

NEW ZEALAND *ASPENIUM* (ASPLENIACEAE: PTERIDOPHYTA) REVISITED – DNA SEQUENCING AND AFLP FINGERPRINTING

L.R. PERRIE¹ & P.J. BROWNSEY

Museum of New Zealand Te Papa Tongarewa, P.O. Box 467, Wellington, New Zealand
(Email: ¹leonp@tepapa.govt.nz)

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ABSTRACT

This paper summarises our continuing study of the evolutionary history of *Asplenium* in New Zealand. Chloroplast DNA sequence data and AFLP DNA-fingerprinting have been used to examine the origins and relationships of the New Zealand *Asplenium* taxa, to test species boundaries, and to further investigate the ancestry of the octoploid *Asplenium* taxa belonging to the Austral group.

INTRODUCTION

In 1863, after three years in New Zealand, Samuel Butler's "we have one very stupid white gentian" (Butler, 1863) was assumedly a frustrated reflection of the difficulty of recognising distinct entities within New Zealand *Gentiana* Moench (there are actually 30 species, and only half have white flowers; Glenn, 2003). Analogous sentiments are apparent from those who first dealt taxonomically with *Asplenium* L. in New Zealand. Joseph Hooker (1855, p.33) wrote "The New Zealand kinds have defied all attempts to be limited by words". Similarly, "[some] are extremely inconstant and pass into one another, thus rendering their classification a work of considerable difficulty" (Thomson, 1882, p.72), and "The New Zealand species present exceptional difficulties to the student, on account of their extreme variability and the manner in which several of them are connected by intermediate forms" (Cheeseman, 1906, p.987). Allan (1961, p.75) stated that the species of New Zealand *Asplenium* were "very ill-defined", and that while many appeared to respond "markedly to environmental conditions...[,] there is also no doubt that hybridism plays an important part".

Indeed, hybridisation has proved to be frequent amongst (some) New Zealand *Asplenium*, and although these hybrids blur the morphological discontinuities between taxa, they are sterile and present little opportunity for introgressive gene flow (Brownsey, 1977a). The identification of hybrids was an integral part of delimiting taxa in Brownsey's (1977b) taxonomic revision of New Zealand *Asplenium*, in which 14 species and four subspecies were recognised. Since that treatment three species have been newly described (Brownsey & Jackson 1984; Brownsey, 1985; Brownsey & de Lange, 1997), some names have changed (Brownsey, 1979; Brownsey, 1999), and the appropriate rank for some taxa has been debated (Ogle, 1985; Brownsey, 1998; Perrie & Brownsey, 2005a), such that a present treatment (Table 1) might recognise 18 species and four subspecies; comprising approximately 10% of the New Zealand fern flora (Brownsey & Smith-Dodsworth, 2000).

This paper will summarise some of our recent discoveries in New Zealand *Asplenium*, but we emphasise that our understanding of the evolution of New Zealand

TABLE 1: A present taxonomic treatment of *Asplenium* in New Zealand compared with that of Brownsey (1977b).

Present Treatment	As treated by Brownsey (1977b)
<i>A. appendiculatum</i> (Labill.) C.Presl subsp. <i>appendiculatum</i>	<i>A. terrestre</i> Brownsey subsp. <i>terrestre</i>
subsp. <i>maritimum</i> (Brownsey) Brownsey	subsp. <i>maritimum</i> Brownsey
<i>A. bulbiferum</i> G.Forst.	<i>A. bulbiferum</i> G.Forst. subsp. <i>bulbiferum</i>
<i>A. gracillimum</i> Colenso	subsp. <i>gracillimum</i> (Colenso) Brownsey
<i>A. chathamense</i> Brownsey	-
<i>A. cimmericum</i> Brownsey et de Lange	-
<i>A. flabellifolium</i> Cav.	<i>A. flabellifolium</i> Cav.
<i>A. flaccidum</i> G.Forst. subsp. <i>flaccidum</i>	<i>A. flaccidum</i> G.Forst. subsp. <i>flaccidum</i>
subsp. <i>aurakiense</i> Brownsey	subsp. <i>aurakiense</i> Brownsey
<i>A. hookerianum</i> Colenso	<i>A. hookerianum</i> Colenso
<i>A. lamprophyllum</i> Carse	<i>A. lamprophyllum</i> Carse
<i>A. lyallii</i> (Hook.f.) T.Moore	<i>A. lyallii</i> (Hook.f.) T.Moore
<i>A. oblongifolium</i> Colenso	<i>A. lucidum</i> Forst.f.
<i>A. obtusatum</i> G.Forst. subsp. <i>obtusatum</i>	<i>A. obtusatum</i> G.Forst. subsp. <i>obtusatum</i>
subsp. <i>northlandicum</i> Brownsey	subsp. <i>northlandicum</i> Brownsey
<i>A. pauperequitum</i> Brownsey et P.J.Jacks.	-
<i>A. polyodon</i> G.Forst.	<i>A. polyodon</i> G.Forst.
<i>A. richardii</i> (Hook.f.) Hook.f.	<i>A. richardii</i> (Hook.f.) Hook.f.
<i>A. scleroprium</i> Hombr.	<i>A. scleroprium</i> Hombr.
<i>A. shuttleworthianum</i> Kunze	<i>A. shuttleworthianum</i> Kunze
<i>A. trichomanes</i> L. subsp. <i>quadrivalens</i> D.E.Mey emend Lovis	<i>A. trichomanes</i> L.
subsp. nov.	-

