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Species Delimitation in *Sciadocladus* (Pterobryellaceae, Bryophyta)

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Abstract

Sciadocladus is a distinctive genus of large dendroid mosses restricted to New Zealand, New Caledonia and the Solomon Islands. Of the two extant species, *S. kerrii* is endemic to New Zealand, while *S. menziesii* is found in New Zealand, New Caledonia and the Solomon Islands. Populations of *S. menziesii* from New Caledonia and the Solomons have been recognised as subspecies *splendidum*, distinct from the New Zealand populations.

Given the geographical isolation of the two subspecies of *S. menziesii*, we hypothesised that the endemic *S. kerrii* could have arisen in situ in New Zealand through speciation from *S. menziesii* subsp. *menziesii*. This would make these two taxa more closely related to each other than *S. menziesii* subsp. *menziesii* is to *S. menziesii* subsp. *splendidum*, challenging the species concept of *S. menziesii*. We tested this hypothesis by conducting a phylogenetic analysis of molecular data from multiple exemplars of *Sciadocladus*, including material from both New Zealand and New Caledonia. The results show all specimens of *S. menziesii* strongly supported as monophyletic, sister to a clade comprising *S. kerrii* specimens, thus rejecting the hypothesis and corroborating the established taxonomy. Implications for interpretation of the phylogeographic history of the genus are discussed.

Key words: ITS2, New Caledonia, New Zealand, pleurocarps, *rbcL*, *trnL-trnF*

Introduction

The genus *Sciadocladus* Kindberg (1899:393), in common with several other genera within the order Hypnodendrales (Brotherus 1909:1166) Bell, Newton, & Quandt (2007:554) such as *Mniodendron* Lindberg (1866:322) and *Dendrohypnum* Hampe (1872:289), has had a convoluted taxonomic history, with several family and genus level rearrangements, validations and recircumscriptions. The genus was first proposed by Lindberg (1862), but no description was given; thus, it was not validly published. Most bryologists of the time adopted the name, with Kindberg (1899) validating it and placing it within the Climaciaceae Kindberg (1897:7). Brotherus (1905, 1909) accommodated the genus in the Hypnodendraceae Brotherus (1909:1166), while Touw (1971) placed its species within a section *Sciadocladus* of the genus *Hypnodendron* (Müller 1851:496) Lindberg (1862:374). Finally, Bell *et al.* (2007) reinstated *Sciadocladus* as a genus within the family Pterobryellaceae Buck & Vitt (1986:33) allied with *Pterobryella* (Müller 1872:182) Jaeger (1877:241), based on a combination of morphological features and genetic data analyses (Bell *et al.* 2007, 2012).

The species in *Sciadocladus* have most recently been revised by Touw (1971) on the basis of morphological characters. These species concepts were subsequently used by Bell *et al.* (2007). Although morphological and genetic data were used, intrageneric structure was not explored as the sampling in that study was designed to resolve ordinal and family level questions rather than intrageneric ones.

An examination of the intrageneric genetic structure of *Sciadocladus* and a revision of morphological characters for species within the genus have been carried out in the light of uncertainties in morphology described below, these being compatible with two potential phylogenetic scenarios with different implications for taxonomy.

As far as the current taxonomy is concerned, the genus consists of two species with overlapping geographical ranges: *Sciadocladus kerrii* (Mitten 1859: 86) Brotherus (1909: 1168). is present in New Zealand (NZ widespread, Stewart Island rare), while *S. menziesii* (Hooker 1818:33) Brotherus (1909:1168) is present in New Zealand, New Caledonia and the Solomon Islands. The latter species was subdivided by Touw (1971) into *S. menziesii* subsp. *menziesii*, occurring in NZ (with dubious records from Norfolk Island and Tasmania); and *S. menziesii* subsp. *splendidum* (Bescherelle

1873:245) Touw (1971:265), occurring in New Caledonia and the Solomon Islands, and previously treated by other authors as a distinct species.

