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ORIGINAL ARTICLE

Phylogeny and systematic position of *Mesoptychia* (Lindb.) A. Evans

Ariane Cailliau · David G. Long · Michelle J. Price · Mathieu Perret

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Abstract A phylogenetic analysis of the leafy liverwort genus *Mesoptychia* (incl. *Leiocolea* and *Hattoriella*), using two chloroplast markers, *rbc*L and *rps*4, was performed to clarify the circumscription of the genus and to establish its systematic position within the Jungermanniales. Ten species and one variety of *Mesoptychia* (out of a total of 17 accepted specific names), together with 85 species from 57 genera representing different lineages of Jungermanniales, were sampled. The results support the monophyly of *Mesoptychia* and its inclusion within the Jungermanniaceae, which itself forms a clade with the Delavayellaceae. *Gymnocolea borealis*, previously treated under *Leiocolea* (= *Mesoptychia*), is nested in the Anastrophyllaceae. The synonymy of *Hattoriella* with *Mesoptychia* is phylogenetically confirmed. Four major clades are identified within *Mesoptychia* and their circumscriptions and distinguishing morphological features are discussed.

Keywords Jungermanniaceae · *Mesoptychia* · *rbc*L · *rps*4 · Phylogeny

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Introduction

The Jungermanniales has undergone considerable systematic reorganization over the past decade (Davis 2004; Heinrichs et al. 2005, 2007; Forrest et al. 2006). Families such as the Jungermanniaceae have been redefined and their delimitation has changed repeatedly in recent years (Hentschel et al. 2007; Crandall-Stotler et al. 2008, 2009; Vilnet et al. 2010). According to the classification of Crandall-Stotler et al. (2009), established using morphological and phylogenetic data, the Jungermanniaceae includes *Eremonotus* Lindb. et Kaal. ex Pearson, *Hattoriella* (Inoue) Inoue, *Jungermannia* L., *Leiocolea* (Müll. Frib.) H. Buch and *Mesoptychia* (Lindb.) A. Evans. Recently, *Hattoriella* and *Leiocolea* were united under *Mesoptychia* (Váňa et al. 2012), based on previous evidence supporting the embedded position of *Mesoptychia* in *Leiocolea* (Yatsentyuk et al. 2004; De Roo et al. 2007; Hentschel et al. 2007; Vilnet et al. 2010, 2011). The limited taxonomic sampling of these studies has so far not provided a clear phylogenetic circumscription of *Mesoptychia* or further insights into its infrageneric relationships. For instance, the phylogenetic position of *Gymnocolea borealis* (Frisvoll et Moen) R.M. Schust., previously treated as *Leiocolea borealis* (Frisvoll et Moen) L. Söderstr. had not been assessed. Additionally, *Mesoptychia fitzgeraldiae* (Paton et A.R. Perry) L. Söderstr. et Váňa, *M. morrisoncola* (Horik.) L. Söderstr. et Váňa, *M. turbinata* (Raddi) L. Söderstr. et Váňa and *M. heterocolpos* var. *arctica* (S.W. Arnell) L. Söderstr. et Váňa had not previously been sequenced. The aim of the present study is to infer the monophyly of *Mesoptychia,* using a larger sampling of species, and to establish its systematic position within the Jungermanniales. The molecular analyses conducted herein contribute to the taxonomic revision of *Mesoptychia* that is currently underway.

Materials and methods

Plant material and sampling

Ten species of *Mesoptychia* and one variety out of 17 specific accepted names by Váňa et al. (2012), together with 57 other species of leafy liverworts were sampled, representing a total of 116 novel sequences. A complete sampling of the species of *Mesoptychia* was not possible due to the unavailability of material or to the poor quality of the DNA obtained from old herbarium specimens. The data set was completed with sequences obtained from Genbank for 39 species from the Jungermanniales (incl. members of the Jungermanniineae, Lophocoleineae, Cephaloziineae and Perssoniellineae) and six representatives of Porellales selected for the outgroup. Voucher data and Genbank accession numbers of the taxa sampled are listed in the Table 1.

DNA extraction, amplification and sequencing

Whole DNA was extracted from silica gel-dried plant tissue, excepting a few samples taken from herbarium specimens, using either a modified miniprep CTAB method of Doyle and Doyle (1987) or the DNA NucleoSpin® Plant II kit, according to the protocol provided by manufacturer. For the CTAB method, tissues were ground in a Qiagen mixer mill and incubated for 30 min at 65 °C in CTAB buffer (0.2 % β-mercaptoethanol and 0.1 % polyvinylpolypyrrolidone). Two chloroform isoamyl alcohol extractions preceded DNA precipitation by ice-cold isopropanol. After being washed with 76 % ethanol and 10 mM ammonium acetate, the resulting pellet was eluted in 50 µl of Tris-EDTA.