Asplenium is far from complete.

RELATIONSHIPS AND BIOGEOGRAPHY

Brownsey (1977a) considered the *Asplenium* species hybridising in New Zealand to comprise a closely related group, dubbed the ‘Austral’ group, and this has been supported by phylogenetic analyses of chloroplast DNA sequence data (Perrie & Brownsey, 2005a). The species in New Zealand that do not hybridise there - *A. flabellifolium*, *A. trichomanes*, *A. pauperequitum*, and *A. polyodon* - have closer affinities elsewhere (the former three with a primarily temperate, northern hemisphere group, and *A. polyodon* with a group that includes *A. aethiopicum*). Interestingly, the Austral group is closely related to the bird’s nest ferns (e.g., *A. australasicum* (J.Sm.) Hook.) and the finely dissected *A. theciferum* (Kunth) Mett., which, as *Loxoscaphe thecifera* (Kunth) T.Moore, is the type of *Loxoscaphe* T.Moore.

Amongst the New Zealand members of the Austral group, three chloroplast subgroups are clearly delimited: the Bulbiferum, Flaccidum, and Obtusatum chloroplast groups (Perrie & Brownsey, 2005a). However, it remains unclear how these groups are related to one another, as different DNA regions support conflicting relationships: {{Bul,Obt}Fla} with 71% bootstrap support from the *rbcL* gene, versus {{Bul,Fla}Obt} with 85% bootstrap support from the *trnL-trnF* intergenic spacer.

Molecular dating, using penalised likelihood of *rbcL* DNA sequence data and a calibration of 140 million years ago for the divergence of *Asplenium* and *Hymenasplenium* Hayata, recovers dates of 45 million years ago or younger for the divergence between each New Zealand *Asplenium* species and its closest non-New Zealand relative in the sample set analysed (Perrie & Brownsey, 2005a). The calibration age is probably conservatively old (for instance, Schneider *et al.* (2004) calculated the *Asplenium-Hymenasplenium* split at just 55 million years ago), so it is likely that the estimated divergence ages are (considerably) younger than actually calculated. In any case, the estimated ages of the divergence are much more recent than the geological separation of New Zealand from Gondwana (c.80 million years ago), implicating multiple events of long-distance dispersal in the origins of New Zealand *Asplenium* (Perrie & Brownsey, 2005a). The entire Austral group appears to have arisen after New Zealand’s isolation. Therefore, the disjunct distributions of each of the seven Austral *Asplenium* taxa that occur in New Zealand and elsewhere requires inference of at least one dispersal event (unless the taxa are actually polyphyletic). Dispersal across the Tasman Sea (c.2000 km) is potentially so common that it would be of considerable interest to investigate the degree of genetic isolation between Australian and New Zealand populations of shared fern species.



Figure 1. Plants from the Kaweka Ranges of *Asplenium hookerianum* that exhibit the broad-pinnuled and narrow-pinnuled morphology that has previously been referred to as *A. hookerianum* and *A. colensoi*, respectively.

