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Evidence for an allopolyploid complex in New Zealand *Polystichum* (Dryopteridaceae)

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Abstract Evidence is provided for the first demonstrated example of allopolyploidy in the New Zealand fern flora. Cytological, morphological, and molecular (AFLP-DNA fingerprinting) analyses indicate that the fern previously known as *Polystichum richardii* constitutes an allopolyploid complex, in which four separate evolutionary lineages are present. These are here recognised as three taxonomic species, with one of these encompassing two subspecies. The two allo-octoploid lineages are accommodated under the reinstated name *P. neozelandicum*, each as a separate subspecies: *P. neozelandicum* subsp. *neozelandicum* and the new combination *P. neozelandicum* subsp. *zerophyllum*. The new combination *P. wawranum* is made for one of the tetraploid lineages, while the name *P. oculatum* is reinstated for the other. *Polystichum richardii* is a later synonym of *P. neozelandicum*, and hence is not a legitimate name for any of the taxa recognised here.

Keywords *Polystichum*; *P. richardii*; *P. neozelandicum* subsp. *neozelandicum*; *P. neozelandicum* subsp. *zerophyllum*; *P. oculatum*; *P. wawranum*; Dryopteridaceae; fern; allopolyploid; AFLP DNA-fingerprinting; taxonomy; species; evolution; lineage; New Zealand flora

INTRODUCTION

The New Zealand endemic fern *Polystichum richardii* (Hook.) J.Smith (≡*Aspidium richardii* Hook.) has long been acknowledged as morphologically variable (e.g., Cheeseman 1906, 1925; Allan 1961; Crookes 1963). Brownsey & 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. The recognition by nineteenth century botanists (Fée 1857; Hooker 1863; Szyszylowicz in Wawra 1888; Colenso 1897) of four species related to *P. richardii* is, at least partially, a reflection of this variation. However, the complex has been treated as a single taxonomic species for some 80 years (e.g., Dobbie 1921; Allan 1961; Brownsey & Smith-Dodsworth 2000). Earlier, Cheeseman (1906, p. 999) listed the proposed species *Aspidium oculatum* Hook., but dismissed it as "probably nothing more than a trivial variety of *A. Richardii*".

Nevertheless, some authors have been uncomfortable with this single species concept. For instance, Cockayne & Allan (1934) and Crookes (1963) indicated that *Polystichum richardii* was a compound species. Clarkson (1991; pers. comm.) recently drew attention to the sympatric nature of some of this variation on the North Island's east

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coast. His suggestion that more than one species was present led to the inception of this study.

Previously, we reported preliminary evidence for the partitioning of *P. richardii* into two groups (Perrie et al. 2000). However, here we report from an expanded sample set that what was previously regarded as a single species is, in fact, an allopolyploid complex in which we recognise three species, with one of these encompassing two subspecies: *P. oculatum* (Hook.) J.B.Armstr., *P. wawranum* (Szyszyl. in Wawra) Perrie comb. nov., *P. neozelandicum* Fée subsp. *neozelandicum*, and *P. neozelandicum* subsp. *zerophyllum* (Colenso) Perrie comb. et stat. nov. *Polystichum richardii* is a later synonym of *P. neozelandicum*, and hence is not a legitimate name for any of the taxa recognised here.

MATERIALS AND METHODS

Morphology

Morphological characters were investigated in 134 samples referable to *P. richardii* (*sensu* Brownsey & Smith-Dodsworth 1989) collected from throughout New Zealand for this study (these have been distributed to AK, CHR, MPN, WAIK, and WELT; further details in Perrie (2001), or available from the senior author on request). Collection details of samples referred to directly in the text are given in Appendix 1 (except types), where details of additional representative specimens are also provided.

The definition and measurement of morphological characters found to be useful in differentiating the taxa recognised here are described in Table 1. Plants with aborted spores were identified as putative hybrids (see below), and excluded from analyses. Box-plot summaries of the variation in the quantitative morphological characters were produced using the program SPSS 10.1.0 (SPSS 2000), as were scatter-plots which were used to investigate whether morphological characters were exhibiting concordant partitioning. This is where the samples are more or less restricted to two diagonally opposite quadrants of the plot. The greater this restriction, the stronger the partitioning. Such concordant partitioning by two independent characters is only likely if two (or more) separate lineages, or assortatively fertilising groups of individuals, are present, therein allowing rejection of the null-hypothesis that "a single evolutionary lineage is present" (see below).

The ranges for quantitative characters given in the taxonomic treatment below are based on 5th and 95th percentiles.

Distribution maps were compiled using the samples collected for this study, together with the collections of AK, CHR, WAIK, and WELT (annotated with determinavit slips).

Cytology

Meiotic chromosome counts were made from young sporangia with a standard acetocarmine methodology (Manton 1950).

Table 1 Definition and measurement of morphological characters.

Character name	Character definition and measurement
Mid-scale width	Average width of five scales from the stipe-rachis junction, measured at their mid length at ×40 magnification (μm).
Max scale width	Average maximum width of five scales from the stipe-rachis junction, measured at ×40 magnification (μm).
Pinnae distance ratio	Distance between the 2nd and 4th most basal pair of primary pinnae, divided by the length of the rachis.
Pinna width ratio	Length divided by width of the longest primary pinna.
Annulus cells	Average number of indurated cells counted in 10 sporangia, observed at ×100 magnification.
Indusia dark centre	Average percentage of surface area occupied by central dark area in 10 indusia, measured at ×100 magnification.
Spore size	Average product of spore exine length and width of 30 spores, measured at ×1000 magnification (μm^2) in a 1:1 solution of glycerol:water.

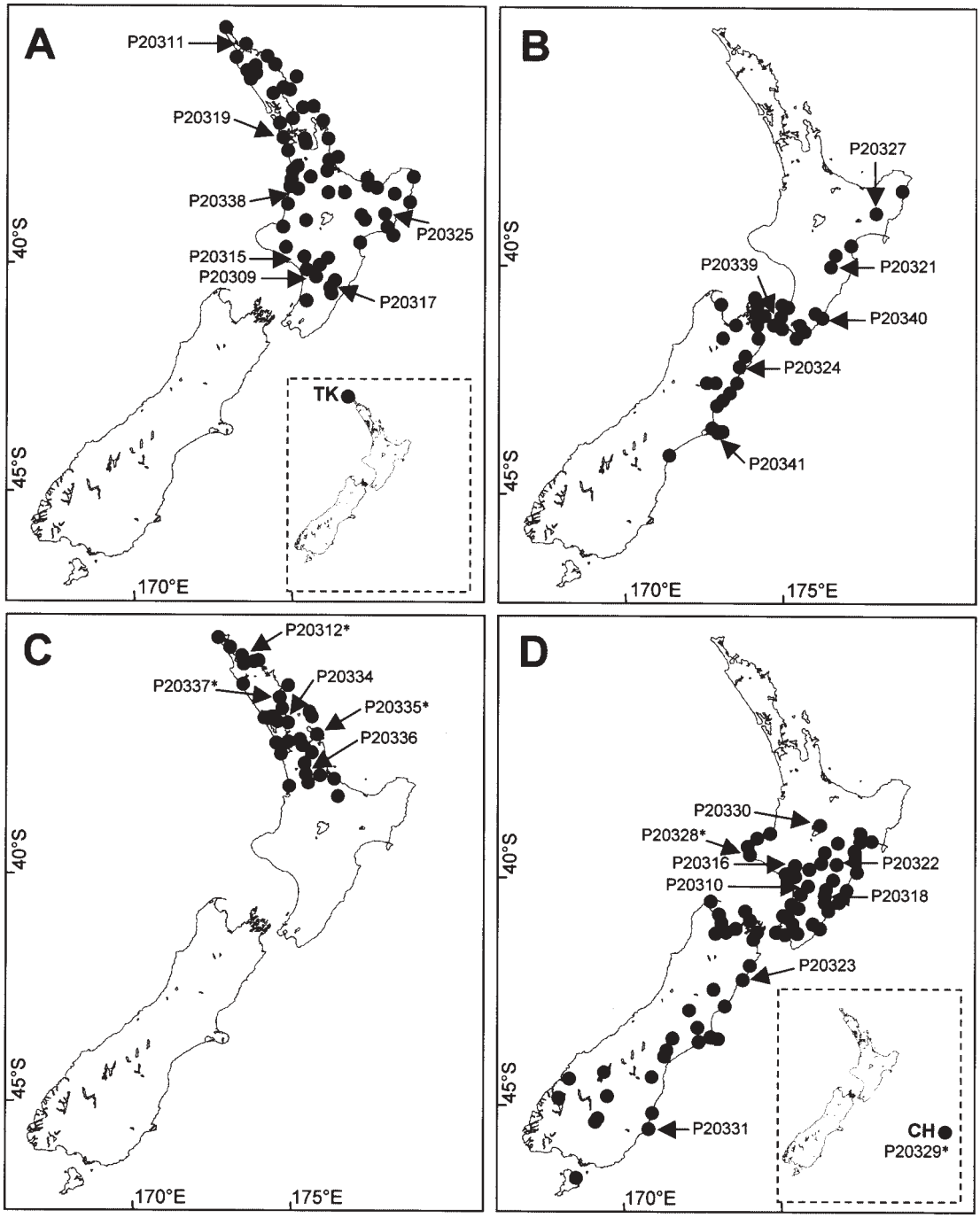


Fig. 1 Distribution of **A**, *Polystichum wawranum*; **B**, *P. oculatum*; **C**, *P. neozelandicum* subsp. *neozelandicum*; **D**, *P. neozelandicum* subsp. *zerophyllum*. Localities for the AFLP samples are indicated. Samples are referenced by their WELT accession number. *, samples included in supplementary AFLP analyses but not in the analysis presented here; TK, Three Kings Islands; CH, Chatham Islands.

