

MULTIGENE EVIDENCE REVEALS THE SYSTEMATIC POSITION OF *PLEUROCLADOPSIS SIMULANS* (C. MASSAL.) R. M. SCHUST. WITHIN *SCHISTOCHILA DUMORT.*, SCHISTOCHILACEAE

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Abstract. The monotypic *Pleurocladopsis*, endemic to Chile, was established by Schuster in 1964 based on an earlier poorly known species *Cephalozia* (?) *simulans* C. Massal. The phylogenetic position of *Pleurocladopsis simulans* had been considered uncertain until it was placed in the family Schistochilaceae on account of the gynoecial and sporophytic characters. It has been assumed that *Pleurocladopsis* represents the starting point of evolution in Schistochilaceae. In the present study, the phylogenetic position and taxonomic status of *Pleurocladopsis simulans* are inferred from phylogenetic analysis of three chloroplast DNA sequence data. The result suggests that the genus was established solely based on the autapomorphic characters, thus obscuring its actual phylogenetic relationship with *Schistochila* and that these characters are later derived rather than ancestral. The result also confirms that the gynoecial and sporophytic characters are important in taxonomy, but they may be not sufficient at the infrafamilial level and at other lower taxonomic levels. In accordance with the results of the present study, *Pleurocladopsis* is synonymised with *Schistochila*, and the new combination *Schistochila simulans* (C. Massal.) Xiao L. He & Yu Sun is made.

Key words: autapomorphy, Chile, DNA sequence, endemics, liverwort, molecular phylogeny, morphology, *Pleurocladopsis simulans*, *Schistochila*, Schistochilaceae, systematics, taxonomy

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INTRODUCTION

The genus *Pleurocladopsis* R. M. Schust. has been thought to be a perfect example demonstrating the importance of the role of gynoecial and sporophytic characters in liverwort taxonomy (Schuster 1971). The monotypic *Pleurocladopsis*, endemic to Chile, was established by Schuster in 1964 (Schuster 1964a) based on an earlier poorly known species *Cephalozia* (?) *simulans* C. Massal. (Massalongo 1885). The phylogenetic position of *Pleurocladopsis simulans* (C. Massal.) R. M. Schust. had been considered uncertain until it was placed in the family Schistochilaceae on account of the gynoecial and sporophytic characters (Schuster 1972). Schuster (1972) assumed that the family Schistochilaceae possibly evolved directly from *Pleurocladopsis*-like ancestors based on the characters present in *Pleurocladopsis*, such as the tristichous and nearly isophyllous leaf organization and the bracteolar antheridia. However, this

assumption has not been tested by evidence other than that derived from morphology. Results from recent studies on the molecular systematics of the liverworts have rejected the presumption that isophylly represents the starting point of leafy liverwort evolution; on the contrary, it has been shown to be a derived character which has evolved several times independently in various leafy lineages (e.g., He-Nygrén *et al.* 2004, 2006).

Massalongo (1885) described a sterile liverwort as *Cephalozia* (?) *simulans* under a new section *Pleurocladotypus* of which the general aspect resembles *Pleuroclada albescens* (Hook.) Spruce within a broadly defined *Cephalozia* (Dumort.) Dumort. Stephani (1898–1924) placed it without question in *Cephalozia*. However, various gametophytic features of the species including erect and nearly isophyllous organization with large underleaves approaching the lateral leaves in size and

shape, the entirely lateral branching, the shallowly bilobed leaves and the leaf cells with coarse, nodose to triradiate trigones are markedly different from those of *Cephalozia* and Cephaloziaceae. These differences had led Schuster (1964a) to assign the species to a new genus *Pleurocladopsis*, but he failed to recognize its position within established families based on available gametophytic characters. Schuster (1966) later placed it tentatively in the family Herbertaceae on account of characters such as tristichous leaf organization, bilobed leaves and well-developed trigones of the leaf cells. The uncertainty of the phylogenetic position of the species persisted until the discovery of fertile material with sporophytes, revealing its close affinity to the complicate-bilobed and distichous Schistochilaceae (Schuster 1971)! Based on the shared characters, including the scattered purplish rhizoids, the presence of a coelocaulis of the gynoeceum, the sporophyte capsule shape and wall thickening patterns, and also spore and elater anatomy, Schuster (1971) conclusively placed *Pleurocladopsis* in Schistochilaceae and later elevated it as an independent subfamily Pleurocladopsidoideae corresponding to another subfamily Schistochiloideae possessing a distichous leaf organization (Schuster 1972; Schuster & Engel 1977, 1985; Hässel de Menéndez & Rubies 2009). A different opinion was offered by Solari (1971), whereby she proposed a new family Pleurocladopsidaceae to accommodate *Pleurocladopsis simulans* C. Massal. alone under the suborder Herbertineae. This arrangement was supported by Grolle (1972).

