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Siphula represents a remarkable case of morphological convergence in sterile lichens

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Abstract: A phylogenetic study using large subunit ribosomal DNA sequence data of several species of *Siphula*, representing all phenotypically recognized species groups, suggests that *Siphula* is not monophyletic. One lineage, including the core group related to *S. ceratites* and the *S. decumbens* group, is placed with members of the *Icmadophilaceae*, whereas a second distinct lineage, comprising *S. complanata*, *S. fragilis* and their relatives, has evolved within the *Coccotremataceae*. To accommodate these species, the new genus *Parasiphula* is described as new to science. *Parasiphula* consists of species restricted to cool to cold latitudes of the Southern Hemisphere. The results show a remarkable case of parallel evolution of lineages that have lost sexual stages and propagate via thallus fragments.

Key words: Bayesian inference, ITS, LSU rDNA, *Parasiphula*, phylogeny, *Siphula*, sterile lichens

Introduction

The classification of sterile lichens was often a problem for lichen taxonomists before the introduction of molecular methods. Classification was reasonable in cases where apparently closely related species with fruiting bodies existed, and morphological or chemical thallus characters were sufficiently complex for a proper placement (e.g. in species pairs of the *Parmeliaceae* and *Physciaceae*). However, species with poor vegetative characters or with unique thallus morphology were placed in a taxonomic group only with considerable uncertainty or remained unclassified (e.g. see Poelt 1973).

In several cases, molecular methods have already helped to clarify these problems and to place sterile lichens into a phylogenetic framework. This has led to some unexpected outcomes. For example, molecular data have shown that most species of the large, strictly sorediate genus *Lepraria* Ach. are apparently related to *Stereocaulon* Hoffm., whereas a few species belong either to the *Verrucariaceae* or *Lecanoraceae* (Ekman & Tønsberg 2002). In other cases, previous generic placement based on morphology has been found to have grouped unrelated taxa from completely different lineages. Thus the crustose lobate species '*Lecanora*' *demissa* (Flot.) Zahlbr. was shown to be related to the *Caloplaca variabilis* (Pers.) Müll. Arg. group (Arup & Grube 1999), while the likewise effigurate '*Lecanora*' *lisbonensis* G. Samp. is now accepted in the monotypic *Coscinocladium* Kunze, which is reinstated as a member of the *Physciaceae* (Crespo *et al.* 2004).

Among the strictly sterile fruticose lichen genera, *Thamnolia* and *Siphula* propagate mainly via thallus fragments that are dispersed by the force of wind or water. Many modern authors, for example Hafellner (1988), consider *Siphula* and *Thamnolia incertae sedis*, although Poelt (1973) recognized the family *Siphulaceae* Reichenb. (also containing *Endocaena*) and placed it near the *Cladoniaceae* on account of its chemistry. The core of *Thamnolia* was shown to be a member of the *Icmadophilaceae* by Stenroos *et al.* (1998) and Platt & Spatafora (2000);

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TABLE 1. *Species groups in* Siphula

Group	Thallus growth	Secondary chemistry
Siphula ceratites (*Siphula* s. str.)	Fruticose thallus	Chromones (siphulin)
S. decumbens	Fruticose to subfoliose, chalky thallus	Depsides (thamnolic, hypothamnolic, baeomycesic, squamatic, barbatic, neothamnolic and/or lactothamnolic acids)
S. complanata	Fruticose to subfoliose thallus	Dibenzofuranes (porphyrilic acid and/or methyl porphyrilate) or lacking substances
S. fragilis	Foliose thallus	Lacking substances or with depsidones (lobaric acid)

the latter studies also included some species of *Siphula*. Both Stenroos & DePriest (1998) and Platt & Spatafora (2000) suggested that *Siphula*, at least with respect to the species analysed, is close to the *Icmadophilaceae*, a view supported by morphological, chemical, ecological and biogeographical data (Kantvilas 1996, 1998, 2002). On the basis of correlations between chemical and morphological characters, four infra-generic groups within *Siphula* were proposed by Kantvilas (2002); see also Table 1. In this study, we present new and unexpected data about the phylogenetic position of all four species groups.