AFLP

Genetic relationships between a sample set of 22 individuals selected to encompass the groups recognised from the morphological and cytological analyses were investigated using AFLP-DNA fingerprinting (Vos et al. 1995; reviewed in Mueller & Wolfenbarger 1999). Of the taxa newly defined in this paper, six samples of *P. oculatum*, seven of *P. wawranum*, seven of *P. neozelandicum* subsp. *zerophyllum*, and two of *P. neozelandicum* subsp. *neozelandicum* were analysed. The geographic origin of these samples is indicated in Fig. 1. Included were individuals from two locally sympatric sites between *P. neozelandicum* subsp. *zerophyllum* and *P. wawranum*, and one locally sympatric site between each of *P. oculatum* and *P. neozelandicum* subsp. *zerophyllum*, and *P. oculatum* and *P. wawranum* (see Appendix 1 for details). Additional samples of *P. neozelandicum* subsp. *neozelandicum* and *P. neozelandicum* subsp. *zerophyllum* included in supplementary AFLP analyses (Perrie 2001) but not directly reported here are also indicated in Fig. 1.

Genomic DNA was extracted from silica-gel-dried or fresh frond tissue using a modified CTAB protocol (Doyle & Doyle 1990). The AFLP protocol was adapted from Vos et al. (1995). Extracted genomic DNA was digested with the restriction enzymes *Eco*RI and *Mse*I for 3 h, with ligation reactions subsequently carried out overnight at 4°C. AFLP PCR was performed in two steps with non-radioactive primers and a simplification of the thermocycling profile as used in Lockhart & McLenachan (1997). AFLP profiles were generated using three primer combinations; Eco-ATA and Mse-CTG, Eco-AAT and Mse-CAG, and Eco-ATA and Mse-CAG. Profiles were visualised by electrophoresis for 3 h at 40 W on 5% polyacrylamide gels, followed by silver staining (Promega 1998).

Bands of a given size were treated as independent characters, and their presence or absence across all samples was scored to produce a binary data matrix. Any phylogenetic interpretation of AFLP banding patterns, whether this is based on ordination, distance, or parsimony analyses, requires that they represent independent characters. This assumption is supported by several studies (e.g., Maughan et al. 1996; Maheswaran et al. 1997; Liu et al. 1998; Koopman et al. 2001; Parsons & Shaw 2001; see Mueller & Wolfenbarger 1999). In this case, given the high chromosome numbers (n 82) of the taxa involved, any given pair of AFLP markers is likely to be segregating on different chromosomes.

Phylogenetic signal in the AFLP data was assessed using split-decomposition (Swofford et al. 1996; Huson 1998; Lockhart et al. 2001), and bootstrapping analyses under parsimony and neighbour-joining (Swofford et al. 1996). In these contexts, internal branches* within the resultant trees (or graphs) are expected to be recovered only where lineage-sorting has engendered concordant partitioning across multiple characters between divergently related, assortatively fertilising groups (Sharbel et al. 2000; Koopman et al. 2001). Strong support for such branches could be used to reject the null-hypothesis that "a single evolutionary lineage is present" (see below).

Split-decomposition is a particularly conservative method in that it will only recover branches in the resultant splits-graph that are relatively well supported. It was implemented using the program Splitstree 2.4 (Huson 1997, 1998) under a parsimony criterion. However, the results obtained with split-decomposition under parsimony differed little from those obtained using a distance criterion under split-decomposition (results not shown).

The program PAUP* 4.08b (Swofford 2001) was used to implement the parsimony and neighbour-joining analyses with bootstrapping (1000 replicates). Both of these tree selection criteria reconstruct fully resolved trees, even when the input data are essentially random. However, when either is implemented with bootstrapping analysis only internal branches with strong support in the data are recovered in the consensus tree summarising the results (see Koopman et al. 2001). Parsimony was implemented with the heuristic search option, and with the tree-bisection-reconnection swapping algorithm and accelerated transformation (ACCTRAN) optimisation in effect. Neighbour-joining employed observed (= p = Hamming) distances.

*The term "branch" (= "edge" in mathematical notation) is used to refer to a link, or internode, that connects two nodes in a graph (or tree) depicting evolutionary relationships. External branches connect a sample of an extant individual (= an external node) with an internal node (= a real or inferred ancestor). Internal branches connect two internal nodes. Each branch corresponds to a "split", which is the bipartitioning of a sample set into two subsets. Splits may correspondingly be described as external or internal (Swofford et al. 1996; Penny & Hendy 2001).

Taxonomic assignment

For a number of seemingly good reasons (Donoghue & Cantino 1988; Ridley 1996), taxonomic delimitation is now commonly based on evolutionary relationships, at least for higher categories. If this principle is extended to the species category, a required (but not necessarily sufficient) criterion might be that taxonomic species constitute separate evolutionary lineages. Indeed, de Queiroz (1998) suggested that this criterion is already generally, if often only implicitly, agreed upon.

The separation between lineages is engendered, at least in sexually outcrossing organisms, by assortative fertilisation. This is the propensity of one group of individuals to breed amongst themselves, rather than with another group(s). Assortative fertilisation may stem from SMRS differentiation and/or allopatry. SMRS (or Specific Mate Recognition System; more or less in the sense of Paterson (1993)) differentiation involves changes in the fertilisation system of one group of individuals such that they are more likely to fertilise one another even when sympatric with related groups. By definition, the geographic separation between allopatric groups results in assortative fertilisation.

In a practical sense, testing directly for assortative fertilisation between groups will often be difficult. Detecting SMRS differentiation directly requires pre-fertilisation mating-competition experiments of the sort summarised by Arnold (1997). Even a determination of allopatry is not as straight-forward as it may seem, as an observation that two groups of adults have non-overlapping distributions does not necessarily mean they should be considered allopatric, especially if their propagules are capable of long-distance dispersal.

However, evidence of lineage separation can also be garnered from analyses of character state variation. That is, the observed patterns of character state variation might be best explained by inferring that assortative fertilisation has occurred between two groups, such that they might be considered separate evolutionary lineages. In instances where such lineages are sympatric, it might be further inferred that the assortative fertilisation between them is a result of SMRS differentiation. This is because lineage separation in sympatry can only be engendered by SMRS differentiation (excluding cases of micro-allopatry). Because of the well-documented phenomenon of negative heterosis, post-fertilisation barriers (e.g., reductions in fitness and/or fertility) do not contribute to lineage separation in sympatry. Sympatric groups of sexually outcrossing organisms separated only by post-fertilisation barriers, such that there is no assortative fertilisation between them, will either merge or one will go extinct depending on the severity of the fertility/fitness reduction in hybrids between the groups (Paterson 1978; Lambert et al. 1984; Spencer et al. 1986; Masters & Spencer 1989), and could hardly be considered separate evolutionary lineages.

In this work, we take a two-step approach to the taxonomic description of biodiversity. Firstly, the null hypothesis that "a single evolutionary lineage is present" is tested using analyses of character state variation. Then, having delimited evolutionary lineages, the second step is the delimitation of these into the taxonomic scheme. Here we recognise lineages for which there is direct or indirect evidence of SMRS differentiation as separate taxonomic species, whilst lineages for which there is no evidence of SMRS differentiation are treated at the subspecific level. An alternative (and defensible in many instances) approach might be to recognise all delimited evolutionary lineages as separate species. In either case, the varietal category is reserved for instances where it is considered "useful" to designate taxonomically biological variation that is not correlated with the boundaries of a delimited lineage.

RESULTS

Cytology and morphology

Variation in the morphological characters found to be useful in differentiating the taxa recognised here is summarised in Table 2, with box-plot summaries presented in Fig. 2. Representative fronds are illustrated in Fig. 3 and 4, scales in Fig. 5, and indusia in Fig. 6.

Plants from both lineages of *P. neozelandicum* were found to have larger spores than either *P. oculatum* or *P. wawranum* (see Fig. 2F), and, as with previous reports (e.g., Barrington et al. 1986) of a correlation between spore size and ploidy level, cytological analysis indicated the presence of two ploidy levels. Plants of the small-spored *P. oculatum* and *P. wawranum* were found to be tetraploid with n = c. 82 bivalents counted at diakinesis (Fig. 7). The base chromosome number in *Polystichum* is x = 41 (Manton 1950). In contrast, samples from the large-spored *P. neozelandicum* lineages were found to be octoploid with n = c. 164 bivalents counted at diakinesis (Fig. 7). It should be noted that the

