

Transfer of the leafy liverwort *Xenochila* from Plagiochilaceae (Lophocoleineae) to Jungermanniaceae (Jungermanniineae)

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Abstract *Xenochila* is a monospecific genus that has not yet been included in molecular phylogenies. Based on morphology it has been aligned with Plagiochilaceae. A chloroplast DNA phylogeny places *Xenochila* within Jungermanniaceae in a robust sister relationship with the monospecific genus *Delavayella*. Though outwardly disparate in form, *Delavayella* and *Xenochila* share the presence of multicellular parenchymatous propagules forming singly from leaf margins of specialized gemmiparous shoots, a papillose gametophyte surface, nearly undifferentiated stem cells, absence of underleaves and bracteoles, weakly to distinctly bifid female bracts that are slightly larger than the leaves, and long cylindrical perianths with a dentate mouth. With the addition of *Xenochila*, Jungermanniaceae include the genera *Delavayella*, *Eremonotus*, *Jungermannia*, *Liochlaena* and *Mesoptychia*.

Keywords Chloroplast DNA · *Delavayella* · Jungermanniales · Marchantiophyta · Molecular phylogeny · *Syzygiella*

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Introduction

Molecular phylogenetic studies greatly improved the classification of liverworts (Crandall-Stotler et al. 2009) and culminated in the recent publication of a global species checklist reflecting current knowledge on systematic relationships (Söderström et al. 2016). However, despite comprehensive efforts to build the liverwort tree of life (e.g., Shaw et al. 2015) it has not yet been possible to include all currently accepted genera in molecular studies.

One such example is the monospecific Asian genus *Xenochila* R.M.Schust. Schuster (1959) described *Xenochila* based on its septate rhizoids and ovoid propagules developing singly from marginal teeth of young leaves, and treated *Xenochila* as a member of Plagiochilaceae. However, Schuster's concept of this family was broad enough to include also *Leptoscyphus* Mitt. (Lophocoleaceae, Hentschel et al. 2006); *Mylia* S.F.Gray (Myliaceae, Heinrichs et al. 2007) and *Syzygiella* Spruce (Adelanthaceae, Feldberg et al. 2010b). Although Schuster's concept of Plagiochilaceae is now largely rejected, *Xenochila* remains accepted as a member of Plagiochilaceae (Inoue 1963; Crandall-Stotler et al. 2009; Singh et al. 2015; Söderström et al. 2016). In the dissenting opinion, Inoue (1963) pointed to morphological similarities of *Xenochila* with *Jamesoniella* (now considered a synonym of *Syzygiella*, Feldberg et al. 2010a) and *Jungermannia* L. This view was shared by Patzak et al. (2016) who considered the undifferentiated stem cells of *Xenochila* and the rhizoids restricted to ventral leaf bases untypical for Plagiochilaceae.

Here we test the different hypotheses by presenting a chloroplast DNA phylogeny of Jungermanniales.

Materials and methods

DNA extraction, PCR amplification and sequencing

Gametophytical plant tissue was isolated from a herbarium specimen of *Xenochila integrifolia* (Mitt.) Inoue (Hepaticae Japonicae Exsiccatae Ser. 15, 750, M) and used to extract genomic DNA with the Invisorb Spin Plant Mini Kit (Invitex, Berlin).

PCR reactions were carried out with 0.25 μ L MyTaq DNA Polymerase (Bioline Reagents Ltd, UK), 10 μ L reaction buffer, 1 μ L of upstream primer (10 μ M), 1 μ L of downstream primer (10 μ M) and 1.5 μ L of template DNA. This mix was filled up with double distilled water to a total volume of 50 μ L. Amplification of *rps4* used the primers *rps5-F* (Nadot et al. 1994) and *trnS-R* (Taberlet et al. 1991). Temperature profile for *rps4* was 92 °C for 10 min, followed by 35 cycles of 92 °C for 60 s, 52 °C for 60 s, and 72 °C for 150 s. A final extension step of 72 °C for 10 min completed the thermal cycle.