Material and Methods

Material

Fresh lichen specimens were collected wherever possible. Additional lichen material for the study was also taken from the herbaria GZU and HO. Voucher specimens used for molecular analyses (see Table 2) are stored in HO. Further details about the specimens are available upon request.

The following sequences were included from Genbank: *Baeomyces heteromorphus* AF113741, *B. rufus* AF113744, *Collema flaccidum* AY424213, *Coccotrema cucurbitula* AF274092 & AF329162, *C. maritimum* AF329164 & AF329165, *C. pocillarium* AF274093 & AF329167, *Dibaeis absoluta* AF113731, *Evernia prunastri* AF113745, *Geoglossum glabrum* AF113738, *Icmadophila ericetorum* AF113729, *Leotia viscosa* AF113737, *Lepolichen coccophorus* AF274096 & AF329169, *Leptogium gelatinosum* AY424212, *Lobaria amplissima* AY424206, *Nephroma bellum* AY424211, *Pannaria conoplea* AY424209, *Pertusaria leioplaca* AY300852, *P. werneriana* AY300856, *Pseudocyphellaria anomala* AY424208, *Psoroma hypnorum* AY424210, *Sclerotinia veratri* AF113739, *Siphula ceratites* AF107557 & AF113723, *S. coriacea* AF113724, *S. pickeringii* AF113725, AF113726 & AF113727, *Solorina saccata* AY424200, *Spathularia flavida* AF113736, *Stereocaulon paschale* AF279413, *Sticta limbata* AY424207, *Stictis radiata* AF113746, *Thamnolia subuliformis* AF113733, *T. vermicularis* AF113732.

DNA extraction, amplification, and sequencing

Total DNA was extracted from individual thalli according to a modified CTAB method (Cubero *et al.* 1999) after inspection for the absence of any contamination by lichenicolous fungi with a stereomicroscope. Extracts of DNA were used for PCR-amplification of the ITS regions including the 5·8S gene or the 5′-end of nuclear large ribosomal subunit rDNA. Primers for amplification of ITS were ITS1F (Gardes & Bruns 1993), ITS4 (White *et al.* 1990), whereas for LSU rDNA, LR0R and LR3 were used (http://www.biology.duke.edu/fungi/mycolab/primers.htm). We used this approach instead of amplifying both ITS and LSU rDNA in a single PCR because the latter failed to give results in several instances. 50 µl PCR mix (10 mM Tris pH 8·3/50 mM KCl/1·5 mM $MgCl_2$/50 µg gelatine) contained 1·25 units of Dynazyme Taq polymerase (Finnzymes, Oulu), 0·2 mM of each of the four dNTPs, 0·5 µM of each primer and *c.* 10–50 ng genomic DNA. Products were cleaned using QIAGEN quick spin columns (Qiagen, Vienna). Both complementary strands were sequenced using the Dye Terminator Cycle Sequencing Ready Reaction Kit (Applera, Vienna) according to the manufacturer's instructions. Sequences were run on an ABI310 automated sequencers (Applera, Vienna). The sequences are submitted to EMBL/GenBank. The list of sequenced taxa together with their Genbank accession numbers is presented in Table 2.

Data analysis

Sequences were aligned using Clustal W as included in the BioEdit Sequence Alignment Editor (Hall 1999). Ambiguously aligned regions were discarded after manual adjustment (i.e. a segment of 50 nucleotides in the ITS1 region). The phylogenetic hypotheses were constructed using a Bayesian approach as implemented