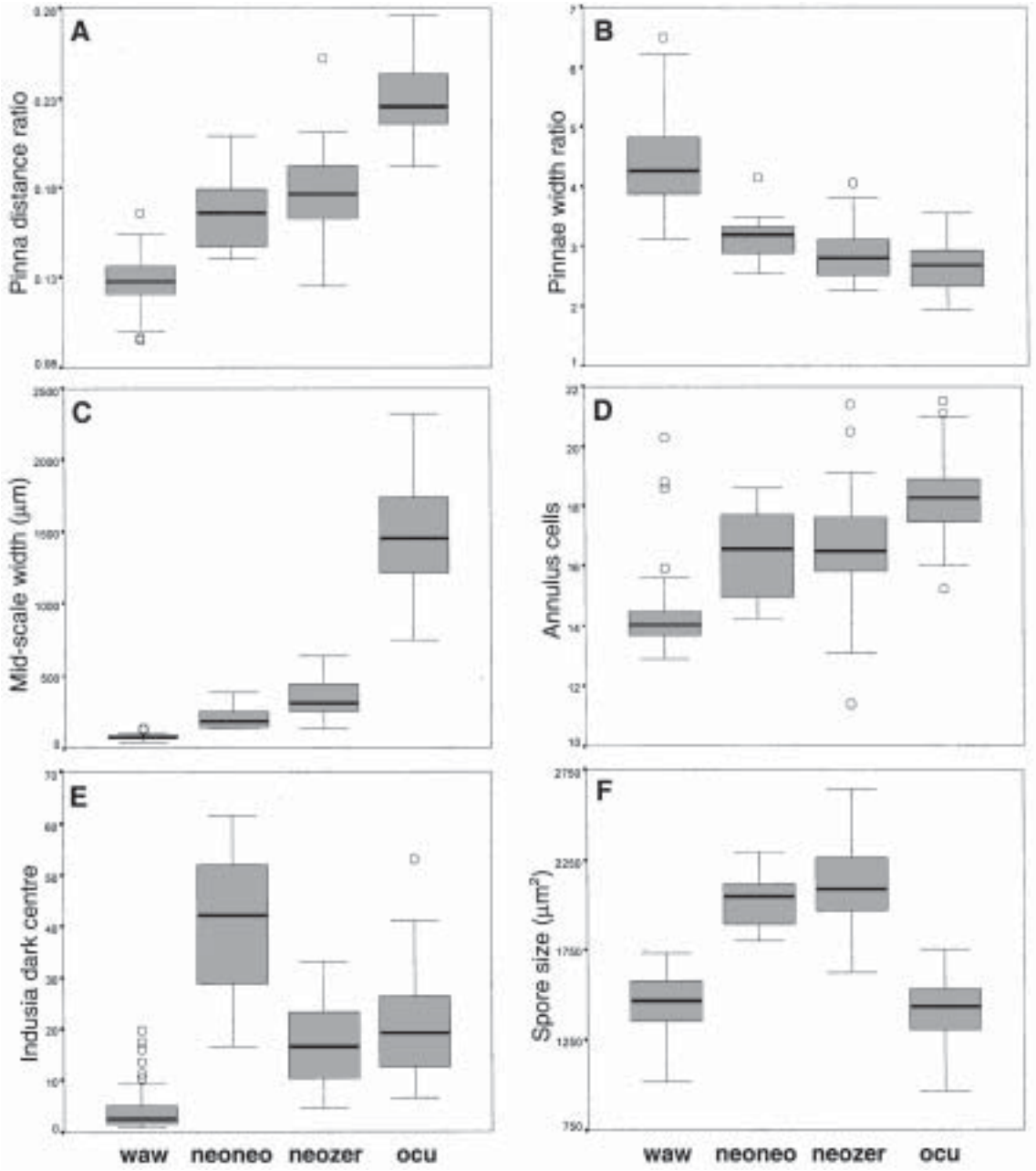


Fig. 2 Box-plot summaries of morphological characters that show differentiation between *Polystichum wawranum* (waw), *P. neozelandicum* subsp. *neozelandicum* (neoneo), *P. neozelandicum* subsp. *zerophyllum* (neozer), and *P. oculatum* (ocu). Characters are defined in Table 1. o, outliers. Numbers of samples analysed (waw, neoneo, neozer, ocu) for: A, B, & C, 43, 16, 52, 23; D, 41, 15, 51, 23; E, 43, 16, 51, 23; F, 39, 11, 38, 20.

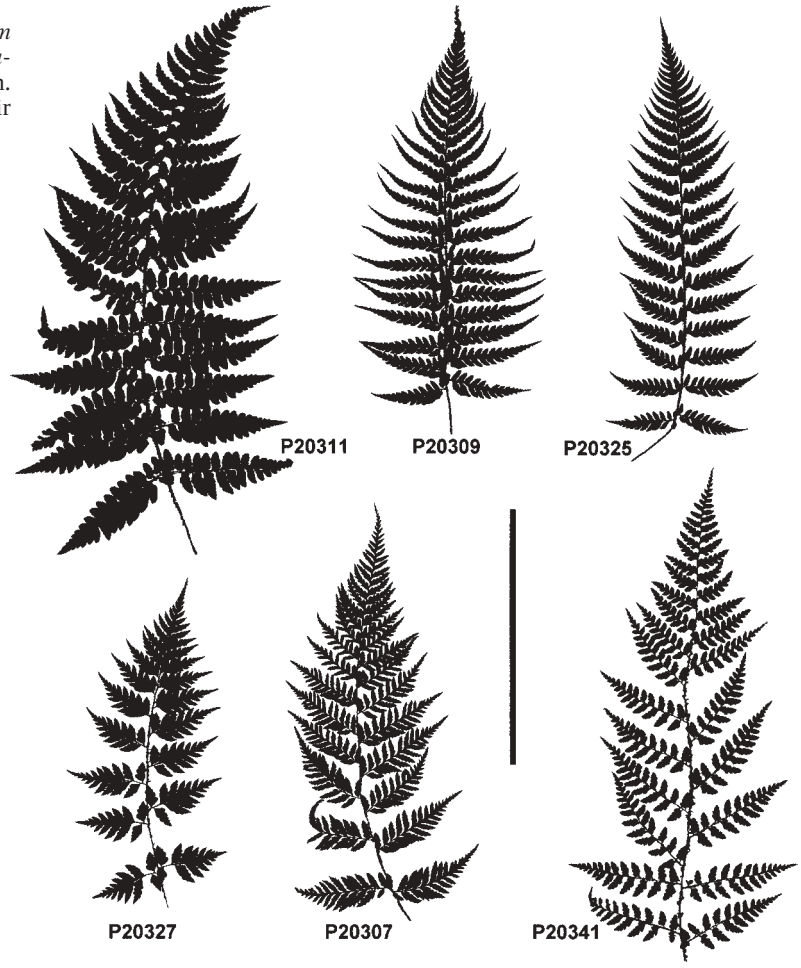


Fig. 3 Fronds of *Polystichum wawranum* (upper) and *P. oculatum* (lower). Scale bar = 20 cm. Samples are referenced by their WELT accession number.

Table 2 Morphological (and cytological) characters that distinguish *Polystichum wawranum*, *P. neozelandicum* subsp. *neozelandicum*, *P. neozelandicum* subsp. *zerophyllum*, and *P. oculatum*. The numbers given below for the quantitative characters are the 5th percentile–median–95th percentile.

Character	*P. wawranum*	*P. neozelandicum* subsp. *neozelandicum*	*P. neozelandicum* subsp. *zerophyllum*	*P. oculatum*
Quantitative characters				
Mid-scale width (µm)	40–75–120	135–185–340	150–310–570	770–1460–2280
Max scale width (µm)	200–300–390	290–420–650	380–580–890	1060–1630–2550
Pinnae distance ratio	0.10–0.13–0.15	0.14–0.17–0.20	0.14–0.18–0.21	0.19–0.23–0.28
Pinna width ratio	3.2–4.3–6.1	2.5–3.2–3.7	2.3–2.8–3.7	2.0–2.7–3.5
Annulus cells	13.0–14.0–18.8	14.2–16.6–18.3	13.1–16.5–19.7	15.4–18.3–21.4
Indusia dark centre	1.0–2.6–17.1%	16.6–42.2–59.0%	6.1–16.5–29.7%	7.0–19.2–50.6%
Spore size (µm^2)	1160–1470–1720	1800–2050–2270	1670–2090–2540	980–1430–1750
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
Primary costae obviously darker than lamina	yes	yes	yes	no

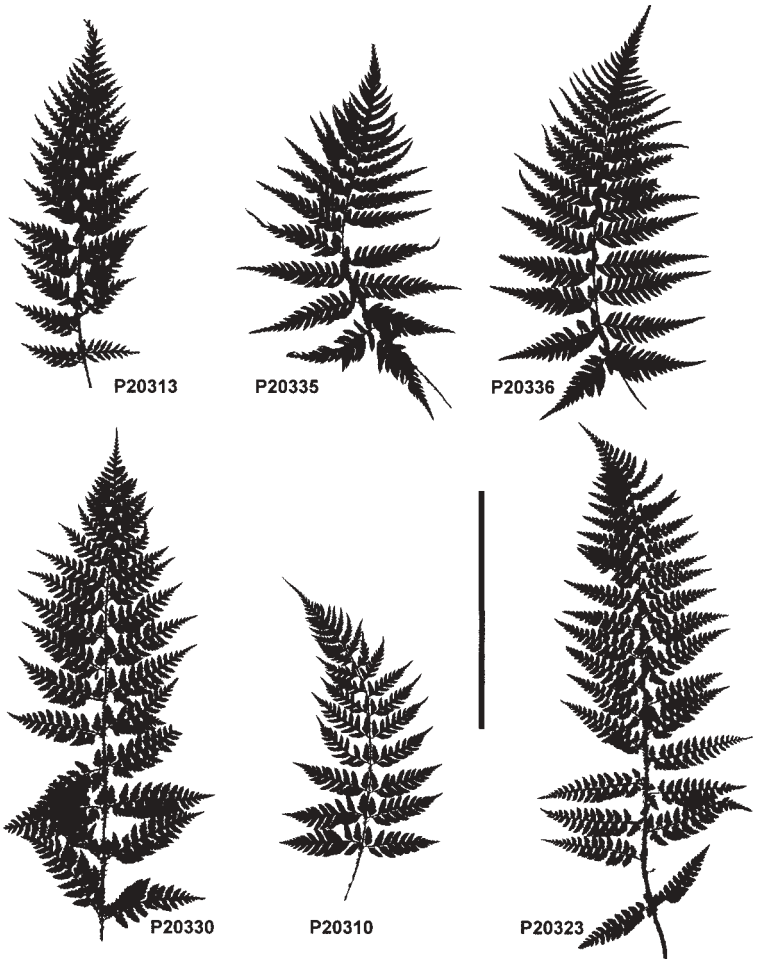


Fig. 4 Fronds of *Polystichum neozelandicum* subsp. *neozelandicum* (upper) and *P. neozelandicum* subsp. *zerophyllum* (lower). Scale bar = 20 cm. Samples are referenced by their WELT accession number.