For amplification of *rbcL* a nested PCR was carried out. Temperature profile for both PCR reactions was 95 °C for 2 min, followed by 30 cycles of 95 °C for 60 s, 51 °C for 50 s, and 72 °C for 90 s. A final extension step of 72 °C for 10 min completed the thermal cycle. The primer pairs used for the nested *rbcL* PCR were *rbcL1-PI-F* and *rbcL-M1390-R* (Wilson et al. 2004) for the first step using genomic DNA, and *rbcL-M38F* (Wilson et al. 2004) and *rbcL-1379-PI-R* (Groth and Heinrichs 2005) in the second PCR seeded with 1.5 μ L of the product from PCR 1.

Sequencing was carried out on an ABI 3730 capillary sequencer using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). Primers used for PCR amplification were also used for sequencing. The new sequences were assembled and edited in CodonCode Aligner 5.0.1 (CodonCode Corp., Dedham, MA, USA).

Phylogenetic analyses

First, the *rbcL* and *rps4* sequences of *Xenochila* were compared with GenBank sequences using the BLASTN program (Altschul et al. 1990). The BLAST searches suggested an affiliation of *Xenochila* to Jungermanniales suborder Jungermanniineae. Based on the phylogenetic hypotheses of Heinrichs et al. (2007), Cailliau et al. (2013), Sun et al. (2014) and Shaw et al. (2015), sequences of Jungermanniales suborders Jungermanniineae, Cephaloziineae and Lophocoleineae were downloaded from Genbank. Two representatives of Jungermanniales suborder Perssoniellineae were chosen as outgroup (Table 1).

All sequences were aligned manually in Bioedit version 7.0.5.2 (Hall 1999). Lacking parts of sequences were coded as missing. Maximum likelihood (ML) inference was carried out using RAxML 8.1.2 (Stamatakis 2014; <http://sco.h-its.org/exelixis/web/software/raxml/#documentation>) as implemented in raxmlGUI 1.5b1 (Silvestro and Michalak 2012; <http://sourceforge.net/projects/raxmlgui/>). The best-fit models of evolution were selected in jModelTest 2 (Darriba et al. 2012) under the Akaike information criterion (Akaike 1973) following the suggestions given by Posada (2008). Initial analyses were carried out for the two chloroplast DNA datasets. Since there was no statistically supported (>70 % bootstrap value) contradiction (Mason-Gamer and Kellogg 1996), the two datasets were concatenated, resulting in an alignment of 1910 nucleotides (Online Resource 1). ML analyses of the concatenated datasets were conducted using the GTR model (Tavaré 1986) with a proportion of invariable characters (I) and among-site rate heterogeneity modeled as a discrete gamma distribution with four rate categories and its estimated parameters (Γ), and a *rps4* and *rbcL* partition. Trees were generated by selecting ten independent runs and the multiparametric bootstrap option autoMRE resulting in 450 bootstrap replicates. ML bootstrap values (BV) of each node were visualized using FigTree 1.4 (<http://beast.bio.ed.ac.uk/figtree>). Clades with bootstrap values (BV) ≥ 70 % were considered to be well supported (Hillis and Bull 1993).

Morphological investigation

Specimens were studied using a Leica M50 dissection microscope and a Carl Zeiss AxioScope A1 compound microscope, the latter equipped with a Canon 60D digital camera. Incident and transmitted light were used simultaneously or separately. The presented illustrations (Fig. 2) are digitally stacked photomicrographic composites of moistened plants obtained with the software package HeliconFocus 6.5.1.

Results

The ML phylogeny (Fig. 1) includes three major clades corresponding to Jungermanniales suborders Cephaloziineae (BV 78), Lophocoleineae (BV 97) and Jungermanniineae (BV 100). *Xenochila integrifolia* is placed in the Jungermanniineae lineage and sister to *Delavayella serrata* Steph. (BV 99); this clade forms a sister relationship with *Liochlaena* Nees (BV 100). The *Delavayella* Steph.-*Liochlaena*-*Xenochila*-clade is placed sister to a clade containing *Eremonotus* and *Jungermannia* (BV 97); these genera form a sister relationship with *Mesoptychia* (Lindb.) A.Evans (BV 97).

