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Reinstatement of Lophocoleaceae (Jungermanniopsida) based on chloroplast gene *rbc*L data: exploring the importance of female involucres for the systematics of Jungermanniales

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Abstract. Maximum likelihood analysis of 113 *rbc*L sequences leads to a well resolved phylogeny of Jungermanniales. All species with perigynia or marsupia are found in one clade, whereas species with coelocaules are placed in several lineages. The broadly circumscribed Geocalycaceae (including Lophocoleaceae) of most recent authors are resolved as polyphyletic. Geocalycaceae genera which develop female involucres without involvement of stem tissue (*Chiloscyphus, Heteroscyphus, Leptoscyphus, Physotheca*) form a robust clade which is placed sister to Plagiochilaceae whereas the genera with involucres originating at least partly from stem tissue (Geocalycaceae s.str., *Geocalyx*, *Harpanthus*, *Saccogyna*) are nested within the paraphyletic Jungermanniaceae. This topology leads to the exclusion of the strictly perianth-bearing species from Geocalycaceae and the reinstatement of Lophocoleaceae. *Campanocolea* is nested within *Chiloscyphus*. *Physotheca* and *Chiloscyphus breutelii* are placed within an unsupported clade with several accessions of *Leptoscyphus*. *Heteroscyphus* forms a paraphyletic grade at the base of *Chiloscyphus*.

Key words: Jungermanniopsida, Jungermanniales, Geocalycaceae, Lophocoleaceae, Plagiochilaceae, female involucres, *rbc*L, phylogeny.

Recent molecular phylogenetic studies have improved our understanding of the deep relationships of Marchantiophyta (Crandall-Stotler et al. 2005, Davis 2004, Heinrichs et al. 2005, He-Nygrén et al. 2004, Lewis et al. 1997). Leafy liverworts (Jungermanniopsida subclass Jungermanniidae) have been resolved as one major clade of hepatics which in turn splits into two main lineages (Davis 2004) corresponding to Porellales and Jungermanniales (Heinrichs et al. 2005). Taking into account results of recent monographic work (Gradstein et al. 2001, Yano and Gradstein 1997), Jungermanniales are the largest lineage of Marchantiophyta with an extant diversity of about 3,000 species in about 250 genera in about 20 families. This order is characterized by succubous or incubous leaves developing usually by means of two initial cells, lack of watersacs, occurrence of ventral branches and non-fasciculate rhizoids, exosporous protonemata, and association with Asco- and Basidiomycota (Heinrichs et al. 2005). Classification of Jungermanniales has been hampered by the low number of phylogenetically informative morphological characters and extensive morphological homoplasy (Crandall-Stotler et al. 2005). Only a few molecular studies of Jungermannialean genera or families have been published to date (e.g. He-Nygrén and Piippo 2003, Heinrichs et al. 2004, Schill et al. 2004). The topologies depicted in these studies are often in conflict with previous morphology-based classifications. Obviously major rearrangements are inevitable to generate a natural classification of this order.

Representatives of Jungermanniales possess several forms of female involucres surrounding the archegonia (Gottsche 1845, 1880; Knapp 1930; Leitgeb 1881). Perianths originate by fusion of two or three modified leaves whereas other structures develop at least partly from stem tissue. These structures have once been subsumed under the term "perigynium" (Schuster 1966). However, modern floras (e.g. Gradstein and Pinheiro da Costa 2003, Paton 1999) usually differentiate between coelocaules, perigynia s.str. and marsupia. Coelocaules are hollow outgrowths characterized by extreme axial growth of the entire shoot tip resulting in complete penetration of the sporophyte into the shoot apex. These structures are frequently associated with the absence of a perianth. Perigynia are tubular structures derived from axial cells that surround and protect the archegonia. They are often terminated by perianths and can be associated with a shoot calyptra (fleshy calyptra whose basal parts originate from stem tissue). Marsupia are multistratose pendent structures derived from stem tissue. They penetrate downwards into the substrate and usually bear rhizoids. Schuster (1966) stated that the delimitation of these structures is sometimes subject to controversy, especially because various combinations and transitions may occur.

Currently most authors adopt a wide concept of Geocalycaceae including Lophocoleaceae and Harpanthaceae (e.g. Crandall-Stotler and Stotler 2000; Grolle 1965, 1983; Grolle and Long 2000; Schuster 1980; Srivastava and Srivastava 2002). In this circumscription, Geocalycaceae include species with a marsupium, a perigynium with a terminal perianth or a trigonous to laterally compressed perianth. In addition the broadly circumscribed Geocalycaceae are characterized by a stem lacking hyalodermis and well differentiated cortical cells, succubous leaves, presence of conspicuous underleaves, which are often connate to the adjacent postical leaf bases, and rhizoids at the base of the underleaves, occasionally scattered over the ventral merophyte (e.g. Paton 1999, Schuster 1980, Srivastava and Srivastava 2002). Other authors (Frahm and Frey 2004, Müller 1951–1958) suggested a different classification in which the exclusively perianth-bearing Lophocoleaceae are separated from the Geocalycaceae/Harpanthaceae, that develop female involucres which originate at least partly from stem tissue.

Schuster and Engel (1982) questioned the separation of Geocalycaceae subfam. Leptoscyphoideae and Plagiochilaceae, based on similarities in perianth shape. On the other hand, Grolle (1962, 1983) regarded the similarities of Leptoscyphoideae and Plagiochilaceae as superficial.

Molecular data may help to resolve the relationships. To date only five of the approximately 25 genera currently assigned to Geocalycaceae (Crandall-Stotler and Stotler 2000, Engel and Gradstein 2003, Gradstein and Pinheiro da Costa 2003) have been included in molecular phylogenetic studies (Davis 2004, He-Nygrén and Piippo 2003, He-Nygrén et al. 2004). Davis (2004) resolved *Harpanthus* Nees, *Lophocolea* (L.) Dumort. and *Chiloscyphus* Corda in three separate lineages. In contrast, He-Nygrén and Piippo (2003) and Heinrichs et al. (2005) resolved *Chiloscyphus* within *Lophocolea*, supporting the broad concept of *Chiloscyphus* advocated by Schuster (1980) and Engel and Schuster (1984), and confirmed the separation of the *Chiloscpyhus* segregate *Heteroscyphus* Schiffn. Following Heinrichs et al. (2005) a broader taxon sampling of Geocalycaceae including genera with marsupia is needed to test the current family concept.

