

MOLECULAR PHYLOGENETICS

Molecular insights into the phylogeny of the leafy liverwort family Lophoziaceae Cavers

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Delimitation and classification of the large, cosmopolitan liverwort family Lophoziaceae is controversial. Many recent workers have included it in Jungermanniaceae, and even in its strictest sense, internal classification has varied widely among different treatments. We analyse variation in DNA sequences of the chloroplast *rps4* gene and the *trnG* intron to provide resolution of phylogenetic relationships in the leafy liverworts with emphasis on the various elements usually placed in Lophoziaceae. The following conclusions are drawn. Lophoziaceae is not closely related to Jungermanniaceae. Lophoziaceae, and perhaps also Cephaloziellaceae, should be included in Scapaniaceae unless many small families are recognised. *Delavayella* and *Blepharidophyllum* are excluded from Scapaniaceae. Jamesonielloideae is a family of its own (Jamesoniellaceae) sister to Adelanthaceae (or a subfamily of Adelanthaceae). The genus *Anastrophyllum* should be split into *Anastrophyllum* and *Sphenolobus*. *Lophozia* is polyphyletic and the genera *Isopaches* and *Schistochilopsis*, and perhaps *Obtusifolium*, should be recognised while *L. sudetica* could be transferred to *Barbilophozia*. *Barbilophozia* s.str. is monophyletic while *Orthocaulis* is polyphyletic with the four sampled species appearing in 3 different clades; their relationships are poorly resolved. *Lophozia silvicola* Buch is clearly separated from *L. ventricosa* and *Jamesoniella oenops* from *J. colorata* at species level.

KEYWORDS: Lophoziaceae, molecular phylogeny, *rps4*, Scapaniaceae, *trnG*

INTRODUCTION

The Lophoziaceae are a large (ca. 280 species), globally distributed family of leafy liverworts including species ranging from narrow endemics to those that are very widespread, rare species to very abundant ones, species with very frequent sexual reproduction to those unknown to produce spores, and species with and without asexual reproduction. Most taxa occur in cool to cold areas and in the tropics mostly in montane or alpine regions. In some environments (e.g., cooler and cold portions of the Northern Hemisphere, and in humus-rich acid habitats in montane tropical and temperate rain forests) the group is ecologically significant.

Classification of the Lophoziaceae is controversial at all levels, including views on the very existence of the family. Individual researchers have adopted radically different circumscriptions, and in particular its distinctness from, and relationship to, Jungermanniaceae has been debated. As with many other treatments, the most complete recent classification of hepatics (Crandall-Stotler & Stotler, 2000) includes Lophoziaceae as a subfamily of the latter. Similarly, Schuster (2002a) does not distinguish the two at family level because of perceived exceptions to any diagnostic characters that can be identified for each

group. On the other hand, Grolle & Long (2000) recognise European Lophoziaceae (including two subfamilies, Lophozioideae and Jamesonielloideae) as distinct from Jungermanniaceae.

The delimitation of subfamilies and genera has also been problematic. Schuster (2002a), who subsumed the family within Jungermanniaceae, writes: “The problem of how to circumscribe subfamilies is also illuminated by the fact that most generalisations used to separate groups ... are exactly that, generalisations; almost all are *transgressed* by one or more exceptions.” He notes that generalisations derived from examination of Holarctic taxa alone may be deceptive and that in Austral areas a very different mix of characters may be seen (Schuster, 2002a). He does suggest that subfamilies Lophozioideae Cavers and Jamesonielloideae Inoue have closest affinity to each other, concordant with their treatment as subfamilies of Lophoziaceae by Grolle & Long (2000).

The same problems exist at generic level; Schuster (2002a) notes that when the criteria used for subdividing Holarctic members of the large and complex genus *Lophozia* are used for taxa from elsewhere, they largely fail for recognising segregate genera in this broader context. This is seen in the use of leaf-lobe number to separate *Barbilophozia* and *Orthocaulis*, a character that is not consist-

ent when including the richer southern hemisphere flora rather than just Eurasian representatives (Schuster, 2002a).

Whilst molecular data have been used to great effect in improving our understanding of evolutionary relationships in many groups of plants and animals including bryophytes (e.g., Hedderson & al., 1996, 1998, 2004; Capesius & Stech, 1997; Cox & Hedderson, 1999; Stech, 1999; Newton & al., 2000), only a few recent studies have addressed higher-level relationships in hepatics (e.g., Davis, 2004; He-Nygrén & al., 2004, 2006; Heinrichs & al., 2005). With respect to Lophoziaceae, several recent studies (e.g., Davis, 2004; Yatsentyuk & al., 2004; Heinrichs & al., 2005; He-Nygrén & al., 2006; Hentschel & al., 2006) have shown that the family Jungermanniaceae s.l. is polyphyletic and that current family and subfamily delimitations are largely artificial. In addition Schill & al. (2004) demonstrated that the large family Scapaniaceae is nested within Lophoziaceae (see also Yatsentyuk & al., 2004; Heinrichs & al., 2005; He-Nygrén & al., 2006) and Heinrichs & al. (2005) formally include Lophoziaceae in Scapaniaceae.

In this paper we provide an analysis of chloroplast DNA variation in leafy liverworts, with particular emphasis on the relationships of Lophoziaceae. Our main objectives are to (1) test the monophyly of Lophoziaceae and the main taxonomic groupings within it, and (2) evaluate the relationship between Jungermanniaceae and Lophoziaceae.

MATERIALS AND METHODS

The 190 exemplars included in the analyses are listed in the Appendix. The nomenclature for families and higher taxonomic levels follows Crandall-Stotler & Stotler (2000) except for Jungermanniaceae and, by definition, also Lophoziaceae where we follow Grolle & Long (2002). Taxa were chosen to represent a wide range of Jungermanniidae with emphasis on the Lophoziaceae and taxa placed near it in most classifications. The sampling attempts as far as possible to represent the major lineages of the Jungermanniidae, at least as these are currently understood. In addition, we included *Riccardia*, *Metzgeria*, *Symphyogyna*, *Haplomitrium* and *Pellia* (Metzgeriidae) as outgroups. Most sequences were generated in the course of this work (35 *rps4* sequences were taken from GenBank); see Appendix for voucher information and GenBank accession numbers. The sequence for *Plicanthus* sp. (taxon 59 in the Appendix) is labelled as *Chandonanthus* sp. in GenBank. However, it most probably belongs to *Plicanthus*, since “true” *Chandonanthus* occurs only in New Zealand. This specimen is from China and therefore almost certainly represents the segregate genus *Plicanthus* (Schuster, 2002b), either *P. hirtellus* or *P. birmensis*.

Total genomic DNA was extracted from herbarium specimens by the method of Edwards & al. (1991). Two

chloroplast regions were sampled, the protein coding *rps4* (Nadot & al., 1994) and the tRNA-Gly (*trnG*) gene intron (Pacak & Szweykowska-Kulińska, 2003). Primers *rps5* and *trnaS* (Nadot & al., 1995) were used to amplify the *rps4* gene whilst primers A and B (Pacak & Szweykowska-Kulińska, 2003) were used to amplify the tRNA-Gly (*trnG*) gene intron. PCR amplification employed 30 cycles of one minute at 94°C (denaturing), one minute at 52°C (annealing), and two minutes at 72°C (extension), preceded by an initial melting step at 94°C and followed by a final extension period of seven minutes at 72°C. Fragments were cleaned with the GFX™ (Amersham Biosciences) PCR DNA and gel band purification kit. Amplification primers, used in conjunction with the ABI Prism™ Dye Terminator Cycle Sequence kit (Version 3.1), were also used as sequencing primers, and sequencing products were resolved on an ABI Prism 3100 genetic analyser.

Sequences were assembled and checked for inaccurate base calling using SeqMan II (Laser Gene System Software, DNASTar, Inc). Assembled sequences were aligned manually using MegAlign (Laser Gene System Software, DNASTar, Inc). The non-coding sequence at the 3' end of the amplified segment of *rps4* was excluded, and *trnG* intron positions that are difficult to align over the wide range of taxa sampled were also excluded from the current analysis.

Parsimony analysis. — Topologies were evaluated under the parsimony criterion using PAUP* 4.0b8a (Swofford, 1998). A heuristic search was conducted with 40,000 replicates of random taxon addition using TBR branch swapping; only one tree was saved for each replicate. All characters were given equal weight and states were unordered. All most parsimonious trees (MPTs) were saved, to a maximum of 10,000. Nodal support was evaluated by the jackknife as implemented in PAUP* 4.0b8a, using 1,000 resampling replicates. For each replicate 10 replicates of random taxon addition using TBR branch swapping were implemented, with a maximum of one tree saved at each replicate. Each replicate had 33.67% of characters deleted, and the “emulate Jac” resampling option was implemented.

Bayesian inference. — Bayesian phylogenetic analyses were conducted using MrBayes v3.0B4 (Huelsenbeck & Ronquist, 2003). We assume a uniform (uninformative) prior for the tree topology, branch lengths, and parameters of the substitution model. We feel this approach is justified given the lack of previous information on the group and the attendant taxonomic uncertainty.

A mixed-model approach (Huelsenbeck & Ronquist, 2003) was used for the substitution process. Hierarchical likelihood-ratio tests, implemented in Modeltest 3.6 (Posada & Crandall, 1998), indicated that each of the data partitions was best fit by the GTR+I+Γ model. This model incorporates separate time-reversible estimates of each

possible substitution type, an estimate of the proportion of sites fixed at invariance, and an estimate of the shape of the gamma distribution to which variable sites are fitted. Parameter values were estimated independently across the two partitions. We used two independent runs of the Metropolis-Coupled Markov Chain Monte Carlo (MCMCMC) approach for sampling parameter values in proportion to their posterior probability. Each analysis used four chains, three heated and one unheated, run for 5×10^6 generations. Model parameters, including trees, were sampled every 500 generations. Plots of the likelihoods of each sample were used to ascertain the number of generations for stationarity to be reached in order to obtain the posterior probability tree set. Trees from the burn-in were excluded.