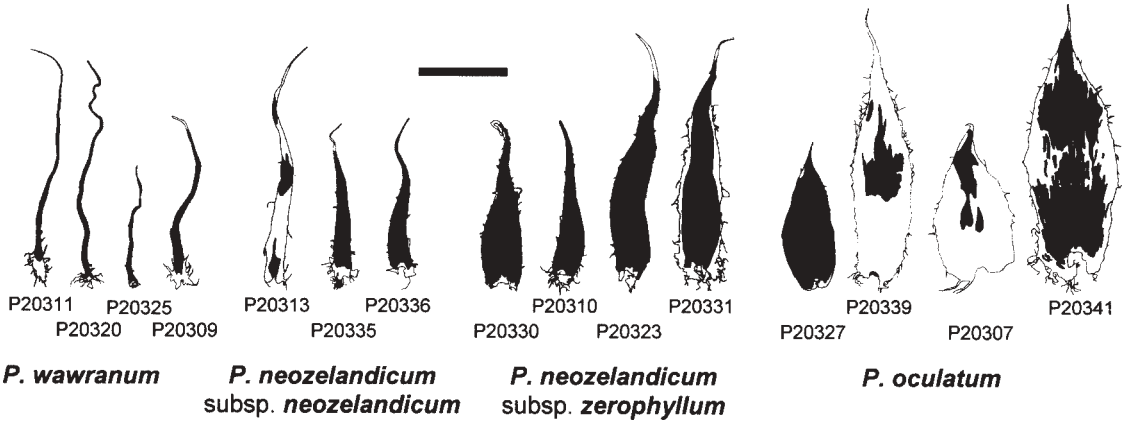


Fig. 5 Scales from the stipe-rachis junction of *Polystichum wawranum*, *P. oculatum*, *P. neozelandicum* subsp. *neozelandicum*, and *P. neozelandicum* subsp. *zerophyllum*. Scale bar = 2000 µm. Samples are referenced by their WELT accession number.

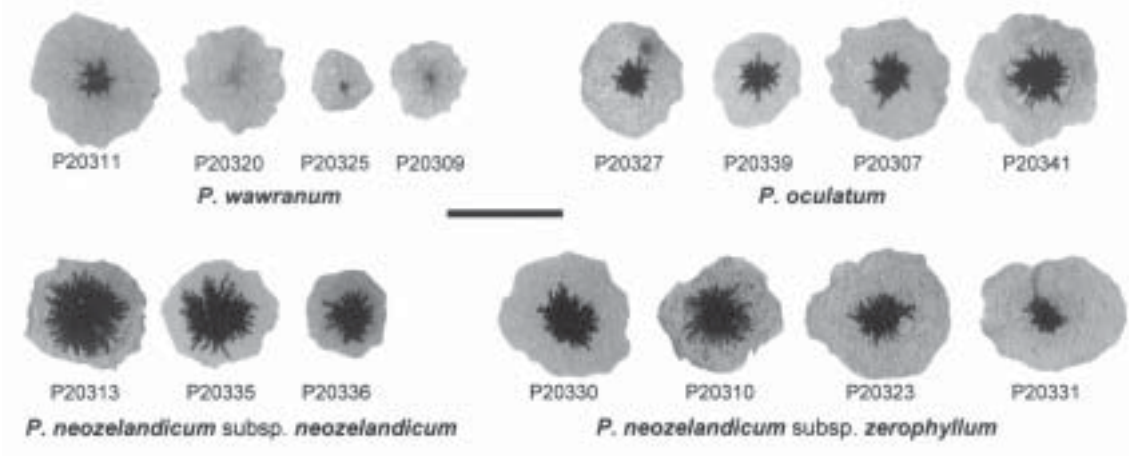


Fig. 6 Representative indusia of *Polystichum wawranum*, *P. oculatum*, *P. neozelandicum* subsp. *neozelandicum*, and *P. neozelandicum* subsp. *zerophyllum*. Scale bar = 1000 μm. Samples are referenced by their WELT accession number.

preparations were not of sufficient quality to exclude the possibility that a small number of multivalents were present.

Morphologically, the small-spored, tetraploid plants fall into two discrete groups (*P. oculatum* and *P. wawranum*). These are concordantly partitioned by a number of pairwise comparisons of assumedly independent morphological characters. This is strongest for the character combination "mid-scale width" and "pinnae distance ratio" (Fig. 8), but all combinations of the characters "mid-scale width" (or "max scale width"), "pinnae distance ratio", "pinna width ratio", "annulus cells", and "indusia dark centre" show some degree of concordant partitioning (Perrie 2001).

In addition to partitioning *P. oculatum* from *P. wawranum*, the characters "mid-scale width" and "pinnae distance ratio" concordantly partition *P. neozelandicum* from *P. oculatum*, and *P. neozelandicum* from *P. wawranum* (Fig. 9). The combinations of "spore size" with "mid-scale width" or "pinnae distance ratio" also concordantly partition *P. neozelandicum* from *P. oculatum* (Perrie 2001), and *P. neozelandicum* from *P. wawranum*, but not *P. oculatum* from *P. wawranum* because of their overlapping spore-size. "Spore size" is plotted against "mid-scale width" for all four taxa in Fig. 10.

The concordance in some of these cases is not absolute, in that there is not necessarily complete restriction of all samples to only two (diagonal) quadrants in the scatter-plots. Nevertheless, the pattern of morphological variation provides support for the recognition of three lineages; the tetraploids *P. oculatum* and *P. wawranum*, and the octoploid *P. neozelandicum*. In contrast, the AFLP analysis (see below) indicates the presence of two octoploid lineages, here named as *P. neozelandicum* subsp. *neozelandicum* and *P. neozelandicum* subsp. *zerophyllum*. Aside from the dark centre of the indusia of the former generally being larger (Fig. 2E), specimens from these two taxa can sometimes be virtually morphologically indistinguishable. However, their morphological extremes are quite different, with the former often tending to resemble *P. wawranum*, and the latter *P. oculatum*.

AFLP analysis

The AFLP results presented here are congruent with those of Perrie et al. (2000), but are based on a broader sample set (see Perrie 2001 for comparison). The primer combinations E-ATA & M-CTG, E-AAT & M-CAG, and E-ATA & M-CAG generated 53, 132, and 126 scorable polymorphic bands, respectively, for a total of 311 characters. Analysis of the AFLP data, with bootstrapping analysis (1000 replicates) under parsimony-based split-decomposition, parsimony, and neighbour-joining, clearly and congruently resolved the sample set into four major groups, corresponding to *P. neozelandicum* subsp. *neozelandicum*, *P.*

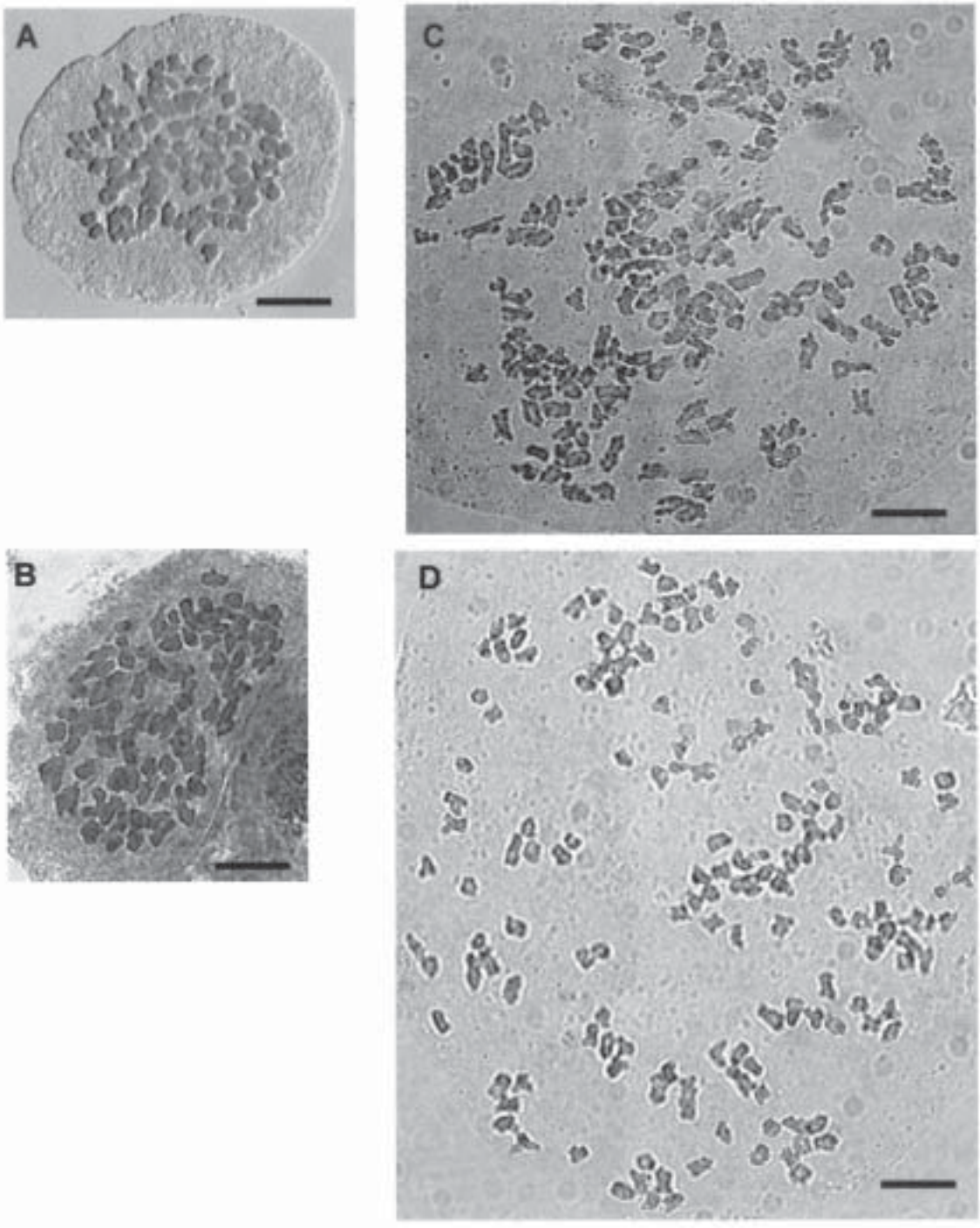
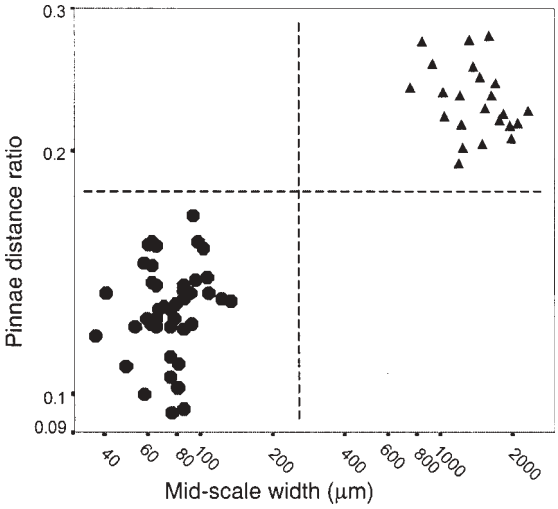