In the present study we explore the different concepts of Geocalycaceae by analyzing sequences of the chloroplast gene *rbc*L of a large taxon set of Jungermanniales, and examine the distribution of the different types of female involucres in this largest order of liverworts.

Materials and methods

Taxon sampling and designation of the outgroup. Forty two chloroplast gene *rbc*L sequences of Jungermanniales as circumscribed by Heinrichs et al. (2005) were newly generated for this study. The ingroup was completed with 69 accessions from GenBank/EMBL. Altogether 33 species of Geocalycaceae were sampled, representing four of the five currently accepted subfamilies, Geocalycoideae, Leptoscyphoideae, Lophocoleoideae and Physothecoideae (Engel and Gradstein 2003). Material of the monospecific Geocalycaceae subfam. Conoscyphoideae (Engel 1987) was not available for molecular study. GenBank/EMBL sequences of *Ptilidium ciliare* (L.) Hampe and *Ptilidium pulcherrimum* (Weber) Vain. (Ptilidiaceae) were designated as outgroup, based on the results of Davis (2004) and Heinrichs et al. (2005).

Specimen data, along with GenBank/EMBL accession numbers, are provided in Table 1. Vouchers related to new sequences are deposited in the herbaria of the University of Jena (JE) or University of Göttingen (GOET).

DNA extraction, PCR amplification and sequencing. Plant tissue from the distal portions of a few shoots was isolated from herbarium specimens. Total genomic DNA was extracted with Invisorb Spin Plant Mini Kit (Invitek, Berlin, Germany).

The polymerase chain reaction was performed in a total volume of 50 μl, including 1 μl template DNA, 1 U Taq-DNA polymerase (BioLine, Berlin, Germany), 5 μl PCR buffer (BioLine, Berlin, Germany), 2 μl $MgCl_2$ (50 mM, BioLine, Berlin, Germany), 1 μl dNTP-mixture (10 mM, Fermentas, St. Leon-Rot, Germany), 2 μl dimethylsulfoxide, 1 μl 3'-primer (10 μM), 1 μl 5'-primer (10 μM), and 36.8 μl double-distilled water. Polymerase chain reactions were carried out using the following program: 120 s initial denaturation at 92 °C, followed by 30 cycles of 60 s denaturation at 92 °C, 50 s annealing at 51 °C, and 90 s elongation at 72 °C. Final elongation was carried out in one step of 10 min at 72 °C. PCR products were subsequently purified using a DNAce Quick Clean purification kit (BioLine, Berlin, Germany). The 5′-primer *rbc*L-1Pl-F and the 3′-primer M1390-R (Wilson et al. 2004) were used to amplify the first 1390 bp of the cp-*rbc*L gene. Sequencing was carried out on a MegaBACE 1000 capillary sequencer using DYEnamic ET Primer DNA Sequencing Reagent (Amersham Biosciences, UK) with the internal primers *rbc*L-170-F, *rbc*L-680Pl-F, *rbc*L-700Pl-R, and *rbc*L-1200-R (Wilson et al. 2004, Groth and Heinrichs 2005).

Phylogenetic analyses. The 113 *rbc*L sequences were aligned manually in BioEdit version 7.0.5.2 (Hall 1999). Lacking parts of sequences were coded as "N" (A, C, G or T). Regions of largely incomplete data were identified and excluded from subsequent analyses, resulting in a dataset including 1,034 homologous sites. The alignment is available upon request. A phylogenetic tree was inferred using maximum likelihood criteria (ML) as implemented in PAUP* version 4.0b10 (Swofford 2000). The nucleotide substitution model with the smallest number of parameters that best fits the data was determined using the hierarchical likelihood test and the Akaike information criterion as implemented in Modeltest 3.06 (Posada and Crandall 1998). The GTR model (Tavaré 1986) was chosen with gamma shape parameter (G) for among site variation and proportion of invariable sites (I) (GTR + I + G) and implemented in PAUP*. A ML analysis was performed as a heuristic search with 3 random addition sequence replicates. The confidence of branching was assessed using 200 non-parametric bootstrap re-samplings (Felsenstein 1985) in ML analysis. A neighbor-joining tree was used as starting tree and the rearrangements per replicate were limited to 2,000. Bootstrap percentage values > 70% were regarded as good support (Hillis and Bull 1993). Posterior probability values (PP) were calculated with a Bayesian inference of phylogeny as implemented in MrBayes (version 3.0b4; Huelsenbeck and Ronquist 2001). The Modeltest 3.06 output values were applied to the command block of MrBayes. Four chains each with two million generations were calculated of which every 100th tree was saved. Trees collected before the likelihood score had stabilized were deleted. A majority rule consensus tree was calculated from the remaining 18,001 trees. Bayesian support, referred to as posterior probabilities, was regarded as significant when exceeding 0.95 (Larget and Simon 1999).