Two chloroplast markers were amplified and sequenced, the barcoding fragment of *rbc*L (Hollingsworth et al. 2009) and the protein-coding gene *rps*4. Primers used for each marker were *rbc*La_f (Kress and Erickson 2007), *rbc*La_rev 5′ GTA AAA TCA AGT CCA CCR CG (Forrest pers. comm.), *rps*5 (Nadot et al. 1994), and *trna*S (Baker in Cox et al. 2000). PCR amplifications were carried out using a 20 µl volume PCR reaction that included: 2 µl FastStart Taq buffer + $MgCl_2$, 0.4 µl dNTP 10 mM each, 1 µl of each primer at 10 mM, 0.15 µl FastStart TaqPolymerase, 0.2 µl BSA 5 %, and 1 µl DNA. Amplified fragments were purified with ExoSAP IT (GE Healthcare). For *rbc*L and *rps*4 amplifications consisted of an initial 4 min pre-melt at 95 °C and 35 cycles of 1 min denaturation at 95 °C, 1 min annealing at 50 °C and 1 min 30 extension at 72 °C, followed by a final extension of 6 min at 72 °C. Cycling reactions were performed using a Biometra® T3 Thermocycler. Sequencing reactions contained 0.5 µl of purified product, 1 µl of one primer 1 mM, 1 µl of BigDye® Terminator v.3.1 buffer, 0.5 µl of BigDye® Terminator v.3.1 (Applied Biosystems) in a total reaction volume of 5 µl. Samples were run on a ABI PRISM 377 automated sequencer (Applied Biosystems). Electropherograms were assembled and edited using Sequencher 4.7 (Gene Codes Corporation, Ann Arbor, MI).

Phylogenetic analyses

Sequence matrices were aligned with MUSCLE (Edgar 2004) and manually adjusted in MacClade 4.08 (Maddison and Maddison 2005). Analyses of each individual marker were performed and compared to verify that they were congruent. Phylogenetic reconstructions were performed using the Maximum parsimony (MP) method in PAUP* version 4.0b10 (Swofford 2002) and the Bayesian inference (BI) in MrBayes version 3.2.1 (Ronquist et al. 2012). Heuristic parsimony searches were conducted with 1,000 random addition sequence replicates using TBR branch swapping and five trees were saved per replicate. Gaps were treated as missing. Parsimony bootstrap support values (BS) were calculated using a heuristic search with 1,000 replicates, each with 10 random addition sequences replicates using TBR branch swapping and five trees saved at each replicate. In the BI, the dataset was partitioned by locus (*rbc*L and *rps*4). For each partition, the selected model was GTR and all parameters were unlinked between the two partitions. Two independent runs with eight chains each were performed on 5×10^6 generations. Trees were sampled every 100 generations. The burn-in period was set at 1.25×10^6 generations, when the stationary likelihood value was reached among the runs (verified on the likelihoods plots of each run). Accordingly, the first 12,500 samples were discarded for each run on the total number of sampled trees. The posterior probability distribution was estimated from the 75,002 remaining trees and then summarized in a majority rule consensus tree.

Results

The combined dataset consisted of 1,131 characters (558 for *rbc*L and 573 for *rps*4) out of which 496 are parsimony-informative (44 %), 109 are variable and 526 are constant. The MP analysis retrieved 395 most parsimonious trees with length = 3292 (Consistency Index = 0.289 and Retention Index = 0.620). The average standard deviation of split frequency between the two runs of the BI was 0.00401. Topologies obtained with the BI and the MP methods are highly congruent. The Bayesian topology is presented in Fig. 1 with the posterior probability values (PP) and MP boostrap values (BS). The Jungermanniales, including *Schistochila appendiculata* Dumort. (Perssoniellineae), is