TABLE 2. *Specimens used in this study and their Genbank Accession numbers*

Isolate	Origin	Gene	Genbank Acc. No.
Siphula ceratites fb114	Norway, Hordaland, *Ekman* 2001 (GZU)	LSU	DQ337618
S. complanata fb109	Tasmania, Drys Bluff, 2002, *Kantvilas* (HO517570)	ITS	DQ337612
S. complanata fb145	Tasmania, Mt Eliza, 2004, *Kantvilas* (HO545950)	ITS	DQ337611
S. decumbens fb141	Tasmania, Red Knoll, 2004, *Kantvilas* (HO526341)	LSU	DQ337615
S. dissoluta fb110	Tasmania, Mt Sarah Jane, 2000, *Kantvilas* (HO 509560)	LSU	DQ337616
S. dissoluta fb113	Tasmania, Hartz Peak, 2001, *Kantvilas* (HO512240)	LSU	DQ337617
S. elixii fb118, sg424	Tasmania, Sentinel Range, 2000, *Kantvilas* (HO509344)	LSU, ITS	DQ337620, DQ337606
S. fastigiata fb116	Tasmania, Mt Sprent, 2000, *Kantvilas* (HO509414)	LSU	DQ337613
S. foliacea fb108	Tasmania, Adamsons Peak, 2001 *Kantvilas* (HO510419)	LSU, ITS	DQ337621, DQ337609
S. fragilis fb119	Tasmania, Mt Lot, 2000, *Kantvilas* (HO509580)	ITS	DQ337607
S. georginae fb111, sg476	Tasmania, Mt Lot, 2000, *Kantvilas* (HO509581)	LSU, ITS	DQ337619, DQ337608
S. jamesii fb143	Tasmania, Red Knoll, 2004, *Kantvilas* (HO526338)	ITS	DQ337610
S. pteruloides fb131	Costa Rica, Cerro de la Muerte, 2003, *Grube* 11796 (GZU)	LSU	DQ337614

in the program MrBAYES (Huelsenbeck & Ronquist 2001). The nucleotide substitution model applied was GTR+G+I. The Markov Chain Monte Carlo (MCMC) analysis was run for 2 000 000 generations, with 4 chains starting from a random tree, and using the default temperature of 0·2. Every hundredth tree was sampled, while the first 50 000 generations were discarded as burn-in. Consensus phylograms showing mean branch lengths were calculated using the *sumt* command in MrBAYES. Phylogenetic trees were drawn using the program Treeview (Page 1996). Topological support was additionally assessed by parsimony bootstrap analysis using heuristic searches with PAUP* 4.0b10 (Swofford 2002). Thousand bootstrap replicates were calculated with 5 random addition sequence replicates each, in addition to the default parameters.

Results

Molecular results

Although we had access to material of 18 of the 25 known species, good sequences could not be obtained for older herbarium specimens (older than about 5 years) with the standard extraction method (30 min in the extraction buffer at 65°C).

A matrix of 537 unambiguously aligned nucleotide position characters was used for the analysis of nuclear LSU rDNA sequence data. The likelihood parameters in the sample of the analysis had the following average values and standard deviations (in brackets): base frequencies $\pi(A)=0{\cdot}22$ $(0{\cdot}02)$, $\pi(C)=0{\cdot}27$ $(0{\cdot}01)$, $\pi(G)=0{\cdot}31$ $(0{\cdot}02)$, $\pi(T)=0{\cdot}21$ $(0{\cdot}01)$, rate matrix $r(AC)=0{\cdot}62$ $(0{\cdot}15)$, $r(AG)=1{\cdot}69$ $(0{\cdot}31)$, $r(AT)=0{\cdot}52$ $(0{\cdot}14)$, $r(CG)=0{\cdot}79$ $(0{\cdot}18)$, $r(CT)=5{\cdot}78$ $(0{\cdot}92)$, $r(GT)=1{\cdot}00$ $(0{\cdot}00)$, gamma shape parameter alpha$=0{\cdot}68$ $(0{\cdot}12)$, portion of invariable sites$=0{\cdot}23$ $(0{\cdot}05)$. The majority-rule consensus tree was calculated from 19 501 trees (Fig. 1); the estimated marginal log likelihood of the tree sample is $-5317{\cdot}84$. The data matrix of ITS data included 524 characters. The likelihood parameters in the sample of the analysis had the following average values and standard deviations (in brackets): base frequencies $\pi(A)=0{\cdot}24$ $(0{\cdot}01)$, $\pi(C)=0{\cdot}27$ $(0{\cdot}01)$, $\pi(G)=0{\cdot}23$ $(0{\cdot}01)$, $\pi(T)=0{\cdot}24$ $(0{\cdot}01)$, rate matrix $r(AC)=0{\cdot}82$ $(0{\cdot}37)$, $r(AG)=2{\cdot}08$ $(0{\cdot}74)$, $r(AT)=1{\cdot}08$ $(0{\cdot}47)$, $r(CG)=0{\cdot}73$ $(0{\cdot}34)$, $r(CT)=3{\cdot}87$ $(1{\cdot}27)$, $r(GT)=1{\cdot}00$ $(0{\cdot}00)$, gamma shape parameter alpha$=20{\cdot}49$ $(14{\cdot}78)$, portion of invariable sites$=0{\cdot}46$ $(0{\cdot}08)$. The majority-rule consensus tree was calculated from 19 501 trees (Fig. 2); the estimated marginal log likelihood of the tree sample is $-1685{\cdot}13$. With both data sets, no topological conflict was found by bootstrapped parsimony analyses.