ASPLENium HOOKERIANUM

AFLP DNA-fingerprinting has been used in conjunction with chloroplast DNA sequencing to investigate some groups in more detail. The morphologically distinct *Asplenium hookerianum* and *A. colensoi* were described by Colenso (1845) in the same publication; the ultimate segments are broad in the former, and narrow in the latter (Figure 1). It is quite common to find sites where both broad and narrow-pinnuled plants grow together, with few if any intermediates, thereby creating the impression of two morphologically discrete groups growing in sympatry. However, genetic variation as assayed by AFLP DNA-fingerprinting and chloroplast DNA sequencing is not concordant with the morphological variation (Perrie & Brownsey, 2005b), and indicates that only one species should be recognised (i.e., *A. hookerianum*).

ASPLENium BULBIFERUM

In part because of the apparent origins of *Asplenium (bulbiferum subsp.) gracillimum* discussed below, we believe that the taxa previously recognised as *A. bulbiferum subsp. bulbiferum* and *A. bulbiferum subsp. gracillimum* (Brownsey, 1977b) should be treated at the specific level as *A. bulbiferum s.s.* (hereafter simply *A. bulbiferum*) and *A. gracillimum* (Perrie & Brownsey, 2005a). Circumscribed as such, the name *A.*

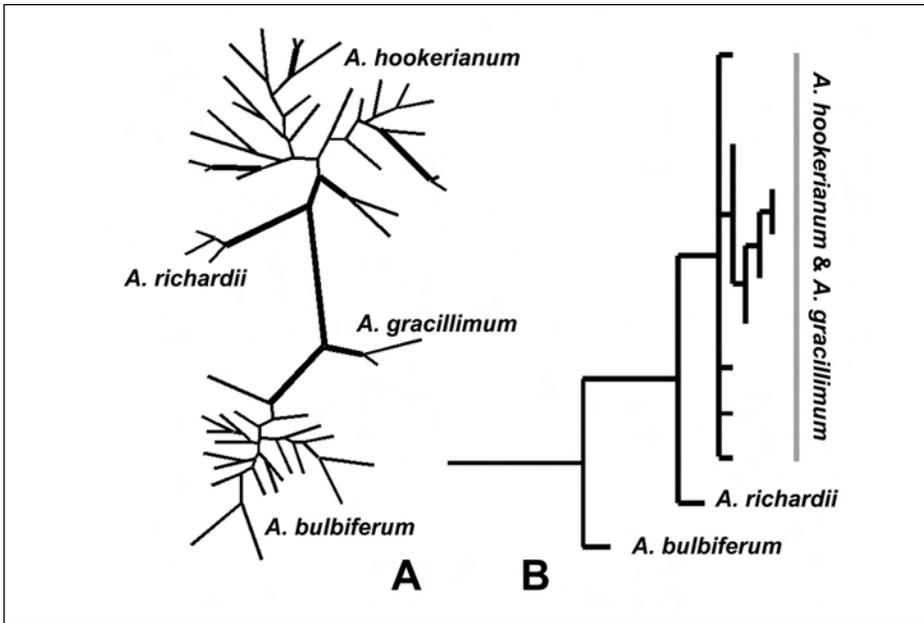


Figure 2. A: Maximum parsimony analysis of 469 AFLP characters for the tetraploids *Asplenium bulbiferum* and *A. hookerianum*, together with the octoploid *A. richardii*, a putative autopolyploid of *A. hookerianum*. Representatives from one of the *A. gracillimum* lineages are included for comparison. One of 55 most parsimonious trees, which differ in the internal arrangement of samples within *A. bulbiferum* and *A. hookerianum*. Thickened branches indicate those with >80% bootstrap support. **B:** One of three most parsimonious trees, differing in the arrangement of the *A. hookerianum* and *A. gracillimum* samples, from chloroplast *trnL-trnF* region DNA sequence data. Rooted using samples from the Flaccidum and Obtusatum chloroplast groups (not shown).

bulbiferum is then restricted to tetraploid plants, and is endemic to New Zealand. Further, it is quite distinct from the plant common in cultivation that is frequently labelled “*A. bulbiferum*” (Perrie *et al.*, 2005). *Asplenium bulbiferum* appears to be less genetically variable than its closest known tetraploid relative, *A. hookerianum*. Only one *trnL-trnF* region haplotype is presently known from *A. bulbiferum*, as opposed to eight in *A. hookerianum*. Also, only 38% of AFLP loci were polymorphic within *A. bulbiferum*, compared to 52% within *A. hookerianum*, despite more populations being sampled from the former.