Gametophytic morphological characters for intrageneric classification are slight, at times only distinguishable in fully grown plants. *Sciadocladus menziesii* often produces distal innovations (reiterations of the primary module), resulting in a series of superimposed dendroid forms of similar size, while *S. kerrii* mostly has simple fronds. Stipe leaves in the two species tend to have slightly different shapes and insertion lines, while there are inconsistent differences in branch leaf shape and the morphology of the papillae on the branch leaves. If sporophytes are present species can be confidently identified, as *S. kerrii* produces sporangia that are distinctly smaller and generally more numerous with apiculate to shortly rostrate opercula (conical opercula in *S. menziesii*). Within *S. menziesii*, morphological characters distinguishing subsp. *splendidum* from subsp. *menziesii* are tenuous, the most reliable being the occurrence of some large teeth near the apices of the stipe leaves in subsp. *splendidum* (uniformly crenulate to serrulate stipe leaves in subsp. *menziesii*), although these are not always found (Touw 1971).

Given the overlap in geographical ranges and difficulties with morphological character-based taxonomy (especially when sporophytes are lacking), an intrageneric and specific morphological revision and genetic analyses are required to confirm and expand knowledge of the species and their genetic structure and confirm whether populations are genetically distinct in different geographical areas. The existence of an endemic species in NZ, together with an endemic subspecies of a more widespread taxon, suggests that *S. kerrii* could have arisen through sympatric speciation from local populations of *S. menziesii* that remain genetically isolated from populations in New Caledonia.

This study aims to use molecular sequence data to determine which of two credible hypotheses best represents relationships between *S. menziesii* and *S. kerrii*, and to ensure that this is adequately reflected in taxonomy. The novel hypothesis tested here is that *S. menziesii* subsp. *menziesii* is in fact more closely related to *S. kerrii* than to *S. menziesii* subsp. *splendidum*, as would be the case if the NZ endemic *S. kerrii* had speciated from populations of *S. menziesii* in NZ after geographic and genetic isolation of the *S. menziesii* subspecies. If this were the case, we would expect the highest-level phylogenetic division within the genus to reflect geography rather than current taxonomy. The alternative is that the geographically and tenuously morphologically distinct subspecies of *S. menziesii* are more closely related to each other than either is to *S. kerrii*, consistent with the current taxonomy (Touw 1971).

Materials and Methods

In order to test if our novel hypothesis better reflects the phylogenetic history of *Sciadocladus* than does the current taxonomy, a molecular dataset was generated with appropriate sampling, combining newly obtained sequences with a dataset generated in previous studies of pleurocarpous mosses (Bell & Newton 2005, Bell *et al.* 2007, 2012). The regions chosen were part of the protein coding chloroplast ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (*rbcL*) region; the chloroplast *trnL-trnF* region (consisting of the *trnL-trnF* intergenic spacer and partial sequences of the *trnL* and *trnF* genes, the former including a group I intron); and the nuclear ribosomal DNA (nrDNA) non-coding internal transcribed spacer 2 (ITS2) region.

Three samples of *S. kerrii* encompassing its geographic range (north and south islands of New Zealand) and five samples of *S. menziesii* (two from New Caledonia, three from New Zealand) were used in this study. Three of these (one *S. kerrii* and two *S. menziesii*) had previously been sequenced (Bell *et al.* 2012). We included specimens from NZ with sporophytes that could be easily identified to the species level, and others without sporophytes that were identified to species based on gametophytic characters only. An outgroup obtained from previous pleurocarp studies (Bell & Newton 2005, Bell *et al.* 2007, 2012) included other Pterobryellaceae (six specimens of four taxa of *Pterobryella*), Hypnodendrales, one sample from the family Racopilaceae Kindberg (1898:85), and four specimens representing distant acrocarp lineages. These outgroups were processed to add sequences from the nuclear ITS2 region where possible. All specimens sampled and further details of the collections are detailed in **Table 1**.

Protocols, troubleshooting strategies and methods used to extract and amplify sequences followed those used in a larger biogeographic and taxonomic study (Sánchez-Ganfornina & Bell pers. comm.; Sánchez-Ganfornina 2018). Similarly, alignment criteria, dataset creation and selection of models for molecular evolution followed the same methods and employed the same software packages as in this larger study.