FIG. 1. Phylogenetic position of *Siphula* species using the 5′ end of nuLSU rDNA. Majority rule consensus tree with average branch lengths based on 19 501 trees from a B/MCMC tree sampling. Posterior probability supports are indicated by increased thickness of the internodes; bootstrap support values equal or higher than 75% are indicated by numbers at the branches. *Leotia* and *Geoglossum* were used as outgroup taxa.

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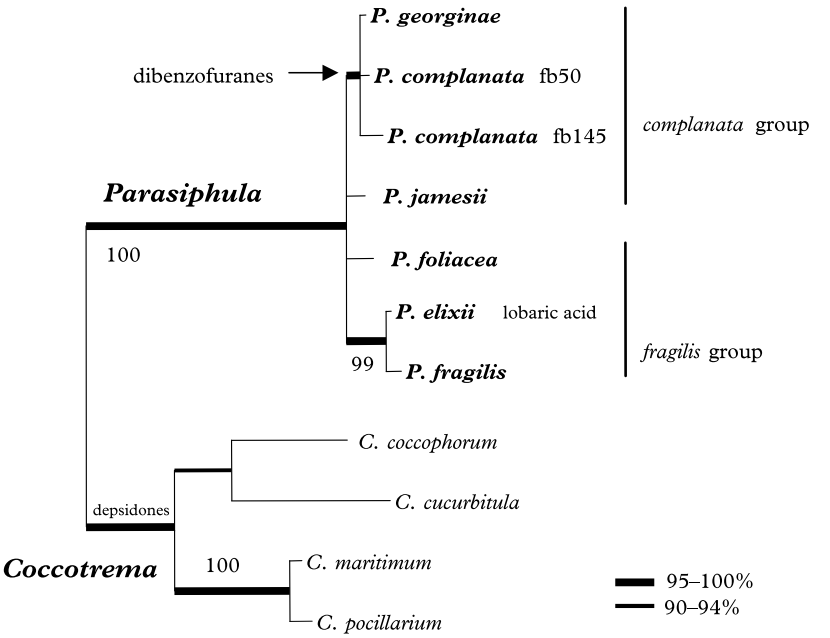

FIG. 2. Phylogenetic hypothesis of *Parasiphula* using ITS sequence data. Majority rule consensus tree with average branch lengths based on 19 501 trees from a B/MCMC tree sampling. Posterior probability supports are indicated by increased thickness of the internodes; bootstrap support values equal or higher than 75% are indicated by numbers at the branches. The tree is rooted by the *Coccotrema* species.

The *Ostropomycetidae* with its selected representatives (including *Baeomycetaceae*, *Coccotremataceae*, *Icmadophilaceae*, *Pertusariaceae* and *Stictidaceae*) are well supported, and also contain all of our sequenced *Siphula* species. The tree shows a significant support for the position of two groups of *Siphula*, the *ceratites* and *decumbens* groups, within the *Icmadophilaceae* together with *Icmadophila ericetorum* and *Dibaeis baeomyces*. However, the two groups are not resolved as monophyletic. *Thamnolia* forms a sister group to this assemblage with high support. A clade termed *Siphula* I in Fig. 1 contains *Siphula ceratites*, the type species, as well as *S. pickeringii* and *S. pteruloides*, whereas *Siphula* clade II includes *S. coriacea*, *S. decumbens*, *S. dissoluta* and *S. fastigiata*. However, *S. elixii* and *S. foliacea*, both members of the *S. fragilis* group, are shown to be unrelated to these groups in the *Icmadophilaceae* and instead form a sister group to *Coccotrema*. The grouping with the *Coccotremataceae* likewise receives high support by posterior probability values. The new genus name *Parasiphula* is introduced below for these species.