RESULTS

Of the 647 *rps4* nucleotide positions included in the alignment, 452 (70%) exhibited variation and 389 (60%) were parsimony-informative across the range of taxa included in our analysis. Of the 828 included characters for the *trnG* intron, 492 (59%) exhibited variation and 388 (47%) were parsimony-informative. In the total DNA matrix there was, therefore, a total of 1,475 characters of which 944 (64%) were variable and 777 (53%) potentially parsimony-informative.

Under the parsimony criterion, 10,000 trees were retained ($L = 4,926$, $CI = 0.3218$, $RCI = 0.2242$). Not all relationships are well-supported (Fig. 1); areas of disagreement are found among accessions of the same species, within clades of closely related species and among some of the deeper nodes of the phylogeny. Within the “core” Lophoziaceae clade (Fig. 1b), a number of strongly supported clades of affiliated species are revealed, but relationships among these clades are for the most part poorly resolved. Overall, relationships are better resolved and more strongly supported under Bayesian inference (Fig. 2); the MCMCMC search required 400,000 and 750,000 generations respectively for each analysis to reach stationarity; and the combined 2,300 trees obtained during these burn-in periods were discarded. The first analysis had a 95% credible set containing 8,741 trees (of 9,201 trees), the second analysis had a 95% credible set containing 8,076 trees (of 8,501 trees). These formed a combined total of 16,817 sampling points for the posterior probability tree set. The median and 95% credible intervals of the model parameters are shown in Table 1.

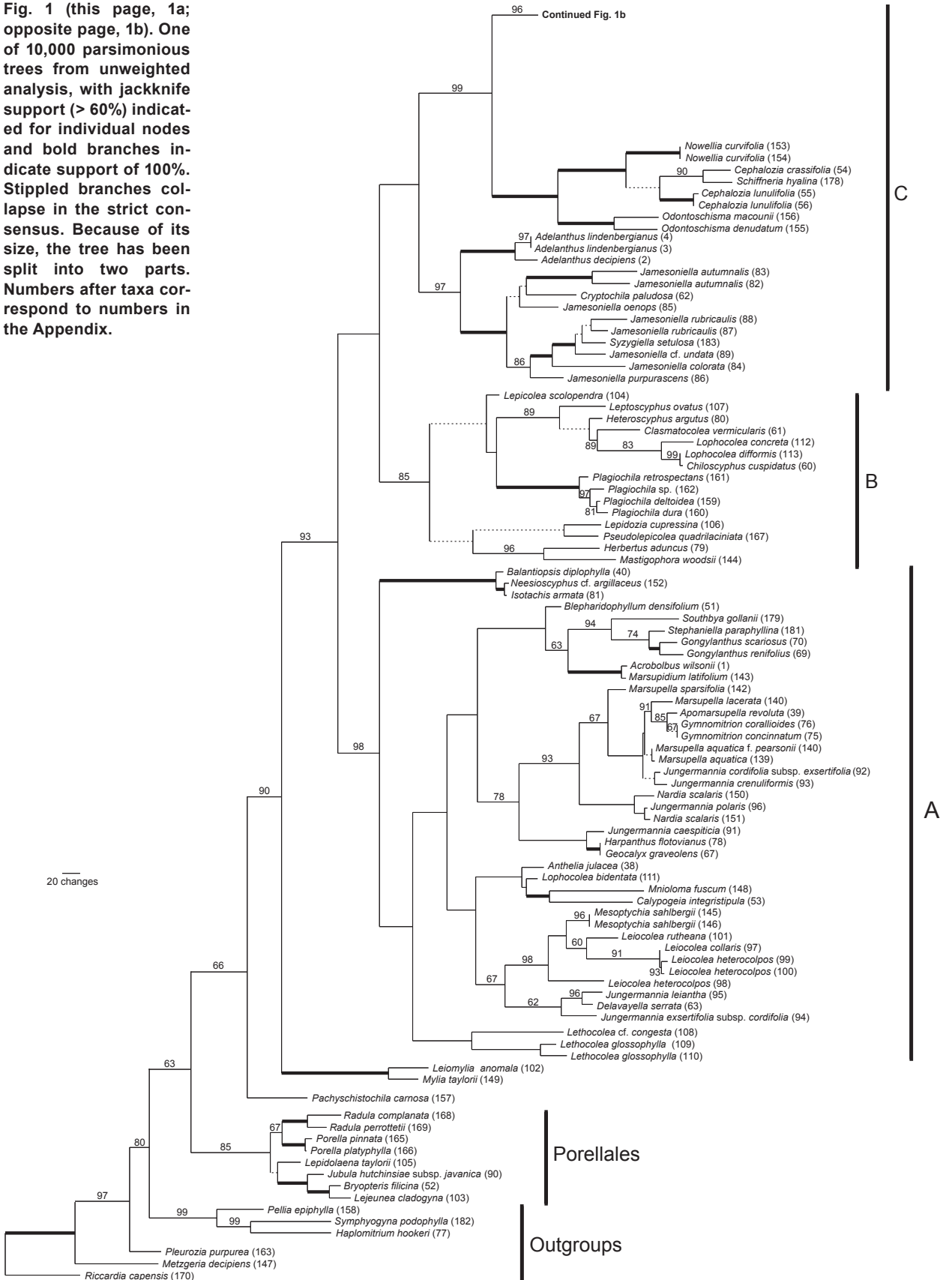
The Jungermanniidae form two clades that, together, are resolved as a poorly supported monophyletic group (jackknife percentage [JK] = 63, posterior probability [PP] = 66). The first clade (Porellales Clade; JK = 85, PP = 99) includes elements of Porellales, Radulales and Lepidolaenaceae (*Lepidolaena taylorii*). The sec-

ond clade (JK = 66, PP = 96) includes Schistochilaceae (*Pachyschistochila carnosus*) as sister to a large clade (JK = 90, PP = 100) of the remaining leafy taxa within which the majority of taxa fall into one of three well-supported, high-level clades. The first of these (Clade A; JK = 98, PP = 100) includes elements of Jungermanniaceae s.str., Gymnomitriaceae, Geocalycaceae (*Lophocolea bidentata*), Balantiopsaceae, Acrobolbaceae, Antheliaceae, Arnelliaceae, Scapaniaceae (*Blepharidophyllum* and *Delavayella*), Mesoptychiaceae, and one genus of Lophoziaceae (*Leiocolea*). It is noteworthy that *Stephaniella* is nested within a strongly supported (JK = 94, PP = 100) Arnelliaceae. The second (Clade B; JK = 85, PP = 90) includes Pseudolepicoleaceae, Plagiochilaceae, Geocalycaceae (*Clasmatocolea*, *Heteroscyphus*, *Lophocolea*, *Leptoscyphus*), Lepidoziaceae (*Lepidozia cupressina*), Lepicoleaceae (*Lepicolea scolopendra*), Herbertaceae (*Herbertus aduncus*) and Mastigophoraceae (*Mastigophora woodsii*). The final group (Clade C; unsupported in the parsimony tree; PP = 100) encompasses all Lophoziaceae except *Leiocolea*, a core group of Scapaniaceae, Cephaloziaceae, Adelanthaceae and Cephaloziellaceae. Ambiguous in its position with respect to Clades A, B and C is a well-supported group (JK = 100, PP = 100) made up of *Myliia taylorii* and the segregate genus *Leiomyliia anomala* (Jungermanniaceae). In the parsimony analysis this is placed sister to the rest whereas in the Bayesian analysis it is sister to Clade A alone (PP = 97). Relationships among clades A, B and C are ambiguous; in the parsimony analysis Clade A is sister to Clades B and C, but this relationship is unsupported by the jackknife and the Bayesian analysis.

Within Clade C, a strongly supported grouping (JK = 97, PP = 100) of *Adelanthus* (Adelanthaceae) sister to a strongly supported (JK = 100, PP = 100) Jamesonielloideae (*Jamesoniella*, *Cryptochila*, *Syzygiella*) is sister to a strongly supported group (JK = 99, PP = 100) comprising the rest of the taxa. The remaining Lophoziaceae, Scapaniaceae and *Cephaloziella* (Fig. 1b) form a well supported group (JK = 96, PP = 99) to which a strongly supported (JK = 100, PP = 100) clade of *Cephalozia*, *Nowellia*, *Schiffneria* and *Odontoschisma* is sister. Within the former group, two strongly-supported clades are resolved. The first (PP = 100) includes most elements of the Lophoziaceae (*Barbilophozia*, *Anastrophyllum*, *Anastrepta*, *Tetralophozia*, *Sphenolobopsis*, *Plicanthus*, *Lophozia*, *Gymnocolea*) and is hereafter denoted the *Anastrophyllum* clade, whilst the second (PP = 100), including Scapaniaceae (*Diplophyllum*, *Scapania*, *Douinia*), some Lophoziaceae (*Lophozia*, *Gymnocoleopsis*, *Gottschelia*, *Tritomaria*) and Cephaloziellaceae (*Cephaloziella*) is hereafter denoted the *Scapania* clade.

Within the *Anastrophyllum* clade a number of well-supported groupings emerge, but the relationships among

Fig. 1 (this page, 1a; opposite page, 1b). One of 10,000 parsimonious trees from unweighted analysis, with jackknife support (> 60%) indicated for individual nodes and bold branches indicate support of 100%. Stippled branches collapse in the strict consensus. Because of its size, the tree has been split into two parts. Numbers after taxa correspond to numbers in the Appendix.