Fig. 7 Acetocarmine preparations of diakinesis. **A**, *Polystichum oculatum*, WELT P20339, *n* = c. 82; **B**, *P. wawranum*, WELT P20314, *n* = c. 82; **C**, *P. neozelandicum* subsp. *zerophyllum*, WELT P20333, *n* = c. 164; **D**, *P. neozelandicum* subsp. *neozelandicum*, WELT P20336, *n* = c. 164. Scale bars = 10 μm.



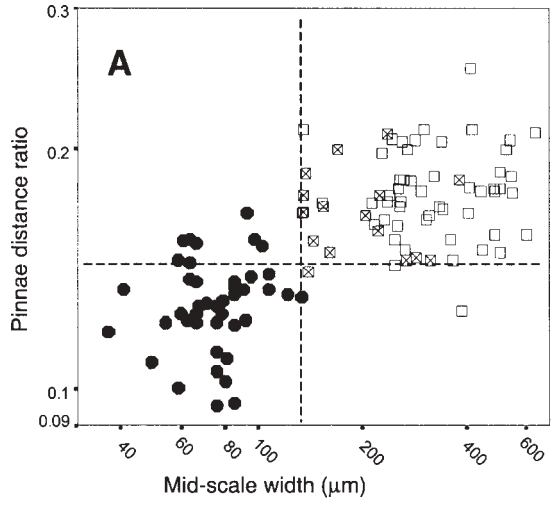


Fig. 8 Scatter-plot of "mid-scale width" against "pinnae distance ratio" for *Polystichum wawranum* (●) and *P. oculatum* (▲). Note that the axes are logarithms. Dashed lines indicate concordant partitioning.

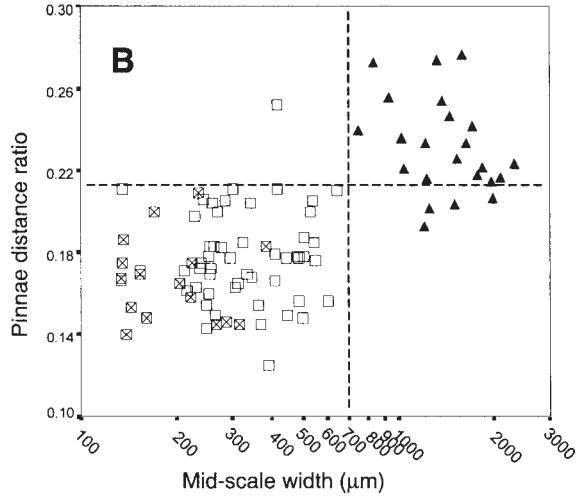


Fig. 9 Scatter-plots of "mid-scale width" against "pinnae distance ratio" for **A**, *Polystichum wawranum* (●) and *P. neozelandicum* (subsp. *neozelandicum* (☒); subsp. *zerophyllum* (□)), and **B**, *P. neozelandicum* and *P. oculatum* (▲). Note that some of the axes are logarithms. Dashed lines indicate partitioning.

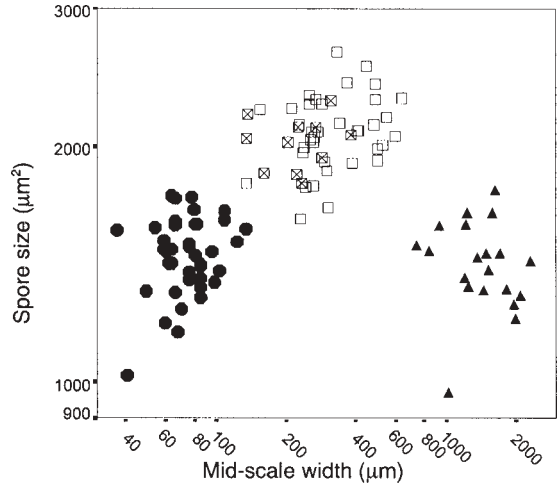


Fig. 10 Scatter-plot of "mid-scale width" against "spore size" for *Polystichum wawranum* (●), *P. neozelandicum* (subsp. *neozelandicum* (☒); subsp. *zerophyllum* (□)), and *P. oculatum* (▲). Note that the axes are logarithms.

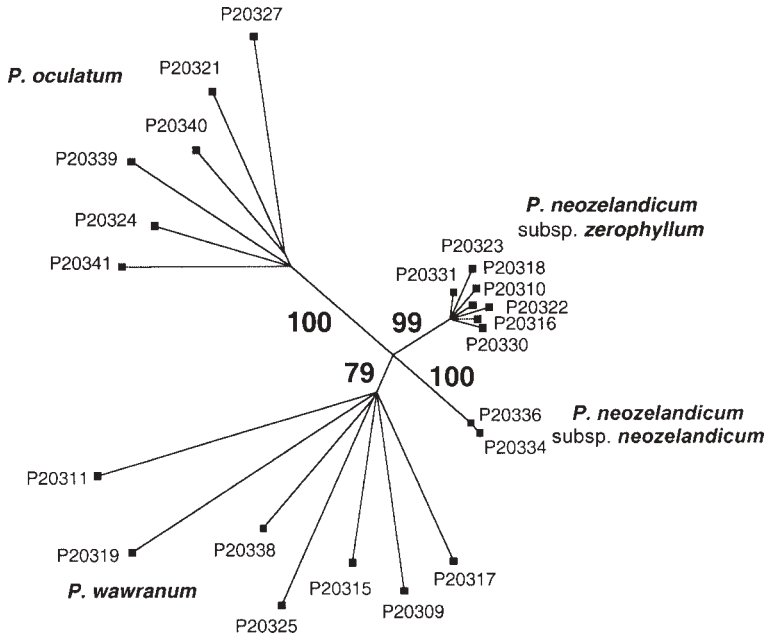


Fig. 11 Parsimony splits-graph of the AFLP data. Bootstrap support (1000 replicates) for the major internal branches is shown. Internal branches recovered within *Polystichum oculatum* and *P. wawranum* received 19% and 11% bootstrap support, respectively. Samples are referenced by their WELT accession number.

neozelandicum subsp. *zerophyllum*, *P. oculatum*, and *P. wawranum*. The splits-graph is illustrated in Fig. 11. Under all three analytical approaches, these groups are each subtended by internal branches with bootstrap support of 99% or 100%, except for *P. wawranum* under split-decomposition where the bootstrap support is 79% (see below).

The recovery of only 79% bootstrap support for *P. wawranum* under split-decomposition appears to be because the two most northerly samples, WELT P20311 and WELT P20319, are less representative than the other samples of the *P. wawranum* genome that contributed to the allopolyploid *P. neozelandicum*. When WELT P20311 and WELT P20319 are excluded from the analysis, the remaining *P. wawranum* samples are grouped with 99% bootstrap support. Further, when *P. neozelandicum* is excluded, all *P. wawranum* samples are separated from all *P. oculatum* samples by an internal branch with 100% bootstrap support.

Supplementary AFLP analysis (Perrie 2001) has indicated that samples from Karikari Peninsula (WELT P20312), near Whangarei (WELT P20337), and Whangapoua on the Coromandel Peninsula (WELT P20335) are virtually identical to the samples of *P. neozelandicum* subsp. *neozelandicum* from near Warkworth (WELT P20334) and Gordonton (WELT P20336) included in the analysis presented here. Similarly, samples of *P. neozelandicum* subsp. *neozelandicum* from Taranaki (WELT P20328) and the Chatham Islands (WELT P20329) are virtually identical to those included in the analysis presented here.

That the groups labelled *P. wawranum*, *P. oculatum*, *P. neozelandicum* subsp. *neozelandicum*, and *P. neozelandicum* subsp. *zerophyllum* are so well supported in analyses of the AFLP data indicates the presence of strong concordant partitioning. This in turn must reflect assortative-fertilisation between these geographically widespread groups of samples, such that they could be termed separate evolutionary lineages.

Distribution

Polystichum wawranum, *P. oculatum*, and *P. neozelandicum* subsp. *zerophyllum* are broadly sympatric with one another over large areas (Fig. 1). The distribution of *P. neozelandicum* subsp. *neozelandicum* does not overlap with those of *P. neozelandicum* subsp. *zerophyllum* and *P. oculatum*, but it is broadly sympatric with *P. wawranum* in the northern third of the North Island. Documented instances of local sympatry between these four taxa are given by Perrie (2001, table 4.1).

DISCUSSION

Taxonomic delimitation

From analysis of both 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 conclusion that four separate evolutionary lineages can be delimited. Morphological variation is congruent with these lineages (but only three lineages can be consistently recognised by morphology alone). Inter-lineage hybridisation (see below) suggests that these lineages are at least partially sexually-outcrossing. Consequently, the concordant partitioning in the morphological and AFLP data must be due to assortative fertilisation.