Table 1. Geographic origins, vouchers, and GenBank/EMBL accession numbers of the taxa investigated. Accession numbers of new sequences are in bold

Taxon	Origin	Voucher	Accession number
Acrobolbus sp.	Ecuador	*Gradstein & Mandl 10130* (GOET)	**DQ312474**
Adelanthus lindenbergianus (Lehm.) Mitt.	Argentina	*Hyvönen 5637a* (H)	AY462285
Anastrophyllum michauxii (F.Weber) H.Buch	U.S.A.	*Sargent s.n.* (ABSH)	AY507390
Anastrophyllum minutum (Schreb.) R.M.Schust.	Spitsbergen	*Hentschel Bryo 0421* (GOET)	**DQ312475**
Balantiopsis cancellata (Nees) Steph.	Chile	*Hyvönen 5946* (H)	AY462286
Balantiopsis diplophylla (Hook.f. & Taylor) Mitt.	New Zealand	*Engel 20853* (GOET)	**DQ312476**
Barbilophozia barbata (Schmidel ex Schreb.) Loeske	Bulgaria	*Hentschel Bryo 0752* (GOET)	**DQ312477**
Barbilophozia hatcheri (A.Evans) Loeske	Spitsbergen	*Hentschel Bryo 0492* (GOET)	**DQ312478**
Bazzania tricrenata (Wahlenb.) Lindb.	Austria	*Heinrichs 4388* (GOET)	AY699990
Blepharostoma trichophyllum (L.) Dumort.	Finland	*He-Nygrén & Piippo 1471* (H)	AY462289
Calypogeia fissa (L.) Raddi	Germany	*Hentschel Bryo 0187* (GOET)	**DQ312479**
Calypogeia integristipula Steph.	Finland	*He-Nygrén & Piippo 1472* (H)	AY462290
Calypogeia muelleriana (Schiffn.) Müll.Frib.		*Crandall-Stotler s.n.*	U87065
Cephalozia bicuspidata (L.) Dumort.	Finland	*Piippo 5655* (H)	AY462291
Cephaloziella divaricata (Sm.) Schiffn.	Germany	*Hentschel Bryo 01159* (GOET)	**DQ312481**
Chaetophyllopsis whiteleggei (Carrington & Pearson) R.M.Schust. ex Hamlin	Australia	*Curnow 4804* (H)	AY462292
Chandonanthus sp.	China	*He-Nygrén 492*	AY462293
Chiastocaulon dendroides (Nees) Carl	Japan	*Kurita 84* (HIRO)	AY699991
Chiloscyphus breutelii (Gottsche) J.J.Engel & R.M.Schust.	Ecuador	*Gradstein & Mandl 10136* (GOET)	**DQ312482**
Chiloscyphus connatus (Sw.) J.J.Engel & R.M.Schust.	Costa Rica	*Gradstein 9404* (GOET)	**DQ312483**
Chiloscyphus cuspidatus (Nees) J.J.Engel & R.M.Schust.	China	*Koponen et al. 48430* (H)	AY149845
Chiloscyphus fragmentissimus (R.M.Schust.) J.J.Engel & R.M.Schust.	Venezuela	*Frahm 97/5/N* (GOET)	**DQ312480**
Chiloscyphus fragrans (Moris & De Not.) J.J.Engel & R.M.Schust.	Azores	*Schwab 113* (JE)	**DQ312484**
Chiloscyphus itoanus (Inoue) J.J.Engel & R.M.Schust.	China	*Piippo 60709* (H)	AY149846
Chiloscyphus japonicus Steph.	China	*Koponen et al. 50238* (H)	AY149847
Chiloscyphus latifolius (Nees) J.J.Engel & R.M.Schust.	Poland	*Jedrzejko & A. Stebel W-58* (H)	AY149842
Chiloscyphus martianus (Nees) J.J.Engel & R.M.Schust.	Ecuador	*Gradstein 10119* (GOET)	**DQ312485**
Chiloscyphus minor (Nees) J.J.Engel & R.M.Schust.	China	*Rao 58428* (H)	AY149843

Chiloscyphus muricatus (Lehm.) J.J.Engel & R.M.Schust.	Australia	*Streimann 51629* (JE)	**DQ312486**
Chiloscyphus pallescens (Ehrh. ex Hoffm.) Dumort.	Poland	*Stebel W-4* (H)	AY149849
Chiloscyphus polyanthos (L.) Corda	Finland	*He-Nygrén & Piippo 1469* (H)	AY149851
Chiloscyphus polychaetus (Spruce) J.J.Engel & R.M.Schust.	Ecuador	*Gradstein & Mandl 10139* (GOET)	**DQ312487**
Chiloscyphus profundus (Nees) J.J.Engel & R.M.Schust.	Finland	*He-Nygrén & Piippo 1470* (H)	AY149852
Chiloscyphus sabuletorum (Hook.f. & Taylor) J.J.Engel & R.M.Schust.	Chile	*Busch et al. Bryo 01396* (JE)	**DQ312488**
Chiloscyphus semiteres (Lehm.) Lehm. & Lindenb.	Australia	*Streimann 58464* (GOET)	**DQ312489**
Dendromastigophora flagellifera (Hook.) R.M.Schust.	New Zealand	*Glenny 8520* (H)	AY462294
Diplophyllum obtusifolium (Hook.) Dumort.	U.S.A.	*Wheeler s.n.* (ABSH)	AY507397
Geocalyx graveolens (Schrad.) Nees	Germany	*Meinunger s.n.* (JE)	**DQ312490**
Gottschea nuda (Horik.) Grolle & Zijlstra	Japan	*Yamaguchi s.n.* (H)	AY462297
Gottschea tuloides (Hook.f. & Taylor) Gottsche, Lindenb. & Nees	New Zealand	*Glenny 8499* (H)	AY462321
Gymnomitrion corallioides Nees	Spitsbergen	*Hentschel Bryo 0458* (GOET)	**DQ312491**
Harpanthus flotovianus (Nees) Nees	Austria	*Heinrichs et al. 4390* (GOET)	**DQ312492**
Herbertus dicranus (Taylor) Trevis.	Nepal	*Long 17542* (H)	AY462300
Herbertus oldfieldianus (Steph.) Rodway	New Zealand	*Stotler & Crandall-Stotler 4580* (ABSH)	AY507404
Herbertus sendtneri (Nees) Lindb.	Bolivia	*Groth s.n.* (GOET)	AY699993
Heteroscyphus aselliformis (Reinw., Blume & Nees) Schiffn.	Indonesia	*Gradstein 10240* (GOET)	**DQ312493**
Heteroscyphus biciliatus (Hook.f. & Taylor) J.J.Engel & R.M.Schust.	New Zealand	*Frahm 20-6* (GOET)	**DQ312494**
Heteroscyphus coalitus (Hook.) Schiffn.	Nepal	*Long 30316* (H)	AY149844
Heteroscyphus cuneistipulus (Steph.) Schiffn.	New Zealand	*Frahm 9–15* (GOET)	**DQ312495**
Heteroscyphus fissistipus (Hook.f. & Taylor) Schiffn.	Ireland	*Long H4064* (JE)	**DQ312496**
Heteroscyphus inflatus (Steph.) S.C.Srivast. & A.Srivast.	Nepal	*Long 30457* (H)	AY149853
Heteroscyphus planus (Mitt.) Schiffn.	Japan	*Mizutani 15828* (H)	AY149850
Heteroscyphus splendens (Lehm. & Lindenb.) Grolle	Papua New Guinea	*Hoffmann 98–749* (H)	AY149854
Heteroscyphus zollingeri (Gottsche) Schiffn.	China	*Koponen et al. 57927* (H)	AY149856
Hygrolembidium acrocladum (Berggr.) R.M.Schust.	Australia	*Curnow 5587* (H)	AY462301
Isotachis humectata (Hook.f. & Taylor) Steph.	Chile	*Hyvönen 5180* (H)	AY462302
Isotachis lyallii (Mitt.) R.M.Schust.	New Zealand	*Engel 21825* (F)	AY608032
Isotachis multiceps (Lindenb. & Gottsche) Gottsche	Panama	*Stotler & Crandall-Stotler 3478* (ABSH)	AY507407