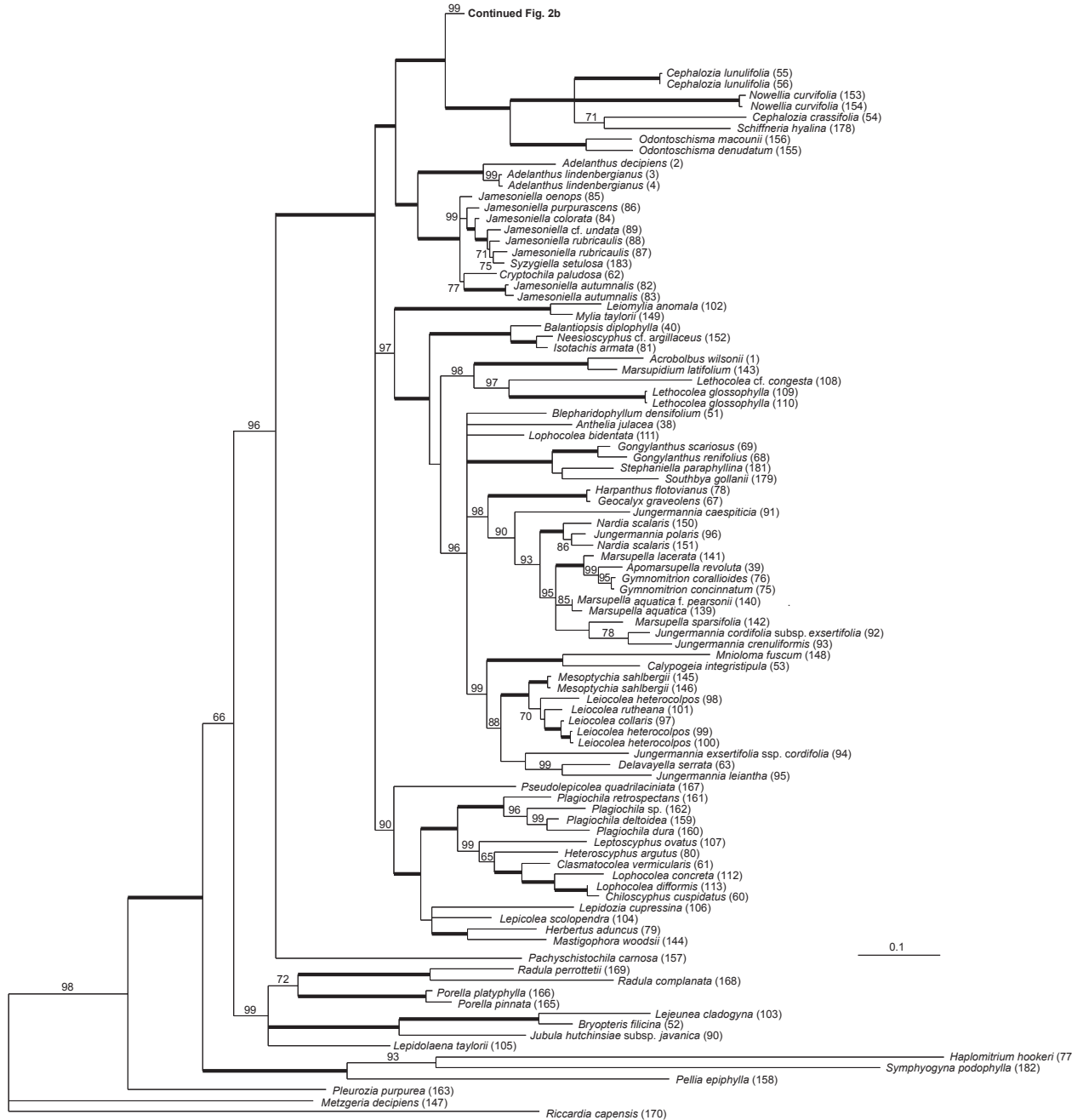
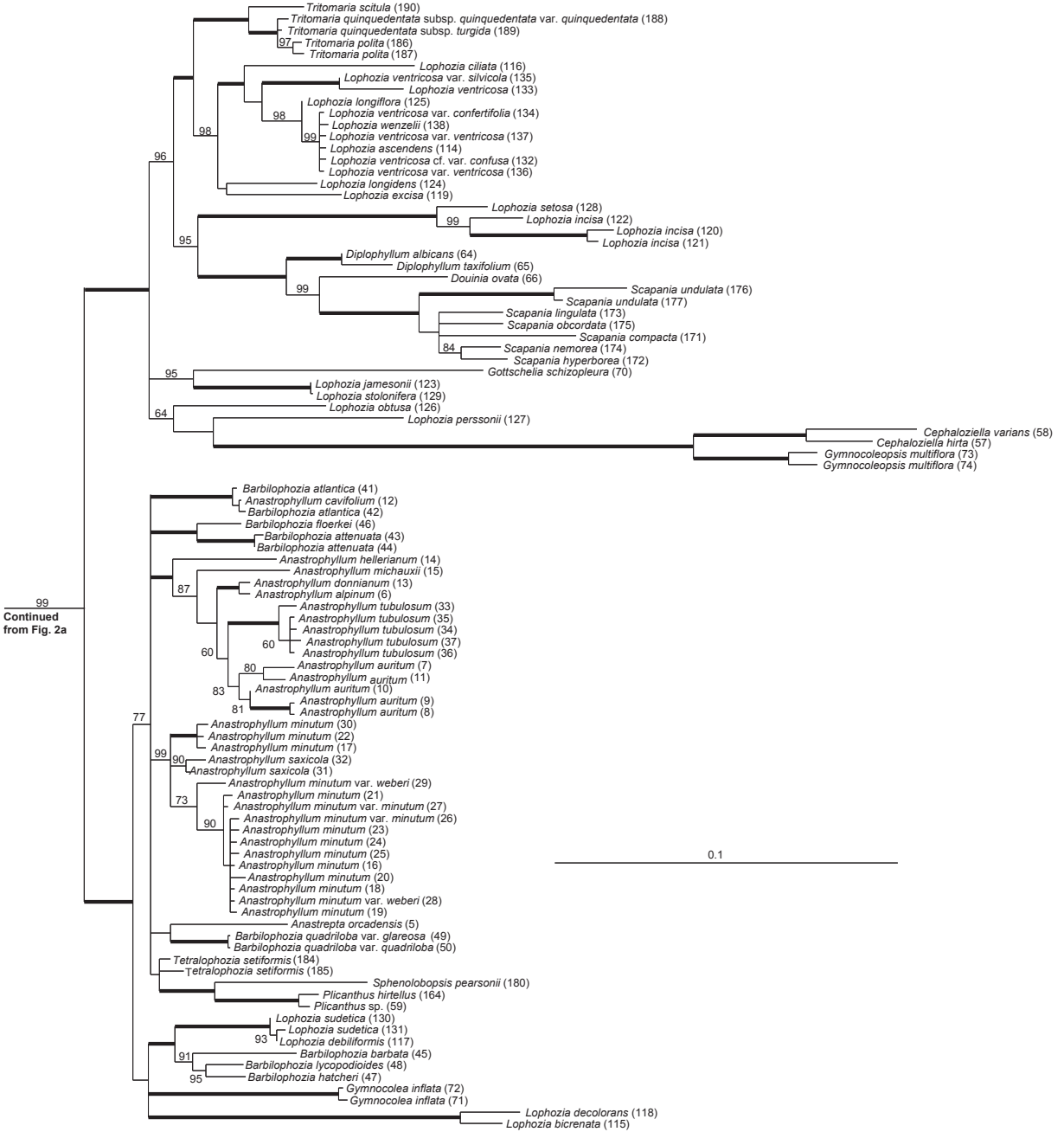


Fig. 2 (this page, 2a; opposite page, 2b). A majority-rule consensus of the trees (excluding burn-in) from the Bayesian analysis. The posterior probabilities from the combined 95% credible tree set are indicated for individual nodes and bold branches indicate probabilities of 100%. Again, the tree has been split into two parts. Numbers after taxa correspond to numbers in the Appendix.

these are not resolved. One of these is a strongly supported group (JK = 100, PP = 100) with the mono-generic *Sphenobolopsis pearsonii* sister to *Plicanthus*. *Barbilophozia hatcheri*, *B. barbata* and *B. lycopodioides* and a strongly supported group (JK = 100, PP = 100) of *Lophozia sudetica* and *L. debiliformis* together form a well-supported group (JK = 79, PP = 100).

In the *Scapania* clade, again a number of well-supported groupings emerge. *Gymnocoleopsis multiflora* is strongly supported (JK = 99, PP = 100) as sister to a strongly supported group (JK = 100, PP = 100) comprising Cephaloziales (*Cephalozia*). The Scapaniaceae (*Diplophyllum*, *Douinia*, *Scapania*) are strongly supported (JK = 100, PP = 100) as monophyletic. Another well sup-



ported group (JK = 76, PP = 100) includes a strongly supported (JK = 100, PP = 100) grouping of sampled *Tritomaria* taxa placed sister to a well supported group (JK = 65, PP = 98) of *Lophozia* taxa including *L. ciliata*, *L. longiflora*, *L. wenzelii*, *L. ascendens*, *L. longidens*, *L. excisa* and the *L. ventricosa* species complex. *Lophozia incisa* and *L. setosa* form a strongly supported group (JK = 100, PP = 100).