Table 1 Voucher specimen information and Genbank accession numbers

Taxon	Origin	Voucher	Accession number	
			*rbc*L	*rps*4
Acrobolbus ciliatus (Mitt.) Schiffn.	China	Long 33833 (E)	JX305531*	JX308552*
Adelanthus lindenbergianus (Lehm.) Mitt.	Argentina	Long 31828 (E)	JX305532*	JX308553*
Anastrophyllum michauxii (F. Weber) H. Buch	USA	Sargent s.n. (ABSH)	AY507390	AY507433
Anastrophyllum minutum (Schreb.) R.M. Schust.	Switzerland	Cailliau et al. 744 (G)	JX305533*	JX308554*
Anthelia julacea (L.) Dumort.	Nepal	Long 31292	DQ026581	–
A. julacea (L.) Dumort.	Canada	Schofield and Schofield 102042 (DUKE)	–	AY608044
Apomarsupella rubida (Mitt.) R.M. Schust.	China	Long 34462 (E)	JX305534*	JX308555*
Arnellia fennica (Gottsche) Lindb.	Finland	Virtanen B070274 (OULU)	JX305535*	JX308556*
Balantiopsis cancellata (Nees) Steph.	Chile	Hyvönen 5946 (H)	AY462286	AY462340
Balantiopsis diplophylla (Hook. f. et Taylor) Mitt.	New Zealand	Engel 20853 (GOET)	DQ312476	–
B. diplophylla (Hook. f. et Taylor) Mitt.	Australia	Streimann 59554 (NY)	–	AY608047
Barbilophozia barbata (Schmidel ex Schreb.) Loeske	Switzerland	Cailliau et al. 730 (G)	JX305536*	JX308557*
Barbilophozia hatcheri (A. Evans) Loeske	Norway	Hentschel Bryo 492 (GOET)	DQ312478	–
B. hatcheri (A. Evans) Loeske	Norway	Söderström 2003/001	–	AM398338
Barbilophozia sp.	Switzerland	Cailliau 850 (G)	JX305573*	JX308594*
Blepharidophyllum densifolium (Hook.) Ångström ex C. Massal.	Argentina	Long 31696 (E)	JX305538*	JX308559*
Calypogeia integristipula Steph.	Finland	He-Nygrén and Piippo 1472	AY462290	–
C. integristipula Steph.	Finland	Söderström and P. Manyanga 2003/090	–	AM398361
Calypogeia muelleriana (Schiffn.) Müll.Frib.	UK	Long 36638 (E)	JX305539*	JX308560*
Cephalozia bicuspidata (L.) Dumort.	UK	Long 36969 (E)	JX305540*	JX308561*
Cephaloziella divaricata (Sm.) Schiffn.	UK	Long 31924 (E)	JX305541*	JX308562*
Cephaloziella hirta Steph.	Australia	Streimann 59793 (NY)	DQ439682	AY608054
Cephaloziella varians (Gottsche) Douin	New Zealand	Engel 21964 (F)	DQ439689	–
C. varians (Gottsche) Douin	Norway	Söderström et al. 2004/365	–	AM398310
Chaetophyllopsis whiteleggei (Carrington et Pearson) R.M. Schust.	Australia	Curnow 4804 (H)	AY462292	AY462346
Cladopodiella fluitans (Nees) H. Buch	Sweden	Cailliau and Hallingbäck 1022 (G)	JX305542*	JX308563*
Delavayella serrata Steph.	China	Long 35978 (E)	JX305543*	JX308564*
Diplophyllum albicans (L.) Dumort.	Switzerland	Cailliau 865 (G)	JX305544*	JX308565*
Diplophyllum obtusifolium (Hook.) Dumort.	USA	Wheeler s.n. [ref. no. 351] (ABSH)	AY507397	AY507439
Gackstroemia magellanica (Lam.) Trevis.	Argentina	Hyvönen 3079 (H)	AY462295	AY462350
Geocalyx caledonicus Steph.	Not given	Qiu and Braggins NZ-03142 (AK, MICH)	DQ787463	DQ787471
Geocalyx graveolens (Schrad.) Dumort.	China	Long 33739 (E)	JX305546*	JX308567*
Gongylanthus ericetorum (Raddi) Nees	Portugal	Long 36610 (E)	JX305547*	JX308568*
Gongylanthus himalayensis Grolle	Bhutan	Long 28706 (E)	JX305548*	JX308569*
G. borealis (Frisvoll et Moen) R.M. Schust.	Sweden	Cailliau and Hallingbäck 1032 (G)	JX305563*	JX308584*
Gymnocolea inflata (Huds.) Dumort.	UK	Cailliau et al. 888 (G)	JX305549*	JX308570*
Gymnomitrion concinnatum (Lightf.) Corda	Canada	Davis 424 (DUKE)	DQ439686	AY608065
Gymnomitrion corallioides Taylor ex Carrington	Norway	Hentschel Bryo 458 (GOET)	DQ312491	–
G. corallioides Taylor ex Carrington	Norway	Söderström et al. 2004/270	–	AM398302
Gymnomitrion crenulatum Gottsche ex Carrington	UK	Long 36682 (E)	JX305550*	JX308571*
Gyrothyra underwoodiana M. Howe	Canada	Forrest 593 (ABSH)	DQ268970	DQ268985
Harpanthus flotovianus (Nees) Nees	Norway	Long 31355 (E)	JX305551*	JX308572*