Maximum Likelihood (ML) analyses were carried out using raxmlGUI v1.3 (Silvestro & Michalak 2012). Three partitions, one for each gene region, were included in 100 runs of a Maximum Likelihood search and bootstrap analysis using the “thorough bootstrap” option with 1,000 repetitions. A maximally likely tree and bootstrap support values were generated.

In parallel with the molecular analyses, herbarium bryophyte observation techniques (Sánchez-Ganforina & Bell pers. comm., Sánchez-Ganforina 2018) were employed to review all specimens morphologically, paying close attention to all characters described to date (Touw 1971), as well as investigating the potential for further informative characters to be discovered.

Results

DNA extraction and amplification was successful in most cases; all samples were successful for *rbcL* and *trnL-trnF*, and four out of eight ingroup samples were successful for ITS2, including representatives of all taxa. Models for molecular evolution used were GTR+I+G for all regions. The optimal (maximally likely) tree from the ML search including bootstrap support values is shown in **Figure 1**.

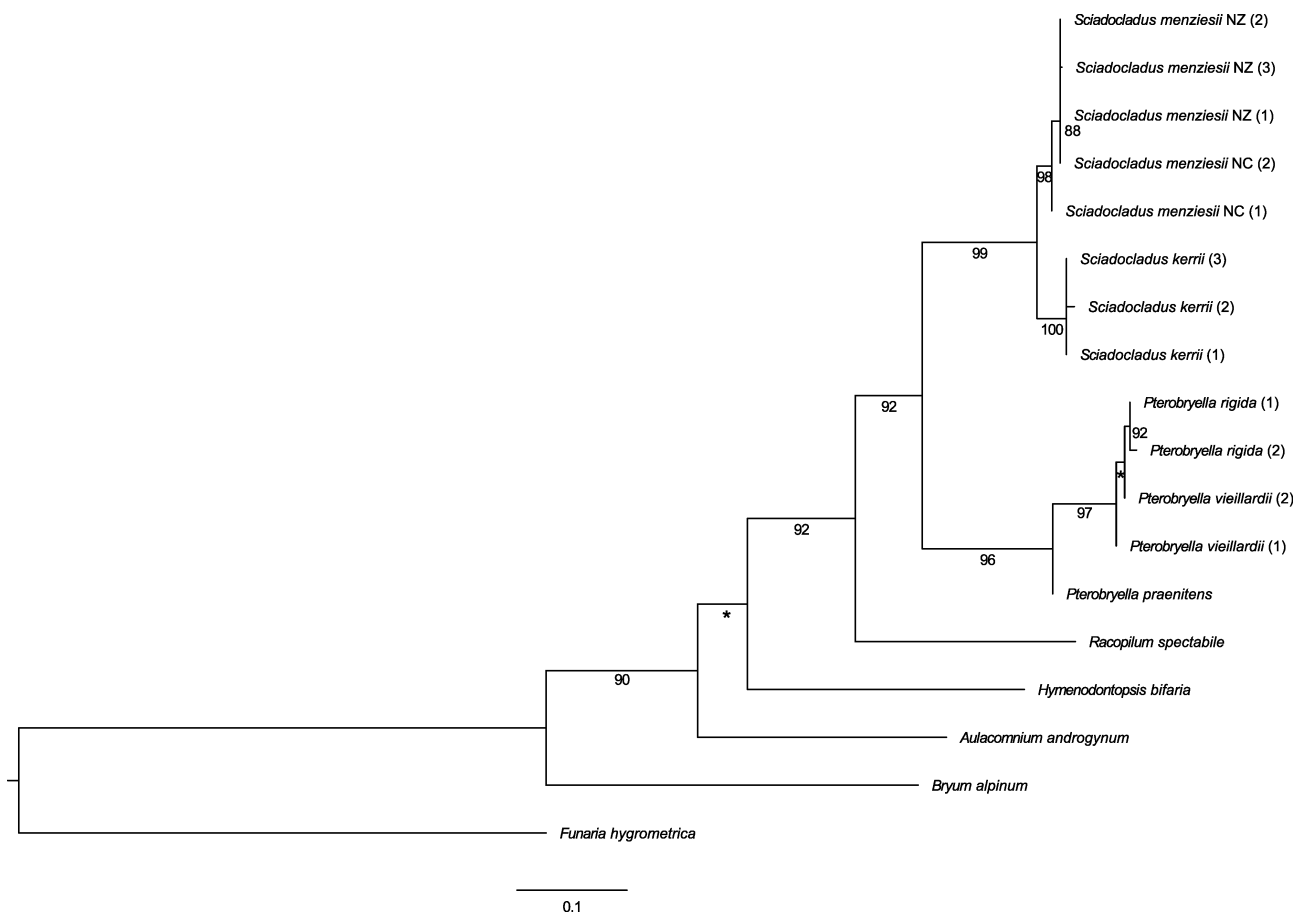


FIGURE 1. Optimal (maximally likely) tree from the ML search including bootstrap support values. Bootstrap support values are shown below branches. Only values over 60% were considered significant, with values over 75% referred to as noteworthy and over 85% as high. When support values were not significant, this is indicated with an asterisk (*).

Sciadocladus kerrii forms a highly supported monophyletic group, as does *S. menziesii*. No subspecific division is supported within *S. menziesii*, with the three NZ specimens (subsp. *menziesii*) forming a well-supported clade together with one of the NC specimens (subsp. *splendidum*), to the exclusion of a second NC exemplar.

No significantly prominent new characters were observed to distinguish gametophytic generations lacking sporophytes in either species. Thus, the only characters able to separate the species are those described by Touw (1971).

Discussion