Further species of the *Coccotrema*-related *Siphula* lineage were investigated using ITS data. These data showed that the *Siphula complanata* group also belongs to the same clade related to the *Coccotremataceae*. *Siphula elixii* and *S. fragilis* form a well supported branch, as do the dibenzofurane-containing *S. georginae* and *S. complanata*. However, *S. jamesii* and *S. foliacea* were not resolved with any of the known species groups.

In some cases, we found sequences that were apparently belonging to an externally unobserved lichenicolous infection. One such example from a *Siphula georginae* specimen is a sequence apparently related with *Sclerotinia* (Fig. 1).

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Taxonomy

Parasiphula Kantvilas & Grube, gen. nov.

Genus novum thallo folioso vel fruticoso, complanato vel subtereti, corticato, rhizinis radicibus similibus affixo, algis viridibus, unicellularibus, plus minusve globosis, ascomatibus conidiomatibusque ignotis, *Siphulae* Fr. affine, praecipue differt materia chemicali qua dibenzofurania vel depsidonia includenti.

Typus generis: *Parasiphula fragilis* (Hook.f. & Taylor) Kantvilas & Grube.

Thallus foliose to fruticose, flattened to subterete, with a well-defined pseudoparenchymatous cortex several cells thick, attached to the substratum by bushy, root-like rhizines; photobiont a unicellular green alga with cells ± globose, 6–*7·5*–10 µm diam.; containing dibenzofuranes, depsidones or lacking secondary lichen substances.

New combinations:

Parasiphula comata (Nyl.) Kantvilas & Grube comb. nov.

Basionym: *Siphula ramalinoides* var. *comata* Nyl. in Crombie, *J. Linn. Soc. Bot.* **15:** 226 (1876).—*Siphula comata* (Nyl.) R. Sant. ex Kantvilas, *Herzogia* **12:** 14 (1996).

Parasiphula complanata (Hook.f. & Taylor) Kantvilas & Grube comb. nov.

Basionym: *Sphaerophoron complanatum* Hook.f. & Taylor, *Hook. Lond. J. Bot.* **3:** 654 (1844).—*Siphula complanata* (Hook.f. & Taylor) R. Sant. in D. J. Galloway, *New Zealand J. Bot.* **21:** 197 (1983).

Siphula subcoriacea Müll. Arg., *Miss. Sci. Cap Horn* 1882–1883, 5 Bot.: 151 (1888).

Siphula patagonica Vainio, *Résult. Voyage S.Y. Belgica, Botan.*: 39 (1903).

Parasiphula elixii (Kantvilas) Kantvilas & Grube comb. nov.

Basionym: *Siphula elixii* Kantvilas, *New Zealand J. Bot.* **32:** 17 (1994).

Parasiphula foliacea (D. Galloway) Kantvilas & Grube comb. nov.

Basionym: *Siphula foliacea* D. Galloway, *New Zealand J. Bot.* **21:** 197 (1983).

Parasiphula fragilis (Hook.f. & Taylor) Kantvilas & Grube comb. nov.

Basionym: *Endocarpon fragile* Hook.f. & Taylor, *Hook. Lond. J. Bot.* **3:** 639 (1844).—*Siphula fragilis* (Hook.f. & Taylor) J. Murray in W.A. Weber, *Lichenes Exsiccati Colo. Fasc 7*, no. 265 (1969).

Parasiphula georginae (Kantvilas) Kantvilas & Grube comb. nov.

Basionym: *Siphula georginae* Kantvilas, *Herzogia* **12:** 12 (1996).

Parasiphula jamesii (Kantvilas) Kantvilas & Grube comb. nov.

Basionym: *Siphula jamesii* Kantvilas, *Nordic J. Bot.* **7:** 585 (1987).