Polystichum wawranum, *P. neozelandicum* subsp. *zerophyllum*, and *P. oculatum* are broadly sympatric with each other over large geographic areas, with numerous local instances of sympatry 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 separation of these lineages is not simply due to geographic isolation, but is likely to involve SMRS, or fertilisation-system, differentiation. Hence, we suggest their recognition as three separate species: *P. wawranum*, *P. neozelandicum*, and *P. oculatum*.

Taxonomic delimitation of the lineage named here as *P. neozelandicum* subsp. *neozelandicum* is less straightforward. Its broad sympatry with *P. wawranum* can be used to infer SMRS differentiation between them, such that they should be regarded as separate species. However, the distribution of *P. neozelandicum* subsp. *neozelandicum* does not overlap with those of *P. neozelandicum* subsp. *zerophyllum* or *P. oculatum*. Morphologically, *P. neozelandicum* subsp. *neozelandicum* is more similar to *P. neozelandicum* subsp. *zerophyllum* (cf. *P. oculatum*), both are octoploid, and they may be derived from the same (allo-) polyploid event. Consequently, the lineage *P. neozelandicum* subsp. *neozelandicum* is better accommodated within the species *P. neozelandicum*, rather than with *P. oculatum*.

Allopolyploidy of *Polystichum neozelandicum*

The octoploid *P. neozelandicum* subsp. *neozelandicum* and *P. neozelandicum* subsp. *zerophyllum* lineages both exhibit little intra-lineage genetic variation, as assayed by AFLP, relative to that found in either of the tetraploid *P. wawranum* or *P. oculatum* lineages. This is depicted by the relatively short length of the external branches to samples of the former pair compared with those to the latter pair in Fig. 11. Such a finding is consistent with the genetic bottlenecking effect expected from a polyploid event, where the genetic variation inherent in one or two individuals (for autopolyploidy and allopolyploidy, respectively) constitutes the founding stock of subsequent derivative polyploid individuals. Interestingly, the *P. neozelandicum* subsp. *zerophyllum* lineage has a greater geographic distribution than those of the genetically more variable tetraploids.

Several pieces of evidence suggest that the octoploid plants have been derived from an allopolyploid hybridisation event between the tetraploid *P. oculatum* and *P. wawranum* lineages (or, more precisely, between ancestors of the extant individuals of these lineages), rather than an autopolyploid event from one of these tetraploids. Firstly, and most strikingly, is the general morphological intermediacy of the *P. neozelandicum* lineages between the two tetraploid lineages (Fig. 2–6; the expected increase in spore size excepted). Morphological intermediacy is consistent with a hybrid (i.e., allopolyploid) origin, but would be unexpected in the case of an autopolyploid.

Secondly, there is a predominantly additive pattern in the AFLP profiles of *P. neozelandicum* relative to those of *P. oculatum* and *P. wawranum* (see Perrie 2001). This is expected in a hybrid scenario (e.g., an allopolyploid origin) with a dominant marker system such as AFLP (Liu et al. 1998; Ayres & Strong 2001; Congiu et al. 2001). Additive profiles are not expected for autopolyploid events. Because of this additive pattern both octoploid lineages fall outside the tetraploid lineages in analyses of the AFLP data (see Fig. 11). In their additive combination of the genomes of both tetraploids, the octoploid lineages are in effect unlike either of their progenitors and hence are recovered as separate groups in the analyses. Autopolyploids, in contrast, would be expected to fall within the diversity of their progenitor lineage (unless, perhaps, they were very old). Indeed, the methods of AFLP

analysis used here would be unlikely to distinguish an autopolyploid and its progenitor as separate lineages.

We emphasise that a literal interpretation of the graphs/trees reconstructed from analyses of the AFLP data (e.g., Fig. 11) does not implicate an allopolyploid origin for the octoploid lineages: they simply appear as separate and distinct lineages. As reported by McDade (1992, 1997), topological position alone is insufficient to identify individuals resulting from hybrid events. However, with the knowledge that some of the lineages are polyploid, the results of the AFLP analyses can be used to distinguish between the very different topological expectations of autopolyploidy and allopolyploidy, as outlined above.

Whether the genetic distinctiveness of *P. neozelandicum* subsp. *neozelandicum* and *P. neozelandicum* subsp. *zerophyllum* indicates two independent allopolyploid origins or a single allopolyploid origin with subsequent divergence is unclear. Soltis & 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 this case, literal interpretation of the AFLP data is ambiguous, as is well depicted by the four-way polytomy in the splits-graph of Fig. 11.

Studies of hybrid formation have inferred multiple origins when both parental types of the uniparentally inherited chloroplast have been found in hybrid individuals (e.g., 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 (Perrie 2001). Both lineages of *P. neozelandicum* share the *P. wawranum* haplotype, rendering this avenue of investigation into the number of allopolyploid origins uninformative. Whether chloroplast inheritance in *Polystichum* is maternal or paternal is unknown, but the former has been found in representatives of other fern genera, e.g., *Pellaea* Link (Gastony & Yatskievych 1992) and *Asplenium* L. (Vogel et al. 1998a). If this were the case also in *Polystichum*, one could conclude that *P. wawranum* was the maternal parent for both lineages of *P. neozelandicum*.

Two independent allopolyploid events, with the respective participation of individuals with differentiated genomes from each progenitor lineage, may explain the genetic distinctiveness of the extant allo-octoploid lineages of *P. neozelandicum* (i.e., two allopolyploid events, of crosses $W_1W_1 \times O_1O_1$, and $W_2W_2 \times O_2O_2$, producing $W_1W_1O_1O_1$, and $W_2W_2O_2O_2$, respectively). However, as discussed below, the possibility of a single allopolyploid event with subsequent genetic diversification should not be discounted.

Table 3 Intra- and inter-lineage genetic variation as measured by the AFLP analysis presented here, as minimum, median, and maximum pairwise distances, with *n*, the number of comparisons made, immediately below.

	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	2, 0.44949 0.324, 0.36	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

The chromosome pairing in tetraploid hybrids between *P. wawranum* and *P. oculatum* has not been investigated, such that it is unknown whether the allopolyploidy implicated for *P. neozelandicum* should be considered "segmental" or "genomic" (Lovis 1977, p. 354). However, studies (e.g., Wagner 1973; Lovis 1977; Barrington 1990) of other *Polystichum* hybrids have, rather unusually relative to other fern genera, almost always found some degree of homoeologous chromosome pairing (see Lovis 1977, p. 333), even when the parental lineages are not considered closely related. Such homoeologous pairing in the offspring of a segmental allopolyploid may lead to tetravalent formation which, with subsequent segregation, could produce greater inter-individual genetic variation amongst the progeny than would be expected for a genomic allopolyploid. *P. neozelandicum* subsp. *neozelandicum* is phenotypically (see above) and genetically (Table 3) more similar to *P. wawranum*, and *P. neozelandicum* subsp. *zerophyllum* to *P. oculatum*. (Wagner in Barrington (1985, p. 24) stated that "hybrid taxa … approach parental extremes in [morphological] variation when growing in a habitat typical of one parent." This statement might also seem to apply here, except that *P. neozelandicum* subsp. *zerophyllum* can often be found growing with *P. wawranum* without any apparent increase in the morphological similarity of the former to the latter.) Although only speculative, differential segregation from a *single* allopolyploid origin, with *P. neozelandicum* subsp. *neozelandicum* inheriting proportionally more of the progenitor *P. wawranum* genome and *P. neozelandicum* subsp. *zerophyllum* more from *P. oculatum*, could account for the aforementioned differential similarity and for the genetic differentiation found between the two allo-octoploid lineages.

In any case, *P. neozelandicum*, as characterised here, represents the first demonstrated example of allopolyploidy in the New Zealand fern flora, although the unpublished work of Braggins (1975) on *Pteris* L. is also compelling. In addition, Brownsey (1977a) and Brownsey & 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 (Manton 1950; Sleep & Reichstein 1967), North America (Wagner 1979; Soltis et al. 1991), and Central America (Barrington 1990).

Relationships

Although the tetraploid lineages *P. wawranum* and *P. oculatum* have been included in the same taxonomic species for almost one hundred years, they are, in fact, very distinct 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*, there is little doubt that *P. wawranum* and *P. oculatum* would have been delimited long ago as separate species on morphological grounds.

There is no evidence that *P. wawranum* and *P. oculatum* are more closely related to each other than to the other tetraploid species of *Polystichum* in New Zealand. In a study of genetic relationships amongst Australasian *Polystichum*, a sister-group relationship between *P. wawranum* and *P. oculatum* was not resolved with chloroplast *rps4–trnS* spacer sequence or with AFLP data (Perrie 2001). 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 (i.e., *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* (G.Forst.) C.Presl. Further, some plants of *P. vestitum* from the Chatham Islands also have marginal projections on their scales (Perrie 2001).

Consequently, any hypothesis of an especially 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 [i.e., allopolyploidy] is not limited by phylogenetic proximity".

TAXONOMIC TREATMENT

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, often almost 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***

Polystichum Roth, *Tent. Fl. Germ. 3*, 31, 69 (1799), *nom. cons.* (Stafleu et al. 1969)

Type species: *Polystichum lonchitis* (L.) Roth (≡ *Polypodium lonchitis* L.).

1. *Polystichum wawranum* (Szyszyl. in Wawra) Perrie, comb. nov. Fig. 2, 3, 5, 6; Table 2

≡ *Aspidium wawranum* Szyszyl. in Wawra, *Itin. princ. Coburgi*, 126, t. 15 (1888), as *A. wawraeanum*. Type: Waitemata, New Zealand, *H. Wawra 242*, 1872–73; holotype in W (Fig. 12).