Table 1 continued

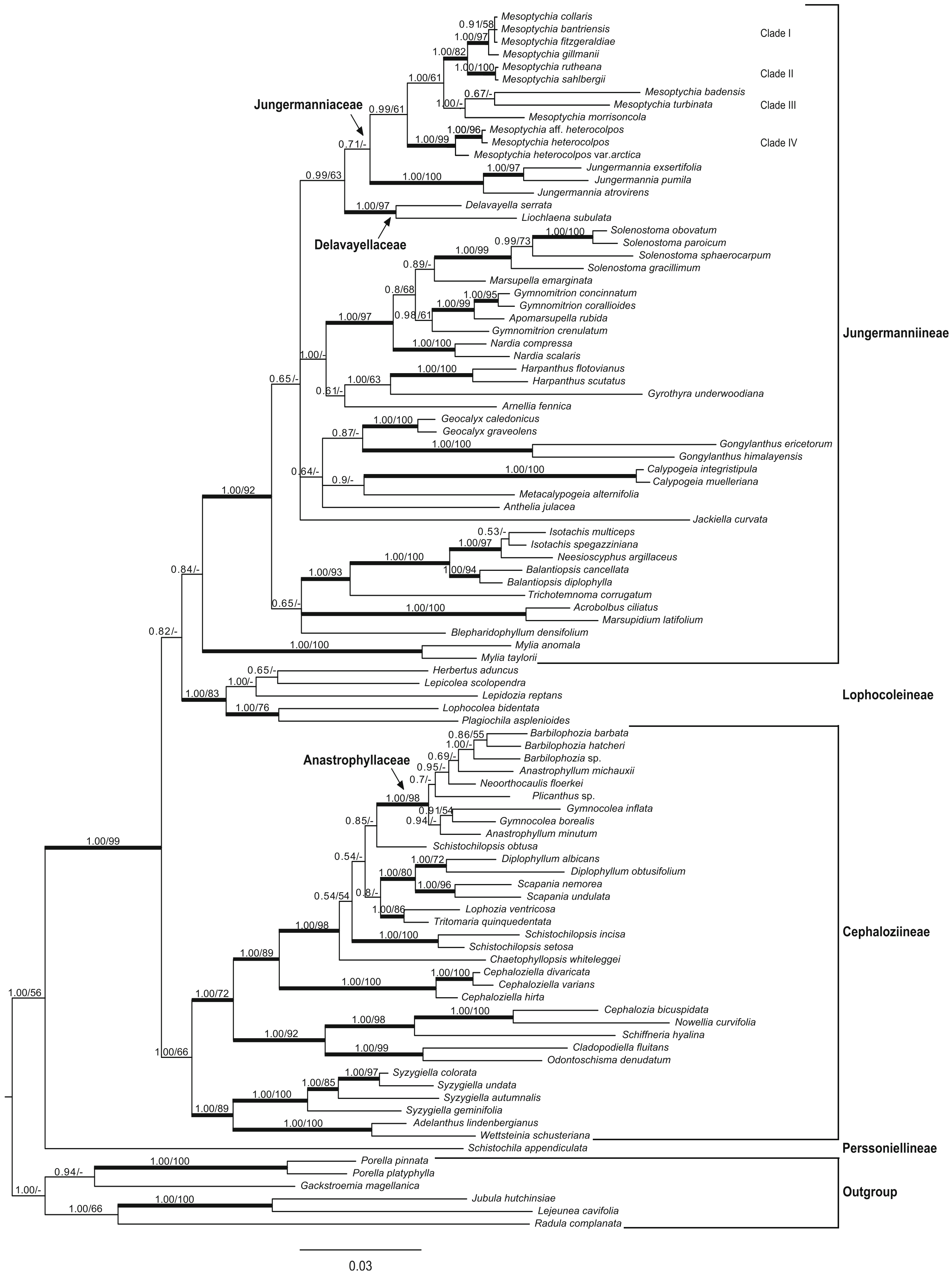
Taxon	Origin	Voucher	Accession number	
			*rbc*L	*rps*4
Harpanthus scutatus (F. Weber et D.Mohr) Spruce	USA	Risk et al. 10341 (DUKE)	DQ787464	–
H. scutatus (F. Weber et D.Mohr) Spruce	USA	Risk et al. 10341 (DUKE)	–	AY608069
Herbertus aduncus (Dicks.) Gray	Japan	Tsubota 4398 (HIRO)	AB476568	–
H. aduncus (Dicks.) Gray	Ireland	Long 33458 (E)	–	AM398291
Isotachis multiceps (Lindenb. et Gottsche) Gottsche	Panama	Stotler and Crandall-Stotler 3478 (ABSH)	AY507407	AY507449
Isotachis spegazziniana C. Massal.	Argentina	Long 31705 (E)	JX305552*	JX308573*
Jackiella curvata Allison et E.A. Hodgs.	Not given	Qiu et al. NZ-03064 (AK, MICH)	DQ787461	DQ787467
Jubula hutchinsiae (Hook.) Dumort. subsp. *javanica* (Steph.) Verd.	Japan	Kodama s.n. (ABSH)	AY507408	AY688794
Jungermannia atrovirens Dumort.	Switzerland	Cailliau 836 (G)	JX305555*	JX308576*
Jungermannia exsertifolia Steph.	UK	Cailliau et al. 886 (G)	JX305556*	JX308577*
Jungermannia pumila With.	UK	Cailliau et al. 885 (G)	JX305557*	JX308578*
Lejeunea cavifolia Steph.	Germany	Heinrichs 3695	AY548102	–
L. cavifolia Steph.	Not given	Qiu 1051 (MICH)	–	DQ787470
Lepicolea scolopendra (Hook.) Bastow	Australia	Streimann 55445 (H)	AY462308	AY462365
Lepidozia reptans (L.) Dumort.	Germany	Hentschel and Wilson Bryo 1601 (GOET)	AM392313	AY608083
Liochlaena subulata (A. Evans) Schljakov	China	Long 35240 (E)	JX305571*	JX308592*
Lophocolea bidentata (L.) Dumort.	UK	Long 37604 (E)	JX305572*	JX308593*
Lophozia ventricosa (Dicks.) Dumort.	Switzerland	Cailliau 807 (G)	JX305576*	JX308597*
Marsupella emarginata (Ehrh.) Dumort.	UK	Cailliau et al. 891 (G)	JX305577*	JX308598*
Marsupidium latifolium R.M. Schust.	Costa Rica	Dauphin 2920 (NY)	AY608034	AY608088