With respect to the taxa of interest to this study, a few differences emerge in comparisons between the Bayesian and Parsimony results. For example the *Barbilophozia*

hatcheri-Lophozia sudetica grouping falls within the *Anastrophyllum* clade in Bayesian analysis, but is placed as sister to the other Lophoziaceae, Scapaniaceae and *Cephaloziella* in the Parsimony tree. Similarly, the *Lophozia incisa* group is found supported (PP = 95) as sister to the Scapaniaceae in the Bayesian analysis, whereas it is found sister to the group of *Gottschelia schizopleura*, *Lophozia jamesonii* and *L. stolonifera* in the parsimony tree. Within Clade A, *Leiocolea* spp. and *Mesoptychia sahlbergii* form a strongly supported group (JK = 98, PP = 100). However while *Leiocolea* itself is resolved as monophyletic, albeit

without support, in the Bayesian analysis, *Mesoptychia* is nested within this genus under the Parsimony criterion. None of these are strong conflicts in the sense that where the two differ the parsimony results are usually poorly supported.

DISCUSSION

trnG and *rps4* provide considerable resolution of phylogenetic relationships in the leafy liverworts. In almost all instances the posterior probabilities recovered were higher than the jackknife levels of support; this can be explained through (1) differences in the concepts of support implicit in the different statistical methods and (2) better performance of an explicit modelling approach. Overall the Bayesian analyses yield better resolution, especially in the core *Lophozia* clade. There is no strong conflict between the two sets of results, and where the two differ the parsimony results are usually poorly supported. Therefore much of the discussion centres on the Bayesian results.

The relationships recovered here correspond well to results of previous studies. The Porellales-Radulales Clade identified here corresponds to the Leafy I clade of Davis (2004), the Porellales-Lepidolaenineae clade of He-Nygrén & al. (2004), the Porellales-Radulales clade (Ahonen, 2004) and the Porellales clade of Heinrichs & al. (2005). The second clade, comprising the remaining

leafy liverworts, corresponds to the Leafy II clade of Davis (2004), the Perssoniellineae-Herbertineae clade of He-Nygrén & al. (2004) and the Jungermanniales clade of Heinrichs & al. (2005).

Mylia forms a deep branch in both Bayesian and parsimony analyses, being either sister to all remaining leafy liverworts or to Clade A. This supports the recent resurrection by Engel & Braggins (2005) of the family Myliaceae and suborder Myliinae to accommodate the genus (see also Hentschel & al., 2006). Their argument was based on the occurrence in *M. taylorii* of unique cell wall characters, namely the presence of perforations over the middle lamella, and the leaf surface having irregular plates or strips of smooth wall material separated by fibril-filled grids. In the same study, these authors erected the genus *Leiomylia*, retained in Jungermanniaceae, to accommodate *M. anomala*, which lacks these cell wall features. However, in our analyses the two *Mylia* species form a very strongly supported grouping, and we would argue that separate generic status is unwarranted. *Trabacellula tumidula* Fulford, usually allocated to the Cephaloziaceae, was also found to possess these cell wall characters and was placed back by Engel and Braggins in its own family Trabacellulaceae, in the new suborder Myliinae (Engel & Braggins, 2005). Inclusion of this taxon in molecular analyses would shed further light on the evolution of what appears to be a unique set of cell wall characteristics and allow determination of whether the absence of these in *Mylia anomala* represents a secondary reversal or the plesiomorphic condition.

The remaining Jungermanniaceae s.l. (sensu Crandall-Stotler & Stotler, 2000; and others) are clearly polyphyletic. Of the Jungermanniaceae s.str. included in this study, all, with the exception of *Mylia*, are found nested within Clade A. However, even in the strict sense the family is not monophyletic with the bulk of the sampled Gymnomitriaceae (ex. *Stephaniella*) located in the same clade with *Nardia* and some of the *Jungermannia* spp. *Jungermannia* itself is also polyphyletic, with *J. polaris* (nested within *Nardia*), *J. caespiticia*, *J. cordifolia* and *J. crenuliformis* placed in a clade containing Gymnomitriaceae and Geocalycaceae whilst *J. exsertifolia* and *J. leiantha* are in a group with *Leiocolea*, Mesoptychiaceae, *Delavayella* and Calypogeiaceae. *Stephaniella*, a genus hitherto placed in Gymnomitriaceae or in a family of its own, is found nested as part of Arnelliaceae. Schuster (2002a) comments that oil-body criteria suggest that *Stephaniella* does not belong in the Gymnomitriaceae. The genus is quite unique in its nearly chlorophyll-free leaves and elaborated paraphyllia (Schuster, 2002a). The placement of *Lophocolea bidentata* is odd, and may be based on a misidentification or contamination. The sequence was retrieved from GenBank and verification of identity is beyond the scope of this study.

Table 1. Parameter values for the first Bayesian analysis.

Parameter	95% Cred. Interval		Median
	Lower	Upper	
TL {all}	11.969000	15.319000	13.335000
r(G ⇌ T) {trnG}	1.000000	1.000000	1.000000
r(C ⇌ T) {trnG}	2.432825	4.556134	3.337974
r(C ⇌ G) {trnG}	0.675148	1.515353	1.012487
r(A ⇌ T) {trnG}	0.235286	0.465749	0.329869
r(A ⇌ G) {trnG}	3.039681	4.716709	3.766826
r(A ⇌ C) {trnG}	0.619415	1.256443	0.886786
r(G ⇌ T) {rps4}	1.000000	1.000000	1.000000
r(C ⇌ T) {rps4}	6.260180	10.475860	7.985856
r(C ⇌ G) {rps4}	1.309878	2.604897	1.836572
r(A ⇌ T) {rps4}	0.205861	0.410085	0.295015
r(A ⇌ G) {rps4}	7.460351	11.572561	9.248623
r(A ⇌ C) {rps4}	1.624953	2.895411	2.167063
pi(A) {trnG}	0.356964	0.409225	0.383303
pi(C) {trnG}	0.126317	0.162729	0.143550
pi(G) {trnG}	0.094024	0.125552	0.108344
pi(T) {trnG}	0.339705	0.391168	0.363824
pi(A) {rps4}	0.326683	0.381278	0.353291
pi(C) {rps4}	0.124268	0.156549	0.140382
pi(G) {rps4}	0.164841	0.199547	0.181862
pi(T) {rps4}	0.298162	0.350048	0.324145
alpha {trnG}	0.534336	0.694894	0.611168
alpha {rps4}	0.489224	0.587253	0.535510
m {trnG}	1.142932	1.336475	1.240365
m {rps4}	0.569395	0.817082	0.692392

The majority of sampled Lophoziaceae (s.str.) fall into a well-supported group that is not particularly closely related to Jungermanniaceae. However, *Leiocolea*, usually classified as Lophoziaceae, does not belong to this clade but is nested (with *Mesoptychia*) in a mixed group of Jungermanniaceae, *Delavayella* (Scapaniaceae) and Calypogeiaceae. Schuster (1969) refers to *Leiocolea* as a closely allied group of species, and suggested that the retention of the taxon as a part of *Lophozia* s.l. results in a “sharply circumscribed and very isolated sub-generic group in *Lophozia*.” However, he retained *Leiocolea* as a part of *Lophozia* s.l. because characters used by others to segregate the genus recur sporadically in taxa of *Lophozia*. Schuster (1969) also comments that the perianth of *Leiocolea* is extremely characteristic, being tubular and terete and quickly constricted near the apex into a small narrow beak. Supporting the position of *Mesoptychia* as sister to *Leiocolea*, the immature perianth of *Mesoptychia* is described as beaked, and there is a strong resemblance in leaf form between *L. rutheana* and *Mesoptychia* with both showing a very oblique line of leaf insertion (Schuster, 1969). Our results (cf. also Yatsenyuk & al., 2004) therefore suggest the inclusion of *Leiocolea* in Mesoptychiaceae.

The subfamilies Jamesonielloideae and Adelantheaceae form a group, with the sampled Jamesonielloideae monophyletic. Inoue (1966) placed the subfamily in Lophoziaceae, whereas Schuster (2002a) regards it as showing both Lophozioideae and Jungermannioideae characters, thus placing all three groups in an expanded Jungermanniaceae. Whilst He-Nyngren & al. (2006) recently elevated Jamesonielloideae to the rank of family, (Jamesoniellaceae), an alternative is to transfer the subfamily to Adelantheaceae, as suggested by Hentschel & al. (2006). Morphologically, the Adelantheaceae have geotropic rhizoidous axes which are sometimes present in Jamesonielloideae, both have ventral to lateral intercalary and, when present, *Frullania*-type terminal branching, both share reduced/ephemeral (absent in some Adelantheaceae) underleaves and both lack a perigynium (Schuster, 2002a).

Jamesoniella itself is not monophyletic because *Syzygiella* and *Cryptochila* are nested within it. Schuster (2002a) regards *Syzygiella* as belonging to a subfamily of Plagiochilaceae because of perfectly opposite leaves (Jamesonielloideae have alternate leaves). Inoue (1966) saw *Syzygiella* as being strongly related to Lophoziaceae by having pluriplicate perianths with the mouth contracted, well-developed bracts and bracteoles that are united at least at the base, a distinct tendency toward reddish or purple pigmentation, and distinct mycorrhizae among the cells of the stem. He placed *Syzygiella* as part of Jamesonielloideae because of small, vestigial (or often totally absent) underleaves, perianths having plicae in the upper half, postical-intercalary branching and a

total absence of vegetative propagulae. Based on *rbcL* sequences, Groth & Heinrichs (2005) also concluded that relationships of *Syzygiella* are with the Lophoziaceae rather than Plagiochilaceae. Schuster (2002a) notes that criteria used to distinguish *Cryptochila* subg. *Acinaria* Grolle (including *C. paludosa*), are all features that recur in *Jamesoniella* and that *Acinaria* could be assigned to *Jamesoniella*. It is also clear that *Jamesoniella colorata* as defined by Grolle (1971) includes more than one species since *J. oenops* (synonymised with *J. colorata* by Grolle, 1971) is well separated from *J. colorata*.

