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

CAILLIAU, Ariane, et al.

Abstract

A phylogenetic analysis of the leafy liverwort genus Mesoptychia (incl. Leiocolea and Hattoriella), using two chloroplast markers, rbcL and rps4, 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.

Reference


DOI: 10.1007/s00606-013-0792-z
Phylogeny and systematic position of *Mesoptychia* (Lindb.)
A. Evans

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Received: 2 November 2012/ Accepted: 6 March 2013/Published online: 2 April 2013
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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. *Gymnocollea 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

**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 *Gymnocollea 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, Cephaloziniinae 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 *rbcL* (Hollingsworth et al. 2009) and the protein-coding gene *rps4*. Primers used for each marker were *rbcLa_f* (Kress and Erickson 2007), *rbcLa_rev 5’amplified* (Hollingsworth et al. 2009) and *rps4* (Nadot et al. 1994), and *trnS* (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 + MgCl2, 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 *rbcL* and *rps4* 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 Big-Dye® Terminator v.3.1 (Applied Biosystems) in a total reaction volume of 5 μl. Samples were run on an 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 (*rbcL* and *rps4*). 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 × 10⁶ generations. Trees were sampled every 100 generations. The burn-in period was set at 1.25 × 10⁵ generations, when the stationary likelihood value was reached among the runs (verified on the likelihood 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 *rbcL* and 573 for *rps4*) 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 bootstrap values (BS). The Jungermanniales, including *Schistochila appendiculata* Dumort. (Perssoniellineae), is
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<td><em>Neoorthocaulis floerkei</em> (F. Weber et D. Mohr) L. Södstr. et al.</td>
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An asterisk indicates newly generated sequences

* In He-Nygren et al. (2006), the rbcL sequence of *S. guminifolia* is referred to under DQ026591 whereas in Genbank the accession number for this sequence is DQ026590. Likewise, for *W. schusteriana* the rbcL sequence is referred to as DQ026593 but the GenBank reference is DQ026592
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). Gymnocollea 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. baniensis (Hook.) L. Söderstr. et Váňa, M. fitzgeraldiae and M. gillmani (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). Gymnocollea 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 trinques, 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. baniensis 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. baniensis 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. baniensis requires taxonomic confirmation. Mesoptychia gillmani is sister to the three above-mentioned species and is morphologically distinguished from them by its parocious 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 trinques and a strongly papillosel cuticle.

Mesoptychia morrisoncola, previously treated under Hattoriella, is sister to M. badensis and M. turbinata (Clade III). The phylogenetic position of Hattoriella is nested within Mesoptychia (see nested 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 trinques: short leaf insertion and the absence of trinques in M. turbinata versus long leaf insertion and the presence of trinques 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. baniensis* 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*.

**Acknowledgments** The first author would like to thank SYN- THESYS for financing her visit to the Royal Botanic Garden Edinburgh, UK (RBGE), RBGE and its staff for their collaboration and supply of specimens, the Conservatoire et Jardin botaniques de la Ville de Genève, Switzerland (CJBG) for the laboratory facilities, the staff of the CJBG laboratory for their assistance, Dr. Yamama Naciri (CJBG) for her advice and support, the Association des Amis du Jardin Botanique for financing field trips in the Swiss Alps, the Bourge Aubrunin Lombard for financing field work in Sweden, the Abisko Scientific Research Station for funding a research visit, Dr. Higuchi (TNS) and Dr. Risto Virtanen (OULU) for specimens, and Drs. Nadir Alvarez (University of Lausanne, Switzerland), Louis Nusbaumer (CJBG) and Camille Truong (CJBG) for their advice and discussions. We thank the two anonymous reviewers for their critical comments.

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