Mesoptychia badensis (Gottsche ex Rabenh.) L. Söderstr. et Váňa	UK	Chamberlain and Flagmeier 242364 (E)	JX305561*	JX308582*
Mesoptychia bantriensis (Hook.) L. Söderstr. et Váňa	Switzerland	Cailliau et al. 770 (G)	JX305562*	JX308583*
M. collaris (Nees) L. Söderstr. et Váňa	Switzerland	Cailliau 800 (G)	JX305558*	JX308579*
M. fitzgeraldiae (Paton et A.R. Perry) L. Söderstr. et Váňa	UK	Rothero 883 (E)	JX305564*	JX308585*
M. gillmanii (Austin) L. Söderstr. et Váňa	UK	Long 24667 (E)	JX305565*	JX308586*
Mesoptychia heterocolpos (Thed. ex Hartm.) L. Söderstr. et Váňa	Switzerland	Cailliau et al. 750 (G)	JX305567*	JX308588*
M. heterocolpos var. *arctica* (S.W. Arnell) L. Söderstr. et Váňa	Sweden	Cailliau and Lönnell 986 (G)	JX305559*	JX308580*
Mesoptychia aff. *heterocolpos*	Sweden	Cailliau and Lönnell 985 (G)	JX305560*	JX308581*
Mesoptychia morrisoncola (Horik.) L. Söderstr. et Váňa	China	Long 33526 (E)	JX305568*	JX308589*
M. rutheana (Limpr.) L. Söderstr. et Váňa	Russia	Konstantinova and Schuster s.n. (G)	JX305569*	JX308590*
Mesoptychia sahlbergii (Lindb. et Arnell) A. Evans	USA	Long 11329 (E)	JX305578*	JX308599*
Mesoptychia turbinata (Raddi) L. Söderstr. et Váňa	Switzerland	Cailliau 844 (G)	JX305570*	JX308591*
Metacalypogeia alternifolia (Gottsche et al.) Grolle	China	Long 33734 (E)	JX305579*	JX308600*
Mylia anomala (Hook.) Gray	France	Cailliau and Burgisser 827 (G)	JX305580*	JX308601*
Mylia taylorii (Hook.) Gray	Norway	Long 38097 (E)	JX305581*	JX308602*
Nardia compressa (Hook.) Gray	Norway	Long 38098 (E)	JX305582*	JX308603*
Nardia scalaris Gray	Switzerland	Cailliau 808 (G)	JX305583*	JX308604*
Neesioscyphus argillaceus (Nees) Grolle	Brazil	Verwimp 11107 (GOET)	DQ312509	–
N. argillaceus (Nees) Grolle	Venezuela	Söderström et al. 2004/022	–	AM398235