Cephaloziaceae is a large family; with only 4 of 15 genera sampled (Crandall-Stotler & Stotler, 2000). The sampled species form a strongly supported clade sister to the main clade containing Lophoziaceae, Scapaniaceae and the sampled Cephaloziellaceae taxa. The genus *Cephalozia* itself appears paraphyletic since *Schiffneria hyalina* is sister to *Cephalozia crassifolia*.

The majority of Scapaniaceae are nested within Lophoziaceae as reported previously (Schill & al., 2004; Yatsenyuk & al., 2004; Heinrichs & al., 2005; He-Nyngren & al., 2006), but *Blepharidophyllum* and *Delavayella* do not belong with the rest of the family. Schuster (1961, 1974, 1984, 1999) has already noted that these are not members of Scapaniaceae (for *Delavayella* see also Schill & al., 2004) remarking that a whole ensemble of differences, including rhizoid dispersion, type of asexual reproduction, leaf symmetry, leaf lobing and shoot apex orientation, separate Scapaniaceae, *Blepharidophyllum* and *Delavayella*, and that these three should go into autonomous families, placing the latter two in Blepharidophyllaceae and Delavayellaceae respectively (Schuster, 1999). Schuster (1974) proposed that Scapaniaceae s.str. is closely related to the “less derivative” Lophoziaceae, and noted that most taxa of the two families share the following features: (1) bilobed leaves with the dorsal lobe tending to be smaller than the ventral, (2) succubous ventral and almost transverse dorsal insertion of the leaves, (3) normally exclusively lateral branching, (4) gemmae produced freely in branched fascicles and of similar form, (5) terminal perianths, (6) androecia relatively unmodified and intercalary, (7) paraphyllia often found with the male bracts, and (8) a multistratose capsule wall.

The main elements of Lophoziaceae fall into two main clades. There is strong support for a clade including mainly *Anastrophyllum* and *Barbilophozia* (the “*Anastrophyllum*” clade). Also included in this clade are *Tetralophozia setiformis*, *Plicanthus*, *Sphenolobopsis pearsonii*, *Anastrepta orcadensis*, *Gymnocolea inflata* and a few *Lophozia* species (*L. bicrenata*, *L. decolorans*, *L. sudetica*, *L. debiliformis*). This group needs more sampling but it is clear that none of the sampled genera with more than one representative is monophyletic. *Anastrophyllum minutum* and *A. saxicola* form a strongly supported clade which

corresponds to the previously recognised genus *Sphenolobus*. The remaining *Anastrophyllum* species except *A. cavifolium* form a separate clade to which this genus name can be applied. *A. cavifolium* seems to be a form of *Barbilophozia*, possibly conspecific with *B. atlantica*. With the present sampling the monophyly of the genus *Anastrophyllum* cannot be rejected. It is also notable that *A. minutum* specimens form two strongly supported clades that form a trichotomy with *A. saxicola*. Three specimens of *A. minutum* from the Southern Hemisphere (Venezuela and South Africa) and one from Norway form a group that is clearly separated from the rest. The species may therefore not be monophyletic and possibly cryptic speciation occurs in this widespread taxon. Alternatively the apparent paraphyly could be due to gene-level coalescent processes. This interesting species complex requires a wider and more detailed study to explore these possibilities.

Barbilophozia s.str. is monophyletic while the group of taxa sometimes placed in the genus or subgenus *Orthocaulis* (Buch) Schust. is polyphyletic. *Lophozia sudetica* (incl. *L. debiliformis*) is sister to *Barbilophozia* and may be transferred to the latter. The two sampled representatives of subg. *Isopaches*, *Lophozia decolorans* and *L. bicrenata*, form a well-supported group. The two remaining members of this subgenus, *L. albovididis* R.M. Schust. and *L. pumicicola* Berggr. have not been sampled, but our results indicate that *Isopaches* should be recognised at generic level.

The second clade of “Lophoziaceae” (the “*Scapania*” clade) is strongly supported but also needs more sampling. Included in this clade are also *Gottschelia*, *Gymnocoleopsis* and *Tritomaria*. Relationships in general are better resolved in this clade than in the “*Anastrophyllum*” clade, but the “backbone” relationships of this group are still unclear.

Our results support the inclusion of Lophoziaceae by Heinrichs & al. (2005) and perhaps also Cephaloziellaceae (type species not sampled) in Scapaniaceae unless many small families are erected. Affinities of *Cephaloziella* have sometimes been assumed to be with the Lophozioidea as Douin’s genus name “*Lophoziella*” testifies (Schuster, 1971, 2002a). Schuster (1971) notes that reduced members of Cephaloziellaceae and of the Lophoziaceae both may have very reduced setae, and that additional plicae occur in the perianth of most *Cephaloziella* and *Cylindrocolea* species, lending the impression that this group is allied to the Lophoziaceae.

Cephaloziella varians and *C. hirta* (Cephaloziellaceae) form a clade sister to *Gymnocoleopsis*. Taxa currently in *Gymnocoleopsis* have previously been assigned to both *Gymnocolea* and *Lophozia* (Schuster, 2002a). However, *Gymnocoleopsis* is not closely associated with *Gymnocolea*, which is found in the “*Anastrophyllum*”

clade. The two differ mainly in that *Gymnocoleopsis* is autoecious, while *Gymnocolea* is dioecious (Schuster, 2002a). *Gymnocoleopsis* also differs from *Lophozia* in the seta being consistently 8 + 4 seriate, a condition also found in some Cephaloziellaceae (Schuster, 1971), the branches are uniformly lateral-intercalary and the capsule walls are bistratose, also the case for most Cephaloziellaceae (Schuster, 1971), with both strata almost equal in height (Schuster, 2002a).

Another strongly supported branch is the Scapaniaceae when *Delavayella* and *Blepharidophyllum* are excluded. The sampled *Scapania* species are monophyletic and sister to *Douinia* and a monophyletic *Diplophyllum*. Clearly, the suggestion by Potemkin (1999) to segregate *Diplophyllum* and *Douinia* (into Diplophyllaceae) as “a group of different origins resulting in a different morphology” is not supported.

The strong support for *Tritomaria* and its sister relationship with a clade comprising species from *Lophozia* (mainly subg. *Lophozia*) is supported morphologically. In both groups, ventral sectors of the stem medulla becoming brown and strongly mycorrhizal, ventral merophytes are narrow and consequently unable to produce underleaves, the gynoeical bracteole is lacking (or reduced), perianths are plicate, and branching is of the *Frullania* and *Radula*-type as well as lateral-intercalary (Schuster, 2002a). *Tritomaria* itself has morphological support by the possession of trilobed leaves, and by having the cuticle of the leaves and stem almost always verruculose to finely ridged (Schuster, 1969).

Lophozia s.l. as delimited by most recent authors is polyphyletic and several of the previously segregated genera should be re-instated. Thus, for example, one of the strongly supported clades includes species sometimes placed in *Schistochilopsis* (*L. setosa* and *L. incisa*) and that genus should probably be recognised although the type species, *S. cornuta* (Steph.) Konstantinova, has not been sampled. It is worth noting that *Lophozia ventricosa* var. *silvicola* is well separated from var. *ventricosa* and should be re-instated as a species. On the other hand, *L. jamesonii* and *L. stolonifera* are suggested by J. Vána (pers. comm.) as possibly conspecific; Schuster (2002a) notes that except for *L. jamesonii* being gemma-free, the two are virtually identical and our results support such a treatment. The generic position of the species is unclear as is the position of *L. perssonii* and *L. obtusa*. The recognition of *Obtusifolium* S. Arn. for the last species could be advocated, but as the clades around it are poorly resolved, more sampling of *Lophozia* taxa is needed.

These suggestions, as well as many relating to higher-level (i.e., supra-familial) relationships within the Jungermanniales as a whole, need to be addressed in the interests of a stable classification that truly reflects common ancestry. However, alterations need to be made in the

context of much wider species sampling. Furthermore, the addition of molecular and anatomical characters would contribute greatly to better understanding the evolution of the diversity in these groups. In addition, hypotheses advanced here need to be tested by data from additional DNA regions. This study has utilised plastid loci; however the plastid genome is inherited as a unit and usually uniparentally, so there is a danger that our current understanding of phylogeny in reality encompasses only the chloroplast history. It is important therefore that future studies include information from morphology, anatomy and other genomes, especially nuclear loci, to facilitate a better understanding of liverwort evolutionary history.

CONCLUSIONS

The current classification of leafy hepatics is highly inconsistent with phylogeny as revealed by chloroplast markers. We highlight the following conclusions from our analyses:

- (1) Lophoziaceae, and perhaps also Cephaloziellaceae, in Scapaniaceae unless many smaller families are recognised. They are not closely related to Jungermanniaceae.
- (2) *Delavayella* and *Blepharidophyllum* should be excluded from Scapaniaceae.
- (3) Jamesonielloideae is a family of its own (Jamesoniellaceae) sister to Adelanthaceae, or should be included in Adelanthaceae.
- (4) The genus *Anastrophyllum* should be split into *Anastrophyllum* and *Sphenolobus*.
- (5) *Lophozia* is polyphyletic and the genera *Isopachis* and *Schistochilopsis*, and perhaps *Obtusifolium*, should be recognised while *L. sudetica* could be transferred to *Barbilophozia*. However, the generic position of many *Lophozia* species is still unclear.
- (6) *Barbilophozia* s.str. is monophyletic, while *Orthocaulis* is polyphyletic with the four sampled species appearing in 3 different clades. However, their relationships to other taxa are poorly resolved.
- (7) *Lophozia silvicola* Buch is clearly separated from *L. ventricosa* at species level.
- (8) *Jamesoniella oenops* is clearly separated from *J. colorata* at species level.
- (9) The generic status of *Leiomylia* is unwarranted.