ASPENIUM GRACILLIMUM

The octoploid *Asplenium gracillimum* (previously *A. bulbiferum* subsp. *gracillimum*) appears to be an allopolyploid between the tetraploids *A. bulbiferum* and *A. hookerianum*. *Asplenium gracillimum* produces bulbils like *A. bulbiferum* (albeit with much less frequency), but has the chloroplast DNA sequence of *A. hookerianum* (Perrie & Brownsey, 2005a). Further, *A. gracillimum* is intermediate between its two putative

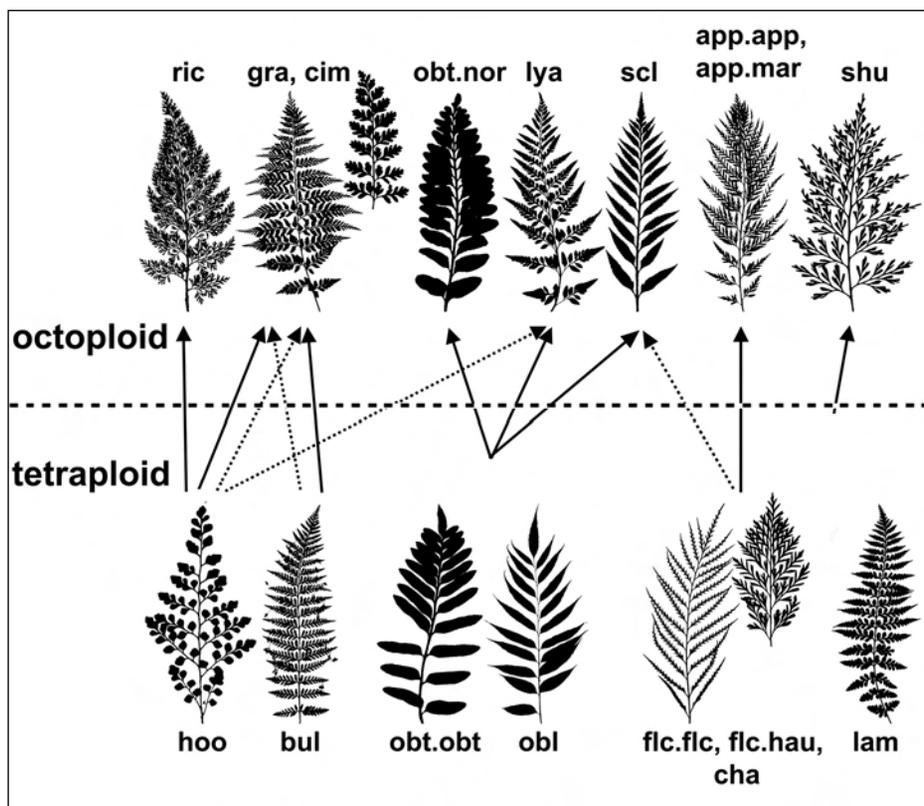


Figure 3. Hypothesised origins for the New Zealand species of the *Asplenium* Austral group. Octoploid taxa subtended by a single arrow are thought to be autopolyploids. Probable allopolyploids are subtended by two arrows. Solid arrows reflect linkages indicated by chloroplast DNA sequences; dashed arrows are linkages inferred from morphology. Arrows not pointing directly to a tetraploid taxon reflect uncertainty. Taxa are represented by the first three letters of their name in Table 1, except ‘flc’ = *flaccidum*. Fronds are not to scale.

parents with regards to frond architecture, scale shape, and habitat.

Reminiscent of the results of Treweek *et al.* (2002) with *Asplenium ceterach* L., chloroplast DNA sequence haplotypes shared by *Asplenium hookerianum* and *A. gracillimum* indicate that the latter has had multiple polyploid origins. Although this work is still preliminary, the different chloroplast DNA sequence haplotypes found in *A. gracillimum* appear to correspond to distinct groups detected with AFLP (Perrie & Brownsey, in prep.). Some of these apparently independently derived lineages of *A. gracillimum* appear to be sympatric, but it is unknown what happens reproductively where they meet.