Table 1. (continued)

Taxon	Origin	Voucher	Accession number
Jamesoniella autumnalis (DC.) Steph.	China	*Koponen et al. 55912* (H)	AY462303
Jungermannia callithrix Lindenb. & Gottsche	Mexico	*Burghardt 4509* (GOET)	**DQ312497**
Jungermannia infusca (Mitt.) Steph.	Japan	*Deguchi s.n.* (GOET)	**DQ312498**
Jungermannia leiantha Grolle	U.S.A.	*Stotler & Crandall-Stotler 107* (ABSH)	AY507409
Jungermannia obovata Nees	Austria	*Heinrichs et al. 4391* (GOET)	**DQ312499**
Jungermannia ovato-trigona (Steph.) Grolle	Colombia	*Gradstein 10007* (GOET)	**DQ312500**
Leiocolea collaris (Nees) Schljakov	Slovakia	*Hentschel Bryo 0864* (GOET)	**DQ312501**
Leiomitra lanata (Hook.) R.M.Schust.	New Zealand	*Glenny 8521*	AY462305
Lepicolea attenuata (Mitt.) Steph.	New Zealand	*Stotler & Crandall-Stotler 4586* (ABSH)	AY507410
Lepicolea ochroleuca (L.f. ex Spreng.) Spruce	Chile	*Hyvönen 2938* (H)	AY462306
Lepicolea pruinosa (Taylor) Spruce	Peru	*Frahm et al. s.n.* (H)	AY462307
Lepicolea scolopendra (Hook.) Dumort. ex Trevis.	Australia	*Streimann 55445* (H)	AY462308
Lepidozia sp.	China	*Koponen et al. 50623* (H)	AY462311
Leptoscyphus amphibolius (Nees) Grolle	Panama	*Dauphin et al. 1578* (GOET)	**DQ312502**
Leptoscyphus expansus (Lehm.) Grolle	Prince-Edward-Isles	*Gremmen 98-124* (JE)	**DQ312503**
Leptoscyphus gibbosus (Taylor) Mitt.	Ecuador	*Schäfer-Verwimp et al. 24296* (GOET)	**DQ312504**
Lophozia ventricosa (Dicks.) Dumort.	Belgium	*Heinrichs 3826* (GOET)	AY699994
Marsupella emarginata (Ehrh.) Dumort.	Germany	*Hentschel Bryo 01638* (GOET)	**DQ312505**
Marsupidium latifolium R.M.Schust.	Costa Rica	*Dauphin 2920* (NY)	AY608034
Mastigophora woodsii (Hook.) Nees	Australia	*Frahm* (CANB 639918)	AY462314
Mylia tylorii (Hook.) Gray	Austria	*Heinrichs et al. 4393* (GOET)	**DQ312506**
Nardia assamica (Mitt.) Amakawa	China	*Koponen et al. 49396* (H)	AY462316
Nardia compressa (Hook.) Gray	Belgium	*Heinrichs et al. 3806* (GOET)	**DQ312507**
Nardia scalaris Gray	Germany	*Hentschel & Wilson Bryo 01618* (GOET)	**DQ312508**
Neesioscyphus argillaceus (Nees) Grolle	Brazil	*Schäfer-Verwimp & Verwimp 11107* (GOET)	**DQ312509**
Nowellia curvifolia (Dicks.) Mitt.	Mexico	*Burghardt 4493* (GOET)	**DQ312510**
Odontoschisma denudatum (Mart.) Dumort.	U.S.A.	*Horn 1809* (DUKE)	AY608036
Pedinophyllum truncatum Inoue	China	*Koponen 46768* (H)	AY149855
Physotheca autoica J.J.Engel & Gradst.	Ecuador	*Gradstein & Nöske 10090* (GOET)	**DQ312511**