ACKNOWLEDGEMENTS

We are grateful to D.G. Long (Edinburgh), J. Váňa (Prague), K. Hassel (Trondheim), M. Ignatov (Moscow) and H. Weibull (Fjärdhundra) for sending us material for this study. J. Váňa and D.G. Long also identified some of our specimens. Financial

support came from the Research Council of Norway and the South African National Research Foundation through grants to L. Söderström & T. Hedderson.

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Appendix. Taxa and DNA regions used for the study. All specimens are in BOL except where stated. Further information on the specimens can be obtained from the authors. Sequences directly retrieved from GenBank are in italics.

Acrobolbus wilsonii Nees, UNITED KINGDOM 2001, *D. Long & Rothero* 29767 (E), AM398297 (*rps4*); *Adelanthus decipiens* (Hook.) Mitt., SOUTH AFRICA 2004, *L. Söderström* 2004/177, AM398307 (*rps4*); *A. lindenbergianus* (Lehm.) Mitt., 1, ARGENTINA 2003, *D. Long* 31828 (E), AM397738 (*trnG*), AM398292 (*rps4*); 2, *AY608042* (*rps4*); *Anastrepta orcadensis* (Hook.) Schiffn., NORWAY 2003, *L. Söderström* 2003/017, AM397771 (*trnG*), AM398339 (*rps4*); *Anastrophyllum alpinum* Steph., NEPAL 2001, *D. Long* 30460 (E), AM397754 (*trnG*), AM398320 (*rps4*); *A. auritum* (Lehm.) Steph., 1, VENEZUELA 2004, *L. Söderström & al.* 2004/028, AM398240 (*rps4*); 2, VENEZUELA 2004, *L. Söderström & al.* 2004/029, AM397703 (*trnG*), AM398243 (*rps4*); 3, VENEZUELA 2004, *L. Söderström & al.* 2004/065, AM397699 (*trnG*), AM398238 (*rps4*); 4, VENEZUELA 2004, *L. Söderström & al.* 2004/111, AM397714 (*trnG*), AM398255 (*rps4*); 5, VENEZUELA 2004, *L. Söderström & al.* 2004/110, AM397701 (*trnG*), AM398241 (*rps4*); *A. cavifolium* (H. Buch & S.W. Arnell) Lammes, NORWAY 2004, *A. Séneca & L. Söderström* 2004/233, AM397742 (*trnG*), AM398301 (*rps4*); *A. donnianum* (Hook.) Steph., UNITED KINGDOM 2000, *D. Long* 30045 (E), AM398319 (*rps4*); *A. hellerianum* (Nees ex Lindenb.) R.M. Schust., SWEDEN 2003, *L. Söderström & P. Manyanga* 2003/081, AM397798 (*trnG*), AM398364 (*rps4*); *A. michauxii* (F. Weber) H. Buch, *AY507433* (*rps4*); *A. minutum* (Schreb.) R.M. Schust., 1, NORWAY 2004, *A. Séneca & L. Söderström* 2004/224, AM397762 (*trnG*), AM398330 (*rps4*); 2, NORWAY 2004, *L. Söderström & al.* 2004/217, AM398326 (*rps4*); 3, NORWAY 2004, *L. Söderström & al.* 2004/271, AM397764 (*trnG*), AM398331 (*rps4*); 4, NORWAY 2004, *L. Söderström & al.* 2004/316, AM397774 (*trnG*); 5, NORWAY 2004, *L. Söderström & al.* 2004/444, AM397763 (*trnG*); 6, NORWAY 2004, *L. Söderström & al.* 2004/205, AM398284 (*rps4*); 7, SOUTH AFRICA 2003, *T. Hedderson* 15437, AM397723 (*trnG*), AM398268 (*rps4*); 8, SPAIN 2004, *M. Infante & al. s.n.*, AM398290 (*rps4*); 9, SVALBARD 2004, *L. Söderström & al.* 2004/327, AM397761 (*trnG*), AM398327 (*rps4*); 10, SVALBARD 2004, *L. Söderström & al.* 2004/387, AM398329 (*rps4*); 11, VENEZUELA 2004, *L. Söderström & al.* 2004/135, AM397712 (*trnG*), AM398253 (*rps4*); *A. minutum* (Schreb.) R.M. Schust. var. *minutum*, 1, NORWAY 2004, *L. Söderström & al.* 2004/202, AM398278 (*rps4*); 2, SWEDEN 2003, *L. Söderström & P. Manyanga* 2003/054, AM397780 (*trnG*), AM398348 (*rps4*); *A. minutum* (Schreb.) R.M. Schust. var. *weberi* (Mart.) Kartt., 1, NORWAY 2003, *L. Söderström* 2003/013, AM397765 (*trnG*), AM398332 (*rps4*); 2, NORWAY 2004, *L. Söderström & al.* 2004/204, AM398279

Appendix. Continued.