Schuster (1964b) suggested the close affinities between Schistochilaceae and Perssoniellaceae and the two families were proposed to constitute the suborder Perssoniellineae. However, the sole species of the Perssoniellaceae, *Perssoniella vitreocincta* Herzog, endemic to New Caledonia, with a series of unique morphological characters, has recently been transferred to *Schistochila* based on DNA sequence evidence. From He and Glenn (2010), who showed that the phylogenetic signal present in morphological data can be diminished by remarkable autapomorphic evolution, and that higher-level taxa whose distinctness rests on autapomorphies should be treated with caution and

that independent evidence of their special systematic position is needed.

In the present study, the phylogenetic position and taxonomic status of *Pleurocladopsis simulans* are inferred from phylogenetic analysis of three chloroplast DNA sequence data. We aim to answer the following questions: Does *Pleurocladopsis simulans* represent an isolated lineage as an archaic species representing the starting point of evolution within Schistochilaceae? Is *Pleurocladopsis* deserving of family rank, that is, as belonging to an independent family, Pleurocladopsidaceae? What is the natural systematic position of the taxon? Is there any phylogenetic information on the systematic position of the species that could not be revealed by morphological evidence alone?

MATERIALS AND METHODS

TAXON SAMPLING FOR PHYLOGENETIC ANALYSES

For the present study, a specimen of *Pleurocladopsis simulans* was collected by the senior author in 2012 at Alberto de Agostini National Park, Cape Horn Biosphere Reserve, Chile. The plant grows on a dripping cliff wall, intermixed with *Schistochila splachnophylla* (Hook. f. & Taylor) Stephani and other bryophyte species. In total, 55 exemplars including *Pleurocladopsis simulans* and other 53 Schistochilaceae taxa as ingroup and *Ptilidium pulcherrimum* (F. Weber) Hampe as outgroup were included for phylogenetic analyses. The selection of the outgroup was done based on the study of He and Glenn (2010). The ingroup included a DNA sequence dataset obtained from three chloroplast DNA regions, *rbcL*, *rps4*, and *trnL-F*, for all 55 species, except *trnL-F* sequences of *Schistochila aligera* (Nees & Blume) J. B. Jack & Stephani, and the *rbcL* sequences of *S. balfouriana* (Hook. f. & Taylor) Stephani, *S. cristata* Stephani, *S. leucophylla* (Lehm.) Stephani, *S. nitidissima* R. M. Schust., *S. parvistipula* Rodway, *S. reflexistipula* J. J. Engel & R. M. Schust., *S. spagazziniana* (C. Massal.) Stephani, *S. sp.* and *S. stratos* (Mont.) A. Evans, which unfortunately could not be obtained for this study. Novel *rps4* sequences for 29 species, *trnL-F* sequences for 28 species and *rbcL* sequences for 20 species were generated in this study. Their sequence accession numbers are in italics in Table 1. Table 1 also provided information of the full species names of the samples used.

Table 1. List of taxa, GenBank accession numbers and sequence sources for *rbcL*, *rps4* and *trnL-F* sequences used in the present study. Accession numbers for novel sequences generated in this study are in Italics.