Table 1 continued

Taxon	Origin	Voucher	Accession number	
			*rbc*L	*rps*4
Neoorthocaulis floerkei (F. Weber et D. Mohr) L. Söderstr. et al.	Switzerland	Cailliau 806 (G)	JX305537*	JX308558*
Nowellia curvifolia (Dicks.) Mitt.	Not given	in Qiu et al. 2006	DQ645976	–
N. curvifolia (Dicks.) Mitt.	USA	Gross 12220	–	AY608094
Odontoschisma denudatum (Mart.) Dumort.	Not given	in Qiu et al. 2006	DQ645977	–
O. denudatum (Mart.) Dumort.	Ireland	Long 29937 (E)	–	AM398325
Plagiochila asplenioides (L.) Dumort.	UK	Long 37615 (E)	JX305584*	JX308605*
Plicanthus sp.	China	He-Nygrén 492	AY462293	AY462347
Porella pinnata L.	USA	Mishler et al. 3773 (DUKE)	U87088	–
P. pinnata L.	USA	Goffinet 4744 (DUKE)	–	AY608101
Porella platyphylla (L.) Pfeiff.	UK	Long 37711 (E)	JX305585*	JX308606*
Radula complanata Dumort.	UK	Long 37710 (E)	JX305586*	JX308607*
Scapania nemorea (L.) Grolle	Switzerland	Cailliau 884 (G)	JX305587*	JX308608*
Scapania undulata (L.) Dumort.	Finland	He-Nygrén and Piippo 1468	AY149840	–
S. undulata (L.) Dumort.	Norway	Söderström et al. 2004/201	–	AM398286
Schiffneria hyalina Steph.	Japan	Mizutani 15961 (H)	AY462327	AY462393
Schistochila appendiculata Dumort.	New Zealand	Glenny 8537	AY462328	–
S. appendiculata Dumort.	New Zealand	Stotler and Crandall-Stotler 4455	–	AY507465
Schistochilopsis incisa (Schrad.) Konstantinova	Germany	Hentschel and Wilson Bryo 1593 (GOET)	AM392308	–
S. incisa (Schrad.) Konstantinova	Sweden	Söderström and Manyanga 2003/079	–	AM398366
Schistochilopsis obtusa (Lindb.) Potemkin	Switzerland	Cailliau 867 (G)	JX305574*	JX308595*
Schistochilopsis setosa (Mitt.) Konstantinova	China	Long 33720 (E)	JX305575*	JX308596*
Solenostoma gracillimum (Sm.) R.M. Schust.	UK	Cailliau et al. 890 (G)	JX305588*	JX308609*
Solenostoma obovatum (Nees) C. Massal.	Switzerland	Cailliau 859 (G)	JX305589*	JX308610*
Solenostoma paroicum (Schiffn.) R.M. Schust.	UK	Long 37819 (E)	JX305590*	JX308611*
Solenostoma sphaerocarpum (Hook.) Steph.	Switzerland	Cailliau 857 (G)	JX305591*	JX308612*
Syzygiella autumnalis (DC.) K. Feldberg et al.	Norway	Long 38130 (E)	JX305554*	JX308575*
Syzygiella colorata (Lehm.) K. Feldberg et al.	South Africa	Gremmen 93–093 (JE)	GQ900307	–
S. colorata (Lehm.) K. Feldberg et al.	South Africa	De Roo s.n.	–	AM398314
Syzygiella geminifolia (Mitt.) Steph.	Tanzania	Pócs et al. 90066/F 3192330 (H)	DQ026590 (DQ026591)[a]	DQ026607
Syzygiella undata (Mont.) K. Feldberg et al.	Bolivia	Churchill et al. 22807 (GOET)	AM392305	–
S. undata (Mont.) K. Feldberg et al.	Venezuela	Söderström et al. 2004/123	–	AM398249
Trichotemnoma corrugatum (Steph.) R.M. Schust.	New Zealand	Glenny 8426	AY462333	AY462399
Tritomaria quinquedentata (Huds.) H. Buch	Germany	Heinrichs 2978 (GOET)	AY700003	–
T. quinquedentata (Huds.) H. Buch	USA	Schofield 106093 (DUKE)	–	AY608119
Wettsteinia schusteriana Grolle	New Zealand	Glenny 8917	DQ026592 (DQ026593)[a]	DQ026610

An asterisk indicates newly generated sequences

[a] In He-Nygrén et al. (2006), the *rbc*L sequence of *S. geminifolia* is referred to under DQ026591 whereas in Genbank the accession number for this sequence is DQ026590. Likewise, for *W. schusteriana* the *rbc*L sequence is referred to as DQ026593 but the GenBank reference is DQ026592