(*rps4*); *A. saxicola* (Schrad.) R.M. Schust., 1, FINLAND 2003, *L. Söderström & P. Manyanga 2003/099*, AM397794 (*trnG*), AM398360 (*rps4*); 2, NORWAY 2004, *L. Söderström & al. 2004/196*, AM398285 (*rps4*); *A. tubulosum* (Nees) Grolle, 1, VENEZUELA 2004, *L. Söderström & al. 2004/030*, AM397697 (*trnG*), AM398237 (*rps4*); 2, VENEZUELA 2004, *L. Söderström & al. 2004/064*, AM397713 (*trnG*), AM398254 (*rps4*); 3, VENEZUELA 2004, *L. Söderström & al. 2004/066*, AM397702 (*trnG*), AM398242 (*rps4*); 4, VENEZUELA 2004, *L. Söderström & al. 2004/078*, AM398257 (*rps4*); 5, VENEZUELA 2004, *L. Söderström & al. 2004/120*, AM397720 (*trnG*), AM398266 (*rps4*); *Anthelia julacea* (L.) Dumort., *AY608044* (*rps4*); *Apomarsupella revoluta* (Nees) R.M. Schust., ICELAND 2004, *A. Séneca & L. Söderström 2004/464*, AM397759 (*trnG*); *Balantiopsis diplophylla* (Hook. f. & Taylor) Mitt., *AY608047* (*rps4*); *Barbilophozia atlantica* (Kaal.) Müll.Frib., 1, NORWAY 2003, *L. Söderström 2003/052*, AM397781 (*trnG*), AM398349 (*rps4*); 2, SWEDEN 2003, *L. Söderström & P. Manyanga 2003/057*, AM397782 (*trnG*), AM398350 (*rps4*); *B. attenuata* (Mart.) Loeske, 1, NORWAY 2003, *L. Söderström 2003/020*, AM397777 (*trnG*), AM398344 (*rps4*); 2, NORWAY 2004, *L. Söderström & al. 2004/208*, AM398282 (*rps4*); *B. barbata* (Schmidel ex Schreb.) Loeske, SWEDEN 1990, *T. Hedderston 8856*, AM398313 (*rps4*); *B. floerkei* (F. Weber & D. Mohr) Loeske, ICELAND 2004, *A. Séneca & L. Söderström 2004/457*, AM397753 (*trnG*), AM398318 (*rps4*); *B. hatcheri* (A. Evans) Loeske, NORWAY 2003, *L. Söderström 2003/001*, AM397770 (*trnG*), AM398338 (*rps4*); *B. lycopodioides* (Wallr.) Loeske, NORWAY 2003, *L. Söderström 2003/019*, AM397766 (*trnG*), AM398333 (*rps4*); *B. quadriloba* (Lindb.) Loeske var. *glareosa* (Jørg.) Lammes, SVALBARD 2004, *L. Söderström & al. 2004/408*, AM397758 (*trnG*), AM398324 (*rps4*); *B. quadriloba* (Lindb.) Loeske var. *quadriloba*, SWEDEN 2003, *L. Söderström & P. Manyanga 2003/61*, AM397808 (*trnG*), AM398375 (*rps4*); *Blepharidophyllum densifolium* (Hook.) Ångstr., ARGENTINA 2003, *D. Long 31696*, AM398306 (*rps4*); *Bryopteris filicina* (Sw.) Nees, *AY608051* (*rps4*); *Calypogeia integristipula* Steph., FINLAND 2003, *L. Söderström & P. Manyanga 2003/090*, AM397795 (*trnG*), AM398361 (*rps4*); *Cephalozia crassifolia* (Lindenb. & Gottsche) Fulford, VENEZUELA 2004, *L. Söderström & al. 2004/060*, AM397746 (*trnG*), AM398309 (*rps4*); *C. lunulifolia* (Dumort.) Dumort., 1, SVALBARD 2004, *L. Söderström & al. 2004/415*, AM397748 (*trnG*), AM398311 (*rps4*); 2, SVALBARD 2004, *L. Söderström & al. 2004/424*, AM397750 (*trnG*), AM398315 (*rps4*); *Cephaloziella hirta* (Steph.) R.M. Schust., *AY608054* (*rps4*); *C. varians* (Gottsche) Steph., SVALBARD 2004, *L. Söderström & al. 2004/365*, AM397747 (*trnG*), AM398310 (*rps4*); *Chandonanthus (Plicanthus) sp.*; *AY462347* (*rps4*); *Chiloscyphus cuspidatus* (Nees) J.J. Engel & R.M. Schust., *AY462348* (*rps4*); *Clasmatocolea vermicularis* (Lehm.) Grolle, VENEZUELA 2004, *L. Söderström & al. 2004/116*, AM397706 (*trnG*), AM398246 (*rps4*); *Cryptochila paludosa* (Steph.) Grolle, SOUTH AFRICA 2003, *T. Hedderston 15333*, AM397729 (*trnG*), AM398275 (*rps4*); *Delavayella serrata* Steph., NEPAL 2001, *D. Long 30522*, AM398305 (*rps4*); *Diplophyllum albicans* (L.) Dumort., NORWAY 2004, *K. Hassel s.n.*, AM397726 (*trnG*), AM398272 (*rps4*); *D. taxifolium* (Wahlenb.) Dumort., FINLAND 2003, *L. Söderström & P. Manyanga 2003/098*, AM397788 (*trnG*), AM398354 (*rps4*); *Douinia ovata* (Dicks.) H. Buch, NORWAY 1996, *T. Hedderston 11792*, AM397786 (*trnG*), AM398353 (*rps4*); *Geocalyx graveolens* (Schrad.) Nees, SWEDEN 2003, *L. Söderström & P. Manyanga 2003/072*, AM398367 (*rps4*); *Gongylanthus renifolius* (Mitt.) Steph., SOUTH AFRICA 2003, *R. de Roo s.n.*, AM397717 (*trnG*), AM398261 (*rps4*); *G. scariosus* (Lehm.) Steph., SOUTH AFRICA 2003, *T. Hedderston 15409*, AM397711 (*trnG*), AM398252 (*rps4*); *Gottschelia schizopleura* (Spr.) Grolle, REUNION 2004, *T. Hedderston 15883*, AM397736 (*trnG*); *Gymnocolea inflata* (Huds.) Dumort., 1, NORWAY 2004, *K. Hassel s.n.*, AM397725 (*trnG*), AM398271 (*rps4*); 2, NORWAY 2004, *A. Séneca & L. Söderström 2004/223*, AM397755 (*trnG*), AM398321 (*rps4*); *Gymnocoleopsis multiflora* (Steph.) R.M. Schust., 1, VENEZUELA 2004, *L. Söderström & al. 2004/035*, AM397710 (*trnG*), AM398251 (*rps4*); 2, VENEZUELA 2004, *L. Söderström & al. 2004/091*, AM397700 (*trnG*), AM398239 (*rps4*); *Gymnomitrium concinnatum* (Lightf.) Corda, *AY608065* (*rps4*); *G. corallioides* Nees, NORWAY 2004, *L. Söderström & al. 2004/270*, AM397743 (*trnG*), AM398302 (*rps4*); *Haplomitrium hookeri* (Sm.) Nees, *AJ251064* (*rps4*); *Harpanthus flotovianus* (Nees) Nees, FINLAND 2004, *L. Söderström & P. Manyanga 2003/095*, AM397791 (*trnG*), AM398357 (*rps4*); *Herbertus aduncus* (Dicks.) Gray, IRELAND 2004, *D. Long 33458* (E), AM397737 (*trnG*), AM398291 (*rps4*); *Heteroscyphus argutus* (Reinw. & Nees) Schiffn., *AY462355* (*rps4*); *Isotachis armata* (Nees) Gottsche, *AY462358* (*rps4*); *Jameisoniella autumnalis* (DC.) Steph., 1, *AJ251066* (*rps4*); 2, SWEDEN 2003, *H. Weibull s.n.*, AM397730 (*trnG*), AM398276 (*rps4*); *J. undata* (Mont.) Steph., VENEZUELA 2004, *L. Söderström & al. 2004/123*, AM397708 (*trnG*), AM398249 (*rps4*); *J. colorata* (Lehm.) Spruce ex Schiffn., SOUTH AFRICA 2003, *R. de Roo s.n.*, AM397749 (*trnG*), AM398314 (*rps4*); *J. oenops* Lindenb. & Gottsche, SOUTH AFRICA 2003, *T. Hedderston s.n.*, AM398233 (*rps4*); *J. purpurascens* Steph., SOUTH AFRICA 2003, *P. Manyanga 89*, AM397693 (*trnG*), AM398232 (*rps4*); *J. rubricaulis* (Nees) Grolle, 1, VENEZUELA 2004, *L. Söderström & al. 2004/023*, AM397707 (*trnG*), AM398247 (*rps4*); 2, VENEZUELA 2004, *L. Söderström & al. 2004/093*, AM397709 (*trnG*), AM398250 (*rps4*); *Jubula hutchinsiae* ssp. *javanica* (Hook.) Dumort. (Steph.) Verd., *AY688794* (*rps4*); *Jungermannia caespiticia* Lindenb., NORWAY 2004, *L. Söderström & al. 2004/451*, AM398288 (*rps4*); *J. cordifolia* subsp. *exsertifolia* (Steph.) Amak., *AY608077* (*rps4*); *J. crenuliformis* Austin, *AY608078* (*rps4*); *J. exsertifolia* ssp. *cordifolia* (Dum.) Vaña, UNITED KINGDOM 1990, *T. Hedderston 8819*, AM397802 (*trnG*), AM398369 (*rps4*); *J. leiantha* Grolle, *AY507451* (*rps4*); *Jungermannia polaris* Lindb., SVALBARD 2004, *L. Söderström & al. 2004/403*, AM398308 (*rps4*); *Leiocolea collaris* (Nees) Schljakov, SWEDEN 2003, *L. Söderström 2003/064*, AM398377 (*rps4*); *L. heterocolpos* (Thed. ex Hartm.) H. Buch, 1, NORWAY 2003, *L. Söderström 2003/014*, AM397769 (*trnG*), AM398337 (*rps4*); 2, NORWAY 2003, *L. Söderström 2003/015*, AM397767 (*trnG*), AM398334 (*rps4*); 3, NORWAY 2003, *L. Söderström 2003/021*, AM397776 (*trnG*), AM398343 (*rps4*); *L. ruteana* (Limpr.) Müll.Frib., NORWAY 2003, *L. Söderström & P. Manyanga 2003/022*, AM397778 (*trnG*), AM398345 (*rps4*); *Leiomylia anomala* (Hook.) J.J. Engel & Braggins, NORWAY 2004, *K. Hassel s.n.*, AM398269 (*rps4*); *Lejeunea cladogyna* A. Evans *AY608079* (*rps4*); *Lepicolea scolopendra* (Hook.) Dumort. ex Trevis. *AY462365* (*rps4*); *Lepidolaena taylorii* (Gottsche) Trevis., *AY462368* (*rps4*); *Lepidozia cupressina* (Sw.) Lindenb., SOUTH AFRICA 2004, *R. de Roo s.n.*, AM397719 (*trnG*), AM398265 (*rps4*); *Leptoscyphus ovatus* (Spruce) Grolle, VENEZUELA 2004, *L. Söderström & al. 2004/127*, AM398248 (*rps4*); *Lethocolea* cf. *congesta* (Lehm.) S. Arnell, SOUTH AFRICA 2003, *T. Hedderston 15301*, AM397721 (*trnG*), AM398267 (*rps4*); *L. glossophylla* (Spruce) Grolle, 1, VENEZUELA 2004, *L. Söderström & al. 2004/047*, AM397704 (*trnG*), AM398244 (*rps4*); 2, VENEZUELA 2004, *L. Söderström & al. 2004/131*, AM397705 (*trnG*), AM398245 (*rps4*); *Lophocolea bidentata* (L.) Dumort., *AY608085* (*rps4*); *L. concreta* Mont., SOUTH AFRICA

Appendix. Continued.