Species	Genbank accession number			Voucher or sequence source
	<i>rbcL</i>	<i>rps4</i>	<i>trnL-F</i>	
<i>Ptilidium pulcherrimum</i> (F. Weber) Hampe	AY302460	AY462388	AY251186	He-Nygrén <i>et al.</i> 2004
<i>Pleurocladopsis simulans</i> (C. Massal.) R. M. Schust.	<i>KF184391</i>	<i>KF184411</i>	<i>KF184440</i>	Chile, He 2928 (H)
<i>Schistochila acuminata</i> Stephani	<i>KF184392</i>	<i>KF184412</i>	<i>KF184441</i>	Malaysia, Bell 19 (H)
<i>Schistochila alata</i> (Lehm.) Stephani	<i>KF184393</i>	<i>KF184413</i>	<i>KF184442</i>	Chile, Hyvönen 2805 (H)
<i>Schistochila aligera</i> (Nees & Blume) J. B. Jack & Stephani	<i>KF184394</i>	<i>KF184414</i>	–	Indonesia, Gradstein 11061 (GOET)
<i>Schistochila antara</i> Grolle	<i>KF184395</i>	<i>KF184415</i>	<i>KF184443</i>	Papua New Guinea, Norris 59752 (H)
<i>Schistochila appendiculata</i> (Hook.) Dumort. <i>ex</i> Trevis.	AY462328	AY462394	AY463596	He-Nygrén <i>et al.</i> 2004
<i>Schistochila berggrenii</i> (J. J. Engel & R. M. Schust.) Xiao-L. He & Glenny	GU733922	GU733964	GU733943	He & Glenny 2010
<i>Schistochila balfouriana</i> (Hook. f. & Taylor) Stephani	–	<i>KF184416</i>	<i>KF184444</i>	New Zealand, He 2262 (H)
<i>Schistochila blumei</i> (Nees) Trevis.	GU733936	GU733978	GU733957	He & Glenny 2010
<i>Schistochila chuldii</i> (R. M. Schust. & J. J. Engel) Xiao-L. He & Glenny	GU733923	GU733965	GU733944	He & Glenny 2010
<i>Schistochila chlorophylla</i> (Col.) J. J. Engel & R. M. Schust.	GU733932	GU733974	GU733953	He & Glenny 2010
<i>Schistochila ciliata</i> (Mitt.) Stephani	GU733937	GU733979	GU733958	He & Glenny 2010
<i>Schistochila colensoana</i> Stephani	GU733924	GU733966	GU733945	He & Glenny 2010
<i>Schistochila conchophylla</i> E. A. Hodgs. & Allison	GU733929	GU733971	GU733950	He & Glenny 2010
<i>Schistochila cristata</i> Stephani	–	<i>KF184417</i>	<i>KF184445</i>	Australia, Streimann 27437 (H)
<i>Schistochila doriae</i> (De Not.) Trevis.	GU733938	GU733980	GU733959	He & Glenny 2010
<i>Schistochila gayana</i> (Gott.) Stephani	GU733939	GU733981	GU733960	He & Glenny 2010
<i>Schistochila glaucescens</i> (Hook.) A. Evans	GU733940	GU733982	GU733961	He & Glenny 2010
<i>Schistochila kirkiana</i> Stephani	GU733941	GU733983	GU733962	He & Glenny 2010
<i>Schistochila lamellata</i> (Hook.) Dumort.	<i>KF184396</i>	<i>KF184418</i>	<i>KF184446</i>	Chile, Hyvönen 5945 (H)
<i>Schistochila laminigera</i> (Hook. f. & Taylor) A. Evans	AY462329	AY462395	AY463586	He-Nygrén <i>et al.</i> 2004
<i>Schistochila lehmanniana</i> (Lindenb.) Car- rington & Pearson	GU733933	GU733975	GU733954	He & Glenny 2010
<i>Schistochila leucophylla</i> (Lehm.) Stephani	–	<i>KF184419</i>	<i>KF184447</i>	Chile, He 3349 (H)
<i>Schistochila monticola</i> R. M. Schust.	<i>KF184397</i>	<i>KF184420</i>	<i>KF184448</i>	New Zealand, Glenny 9923 (H)
<i>Schistochila muricata</i> E. A. Hodgs. & Allison	<i>KF184398</i>	<i>KF184421</i>	<i>KF184449</i>	New Zealand, Glenny 10243 (H)
<i>Schistochila nitidissima</i> R. M. Schust.	–	<i>KF184422</i>	<i>KF184450</i>	New Zealand, Glenny 10505 (H)
<i>Schistochila nivicola</i> (R. M. Schust. & J. J. Engel) Xiao-L. He & Glenny	GU733925	GU733967	GU733946	He & Glenny 2010
<i>Schistochila nobilis</i> (Hook.) Trevis.	<i>KF184399</i>	<i>KF184423</i>	<i>KF184451</i>	New Zealand, He 2174 (H)
<i>Schistochila nuda</i> Horik.	AY462297	AY462351	AY463558	He-Nygrén <i>et al.</i> 2004
<i>Schistochila parvistipula</i> Rodway	–	<i>KF184424</i>	<i>KF184452</i>	New Zealand, Glenny 8903 (H)

Table 1. Continued.