0.91/58
Mesoptychia collaris
Mesoptychia bantriensis
1.00/97
Mesoptychia fitzgeraldiae
1.00/82
Mesoptychia gillmanii
Clade I
1.00/100
Mesoptychia rutheana
1.00/61
Mesoptychia sahlbergii
Clade II
0.67/-
Mesoptychia badensis
Jungermanniaceae
0.99/61
1.00/-
Mesoptychia turbinata
Clade III
Mesoptychia morrisoncola
1.00/96
Mesoptychia aff. heterocolpos
0.71/-
1.00/99
Mesoptychia heterocolpos
Clade IV
Mesoptychia heterocolpos var.arctica
1.00/97
Jungermannia exsertifolia
0.99/63
1.00/100
Jungermannia pumila
Jungermannia atrovirens
1.00/97
Delavayella serrata
Liochlaena subulata
Delavayellaceae
1.00/100
Solenostoma obovatum
0.99/73
Solenostoma paroicum
1.00/99
Solenostoma sphaerocarpum
0.89/-
Solenostoma gracillimum
Marsupella emarginata
0.8/68
1.00/95
Gymnomitrion concinnatum
1.00/99
Gymnomitrion corallioides
1.00/97
0.98/61
Apomarsupella rubida
Gymnomitrion crenulatum
Jungermanniineae
1.00/100
Nardia compressa
1.00/-
Nardia scalaris
1.00/100
Harpanthus flotovianus
0.65/-
1.00/63
Harpanthus scutatus
0.61/-
Gyrothyra underwoodiana
Arnellia fennica
1.00/100
Geocalyx caledonicus
0.87/-
Geocalyx graveolens
1.00/100
Gongylanthus ericetorum
Gongylanthus himalayensis
0.64/-
1.00/100
Calypogeia integristipula
0.9/-
Calypogeia muelleriana
1.00/92
Metacalypogeia alternifolia
Anthelia julacea
Jackiella curvata
0.53/-
Isotachis multiceps
1.00/97
Isotachis spegazziniana
1.00/100
Neesioscyphus argillaceus
0.84/-
1.00/93
1.00/94
Balantiopsis cancellata
Balantiopsis diplophylla
Trichotemnoma corrugatum
0.65/-
1.00/100
Acrobolbus ciliatus
Marsupidium latifolium
0.82/-
Blepharidophyllum densifolium
1.00/100
Mylia anomala
Mylia taylorii
0.65/-
Herbertus aduncus
1.00/-
Lepicolea scolopendra
1.00/83
Lepidozia reptans
Lophocoleineae
1.00/76
Lophocolea bidentata
Plagiochila asplenioides
0.86/55
Barbilophozia barbata
1.00/-
Barbilophozia hatcheri
Anastrophyllaceae
0.69/-
Barbilophozia sp.
0.95/-
Anastrophyllum michauxii
0.7/-
Neoorthocaulis floerkei
1.00/98
Plicanthus sp.
0.91/54
Gymnocolea inflata
0.85/-
0.94/-
Gymnocolea borealis
Anastrophyllum minutum
1.00/99
Schistochilopsis obtusa
0.54/-
1.00/72
Diplophyllum albicans
1.00/80
Diplophyllum obtusifolium
0.54/54
0.8/-
1.00/96
Scapania nemorea
Scapania undulata
1.00/86
Lophozia ventricosa
1.00/98
Tritomaria quinquedentata
1.00/100
Schistochilopsis incisa
Cephaloziineae
1.00/89
Schistochilopsis setosa
Chaetophyllopsis whiteleggei
1.00/100
Cephaloziella divaricata
1.00/100
Cephaloziella varians
1.00/56
1.00/72
Cephaloziella hirta
1.00/100
Cephalozia bicuspidata
1.00/98
Nowellia curvifolia
1.00/92
Schiffneria hyalina
1.00/99
Cladopodiella fluitans
1.00/66
Odontoschisma denudatum
1.00/97
Syzygiella colorata
1.00/85
Syzygiella undata
1.00/100
Syzygiella autumnalis
1.00/89
Syzygiella geminifolia
1.00/100
Adelanthus lindenbergianus
Wettsteinia schusteriana
Schistochila appendiculata
Perssoniellineae
1.00/100
Porella pinnata
0.94/-
Porella platyphylla
1.00/-
Gackstroemia magellanica
Outgroup
1.00/100
Jubula hutchinsiae
1.00/66
Lejeunea cavifolia
Radula complanata
0.03

◀ **Fig. 1** Bayesian 50 % majority rule consensus tree of the Jungermanniales resulting from the combined analyses of *rbc*L and *rps*4. *Gray boxes* represent members of *Mesoptychia* (Clades I–IV) and *G. borealis*. *Values* above the branches are the Bayesian posterior probability (PP) and Maximum parsimony bootstrap values (BS), when higher than 50 %. Branches in *bold* have PP ≥0.95 and BS ≥80

supported with PP = 1.00 and BS = 56 although the relationships between the sub-orders are not fully resolved. The Cephaloziineae is well supported by the BI (PP = 1.00, BS = 66), the Lophocoleineae forms a consistent clade in both analyses (PP = 1.00, BS = 83) and the Jungermanniineae, including *Mylia*, is unresolved (PP = 0.84). The Jungermanniaceae and Delavayellaceae form a single clade (PP = 0.99, BS = 63). *Gymnocolea borealis* is nested in the Anastrophyllaceae (PP = 1.00, BS = 98), where it is sister to *G. inflata* (Huds.) Dumort. The genus *Mesoptychia* constitutes a well-supported lineage according to the BI (PP = 0.99), whereas the corresponding MP support is low (BS = 61). Four groups are distinguished within *Mesoptychia,* named Clade I, II, III, and IV, respectively. Clade I (PP = 1.00 and BS = 97) is composed of *M. collaris* (Nees) L. Söderstr. et Váňa*, M. bantriensis* (Hook.) L. Söderstr. et Váňa, *M. fitzgeraldiae* and *M. gillmanii* (Austin) L. Söderstr. et Váňa. Clade II (PP = 1.00, BS = 100) includes *M. sahlbergii* (Lindb. et Arnell) A. Evans, the type species of *Mesoptychia*, and *M. rutheana* (Limpr.) L. Söderstr. et Váňa. Clade III comprises *M. badensis* (Gottsche ex Rabenh.) L. Söderstr. et Váňa, *M. turbinata* and *M. morrisoncola* according to the BI (PP = 1.00). Clade IV, containing *M. heterocolpos* (Thed. ex Hartm.) L. Söderstr. et Váňa, *M.* aff. *heterocolpos* and *M. heterocolpos* var. *arctica*, is well-supported (PP = 1.00 and BS = 99) and forms a sister group to the other species of *Mesoptychia*.