The epithet *wawraeanum* in the original publication has an incorrectly formed termination, and requires correction under ICBN Art. 60 and Rec. 60C. 1(c) (Greuter et al. 2000).

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 base; those from the stipe-rachis junction 40–120 μm wide at mid length; usually dark brown, but often 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 the stipe and rachis scales appear to be underlain by a 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 blackish blue. Primary pinnae in 18–35 pairs, the longest 55–140 × 13–35 mm. Secondary pinnae usually adnate, but becoming free and 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 undulate and/or scalloped, margins; often deciduous (falling with soral maturity), and sometimes almost fugaceous; central dark area usually insignificant (1–17% surface area, and usually < c.10%). 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, WELT P20314 (Fig. 7); *n* = c. 82, WELT P20308.

HABITAT AND DISTRIBUTION: New Zealand endemic; in the North Island from Cape Reinga to near Otaki in the west and Pahiatua in the east; also Three Kings Islands (Fig. 1). Ranges from scrubby coastal rocks to montane forest. Usually grows in relatively open conditions on sloping substrates such as hillsides or banks between stream terraces, and has extended into anthropogenic habitats such as road cuttings; but sometimes under dense shade and/or on alluvial terraces.

COMMENTS: *Polystichum wawranum* is distinguished by its hair-like scales, closely inserted and relatively long narrow pinnae, indusia mostly lacking obvious dark centres, and relatively small spores. It is likely to be confused only with *P. neozelandicum* subsp. *neozelandicum*, with the later being distinguished by its wider scales, indusia with larger dark centres, and larger spores (see Table 2).

Fig. 12 Holotype in W of *Aspidium wawranum* Szyszyl. in Wawra. Left-hand label reads "*Aspidium Wawraeanum* nov. sp. det Dr. Ign. de Szyszylowicz". Right-hand label reads "Riese d. Prinz. Phil. n. Ang. v. S.-Coburg um die Welt, 1872–73, No. 242, Neu Seeland, Waitemata. Coll. Dr. H. Wawra".

2. *Polystichum oculatum* (Hook.) J.B.Armstr., *Trans. New Zealand Inst. 13*, 364 (1881)
Fig. 2, 3, 5, 6; Table 2

≡ *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: Akaroa, *E. F. A. Raoul*, no date recorded; lectotype (here designated; lower specimen) in K (Fig. 13).

TYPIFICATION: Hooker (1863) listed two collections with his original description of *Aspidium oculatum*; (Wairarapa) "Northern Island, Rev. W. Colenso" and (Akaroa) "Middle Island, Raoul". Both collections, held in K, have been viewed and found to be consistent with the description. The accompanying illustration (Hooker 1863, t. 228) is clearly based on the specimen of Colenso. However, the character states exhibited by this specimen do not allow it to be identified unambiguously. Consequently, the lower, larger, and more fertile frond of the Raoul

Fig. 13 Lectotype (larger, lower-left specimen) in K of *Aspidium oculatum* Hook. The lower-left label reads "Akaroa, Raoul". Scale bar = 10 cm.

collection, whose identity is unambiguous, is selected as the lectotype of *Aspidium oculatum* Hook. (see Fig. 13).

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 mid length; those from the stipe-rachis junction 770–2280 µm (usually > c. 1000 µm) wide at 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

Fig. 14 Holotype in P of *Polystichum neozelandicum* Fée. Label reads "*Polystichum Coriaceum* Sw. N^elle Zélande. S. Mossman. 1854. N^o. 617".

almost concolorous with blackish blue primary and secondary costae. Primary pinnae in 11–22 pairs, the longest 43–105 × 16–43 mm. Secondary pinnae stalked and free 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 (5–50% surface 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. 82, WELT P20339 (Fig. 7). Brownlie's (1958) count of n = 82 for "*P. richardii*" is from a plant of *P. oculatum* (CHR 383123).

HABITAT AND DISTRIBUTION: New Zealand 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 (Fig. 1). Lowland forest and scrub margins, usually on sloping substrates such as hillsides and has extended into anthropogenic habitats such as road cuttings.

COMMENTS: *Polystichum oculatum* is distinguished by its broad, often pentagonal scales, widely inserted and relatively broad pinnae, indusia with obvious dark centres, and relatively small spores. With their substantial geographic overlap and gross morphological similarity, *P. oculatum* might be confused with *P. neozelandicum* subsp. *zerophyllum*, but the latter can be distinguished by its narrower scales and larger spores (see Table 2). The often stark contrast in colour between the primary costae (blackish blue) and the remaining lamina (forest green) in *P. neozelandicum* subsp. *zerophyllum* compared with the more uniform colouring (blackish blue to dark blue-green) in *P. oculatum* can be a useful initial field character. Hybrids may further complicate identification, although these can be recognised by their aborted spores.

3. *Polystichum neozelandicum* Fée, *Mém. Soc. Sci. Nat. Strasbourg 5*: 99 (1857) (as *P. neozelandicum*) Fig. 2, 4, 5, 6; Table 2

TYPE: "N^{elle} Zélande", *S. Mossman 617*, 1854; holotype in P (Fig. 14).

The hyphenation of *neo-zelandicum* in the original publication is an orthographic error under ICBN Art. 60.9, and is corrected by deletion of the hyphen (Webb & Edgar 1999).

DESCRIPTION: Rhizomes short, erect. Stipes 100–420 mm long. Stipes and rachises moderately to densely scaly. Scales obviously scale-like to the naked-eye; usually acicular-lanceolate; usually widest in the basal third of length; those from the stipe-rachis junction usually 135–570 µm wide at 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 blackish blue. Primary pinnae in 11–25 pairs, the longest 45–120 × 5–38 mm. Secondary pinnae stalked and free 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 (5–55% 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).

3a. *Polystichum neozelandicum* Fée subsp. ***neozelandicum*** Fig. 2, 4, 5, 6; Table 2

= *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: "Wyran River, Hook. fil." [Waikare River, Bay of Islands, J. D. Hooker], no date recorded; lectotype (here designated; rhizome-bearing material) in K (Fig. 15).

TYPIFICATION: Hooker (1863) listed three collections 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 collection has not been located, but the latter two (both in K) have been examined, with the Hooker collection found to comprise a sheet with four separate specimens. The specimen of Colenso and all of the separate specimens on the sheet of Hooker are equally consistent with the protologue, except in its reference to the rhizome in both the description (as "caudex", Hooker 1863, p. 23) and illustration (Hooker 1863, t. 222). The rhizome (or part thereof) is not present on the specimen of Colenso, but is found on one of the specimens on the sheet of Hooker, and this rhizome-bearing specimen is consequently selected as the lectotype of *Aspidium richardii* Hook. (see Fig. 15). This specimen is clearly synonymous with the lineage recognised here as *P. neozelandicum* subsp. *neozelandicum*.

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

DIAGNOSIS: Indusia with central dark area often very large (15–60% surface area, and usually > c. 30%).

CHROMOSOME NUMBER: Octoploid; *n* = c. 164, WELT P20336 (Fig. 7).

HABITAT AND DISTRIBUTION: New Zealand endemic; from Northland to Kawhia and the Bay of Plenty

Fig. 15 Lectotype (the rhizome-bearing piece) in K of *Aspidium richardii* Hook. Label in upper-left corner reads: "334 New Zealand. Rocky shore of an island in the Wyran [Waikare] river". Collected by J. D. Hooker.

(Fig. 1). Found on hillsides and banks, from coastal to lowland forest and scrub, usually in well-lit conditions.

COMMENTS: *Polystichum neozelandicum* subsp. *neozelandicum* is distinguished by its acicular-lanceolate scales, indusia with obvious dark centres, and relatively large spores. It might be confused with either *P. wawranum* (see notes under this species) or *P. neozelandicum* subsp. *zerophyllum*. The distributions of *P. neozelandicum* subsp. *neozelandicum* and subsp. *zerophyllum* do not overlap, and the dark centre of the indusia is usually larger in the former (see Table 2).

3b. *Polystichum neozelandicum* Fée subsp. ***zerophyllum*** (Colenso) Perrie, comb. et stat. nov.
Fig. 2, 4, 5, 6; Table 2

≡ *Aspidium zerophyllum* Colenso, *Trans. New*

Fig. 16 Lectotype (AK 139720) of *Aspidium zerophyllum* Colenso. "*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".

Zealand Inst. 29, 418 (1897); ≡ *Polystichum zerophyllum* (Colenso) C.Chr., *Index Fil.*, 98 (1905), 589 (1906); ≡ *Polystichum aculeatum* (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 (Fig. 16).

TYPIFICATION: 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.*" Colenso collected numerous specimens consistent with the lineage here recognised as *P. neozelandicum* subsp. *zerophyllum*. There are at least 6 such specimens in AK and 13 in WELT, 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 Fig. 16).

DIAGNOSIS: Indusia with central dark area moderately sized (5–30% surface area).

CHROMOSOME NUMBER: Octoploid; n = c. 164, WELT P20333 (Fig. 7); n = c. 164, WELT P20332.

HABITAT AND DISTRIBUTION: New Zealand 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 (Fig. 1). Usually found in well-lit conditions on sloping substrates such as hillsides or banks between stream terraces, and has extended into anthropogenic habitats such as road cuttings. Ranges from the coast to lower montane forest and scrub.

COMMENTS: *Polystichum neozelandicum* subsp. *zerophyllum* is distinguished by its acicular-lanceolate scales, indusia with obvious dark centres, and relatively large spores. It might be confused with either *P. neozelandicum* subsp. *neozelandicum* or *P. oculatum* (see notes under these taxa).

Incertae sedis

Aspidium coriaceum (Sw.) Sw. var. *acutidentatum* A.Rich., *Essai Fl. New Zealand*, 71 (1832). Type: not located.