Species	Genbank accession number			Voucher or sequence source
	<i>rbcL</i>	<i>rps4</i>	<i>trnL-F</i>	
<i>Schistochila pellucida</i> R. M. Schust. & J. J. Engel	KF184400	KF184425	KF184453	New Zealand, Glenny 7283 (H)
<i>Schistochila pinnatifolia</i> (Hook.) R. M. Schust.	GU733930	GU733972	GU733951	He & Glenny 2010
<i>Schistochila philippinensis</i> (Mont.) J. B. Jack & Stephani	KF184401	KF184426	KF184454	Sri Lanka, He 2051 (H)
<i>Schistochila pluriciliata</i> R. M. Schust. & J. J. Engel	KF184402	KF184427	KF184455	New Zealand, Glenny 9815 (H)
<i>Schistochila pseudociliata</i> R. M. Schust.	GU733934	GU733976	GU733955	He & Glenny 2010
<i>Schistochila quadrifida</i> A. Evans	KF184403	KF184428	KF184456	Chile, Hyvönen 2744 (H)
<i>Schistochila reflexa</i> (Mont.) Stephani	KF184404	KF184429	KF184457	Argentina, Hyvönen 3045 (H)
<i>Schistochila reflexistipula</i> J. J. Engel & R. M. Schust.	–	KF184430	KF184458	Chile, He 2943 (H)
<i>Schistochila repleta</i> (Hook. f. & Taylor) Stephani	KF184405	KF184431	KF184459	New Zealand, Glenny 9722 (H)
<i>Schistochila schultzei</i> Stephani	KF184406	KF184432	KF184460	Papua New Guinea, Hoffman 90-92 (H)
<i>Schistochila sciophila</i> R. M. Schust.	KF184407	KF184433	KF184461	Chile, Larrain & Andrus 31210 (H)
<i>Schistochila sciurea</i> (Nees) Schiffn.	GU733935	GU733977	GU733956	He & Glenny 2010
<i>Schistochila</i> sp.	–	KF184434	KF184462	Chile, Engel 11872 (H)
<i>Schistochila spegazziniana</i> (C. Massal.) Stephani	–	KF184435	KF184463	Chile, He 3292 (H)
<i>Schistochila sphagnoides</i> (Schwägr.) Stephani	KF184408	KF184436	KF184464	Madagascar, Pócs 9481/N (H)
<i>Schistochila splachnophylla</i> (Hook. f. & Taylor) Stephani	KF184409	KF184437	KF184465	Chile, He 3213 (H)
<i>Schistochila stratosa</i> (Mont.) A. Evans	–	KF184438	KF184466	Chile, Larrain & Vargas 26565 (H)
<i>Schistochila subhyalina</i> R. M. Schust.	GU733926	GU733968	GU733947	He & Glenny 2010
<i>Schistochila tasmanica</i> Stephani	GU733942	GU733984	GU733963	He & Glenny 2010
<i>Schistochila trispiralis</i> R. M. Schust.	GU733927	GU733969	GU733948	He & Glenny 2010
<i>Schistochila tuloides</i> (Hook. f. & Taylor) R. M. Schust.	AY462321	AY462381	AY463581	He-Nygrén <i>et al.</i> 2004
<i>Schistochila virescens</i> R. M. Schust.	GU733928	GU733970	GU733949	He & Glenny 2010
<i>Schistochila vitreocincta</i> (Herzog) Xiao-L. He & Glenny	GU733931	GU733973	GU733952	He & Glenny 2010
<i>Schistochila volans</i> Grolle	KF184410	KF184439	KF184467	Papua New Guinea, De Sloover 43.056 (H)