Discussion

The resolution of the Jungermanniineae, Lophocoleineae, Cephaloziineae, and Perssoniellineae is congruent with previous studies (Forrest et al. 2006; He-Nygrén et al. 2006; Heinrichs et al. 2007; Hentschel et al. 2007). *Gymnocolea borealis*, previously combined under *Leiocolea* by Söderström (1981), is resolved in the Anastrophyllaceae. Its affinity with *G. inflata* agrees with Schuster (1986), who first identified morphological similarities between these two species (plicate perianth, *Frullania* branching-type, non-convex trigones, distant almost horizontally orientated leaves, obtuse lobes and female bracts smaller than the leaves).

The clustering of *Mesoptychia, Jungermannia* and the Delavayellaceae in a single monophyletic group is consistent with previous studies (De Roo et al. 2007; Heinrichs et al. 2007; Crandall-Stotler et al. 2009; Vilnet et al. 2010, 2011). The ten species and one variety of *Mesoptychia* included herein form a monophyletic lineage within the Jungermanniaceae. Four major clades are resolved within *Mesoptychia*. Within Clade I, *M. collaris, M. bantriensis* and *M. fitzgeraldiae,* form a monophyletic group. Morphologically, *M. collaris* and *M. bantriensis* are essentially differentiated by the size of their mid-leaf cells and by the degree of the division of their leaves (Smith 1990; Paton 1999; Nebel and Philippi 2005; Frey et al. 2006). These characters are variable, between different specimens and sometimes even within the same specimen, as mentioned by Váňa and Hubáčková (1989), who suggested that *M. collaris* and *M. bantriensis* were conspecific. *Mesoptychia fitzgeraldiae*, characterized by its dentate bract and sometimes dentate leaf margins, was considered to be close to both *M. collaris* and *M. bantriensis* when it was described (Paton and Perry 1995). These three species appear to represent a range of variability within one taxon and the possible synonymy of *M. collaris* and *M. fitzgeraldiae* with *M. bantriensis* requires taxonomic confirmation. *Mesoptychia gillmanii* is sister to the three above-mentioned species and is morphologically distinguished from them by its paroicous sexual condition and its frequent expression of sexual organs (Paton 1999; Frey et al. 2006). *Mesoptychia rutheana* and *M. sahlbergii* (Clade II) are the largest species in the genus (plants up to 55 mm long and 5 mm wide) and they share the morphological characters of large and ciliated underleaves, conspicuous trigones and a strongly papillose cuticle.

Mesoptychia morrisoncola, previously treated under *Hattoriella*, is sister to *M. badensis* and *M. turbinata* (Clade III). The phylogenetic position of *Hattoriella* as nested within *Mesoptychia* is confirmed herein (Yatsentyuk et al. 2004). *Mesoptychia morrisoncola*, *M. badensis* and *M. turbinata* all lack underleaves. The latter two species, amongst the smallest in the genus (plants up to 15 mm long and 2.5 mm wide), are morphologically very similar. However, they represent two distinct species that can be identified by differences in their leaf insertion and the presence/absence of trigones: short leaf insertion and the absence of trigones in *M. turbinata* versus long leaf insertion and the presence of trigones in *M. badensis* (Paton 1999; Frey et al. 2006). Clade IV, composed of *M. heterocolpos, M.* aff. *heterocolpos* and *M. heterocolpos* var. *arctica* ("*M. heterocolpos*" clade), is sister to the rest of the *Mesoptychia* species (*Mesoptychia s.s.*) which is in agreement with the findings of Yatsentyuk et al. (2004) and De Roo et al. (2007). The distinction of the "*M. heterocolpos*" clade from *Mesoptychia s.s.* is supported morphologically by the production of gemmae, a feature that is not seen in any other species of *Mesoptychia* (Smith 1990; Paton 1999; Nebel and Philippi 2005; Frey et al. 2006). The specimen identified as *M.* aff. *heterocolpos* has morphological characteristics that overlap with both *M. heterocolpos* and *M. heterocolpos var. arctica*: it has gemmiferous shoots as *M. heterocolpos* and it has the red coloration of *M. heterocolpos* var. *arctica* as described by Arnell (1956) and mentioned in Damsholt (2002). *Mesoptychia* aff. *heterocolpos* is resolved herein as sister to *M. heterocolpos*, underlining the uncertainty of the morphological character traits previously used to separate *M. heterocolpos* and *M. heterocolpos* var. *arctica*. The question of whether *M. heterocolpos* var. *arctica* and *M. heterocolpos* should be considered as single or separate entities, as well as the possible segregation of this clade from *Mesoptychia s.s.*, requires a more complete within-genus sampling and a detailed morphological investigation.

Findings outlined within the present phylogenetic analyses, specifically the taxonomic hypotheses concerning the synonymy of *M. collaris, M. fitzgeraldiae* with *M. bantriensis* from Clade I and the systematic rank of "*M. heterocolpos"* (Clade IV), are currently being treated in the context of a systematic revision of *Mesoptychia*.

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