Plagiochila alternans Lindenb. & Gottsche	Bolivia	*Heinrichs et al. 4178* (GOET)	AY699995
Plagiochila asplenioides (L.) Dumort.	Germany	*Heinrichs & Groth 4339* (GOET)	AY699996
Plagiochila ovata (L.) Dumort.	Costa Rica	*Heinrichs et al. 4158* (GOET)	AY699997
Plagiochila porelloides (Torr. ex Nees) Lindenb.	Germany	*Heinrichs & Groth 4340* (GOET)	AY699998
Plagiochila ramosissima (Hook.) Lindenb.	New Zealand	*Engel et al. 11107* (GOET)	**DQ312512**
Plagiochilion conjugatum (Hook.) R.M.Schust.	New Zealand	*Schäfer-Verwimp & Verwimp 14080* (GOET)	**DQ312513**
Plagiochilion mayebarae S.Hatt.	Japan	*Ohnishi 5588* (HIRO)	AY699999
Plagiochilion oppositum (Reinw., Blume & Nees) S.Hatt.	Indonesia	*Schäfer-Verwimp 20935* (GOET)	AY700000
Ptilidium ciliare (L.) Hampe	Canada	*Schofield 103486* (DUKE)	AY608038
Ptilidium pulcherrimum (Weber) Vain.	Finland	*Ahonen & Engblom 7* (H)	AY302460
Saccogyna viticulosa (L.) Dumort.	Tenerife	*Gradstein 9977* (GOET)	**DQ312514**
Scapania nemorea (L.) Grolle	U.S.A.	*Stotler & Crandall-Stotler s.n.* (ABSH)	AY507423
Scapania undulata (L.) Dumort.	Finland	*He-Nygrén & Piippo 1468* (H)	AY149840
Schiffneria hyalina Steph.	Japan	*Mizutani 15961* (H)	AY462327
Schistochila appendiculata (Hook.) Dumort. ex Trevis.	New Zealand	*Glenny 8537* (H)	AY462328
Schistochila laminigera (Hook.f. & Taylor) A.Evans	Chile	*Hyvönen 5888* (H)	AY462329
Syzygiella anomala (Lindenb. & Gottsche) Steph.	Costa Rica	*Gradstein & Mues 9657* (GOET)	AY700001
Syzygiella perfoliata (Sw.) Spruce	Brazil	*Gradstein 9827* (GOET)	AY700002
Temnoma pilosum (A.Evans) R.M.Schust.	Chile	*Hyvönen 5084* (H)	AY462330
Triandrophyllum subtrifidum (Hook. & Taylor) Fulford & Hatcher	Chile	*Hyvönen 5334* (H)	AY462331
Trichocolea tomentella (Ehrh.) Dumort.	China	*He-Nygrén 1137* (H)	AY462332
Trichocolea tomentosa (Sw.) Gottsche	Ecuador	*Davis 368* (DUKE)	AY608040
Trichotemnoma corrugatum (Steph.) R.M.Schust.	New Zealand	*Glenny 8426* (H)	AY462333
Tritomaria quinquedentata (Huds.) H.Buch	Germany	*Heinrichs 2978* (GOET)	AY700003
Tylimanthus setaceo-ciliatus Steph.	Ecuador	*Gradstein & Mandl 10147* (GOET)	**DQ312515**
Vetaforma dusenii (Steph.) Fulford & J.Taylor	Chile	*Engel 11423* (H)	AY462335

Character reconstruction. Five types of female involucres were scored as character states of a single character (perianth, perigynium with perianth, perigynium without perianth, marsupium and coelocaule) according to the species descriptions in Engel and Schuster (1984), Damsholt (2002), Gradstein et al. (2001), Gradstein and Pinheiro da Costa (2003), Grolle (1960, 1964), Grolle and Zijlstra (1984), Hatcher (1960, 1961), Paton (1999), Schuster (1966, 1969, 1972, 1974, 1987, 2000, 2002), Schuster and Engel (1977), Váňa (1973b, 1973c, 1974a, 1974b), Wigginton (2004) and Zhu and So (1996). Question marks were scored for those taxa with ambiguous or lacking information about character states (*Dendromastigophora* R.M.Schust., *Trichotemnoma* R.M.Schust.). The evolution of these character states was reconstructed using the topology recovered in the ML analyses of the *rbc*L data set employing a maximum parsimony approach as implemented in MacClade 4.0 PPC (Maddison and Maddison 2000).

Results

The maximum likelihood analysis resulted in a single optimal topology (−ln = 14448.36238) which is presented in Fig. 1. The robust Schistochilaceae lineage is sister to a clade comprising all other members of Jungermanniales. Geocalycaceae are found to be polyphyletic. Strictly perianth-bearing species (Geocalycaceae subfam. Leptoscyphoideae, Lophocoleoideae, Physothecoideae, represented by the genera *Chiloscyphus*, *Heteroscyphus*, *Leptoscyphus* Mitt. and *Physotheca* J.J.Engel & Gradst.) are separated from the marsupium-bearing and perigynium-bearing (with or without perianth) species (Geocalycaceae subfam. Geocalycoideae, represented by the genera *Geocalyx* Nees, *Harpanthus* and *Saccogyna* Dumort.), leading to the reinstatement of Lophocoleaceae. The well-supported families Lophocoleaceae and Plagiochilaceae are placed in a robust sister relationship. An unsupported clade with three accessions of *Leptoscyphus*, *Chiloscyphus breutelii* (Gottsche) J.J.Engel & R.M.Schust. and *Physotheca autoica* J.J.Engel & Gradst. is placed sister to the remainder of Lophocoleaceae. *Heteroscyphus* forms a parapyletic grade at the base of the weakly supported *Chiloscyphus* lineage. *Chiloscyphus polychaetus* (Spruce) J.J.Engel & R.M.Schust. is nested in a robust clade with several *Heteroscyphus* species. *Chiloscyphus fragmentissimus* (R.M.Schust.) J.J.Engel & R.M.Schust. (syn.: *Campanocolea fragmentissima* (R.M.Schust.) R.M.Schust.) is resolved within the crown group of this genus.

The monophyly of the Plagiochilaceae genera *Plagiochila* (Dumort.) Dumort. and *Plagiochilion* S.Hatt. is supported by the ML topology albeit without support.