DNA EXTRACTION, PCR AMPLIFICATION AND SEQUENCING

DNA was extracted from dry herbarium material, using the Invisorb Spin Plant Mini Kit (Invitex, Berlin, Germany). DNA amplification and sequencing were performed using the protocol in He-Nygrén *et al.* (2004). Of the three molecular regions examined, *rbcL* and *rps4* are

protein-coding genes so there is no length variation in the sequences obtained. In the present study, the length of *rbcL* sequences was 1000 bp, and of *rps4* 573 bp. The *trnL-F* region contains a partial sequence of the leucine transfer RNA_(UAA), an intergenic spacer, and a partial sequence of phenylalanine tRNA_(GAA). The length of the sequences varied from 446 bp (*Schistochila tuloides*) to 589 bp (*Schistochila splachnophylla* largely due to

differences in the length of the variable regions and the intergenic spacer. Alignment of the *trnL-F* region was done using MUSCLE (Edgar 2004), and the length of the aligned characters was 791 bp.

PHYLOGENETIC ANALYSES

The dataset of the 55-exemplar *rbcL*, *rps4*, and *trnL-F* sequence matrix was analyzed using Bayesian inference. In total, 2409 aligned characters were included in the combined dataset. For the phylogenetic analyses, using MrBayes version 3.2.1 (Huelsenbeck & Ronquist 2001; Ronquist 2004; Ronquist *et al.* 2012), the combined data were partitioned into three sets corresponding to the *rbcL*, *rps4* and *trnL-F* regions. Three separate runs, each including 10 million generations with four chains and sampling trees and parameters every 100th generation, were completed. Compartments were unlinked to allow the parameters to vary independently. The analyses were performed under the GTR+I+G model which was used for each of the three partitions within each data set based on the estimates using MrModeltest v. 2.3 (Nylander 2004; Swofford 2002). All sample points prior to stability were discarded as burn-in values, and the remaining points were used to generate a 50% majority consensus tree.