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 has not been available for examination. Its synonymy with the taxa recognised here is uncertain. However, *Aspidium coriaceum* (Sw.) Sw. is a later synonym of *Polypodium adiantiforme* G.Forst. (≡ *Rumohra adiantiformis* (G.Forst.) Ching), and the epithet *acutidentatum* has no priority at either the specific or the subspecific level (ICBN Art. 11.2, Greuter et al. 2000).

Hybrids

Hybrids between fern species often have aborted spores (e.g., Brownsey 1977a,b, 1985; Lovis 1977; Haufler 1996; Vogel et al. 1998b; but see Brownsey 1981; Mayer & Mesler 1993). Plants with aborted spores and intermediate morphology (e.g., WELT P20343, WELT P20344, WELT P20346) 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 specimens WELT P20342 and WELT P20351 are thought to be hybrids between *P. vestitum*, and *P. oculatum* and *P. wawranum*, respectively.

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 in the field the tetraploid hybrid between *P. wawranum* and *P. oculatum* from the allo-octoploid *P. neozelandicum*. The specimens WELT P20345 and CHR 290353 represent the back-cross hybrid between *P. wawranum* and *P. neozelandicum* subsp. *zerophyllum*, and CHR 290304 between *P. wawranum* and *P. neozelandicum* subsp. *neozelandicum*. Specimens from Napenape (WELT P20347, WELT P20348, WELT P20349, WELT P20350) may be hybrids from the back-cross between *P. oculatum* and *P. neozelandicum* subsp. *zerophyllum*. All have aborted spores, but evidence of morphological intermediacy for these specimens 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*, *P. neozelandicum* subsp. *neozelandicum*, 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.

Conservation

The taxa reported here are all, at least relatively, widespread. None is likely to merit listing within one of the risk categories of de Lange et al. (1999). *Polystichum wawranum* and *P. neozelandicum* subsp. *zerophyllum* are frequently protected within reserves. Whether this is also the case for *P. neozelandicum* subsp. *neozelandicum*, or for *P. oculatum* in the northern part of its distribution, remains to be established.

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Appendix 1 Collection details of all samples referred to in text by WELT accession numbers, together with additional representative specimens from AK and CHR. Samples denoted * were included in the AFLP analysis reported here. Superscript letters indicate locally sympatric sites.

Polystichum wawranum: Karikari Peninsula, Whangatupere Bay, *L. R. Perrie rKar6*, Jan 1999, WELT P20311*; Bay of Islands, Motuarohia I., *R. E. Beever*, 7 Jan 1980, AK 156983; Hauraki Gulf, Tiritiri Matangi I., *A. E. Esler 3259*, 18 Dec 1970, CHR 223509; Auckland, Piha, *L. R. Perrie rPih1 & L. D. Shepherd*, Jan 2000, WELT P20319*; Auckland, Piha, *L. R. Perrie rPih3 & L. D. Shepherd*, Jan 2000, WELT P20320; Raglan, Mt Karioi, *R. O. Gardner 2243*, 6 Jan 1979, CHR 353565; Waikato, Waitomo, *L. R. Perrie rWto1 & L. D. Shepherd*, Dec 1999, WELT P20338*; Whakatane, Motuhore (Whale) I., *R. J. Lusk*, 1 Jan 1986, AK 174067; Gisborne, Pehiri, *L. R. Perrie rSte3 & D. King*, 9 Jun 1997, WELT P20325*[A]; Wanganui, Pungarehu, *L. R. Perrie rWan6 & L. D. Shepherd*, 1999, WELT P20315*; Wanganui, Pungarehu, *L. R. Perrie rWan7 & L. D. Shepherd*, 1999, WELT P20314; Manawatu, Pohangina, *L. R. Perrie rPoh1 & M. F. Large*, 19 Mar 1997, WELT P20308[B]; Manawatu, Pohangina, *L. R. Perrie rPoh5 & L. D. Shepherd*, 1999, WELT P20309*[B]; Wairarapa, Ngapaeruru, *L. R. Perrie rNga3 & L. D. Shepherd*, 2000, WELT P20317*[C].

Polystichum oculatum: Gisborne, Pehiri, *L. R. Perrie rSte4 & D. King*, 9 Jun 1997, WELT P20327*[A]; Hawke's Bay, Wakarara, *L. R. Perrie rWak5 & L. D. Shepherd*, 1999, WELT P20321*[D]; Wairarapa, Castlepoint, *L. R. Perrie rCas1 & M. F. Large*, 1997, WELT P20340*; Wellington, Makara, *L. R. Perrie rWel8 & L. D. Shepherd*, Sep 2000, WELT P20339*; Nelson/Marlborough, Graham River, *T. F. Cheeseman*, Jan 1881, AK 138279; Marlborough, Rarangi, *L. R. Perrie rMaa2*, Dec 1998, WELT P20307; Marlborough, Leatham, *D. R. Given*, 14 Apr 1990, CHR 512429; Kaikoura, *L. R. Perrie rKak5*, Feb 1999, WELT P20324*; Lyttelton, Mt Pleasant, *G. Brownlie*, 26 Jul 1952, CHR 383123; Lyttelton Harbour, Quail I., *E. M. Chapman*, 22 Mar 1977, CHR 325363; Banks Peninsula, Kaituna, *L. R. Perrie rKai1 & M. F. Large*, Dec 1998, WELT P20341*.

Polystichum neozelandicum subsp. *neozelandicum*: Karikari Peninsula, Whatuwhiwhi, *L. R. Perrie rKar9*, Jan 2000, WELT P20312; Karikari Peninsula, Maitai Bay, *L. R. Perrie rKar10*, Jan 2000, WELT P20313; Hokianga Cove, Te Moho Rock, *A. E. Wright*, 29 Nov 1989, AK 189744; Warkworth, *L. R. Perrie rWkw1 & L. D. Shepherd*, Dec 1999, WELT P20334*; Whangarei, Mt Mania, *L. R. Perrie rMan1*, Jan 2000, WELT P20337; near Great Barrier I., Whangarara I., *A. E. Wright*, 30 Dec 1984, AK 171179; Manukau Harbour, Green Bay, *H. Carse*, no date, CHR 290303A; Coromandel Peninsula, Whangapoua, *L. R. Perrie rCor3 & J. Armstrong*, Jan 2001, WELT P20335; Thames, Matatoki, *H. Carse*, Jan 1929, CHR 290360A; Waikato, Gordonton, *L. R. Perrie rPmm1 & T. Dugdale*, 3 Apr 1997, WELT P20336*.

Polystichum neozelandicum subsp. *zerophyllum*: Taupo, Opepe, *L. R. Perrie rOpe1 & L. D. Shepherd*, Apr 2000, WELT P20330*; Napier, *L. R. Perrie rWhi3*, 12 Jun 1997, WELT P20333; Taranaki, Opunake, *C. Ryan (L. R.. Perrie rTar3)*, 1999, WELT P20328; Wanganui, Pungarehu, *L. R. Perrie rWan5 & L. D. Shepherd*, 1999, WELT P20316*; Hawke's Bay, Wakarara, *L. R. Perrie rWak1 & L. D. Shepherd*, 1999, WELT P20322*[D]; Manawatu, Pohangina, *P. Hynes*, 28 Jan 1967, AK 113572; Manawatu, Pohangina, *L. R. Perrie rPoh6 & L. D. Shepherd*, 1999, WELT P20310*[B]; Wairarapa, Ngapaeruru, *L. R. Perrie rNga6 & L. D. Shepherd*, 2000, WELT P20318*[C]; Wairarapa, Ruakokoputuna, *L. R. Perrie rRkk1 & D. Havell*, 2000, WELT P20332; Nelson, *A. E. Wright*, 7 Dec 1979, AK 151470; Kaikoura, *L. R. Perrie rKak1*, Feb 1999, WELT P20323*; north-west Nelson, Riwaka River, *A. P. Druce*, Nov 1974, CHR 278035; Dunedin, Flagstaff, *L. R. Perrie rDun1*, 13 Dec 1998, WELT P20331*; Stewart I., Ackers Point, *H. D. Wilson*, 25 Mar 1980, CHR 368809; Chatham I., Plumtree Bush, *L. R. Perrie rCha3*, Feb 1999, WELT P20329.

Hybrids: *Polystichum wawranum* × *P. neozelandicum* subsp. *neozelandicum*: Auckland, Titirangi, *H. Carse*, no date, CHR 290304.

Polystichum wawranum × *P. neozelandicum* subsp. *zerophyllum*: Taranaki, Tarata, *H. Carse*, Jan 1916, CHR 290353; Wairarapa, Coonoor, *L. R. Perrie rWeb1 & D. Havell*, 1999, WELT P20345.

? *Polystichum oculatum* × *P. neozelandicum* subsp. *zerophyllum*: Canterbury, Napenape, *L. R. Perrie rNap13 & M. F. Large*, Dec 1998, WELT P20350; Canterbury, Napenape, *L. R. Perrie rNap15 & M. F. Large*, Dec 1998, WELT P20349; Canterbury, Napenape, *L. R. Perrie rNap16 & M. F. Large*, Dec 1998, WELT P20347; Canterbury, Napenape, *L. R. Perrie rNap18 & M. F. Large*, Dec 1998, WELT P20348.

Polystichum wawranum × *P. vestitum*: Manawatu, Pohangina, *L. R. Perrie & L. D. Shepherd*, Dec 2001, WELT P20351.

Polystichum oculatum × *P. vestitum*: Banks Peninsula, Summit Rd, *L. R. Perrie rXvBan9 & M. F. Large*, Dec 1998, WELT P20342.

Polystichum neozelandicum subsp. *zerophyllum* × *P. vestitum*: Kaweka Ranges, Mahaku Rd, *L. R. Perrie rXvKaw2 & L. D. Shepherd*, 1999, WELT P20344; Kaikoura, *L. R. Perrie rXvKak2*, Feb 1999, WELT P20346; Canterbury, Peel Forest, *L. R. Perrie rXvPee2 & M. F. Large*, Dec 1998, WELT P20343.