Accessions of Adelanthaceae, Cephaloziaceae, Cephaloziellaceae and Scapaniaceae form a well-supported clade sister to a lineage containing *Geocalyx graveolens* (Schrad.) Nees, *Harpanthus flotovianus* (Nees) Nees and *Saccogyna viticulosa* (L.) Dumort. and several other taxa of Jungermanniales which develop female involucres at least partly from stem tissue. Inter alia the latter clade includes species currently assigned to Jungermanniaceae, as well as the robust Acrobolbaceae, Balantiopsidaceae, Calypogeiaceae, and Gymnomitriaceae. Representatives of Jungermanniaceae are placed in several lineages. *Geocalyx* is resolved sister to a weakly supported clade with *Leiocolea collaris* (Nees) Schljakov, *Jungermannia leiantha* Grolle and *J. ovato-trigona* (Steph.) Grolle. *Harpanthus* and *Saccogyna* form a paraphyletic grade at the base of an unsupported clade with Gymnomitriaceae, *Jungermannia callitrix* Lindenb. & Gottsche, *J. infusca* (Mitt.) Steph. and *J. obovata* Nees, as well as *Nardia compressa* (Hook.) Gray and *N. scalaris* Gray, the latter in a robust sister relationship. *Nardia assamica* (Mitt.) Amakawa is resolved in a separate clade, within the Cephaloziaceae.

The presence of a perianth is plesiomorphic in Jungermanniales. Coelocaule structures evolved independently in five clades: (1) a clade corresponding to Schistochilaceae, (2) a clade corresponding to Trichocoleaceae, (3) a clade comprising Lepicoleaceae and Vetaformaceae, (4) *Mastigophora* within Herbertaceae, and (5) Pseudolepicoleaceae. Species with marsupia or perigynia (with or

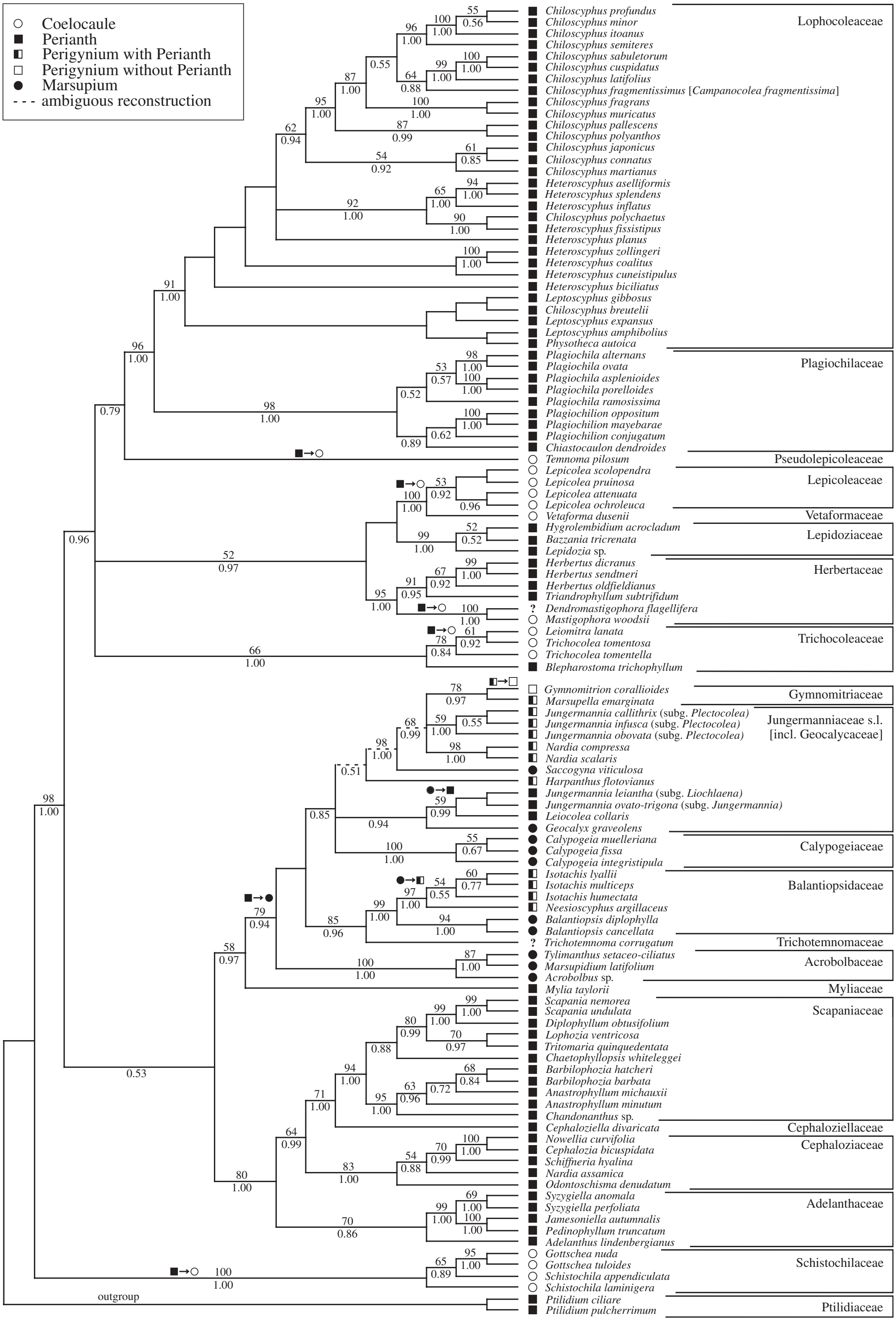

Fig. 1. Jungermanniales topology recovered in ML analyses. Bootstrap percentage values (> 50) above and posterior probabilities below branches. Types of female involucres and character state changes are indicated

without perianths) are found in a single clade consisting of representatives of the families Acrobolbaceae, Balantiopsidaceae, Calypogeiaceae, Gymnomitriaceae, Jungermanniaceae, and Trichotemnomaceae. According to the *rbc*L-topology taxa bearing perigynia with or without perianths evolved from marsupium-bearing taxa. For a clade consisting of Gymnomitriaceae, *Jungermannia* subg. *Plectocolea* (Mitt.) Amakawa, *Nardia* Gray, *Saccogyna* and *Harpanthus* an unambiguous character reconstruction was not possible due to the largely unresolved topology.