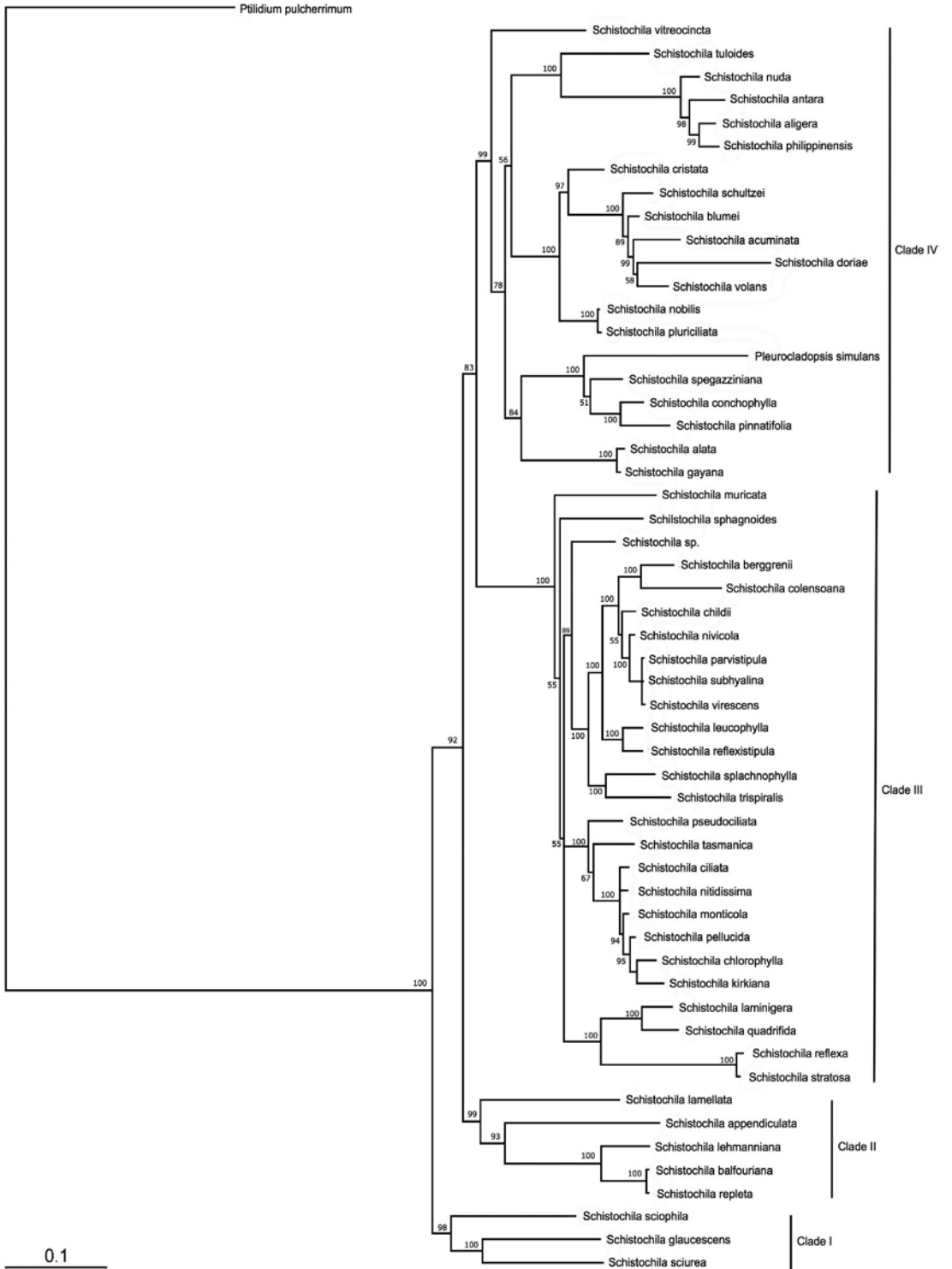
RESULTS

All three sequenced regions were initially analyzed separately. Congruence between datasets was evaluated by visual comparison of the topologies and levels of clade support arrived at the Bayesian analysis. Bayesian inferences based on the combined data set resolve *Pleurocladopsis simulans* as nested within a well-supported clade consisting of *Schistochila spegazziniana*, and closely related *S. conchophylla* and *S. pinnatifolia* (Fig. 1). They together were grouped in a well-supported clade including the South American sister group *S. alata* and *S. gayana*, the New Zealand sister group *S. nobilis* and *S. pluriciliata*, as well as all the tropical southeastern Asian and Oceanian species (clade IV). *P. simulans* and all the species of *Schistochila* formed a monophyletic group, including clade IV and other three well-supported groups, namely clades I, II, and III (Fig. 1). Clade I includes *Schistochila sciophila*, *S. glaucescens* and *S. sciurea*, and clade II *S. lamellata*, *S. appendiculata*, *S. lehman-*

niana, *S. balfouriana*, as well as *S. repleta*. The remaining *Schistochila* species constitutes clade III. The phylogenetic relationships of the family Schistochilaceae were not fully resolved, but the close affinity between *Pleurocladopsis simulans* and *Schistochila spegazziniana*, *S. conchophylla* and *S. pinnatifolia* was clearly determined.

DISCUSSION

The result of the present study resolved *Pleurocladopsis simulans* within the genus *Schistochila*, with close affinity to the South American *Schistochila spegazziniana*, and the Australasian *S. conchophylla* and *S. pinnatifolia*. Interestingly, they fall in the group (clade IV) containing all the southeastern Asian and Oceanian species including *S. vitreocincta*, and only a few of the Southern Hemisphere, and according to our study it is these species that exhibit the most diverse morphology in the family. Schuster (1971) already pointed out that *P. simulans* shares the unkeeled leaves and the beaked sporophyte capsule with *S. spegazziniana*. The species in clade IV have evolved enormous trigones of cells, larger spores and tortuous elaters, in contrast to the small spores and rigid and non-tortuous elaters in the species of clades I and II, and some of clade III. Reduction of the underleaf and evolution of epiphytism have also occurred in many of the species in clade IV. The phylogeny of the family in the present study suggests that these mentioned characters were derived later. Thus our result does not support the longstanding presumption that *Pleurocladopsis* represents the starting point of evolution in Schistochilaceae. Rather, it uncovers that the genus was established solely based on autapomorphic characters, thus obscuring its actual phylogenetic relationship with *Schistochila* and that these characters are later derived rather than ancestral. The remarkable autapomorphic evolution in *Pleurocladopsis simulans* had hindered Massalongo, Stephani as well as Schuster from understanding the species. Our result also confirms that the gynoecial and sporophytic characters are important in leafy liverwort taxonomy, but they may not be sufficient at infrafamilial level and at other lower taxonomic levels.