Discussion

Siphula presents a bewildering range of morphological variation that complicates the delimitation of well-defined taxa. Some of this variation is interpretable in terms of habitat factors but some is not. *Siphula* also displays a range of discrete chemical spectra, comprising depsides, depsidones, dibenzofuranes and chromones, whereas some taxa contain no secondary lichen substances. With the idea in mind that *Siphula* is a monophyletic group of closely related species, we began our molecular investigation by sequencing ITS. However, significant problems arose in aligning the species of the core group around the type species, *Siphula ceratites*, with sequences of other species groups. While the sequences within groups were rather similar, we were unable to align species in the *ceratites* and *decumbens* groups with those of the *fragilis* and *complanata* groups. A further hint of the previously underestimated diversity was the recent finding of Stenroos *et al.* (2002), using small subunit rDNA, that the *Siphula ceratites* group, including *S. carassana* and *S. pickeringii* (the latter as *S. polyschides*), does not form a monophyletic group with *S. decumbens*, including *S. coriacea* and *S.* cf. *fastigiata*. We therefore decided to use the more conserved nuclear large subunit ribosomal

DNA to find the lichen groups most closely related to *Siphula*. The investigation of the systematic position of *Siphula* has produced exciting and unanticipated results. We have found that there are two distinct lineages in *Siphula*. The first consists of the type species, *S. ceratites*, plus the depside-containing species referred to as the *decumbens* group. This lineage is allied with the *Icmadophilaceae* as exemplified by their placement in a group together with *Thamnolia*, *Dibaeis* and *Icmadophila*, confirming the work of earlier authors (Platt & Spatafora 2000).

Our LSU data, however, could not confirm a monophyletic lineage as a sister group to either *Thamnolia*, *Dibaeis* or *Icmadophila*. While the clade named ''*Siphula* I'' appears as a sister group to *Icmadophila*, clade ''*Siphula* II'' forms a sister group to *Dibaeis*. The strictly sterile genus *Thamnolia* is clearly supported as a basal taxon to these assemblages. Surprisingly, the Pacific taxon, *S. pickeringii*, was placed as closely related to the *ceratites* group. On the basis of its morphology (narrow, chalky white lobes), anatomy (no cortex) and chemistry (baeomycesic and squamatic acids), this species was expected to sit comfortably in the *decumbens* group. We need to examine this further with additional collections. Its current placement may indicate that the distinction between these two clusters is not as strong as it may appear. In previous work (Kantvilas 2002; Kantvilas & Elix 2002), some neotropical taxa, notably *S. pteruloides*, were difficult to classify because they were chemically variable and combined the production of chromones, typical of *S. ceratites*, with depsides, typical of the *S. decumbens* group. Our molecular data now indicate that *S. pteruloides* is closely related to *S. ceratites*, and that the chromone producing group and the depside producing group are closely related.

The second lineage is rather distant from *Siphula* s. str. and is grouped together with the *Coccotremataceae* in our phylogeny. This lineage of *Siphula* consists of the *fragilis* and *complanata* groups. To accommodate these taxa, the new genus, *Parasiphula*, with *P. fragilis* as the type, is erected. All species in these morphologically and chemically defined groups are strictly cool to cold temperate Southern Hemisphere species. This lineage represents a remarkable case of parallel evolution.

Parasiphula is superficially very similar to *Siphula* s. str. and, given that in both genera neither ascomata nor conidiomata are known, their delimitation primarily by chemical means is unfortunately inevitable at this stage. Thus whereas *Siphula* s. str. contains either depsides (most frequently thamnolic, baeomycesic, squamatic or hypothamnolic acids) or chromones, *Parasiphula* contains dibenzofuranes (methyl porphyrilate and/or porphyrilic acid) or depsidones (lobaric acid); in addition, several taxa of *Parasiphula* lack any detectable substances. Both genera have root-like, bushy rhizines as the principal mode of attachment. Although both *Siphula* and *Parasiphula* tend to have very brittle lobes, those of *Siphula* tend to be distinctly chalky in colour and texture, whereas species of *Parasiphula* are rather dull-coloured with tints of beige, brown or ivory. In *Parasiphula*, there is a very well developed, multi-layered cortex of rather large, pseudoparenchymatous cells. Although a similar cortex appears to be present in *Siphula ceratites*, the type species of *Siphula*, and in *S. pteruloides*, no species of the *decumbens* group develops such a cortex, and instead the outermost part of the lobes is a rather poorly defined layer of interwoven hyphae and crystalline inclusions.

