vCha10*, *vCha12*, *vCha8*, *vCha7*, *vCha16*, *vCha17*, *vCha19*}{complement}		0.0048	0.0224 (0.0094)
{*vDun1*, *vSou6*, *vAnt2*}{complement}		0.0044	0.0699 (0.0293)
{*vCha18*, *vCha10*, *vCha12*}{complement}		0.0043	0.0231 (0.0097)
{*vCha7*, *vCha16*}{complement}		0.0041	0.0205 (0.0086)
{*vDun1*, *vSou6*}{complement}		0.0036	0.0493 (0.0206)
{*vCha11*, *vCha12*}{complement}		0.0036	0.0427 (0.0179)
{*vCha7*, *vCha16*, *vCha17*}{complement}		0.0035	0.0150 (0.0063)
{*vCha10*, *vCha11*, *vCha12*, *vCha18*, *vCha8*, *vCha7*, *vCha16*, *vCha17*, *vCha19*} {complement}	{All Chatham Island samples} {complement}	0.0034	0.0339 (0.0142)
{*vDun1*, *vSou5*, *vSna1*, *vAki5*, *vAnt2*} {complement}		0.0033	0.0535 (0.0224)
{*vDun1*, *vSou6*, *vSou5*, *vSna1*, *vAki5*, *vAnt2*} {complement}		0.0032	0.0161 (0.0067)
{*vCha7*, *vCha19*}{complement}		0.0030	0.0096 (0.0040)
{*vCha7*, *vCha16*, *vCha17*, *vCha19*} {complement}		0.0028	0.0097 (0.0041)
{*sTea1*, *sAka1*, *vBel1*}{complement}		0.0027	0.0699 (0.0293)
{*vDun1*, *vAnt2*}{complement}		0.0025	0.0471 (0.0197)

Appendix 3.4: Appendices from Chapter Six.

Appendix 3.4.1

The parsimony-informative splits occurring at more than one character in the AFLP data-set discussed in Chapter Six.

Split	Number of characters
{*aFal1*, *aFor1*}{complement}	3
{*cTar1*, *cKak1*}{complement}	2
{*aFal1*, *aFor1*, *oLen1*, *oSet1*}{complement}	2
{*aAus1*, *aFor1*, *oLen1*, *oSet1*}{complement}	2
{*hMoo1*, *hWhi1*}{complement}	2
{*aPro2*, *aAus1*}{complement}	2

Appendix Four: Files on Accompanying CD.

The following is a list and description of data files (in Macintosh format) on the accompanying CD.

File name	Description
Sam-neoCom	Full collection details of samples collected for this study from the *Polystichum neozelandicum* complex (ie. *P. neozelandicum* subsp. *neozelandicum*, *P. neozelandicum* subsp. *zerophyllum*, *P. oculatum*, & *P. wawranum*; see Appendix 2.1.1); Excel table.
Sam-vestitum	Full collection details of samples collected for this study from *Polystichum vestitum* (including the 'divergent Chathams' plants; see Appendix 2.1.2); Excel table.
Sam-cys&sil	Full collection details of samples collected for this study from *Polystichum cystostegia* and *P. silvaticum* (see Appendix 2.1.3); Excel table.
Sam-hybrids	Full collection details of samples collected for this study from *Polystichum* hybrids (see Appendix 2.1.4); Excel table.
Sam-overseas	Full collection details of samples collected for this study from non-New Zealand *Polystichum* species (see Appendix 2.1.5); Excel table.
Morph-NeoCom	Morphological data for samples from the *Polystichum neozelandicum* complex; Excel table.
AFLP-NeoCom.nex	Nexus file of the AFLP data for the *Polystichum neozelandicum* complex; nexus text file.
AFLP-NeoCom.spe	Spectrum file of the AFLP data for the *Polystichum neozelandicum* complex; spectrum text file.
AFLP-PerrieEtal2000.nex	Nexus file of the AFLP data from Perrie et al. (2000); nexus text file.
Morph-Ves	Morphological data for samples from the *Polystichum vestitum*; Excel table.
AFLP-Ves-NS.nex	Nexus file of the AFLP data for *Polystichum vestitum* from the 'North & South Island' sample set; nexus text file.
AFLP-Ves-CI.nex	Nexus file of the AFLP data for *Polystichum vestitum* from the 'Chatham Islands' sample set; nexus text file.
AFLP-Ves-CMB.nex	Nexus file of the AFLP data for *Polystichum vestitum* from the 'Combined' sample set; nexus text file.
AFLP-Ves-CMB.spe	Spectrum file of the AFLP data for *Polystichum vestitum* from the 'Combined' sample set; spectrum text file.
AFLP-Ves-CMB-II.nex	Nexus file of the AFLP data for *Polystichum vestitum* from the 'Combined' sample set, with only the E-AAT & M-CTG, E-ATA & M-CAG, and E-AGC & M-CTG primer combinations; nexus text file.
AFLP-Ves-CMB-III.nex	Nexus file of the AFLP data for *Polystichum vestitum* from the 'Combined' sample set, with only the E-AAT & M-CAG primer combination; nexus text file.
SpacerFull.aln	Complete sequence alignment of the *rps4-trnS* spacer region; text file.
SpacerNoGaps.nex	Nexus file of the *rps4-trnS* spacer region with all gaps excluded; nexus text file.
SpacerGapsIn.nex	Nexus file of the *rps4-trnS* spacer region with gaps coded as characters; nexus text file.
AFLP-SWPac.nex	Nexus file of the AFLP data for the south-western Pacific *Polystichum* sample set; nexus text file.
AFLP-SWPac.spe	Spectrum file of the AFLP data for the south-western Pacific *Polystichum* sample set; spectrum text file.
rbcL-PolDry.aln	Alignment file of *rbcL* sequence for *Dryopteris* and SW Pacific *Polystichum*; alignment text file.
AFLP-sXvAka1.nex	Nexus file of the AFLP data used to construct the graph in Appendix 3.3.1 depicting the genetic intermediacy of the putative *Polystichum silvaticum* x *vestitum* hybrid *sXvAka1* (also includes *vAki1* data); nexus text file.