2004, *R. de Roo s.n.*, AM398262 (*rps4*); *L. difformis* Nees, SOUTH AFRICA 2000, *T. Hedderson 13427*, AM397784 (*trnG*), AM398352 (*rps4*); *Lophozia ascendens* (Warnst.) R.M. Schust., SWEDEN 2003, *L. Söderström & P. Manyanga 2003/077*, AM397796 (*trnG*); *L. bicrenata* (Schmidel ex Hoffm.) Dumort., FINLAND 2003, *L. Söderström & P. Manyanga 2003/100*, AM397789 (*trnG*), AM398355 (*rps4*); *L. ciliata* Damsh. & al., SWEDEN 2003, *L. Söderström & P. Manyanga 2003/084*, AM397797 (*trnG*), AM398363 (*rps4*); *L. debiliformis* R.M. Schust. & Damsh., NORWAY 2003, *K. Hassel s.n.*, AM397806 (*trnG*), AM398373 (*rps4*); *L. decolorans* (Limpr.) Steph., INDIA [year unknown], *D. Long 22566* (E), AM398300 (*rps4*); *L. excisa* (Dicks.) Dumort., NORWAY 2003, *L. Söderström & P. Manyanga 2003/030*, AM397804 (*trnG*), AM398371 (*rps4*); *L. incisa* (Schrad.) Dumort., 1, NORWAY 1996, *T. Hedderson 11802*, AM397785 (*trnG*); 2, SWEDEN 2003, *L. Söderström & P. Manyanga 2003/079*, AM397800 (*trnG*), AM398366 (*rps4*); 3, VENEZUELA 2004, *L. Söderström & al. 2004/129*, AM397694 (*trnG*), AM398234 (*rps4*); *L. jamesonii* (Mont.) R.M. Schust., VENEZUELA 2004, *L. Söderström & al. 2004/043*, AM397696 (*trnG*), AM398236 (*rps4*); *L. longidens* (Lindb.) Macoun, NORWAY 2003, *L. Söderström 2003/016*, AM397772 (*trnG*), AM398340 (*rps4*); *L. longiflora* (Nees) Schiffn., SWEDEN 2003, *L. Söderström & P. Manyanga 2003/082*, AM397799 (*trnG*), AM398365 (*rps4*); *L. obtusa* (Lindb.) A. Evans, FINLAND 2003, *L. Söderström 2003/094*, AM397793 (*trnG*), AM398359 (*rps4*); *L. perssonii* H. Buch & S.W. Arn., NORWAY 2003, *L. Söderström & P. Manyanga 2003/036*, AM397807 (*trnG*), AM398374 (*rps4*); *L. setosa* (Mitt.) Steph., BHUTAN [year unknown], *D. Long 28644* (E), AM397752 (*trnG*), AM398317 (*rps4*); *L. stolonifera* R.M. Schust., VENEZUELA 2004, *L. Söderström & al. 2004/130*, AM397756 (*trnG*), AM398322 (*rps4*); *L. sudetica* (Nees ex Huebener) Grolle, 1, FINLAND 2003, *L. Söderström & P. Manyanga 2003/096*, AM397792 (*trnG*), AM398358 (*rps4*); 2, NORWAY 2003, *L. Söderström & P. Manyanga 2003/049*, AM397783 (*trnG*), AM398351 (*rps4*); *L. ventricosa* (Dicks.) Dumort., *AY462369* (*rps4*); *L. ventricosa* (Dicks.) Dumort. var. *confertifolia* (Schiffn.) Husn., FRANCE 2004, *J. Vaña s.n.*, AM397734 (*trnG*), AM398287 (*rps4*); *L. ventricosa* (Dicks.) Dumort. var. *confusa*, CANADA 1986, *T. Hedderson 5008*, AM397801 (*trnG*), AM398368 (*rps4*); *L. ventricosa* (Dicks.) Dumort. var. *silvicola* (H. Buch) E.W. Jones, NORWAY 2003, *L. Söderström 2003/018*, AM398336 (*rps4*); *L. ventricosa* (Dicks.) Dumort. var. *ventricosa*, 1, SWEDEN 2003, *L. Söderström & P. Manyanga 2003/048*, AM397805 (*trnG*), AM398372 (*rps4*); 2, SWEDEN 2003, *L. Söderström & P. Manyanga 2003/070*, AM397790 (*trnG*), AM398356 (*rps4*); *L. wenzelii* (Nees) Steph., NORWAY 2003, *L. Söderström & P. Manyanga 2003/024*, AM398346 (*rps4*); *Marsupella aquatica* (Lindenb.) Schiffn., *AY608087* (*rps4*); *M. aquatica f. pearsonii* (Schiffn.) Schljakov, NORWAY 2004, *L. Söderström & al. 2004/198*, AM398281 (*rps4*); *M. lacerata* (Steph.) Vaña, SOUTH AFRICA 2001, *T. Hedderson 13613*, AM397728 (*trnG*), AM398274 (*rps4*); *M. sparsifolia* (Lindb.) Dumort., SOUTH AFRICA 2003, *T. Hedderson 15338*, AM397722 (*trnG*); *Marsupidium latifolium* R.M. Schust., *AY608088* (*rps4*); *Mastigophora woodsii* (Hook.) Nees, UNITED KINGDOM [year unknown], *Rothero 11005* (E), AM397741 (*trnG*), AM398298 (*rps4*); *Mesopotychia sahlbergii* (Lindb.) A. Evans, 1, EAST SIBERIA 2004, *Ignatov s.n.*, AM398323 (*rps4*); 2, EAST SIBERIA 2004, *Ignatov s.n.*, AM397757 (*trnG*), AM398328 (*rps4*); *Metzgeria decipiens* (C. Massal.) Schiffn., SOUTH AFRICA 2004, *R. de Roo s.n.*, AM398259 (*rps4*); *Mnioloma fuscum* (Lehm.) R.M. Schust., SOUTH AFRICA 2004, *R. de Roo s.n.*, AM397718 (*trnG*), AM398263 (*rps4*); *Mylia taylorii* (Hook.) Gray, NORWAY 2004, *K. Hassel s.n.*, AM397724 (*trnG*), AM398270 (*rps4*); *Nardia scalaris* Gray, 1, *AY608092* (*rps4*); 2, NORWAY 2004, *K. Hassel s.n.*, AM397727 (*trnG*), AM398273 (*rps4*); *Neesioscyphus argillaceus* (Nees) Grolle, VENEZUELA 2004, *L. Söderström & al. 2004/022*, AM397695 (*trnG*), AM398235 (*rps4*); *Nowellia curvifolia* (Dicks.) Mitt., 1, *AY608094* (*rps4*); 2, UNITED KINGDOM 2001, *D. Long 29513* (E), AM398293 (*rps4*); *Odontoschisma denudatum* (Mart.) Dumort., IRELAND 2001, *D. Long 29937* (E), AM397760 (*trnG*), AM398325 (*rps4*); *O. macounii* (Austin) Underw., SVALBARD 2004, *L. Söderström & al. 2004/431*, AM397744 (*trnG*), AM398303 (*rps4*); *Pachyschistochila carnosus* (Mitt.) R.M. Schust. & Engel, ARGENTINA 2003, *D. Long 31755* (E), AM398296 (*rps4*); *Pellia epiphylla* (Gottsche) Limpr., *AY330479* (*rps4*); *Plagiochila deltoidea* Lindenb. *AY547699* (*rps4*); *P. dura* De Notaris, *AY547700* (*rps4*); *P. retrospectans* (Nees ex Spreng.) Lindenb., *AY547721* (*rps4*); *P. sp.*, SOUTH AFRICA 2003, *R. de Roo 12k*, AM397716 (*trnG*), AM398260 (*rps4*); *Pleurozia purpurea* Lindb., *AY608100* (*rps4*); *Plicanthus hirtellus* (Weber) R.M. Schust., NEPAL 2001, *D. Long 30335*, AM397745 (*trnG*), AM398304 (*rps4*); *Porella pinnata* L., *AY608101* (*rps4*); *P. platyphylla* (L.) Pfeiff., *AY462387* (*rps4*); *Pseudolepicolea quadrilaciniata* (Sull.) Fulf. & J. Taylor, ARGENTINA 2003, *D. Long 31658* (E), AM398299 (*rps4*); *Radula complanata* (L.) Dumort., UNITED KINGDOM 2001, *D. Long 29904* (E), AM397740 (*trnG*), AM398295 (*rps4*); *R. perrottetii* Gottsche ex Steph., *AY608105* (*rps4*); *Riccardia capensis* S.W. Arnell, SOUTH AFRICA 2003, *R. de Roo s.n.*, AM398264 (*rps4*); *Scapania compacta* (Roth) Dumort., NORWAY 2002, *L. Söderström 2002/160*, AM398312 (*rps4*); *S. hyperborea* Jørg., NORWAY 2004, *L. Söderström & al. 2004/191*, AM397732 (*trnG*), AM398283 (*rps4*); *S. lingulata* H. Buch, NORWAY 2004, *L. Söderström & al. 2004/199*, AM398280 (*rps4*); *S. nemorea* (L.) Grolle, NORWAY 2004, *L. Söderström & al. 2004/200*, AM397731 (*trnG*); *S. obcordata* (Berggr.) S.W. Arnell, SVALBARD 2004, *L. Söderström & al. 2004/379*, AM397735 (*trnG*), AM398289 (*rps4*); *S. undulata* (L.) Dumort., 1, CANADA 1985, *T. Hedderson 3432*, AM397787 (*trnG*); 2, NORWAY 2004, *L. Söderström & al. 2004/201*, AM397733 (*trnG*), AM398286 (*rps4*); *Schiffneria hyalina* Steph., *AY462393* (*rps4*); *Southbya gollanii* Steph., NEPAL 2001, *D. Long 30537* (E), AM397739 (*trnG*), AM398294 (*rps4*); *Sphenolobopsis pearsonii* (Spruce) R.M. Schust., UNITED KINGDOM 2004, *D. Long 33507* (E), AM397751 (*trnG*), AM398316 (*rps4*); *Stephaniella paraphyllina* J.B. Jack, VENEZUELA 2004, *L. Söderström & al. 2004/030b*, AM397698 (*trnG*); *Symphogyna podophylla* (Thunb.) Mont. et Nees, SOUTH AFRICA 2004, *R. de Roo s.n.*, AM398258 (*rps4*); *Syzygiella setulosa* Steph., VENEZUELA 2004, *L. Söderström & al. 2004/073*, AM397715 (*trnG*), AM398256 (*rps4*); *Tetralophozia setiformis* (Ehrh.) Schljakov, 1, NORWAY 2004, *L. Söderström & al. 2004/195*, AM398277 (*rps4*); 2, SWEDEN 2003, *L. Söderström & P. Manyanga 2003/056*, AM397803 (*trnG*), AM398370 (*rps4*); *Tritomaria polita* (Nees) Jørg., 1, NORWAY 2003, *L. Söderström & P. Manyanga 2003/037*, AM397775 (*trnG*), AM398342 (*rps4*); 2, SWEDEN 2003, *L. Söderström 2003/063*, AM398376 (*rps4*); *T. quinquentata* (Huds.) H. Buch ssp. *quinquentata* var. *quinquentata*, NORWAY 2003, *L. Söderström 2003/002*, AM397768 (*trnG*), AM398335 (*rps4*); *T. quinquentata* (Huds.) H. Buch ssp. *turgida* (Lindb.) Damsh., NORWAY 2003, *L. Söderström 2003/012*, AM397773 (*trnG*), AM398341 (*rps4*); *T. scitula* (Taylor) Jørg., NORWAY 2003, *L. Söderström & P. Manyanga 2003/028*, AM397779 (*trnG*), AM398347 (*rps4*).