Siphula s. str. is a rather widespread genus with representatives on all continents, but with centres of speciation in tropical America, southern Africa and the Mascarene Islands, southern South America and Australasia (Kantvilas 2002). Species range from austral to tropical latitudes, becoming increasingly montane in the latter, as well as occurring in the boreal zone (*S. ceratites*). In contrast, *Parasiphula* is a strictly cool to cold temperate, Southern Hemisphere genus, occurring mostly in treeless, windswept, wet environments, on peaty soil, often submerged in shallow pools or at the fringes of small lakes. The most closely related genus

to *Parasiphula* appears to be *Coccotrema*. *Coccotrema* is an unusual, small, cool temperate genus with centres of speciation in the Pacific North-West of North America (Brodo 1973), southern South America (Messuti & Vobis 2002) and Tasmania (G. Kantvilas, unpublished data). Despite its superficial resemblance to *Pertusaria*, it is placed in its own family on the basis of the structure of the ascomata and asci, and the presence of cephalodia (Lumbsch *et al.* 1994; Messuti 1996; Schmitt *et al.* 2001). Most are epiphytic but in alpine Tasmania there are taxa that encrust damp, peaty soil, a habitat also colonized by taxa of *Parasiphula*. Thus these unusual, seemingly unrelated lichens do display some degree of biogeographical and ecological overlap. One species of *Coccotrema*, *C. coccophorum*, was formerly included in a separate genus, *Lepolichen*, until Schmitt *et al.* (2001) showed that it cannot be separated from *Coccotrema*. This species forms thalli with terete lobes and fibrillar rhizinae. We suggest that *Parasiphula* be included in the *Coccotremataceae*, thus widening the concept of that family considerably by the addition of a sterile lineage. This placement of *Parasiphula* is also supported by preliminary mtSSU data (data not shown). Thus the *Coccotremataceae* in this wider sense includes species with highly divergent growth forms, ranging from crustose to foliose to fruticose, and species that, in addition to β-orcinol depsides, also produce dibenzofuranes. Within *Parasiphula*, the two phenotypic groups as previously defined are partly supported. The species with dibenzofuranes (i.e. the *complanata* group) appear as a monophyletic group, while the species in the *fragilis* group are not well resolved. Further molecular data are needed to test the monophyly of this group.

An extensive comparative morphological, anatomical and chemical overview of other members of the *Icmadophilaceae* and *Coccotremataceae* is outside the scope of the present study. However, the available chemical data support our phylogenetic results in so far as that depsides, in particular thamnolic, baeomycesic and squamatic acids, are especially common in other taxa of the *Icmadophilaceae* (Rambold *et al.* 1993), whereas the *Coccotremataceae* commonly contain depsidones (Messuti & Vobis 2002). However, the relationships of the *Coccotremataceae* and *Icmadophilaceae* with the *Pertusariales* s.l. are still poorly understood. A recent study also shows an unsupported placement of both families in *Pertusariales* and unexpected sister-group relationship of *Aspicilia* with the *Icmadophilaceae* and some *Pertusaria* species (Wedin *et al.* 2005). It will be interesting to see if this receives further support by the analysis of further genes. Since the usually crustose genus *Aspicilia* can also contain fruticose forms (Sanders 1999), this could indicate, that a general potential exists here to evolve fruticose growth forms from primarily crustose ancestors, with a convergent subsequent loss of sexual reproduction in *Siphula*, *Parasiphula*, and *Thamnolia*.

The current species-level classification of *Siphula* (including *Parasiphula*) is based on chemical characters, correlated with thallus morphology and biogeographic distribution. This has proved to be a practical means of defining the 25 species currently known. Obviously, our investigations at this stage cannot be used to assess species delimitations. However, we need to explore this further, especially to ascertain whether species in *Siphula* should be based, as now, on chemically discrete entities that vary morphologically, or whether one should recognize as species essentially morphologically-defined entities that comprise several chemical races. That is, are individuals that are morphologically similar but have different chemistries more closely related to each other genetically than are individuals that are morphologically different but have the same chemistry, or are there genetic lineages that are polymorphic for each phenotypic character complex?

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