The results of our investigations can be addressed in the light of intrageneric and intraspecific taxonomy as well as evolutionary processes within the southeastern Australasian region in the last 70 million years. The latter is facilitated by our larger study of Hypnodendrales structure and evolution that produced divergence age estimates for selected nodes within the Pterobryellaceae (Sánchez-Ganfornina & Bell pers. comm., Sánchez-Ganfornina 2018).

Sciadocladus is a well-defined and well-supported genus, estimated to have diverged from its sister genus *Pterobryella* approximately 67.04 Ma. *Sciadocladus kerrii* and *S. menziesii* are estimated to have diverged from each other approximately 19.62 Ma (Sánchez-Ganfornina & Bell pers. comm.). *Sciadocladus kerrii* and *S. menziesii* are shown here to be highly supported as monophyletic, corroborating the current species concepts for both taxa. Within *S. menziesii*, no subspecific groupings are shown to be consistent either in the analyses or in terms of geographical differentiation, and there are only low levels of intraspecific variation. Therefore, members of the *S. menziesii* subspecies are clearly more closely related to each other than any are to *S. kerrii*, essentially confirming the species circumscriptions in the latest treatment (Touw 1971).

The evolutionary history and likely dispersal pathways for these taxa are interesting, showing particular patterns deeply linked to the labyrinthine geological history of the area. Although the processes of formation of the extant New Zealand and New Caledonia island groups have been highly controversial (Ladiges & Cantrill 2007), it now seems likely that both were entirely submerged at various points in the Paleocene and Eocene, i.e. considerably later than the divergence of the *Sciadocladus* and *Pterobryella* lineages. This might suggest that *Sciadocladus* (now only found in these island groups) could have originally occurred on the Australian landmass and dispersed to New Zealand and New Caledonia in the later Eocene, subsequently becoming extinct in Australia as aridification and other environmental factors drove rainforest biomes towards the extreme western and eastern coastal fringes. There are, however, a number of taxa in the New Zealand flora with no apparent record of occurrence in Australia, the ultimate origins of which remain controversial and with continuous persistence in the region still not definitely rejected (e.g. Knapp *et al.* 2007).