Discussion

According to the *rbc*L topology (Fig. 1), the broadly circumscribed Geocalycaceae as accepted by many recent authors are polyphyletic. The strict separation of the species which exclusively develop perianths from the marsupia- and perigynium/perianth-bearing species leads to the reinstatement of Lophocoleaceae. Lophocoleaceae are characterized by succubous, undivided to bilobed, entire or toothed, almost horizontally inserted leaves, thin-walled cells, conspicuous underleaves often connate to the leaves, *Frullania*-type and intercalary branching (lateral and ventral), stems without hyalodermis and well developed cortex, rhizoids in bundles from underleaf bases or rarely scattered, gametoecia on leading shoots or on short branches, and a 0–3-keeled perianth.

Chiloscyphus represents the crown group of Lophocoleaceae. The generitype *Chiloscyphus polyanthos* (L.) Corda is nested in a robust subclade with species originally described in *Lophocolea*, supporting the inclusion of *Lophocolea* in *Chiloscyphus* (Engel and Schuster 1984, He-Nygrén and Piippo 2003). Several hundred binomials have been described in this genus (Engel and Schuster 1984, Piippo 1985), which are assigned to numerous subgenera or sections (e.g. Engel 1992, 1999; Engel and Schuster 1984; Grolle 1968; Schuster 1980; Hässel de Menéndez 1996, 2001; Srivastava and Srivastava 2002). Our limited taxon sampling allows only for some preliminary comments on the infrageneric classification of *Chiloscyphus*. *Chiloscyphus* subg. *Chiloscyphus* [*C. pallescens* (Hoffm.) Dumort., *C. polyanthos*] is resolved as monophyletic. This subgenus is characterized by usually entire, unlobed, alternate leaves which are rarely united with the bifid underleaves, always terminal gynoecia on strongly abbreviated lateral branches, female bracts and bracteoles usually smaller than the vegetative leaves, as well as inconspicuous trigonous perianths not or hardly longer than the calyptra (Engel and Schuster 1984, Schuster 1980).

The robust sister clade of *Chiloscyphus* subg. *Chiloscyphus* is here assigned to *C.* subg. *Lophocolea* (Dumort.) J.J.Engel & R.M.Schust. [incl. *C.* subg. *Microlophocolea* (Spruce) J.J.Engel, *C.* subg. *Fragillifolius* (R.M.Schust.) J.J.Engel & R.M.Schust.]. Species of this clade have broad-based, bidentate or bilobed, alternate leaves, gynoecial branches of variable length, and usually sharply trigonous perianths frequently with winged keels (Engel and Schuster 1984, Schuster 1980). *Campanocolea fragmentissima* (R.M.Schust) R.M.Schust. was separated from *Chiloscyphus* based on its almost dichotomous branching pattern and asexual reproduction by caducous lateral leaves (Schuster 1997). Otherwise it fits well into *Chiloscyphus* subg. *Lophocolea*. According to the molecular topology *Campanocolea fragmentissima* is a derived species of *Chiloscyphus* subg. *Lophocolea* rather than a monospecific genus.

The robust sister relationship of the subgenera *Chiloscyphus* and *Lophocolea* is supported by the shared occurrence of uniseriate antheridial stalks, extensive lack of secondary pigmentation, mostly alternate leaves, as well as underleaves which are at most inconspicuously united with the leaves (Bednarek-Ochyra et al. 2000, Engel and Schuster 1984, Schuster 1980). The uniseriate condition of the antheridial stalks of both subgenera is a taxonomic informative character because in other subgenera the antheridial stalks are bi- to oligoseriate. The latter condition is also present in the genera *Leptoscyphus*, *Physotheca* and *Heteroscyphus*.

Engel and Schuster (1984) placed *Chiloscyphus* sect. *Bicornuti* (Spruce) J.J.Engel & R.M.Schust. in *C.* subg. *Lophocolea*, and included species with biseriate antheridial stalks. The corresponding species *Chiloscyphus martianus* (Nees) J.J.Engel & R.M.Schust. and *C. connatus* (Sw.) J.J.Engel & R.M.Schust. are here resolved in a separate lineage (see also He-Nygrén and Piippo 2003) indicating that they do not belong to *C.* subg. *Lophocolea*. Based on the outcome of the phylogenetic analysis and the morphological differences these species could be assigned to *Chiloscyphus* subg. *Connati* (Lindenb.) J.J.Engel. This subgenus is characterized by large underleaves which are connected to the subopposite leaves. Furthermore the antheridial stalks are biseriate (Engel 1999). *Chiloscyphus japonicus* Steph., which is also resolved in the *C. martianus*/*C. connatus* clade, does not fit well in this group.

Chiloscyphus polychaetus is nested in a robust clade with several accessions of *Heteroscyphus*. The leaf cell walls of *Heteroscyphus* are usually provided with distinct trigones. Furthermore this genus stands out by its reniform underleaves often connected with both adjacent leaves, and spicate, slender androecia on short ventral branches (Engel 1991, Engel and Schuster 1984, Schiffner 1910). Male plants of *C. polychaetus* have not yet been described (Fulford 1976, Spruce 1885). The reniform, multiciliate underleaves which are connected to the leaves, and the large trigones of *C. polychaetus* strongly resemble *Heteroscyphus*. Already Engel and Schuster (1984) pointed out difficulties in the delimitation of the *Chiloscyphus* segregate *Heteroscyphus*. In the *rbc*L topology this genus is resolved as paraphyletic. A broader taxon sampling and the extension of the marker set is necessary to decide on the status of *Heteroscyphus*.