Recently, it has been shown that many genera thought to be stenotypic and established based on series of unique morphological characters are wrongly placed and that those characters are later derived rather than ancestral. *Perssoniella* of Schistochilaceae (He & Glenny 2010), *Amphilophocolea* (Engel *et al.* 2009) and *Cyanolophocolea* (Engel & He 2010) of Lophocoleaceae are examples of temperate Southern Hemisphere genera.

The family Schistochilaceae encompasses ca 80 species and more than two-thirds of the extant species of the family occur in southern South America and temperate to subantarctic Australasia. It has been assumed that the Schistochilaceae originated in Gondwanaland and its dispersal has been predicated on continental drift events (Schuster 1979, 1982; Schuster & Engel 1977, 1985). Therefore the family has been thought to be extremely old, the extant taxa possibly being survivors from the beginning of Mesozoic which have undergone little change since the beginning of the Tertiary due to being associated with relatively stable higher plant communities. The origin and biogeography of the family will be analysed and discussed in a separate paper.

TAXONOMIC TREATMENT

In accordance with the results of our study, the following nomenclatural changes are proposed:

Schistochila Dumort.

Recueil d'Observations sur les Jungermanniacées: 15. 1835. – TYPE: *Schistochila appendiculata* (Hook.) Dumort. *ex* Trevis.

= *Paraschistochila* R. M. Schust., J. Hattori Bot. Lab **26**: 259. 1963. – TYPE: *Paraschistochila pinnatifolia* (Hook.) R. M. Schust.

= *Pachyschistochila* R. M. Schust. & J. J. Engel, *Phytologia* **50**: 177. 1982. – TYPE: *Pachyschistochila splachnophylla* (Hook. f. & Taylor) R. M. Schust. & J. J. Engel

= *Perssoniella* Herzog, *Arkiv för Botanik utgivet av K. Svenska Vetenskapsakademien* **2**: 268. 1952. – TYPE: *Perssoniella vitreocincta* Herzog

= *Schistochilaster* H. A. Mill., *Phytologia* **20**: 317. 1970. – TYPE: *Schistochilaster aligera* (Nees) H. A. Miller

= *Tegulifolium* Hässel, *Bol. Soc. Argent. Bot.* **15**: 252. 1973. – TYPE: *Tegulifolium spegazzinianum* (C. Massal.) Hässel

= *Pleurocladopsis* R. M. Schust., *Nova Hedwigia* **8**: 279. 1964., *syn. nov.* – TYPE: *Pleurocladopsis simulans* (C. Massal.) R. M. Schust.

Schistochila simulans* (C. Massal.) Xiao L. He & Yu Sun, *comb. nov.

Cephalozia simulans C. Massal., *Nuovo Giorn. Bot. Ital.* **17**: 236. 1885.

SPECIMEN EXAMINED: CHILE, PROV. ANTÁRTICA CHILENA, Comuna Cabo de Hornos, Parque Nacional Alberto de Agostini, South-Central coast of Isla Gordon, Caleta Caracoles, NW of Estero Fouque along Brazo Sudoeste of Beagle Channel at upland lake, 55°02.038'S, 69°37.015'W, on dripping cliff wall on ENE shore of smaller lake, *X. He 2928* (H).

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Fig. 1. Phylogenetic relationship of *Pleurocladopsis simulans* (C. Massal.) R. M. Schust. based on a combined Bayesian analysis of *rbcl*, *rps4* and *trnL-F* sequence datasets from 55 exemplars. A 50% majority-rule consensus tree is presented. Bayesian posterior probabilities ≥ 0.95 are indicated. Scale bar represents 0.1 substitutions/site.

- R. M. Schust. together with comments on the status of *Tetracymbaliella* Grolle and *Lamellocolea* R.M. Schust. *Phytotaxa* **9**: 41–52.
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