To conclude, species circumscriptions within *Sciadocladus* are confirmed through molecular and morphological methods, corroborating the most recent taxonomic treatment (Touw 1971). There is no evidence from our molecular analysis to indicate that populations of *Sciadocladus menziesii* from NZ and NC should be recognised as distinct subspecies, although more sampling would be required to definitively address this. The biogeography of the genus raises interesting questions in the context of the geological, ecological and climatological history of the region.

TABLE 1. Taxa sampled. Data is presented in the following sequence: specimen label, voucher/reference, GenBank accession codes for *rbcL*, *trnL-trnF*, ITS2. For newly generated sequences, voucher information (collector, collection number, herbarium code, region, all separated by semicolons) is provided. For previously published sequences, references to original publications are provided. Hyphens in GenBank accession codes indicate samples for which that specific gene region was not successfully sequenced.

Specimen label	Voucher/Reference	GenBank accession codes for <i>rbcL</i> / <i>trnL-trnF</i> / ITS2
<i>Sciadocladus menziesii</i> NC (1)	Bell & Newton (2005)	AY524439 / AY524495 / -
<i>Sciadocladus menziesii</i> NC (2)	Neil E. Bell; Bell 07.11.08.029; E; NC	OR866025 / OR866030 / OR911949
<i>Sciadocladus menziesii</i> NZ (1)	Neil E. Bell; Bell 19.11.17.005; E; NZ	OR866022 / OR866027 / -
<i>Sciadocladus menziesii</i> NZ (2)	Neil E. Bell; Bell 19.11.17.002; E; NZ	OR866023 / OR866028 / -
<i>Sciadocladus menziesii</i> NZ (3)	Neil E. Bell; Bell 20.11.17.005; E; NZ	OR866024 / OR866029 / OR911948
<i>Sciadocladus kerrii</i> (1)	Bell & Newton (2005)	AY524470 / AY524442 / -
<i>Sciadocladus kerrii</i> (2)	Neil E. Bell; Bell 12.11.17.002; E; NZ	OR866021 / OR866026 / OR911946
<i>Sciadocladus kerrii</i> (3)	Neil E. Bell; Bell 04.03.08.001; E; NZ	JX021632 / JX021661 / OR911947
<i>Pterobryella vieillardii</i> Müller (1878:66–67) (1)	Bell <i>et al.</i> (2012)	JX021630 / JX021659 / -
<i>Pterobryella vieillardii</i> (2)	Neil E. Bell; Bell 04.11.08.004; H; NC	JX021631 / JX021660 / OR911944
<i>Pterobryella rigida</i> (Mitten 1873:401) Touw (1971:344) (1)	Neil E. Bell; Bell 04.11.08.005; H; NC	JX021628 / JX021657 / OR911945
<i>Pterobryella rigida</i> (2)	Bell <i>et al.</i> (2012)	JX021629 / JX021658 / -

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TABLE 1. (Continued)

Taxon	Voucher/Reference	GenBank accession codes for <i>rbcL</i> / <i>trnL-trnF</i> / ITS2
<i>Pterobryella praenitens</i> (Hampe 1874:671) Müller (1878: 65)	Bell & Newton (2005)	AY524455 / AY524511 / -
<i>Racopilum spectabile</i> Reinwardt & Hornschuch (1829: 721)	Bell & Newton (2005)	AY524450 / AY524506 / -
<i>Hymenodontopsis bifaria</i> (Hooker 1818: 57) Bell, Newton & Quandt (2007:555)	Bell & Newton (2004), O'Brien (2007)	AY631195 / AY857805 / -
<i>Aulacomnium androgynum</i> (Hedwig 1801:178–179) Schwägrichen (1827:2)	Bell & Newton (2004), O'Brien (2007)	AY631174 / AY857795 / -
<i>Bryum alpinum</i> Withering (1801:824)	Bell & Newton (2004), Cox & Hedderson (1999)	AY631176 / AF023738 / -
<i>Funaria hygrometrica</i> Hedwig (1801:172)	Goffinet <i>et al.</i> (1998), Cox & Hedderson (1999)	AF005513 / AF023716 / -

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