Inclusion of *Leptoscyphus* in the molecular investigation provides new evidence to resolve the conflicting hypotheses regarding its systematic position (Grolle 1983, 1995; Schuster and Engel 1982). According to the *rbc*L topology *Leptoscyphus* is a member of Lophocoleaceae with good support. *Leptoscyphus* is characterized by laterally compressed perianths, brownish color, leaf cells with large trigones, and underleaves usually connected with leaves, as well as the usually entire leaf margins (Fulford 1976, Grolle 1962, Hässel de Menéndez 2001, Schuster 1980). *Chiloscyphus breutelii*, a species with ciliately toothed leaves, and *Physotheca autoica* of the monospecific Geocalycaceae subfam. Physothecoideae are nested within *Leptoscyphus*, albeit without support. *Physotheca autoica* is distinguished from *Leptoscyphus* in branching by repeated innovations, an inflated, bladder-like perianth, 7–8-stratose capsule walls and large echinate spores (Engel and Gradstein 2003). The molecular topology provides some evidence that these characters may be the autapomorphic character states of a species related to the *Leptoscyphus* complex. Engel and Gradstein (2003) already pointed out similarities of *Physotheca* and *Leptoscyphus*. Despite this unambiguous evidence we argue that the transfer of *Physotheca* and *Chiloscyphus breutelii* to *Leptoscyphus* should await the results of a study including more variable markers and further related genera such as *Clasmatocolea* Spruce, *Conoscyphus* Mitt., *Pachyglossa* Herzog & Grolle, *Pigafettoa* C.Mass., and *Stolonivector* J.J.Engel. If the *rbc*L topology proves well-supported in further studies, *Heteroscyphus* and *Leptoscyphus* may also be subsumed into a very broadly defined genus *Chiloscyphus*.

Geocalyx, *Harpanthus*, and *Saccogyna* are resolved in a largely polytomous clade with Calypogeiaceae, Gymnomitriaceae as well as several species currently assigned to Jungermanniaceae (Crandall-Stotler and Stotler 2000). The majority of related species develop female involucres which originate at least partly from stem tissue. Heinrichs et al. (2005) already pointed out that no published concept of Jungermanniaceae is in accordance with the *rbc*L topology. This finding is corroborated by the present study. *Jungermannia* subg.

Plectocolea (Mitt.) Amakawa and the *Jungermannia* subgenera *Liochlaena* (Nees) S.W.Arnell and *Jungermannia* are placed in separate lineages. The molecular data provide some evidence that the broad genus concept of most recent authors (e.g. Damsholt 2002, Váňa 1973a) does not reflect ancestral relationships. Therefore reinstatement of the genera *Plectocolea* Nees (Yatsentyuk et al. 2004) or *Solenostoma* Mitt. may be necessary. However, to arrive at a definite conclusion a broader taxon sampling including the generitype *Jungermannia atrovirens* Dumort. is needed as well as additional molecular markers. Yatsentyuk et al. (2004) resolved *Leiocolea* (Müll.Frib.) H.Buch sister to *Jungermannia* or in an isolated position, depending on different gap-coding procedures in their *trn*L-*trn*F-dataset. This genus is currently placed within Lophoziaceae rather than Jungermanniaceae (Crandall-Stotler and Stotler 2000) or treated as a subgenus of *Lophozia* (Dumort.) Dumort. (e.g. Damsholt 2002). In the present study *Leiocolea* is resolved within the Jungermanniaceae clade, indicating that *Leiocolea* and *Lophozia* are not closely related. Extension of the Jungermanniales taxon sampling of Heinrichs et al. (2005) corroborates the finding that the Lophoziaceae concept of Inoue (1966) is not reflected in the molecular topology. As already demonstrated by Heinrichs et al. (2005), Schill et al. (2004) and Yatsentyuk et al. (2004) Scapaniaceae are nested within Lophoziaceae subfam. Lophozioideae, leading to a broad family concept of Scapaniaceae (Heinrichs et al. 2005). In the *rbc*L topology Lophoziaceae subfam. Jamesonielloideae are separated from Scapaniaceae by Cephaloziaceae and Cephaloziellaceae. The position of *Adelanthus* Mitt. within the Jamesonielloideae clade leads to the adoption of the name Adelanthaceae for this clade. To date this family is restricted to *Adelanthus* Mitt. and *Wettsteinia* Schiffn. (Grolle 1972), two genera with dorsal leaf margins often curved towards the stem apex. *Jamesoniella* (Spruce) F.Lees, *Syzygiella* Spruce and *Pedinophyllum truncatum* Inoue lack this type of leaf margin. However, at least *Adelanthus*, *Jamesoniella* and *Syzygiella* share the frequent occurrence of ventral branches (Grolle 1971, 1972; Inoue 1966).

The systematic position of a few taxa remains unclear. According to the *rbc*L tree, *Mylia* Gray represents an isolated lineage. Placement within Jungermanniaceae (subfam. *Mylioideae*, Grolle 1962) is not supported by the molecular data. Instead the *rbc*L topology supports the reinstatement of family Myliaceae advocated by Engel and Braggins (2005) based on cell wall morphology. The poorly known species *Nardia assamica* is resolved outside the generitype lineage comprising *Nardia compressa* and *N. scalaris*. For this species neither Amakawa (1963) nor Cao (2000) described marsupia, typically found in other species of *Nardia*. The systematic position of *Nardia assamica* needs verification by further sequences from other accessions.

Mapping the different types of female involucres on the *rbc*L topology supports the occurrence of peryginia and marsupia as a character of considerable importance for the systematics of Jungermanniales. All species which develop marsupia or perigynia (with or without perianths) are placed in a single clade. This finding corroborates the idea that perigynium and marsupium are related structures (Paton 1999). A closer relationship of the "Jungermanniae Geocalyceae" has already been proposed by Gottsche (1845, 1880) but was rejected by Spruce (1882) and later authors. *Gymnomitrion coralloides* Nees is the single species included in the study which has a perigynium without a terminal perianth. The *rbc*L topology suggests that the ancestor of *Gymnomitrion* Corda was provided with a perigynium with perianth. The occurrence of exclusively perianth-bearing taxa in the "perigynium/marsupium" clade is restricted to the *Leiocolea*/*Jungermannia ovato-trigona*/*J. leiantha*-lineage. According to this topology a single reversal to female involucres lacking stem tissue has taken place. However, this conclusion should be confirmed with a much more exhaustive taxon sampling. Species with coelocaules are diffusely distributed and occur in five different clades. Obviously this structure has evolved several times independently. Mapping perigynia and coelocaules on the *rbc*L tree indicates that these structures are not homologous.

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