ASPENIUM CIMMERIORUM

The recently described *Asplenium cimmeriorum* has a conservation status of 'sparse', being known only from limestone regions in the north-west of the South Island and around Waitomo in the North Island (Brownsey & de Lange, 1997). Chloroplast DNA sequences indicate that the north and south populations of *A. cimmeriorum* have independent derivations; one with an *A. hookerianum*-like chloroplast and the other with an *A. bulbiferum*-like chloroplast (Perrie, de Lange, & Brownsey, in prep.). AFLP also recovers them as distinct groups (with both also distinct from *A. gracillimum*). This suggests a need to provide independent conservation assessments, with the northern Waitomo plants being much less common than those in the south.

Both groups of *Asplenium cimmeriorum* appear to be allopolyploids between *A. bulbiferum* and *A. hookerianum* (like *A. gracillimum*). This situation presents the problem of how to deal taxonomically with multiple lineages of independent but effectively equivalent origins, some of which are ecologically and/or morphologically recognisable (e.g., *A. cimmeriorum* from *A. gracillimum*) while others are not (e.g., the cryptic lineages within *A. gracillimum*).

ASPENIUM RICHARDII

The octoploid *Asplenium richardii* is possibly an (old) autopolyploid of a narrow-pinnuled plant of *A. hookerianum*. In maximum parsimony analysis of AFLP data, *A. richardii* falls just outside the diversity found in extant *A. hookerianum*, which contrasts with the putatively allopolyploid lineages of *A. gracillimum* that lie intermediate between *A. bulbiferum* and *A. hookerianum* (Figure 2; see Perrie *et al.* (2003a) for discussion of AFLP data and type of polyploidy). The chloroplast DNA sequences of *A. richardii* are allied to, but nevertheless distinct from, those found in extant *A. hookerianum* (Figure 2). This suggests that if *A. richardii* is an autopolyploid from a narrow-pinnuled, *A. hookerianum*-like plant, then it is perhaps not of recent origin. That is, sufficient subsequent time has passed since its origin that the *A. richardii* chloroplast DNA sequences have (a) been lost from the extant populations of *A. hookerianum* and/or (b) acquired apomorphic changes.

AUSTRAL GROUP OCTOPOLOID ORIGINS

Chloroplast DNA sequences have revealed the origins of the octoploid, New Zealand taxa from the Austral group with varying degrees of precision (Perrie & Brownsey, 2004, 2005a), and our present knowledge is summarised in Figure 3. For instance, *Asplenium hookerianum* and *A. gracillimum* actually share *trnL-trnF* region haplotypes (Perrie & Brownsey, 2005a; Perrie & Brownsey, in prep.), while more than 2000 base-pairs from four chloroplast loci are unable to differentiate between *A. oblongifolium* and

A. obtusatum subsp. *obtusatum* as the chloroplast parents of *A. lyallii* and *A. scleroprium* (Perrie & Brownsey, 2004). Some of the octoploids are thought to have autopolyploid origins: *A. appendiculatum* (from an *A. flaccidum*-like or *A. chathamense*-like progenitor), *A. obtusatum* subsp. *northlandicum* (possibly from *A. obtusatum* subsp. *obtusatum*), and *A. richardii* (see above). Others are probably allopolyploids: *A. cimmeriorum*, *A. gracillimum* (see above), *A. lyallii* (between something in the *Obtusatum* chloroplast group and possibly *A. hookerianum*), and *A. scleroprium* (between something in the *Obtusatum* chloroplast group and something like *A. flaccidum*). *Asplenium shuttleworthianum* has chloroplast DNA sequences that nest within the *Flaccidum* chloroplast group, but no close tetraploid is known.

CONCLUSION

The extant *Asplenium* of New Zealand are derived from disparate groups within the genus. Long-distance dispersal has seemingly played a major role in shaping the distributions of *Asplenium* in the south-west Pacific, as is the case for much of the flora of New Zealand (Winkworth *et al.* 2002; Perrie *et al.* 2003b). Genetic data has allowed the further unravelling of the histories of some of the Austral octoploids and a resolution of some taxonomic problems (e.g., *A. hookerianum*), but it also has unveiled new avenues for investigation (e.g., the multiple, cryptic groups within *A. gracillimum*). Many issues within New Zealand *Asplenium* remain to be tackled, such as clarifying the boundaries between some taxa (e.g., Perrie & Brownsey, 2004) and establishing the most appropriate ranks for others (Ogle, 1987).

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