Table 1 Taxa used in the present study, including information about the origin of the studied material, voucher information, as well as GenBank accession numbers

Taxon	Voucher	<i>rps4</i>	<i>rbcL</i>
<i>Acrobolbus ochrophyllus</i> (Hook.f. & Taylor) R.M.Schust.	Chile, <i>J.J. Engel 26633</i> (F)	KF851387	KF852285
<i>Albiellopsis parvifolius</i> (Steph.) R.M. Schust.	Japan, <i>Honshu, B. Shaw 8240</i> (DUKE)	KF851505	KF852411
<i>Anthelia julacea</i> (L.) Dumort.	Scotland, <i>D.G. Long 31292</i> (E)	KF851384	KF852280
<i>Arnellia fennica</i> (Gottsche) Lindb.	Finland, <i>B. Shaw 1250</i> (DUKE)	KF851493	KF852402
<i>Bazzania exempta</i> J.J.Engel	New Zealand, <i>P.J. de Lange 7881</i> (DUKE)	KF943540	KF943623
<i>Blepharidophyllum vertebrale</i> (Taylor ex Gottsche, Lindenb. et Nees) Ångstr. ex C.Massal.	New Zealand, <i>J.J. Engel and M. von Konrat s.n.</i> (F)	KF851454	KF852363
<i>Brevianthus flavus</i> (Grolle) J.J.Engel & R.M.Schust.	Tasmania, <i>Renner and Brown 5927</i> (NSW)	KT992643	KT992570
<i>Calypogeia muelleriana</i> (Schiffn.) Müll.Frib.	USA, <i>E.C. Davis 130</i> (DUKE)	AY608052	JF316291
<i>Cephalozia bicuspidata</i> (L.) Dumort.	Russia, <i>N.A. Konstantinova s.n.</i> (F)	KF943435	KF943563
<i>Cephalozia divaricata</i> (Sm.) Schiffn.	Czech Republic, <i>P. Sova s.n.</i> (DUKE)	KF851489	KF852399
<i>Chiasocaulon dendroides</i> (Nees) Carl	Malaysia, <i>O. Dürhammer 26</i> (GOET)	KT992641	KT992568
<i>Chiloscyphus martianus</i> (Nees) J.J.Engel & R.M.Schust. [<i>Cryptolophocolea martiana</i>]	São Tomé Island, <i>Shevock 39856</i> (JE)	KT992656	KT992584
<i>Cuspidatula monodon</i> Steph.	New Zealand, <i>J.J. Engel and M. von Konrat 28380</i> (F)	KF851455	KF852364
<i>Delavayella serrata</i> Steph.	(I) China, <i>D.G. Long 34809</i> (E)	KF851377	KF852269
<i>Delavayella serrata</i>	(II) China, <i>Long 35978</i> (E)	JX308564	JX305543
<i>Eocalypogeia quelpartensis</i> (S.Hatt. & Inoue) R.M.Schust.	Japan, <i>T. Furuki 20233</i> (HIRO)	AB476605	AB476566
<i>Eremonotus myriocarpus</i> (Carrington) Pearson	UK: Scotland, <i>B. Buryová 165/93</i> (DUKE)	KF851445	KF852354
<i>Geocalyx caledonicus</i> Steph.	New Zealand, <i>J.J. Engel and M. von Konrat 28342</i> (F)	KF943470	KF943584
<i>Goebelobryum unguiculatum</i> (Hook.f. & Taylor) Grolle	New Zealand: North Island, <i>Y. Qiu, M.A.M. Renner and R.M. Schuster NZ-03054</i> (AK)	JF513487	JF513466
<i>Gottschelia schizopleura</i> (Spruce) Grolle	Malaysia, <i>D.G. Long 36922</i> (E)	KF851453	KF852362
<i>Gymnomitrium alpinum</i> (Gottsche ex Husn.) Schiffn.	Scotland, <i>D.G. Long 38270</i> (DUKE)	KF851500	KF852406
<i>Gymnomitrium concinnum</i> (Lightf.) Corda	Svalbard, <i>N.A. Konstantinova A.N. Savchenko K158/8-07</i> (F)	KF943428	KF943557
<i>Harpanthus flotovianus</i> (Nees) Nees	Norway, <i>D.G. Long and D.B. Schill 31355</i> (E)	KF851379	KF852272
<i>Herbertus juniperoideus</i> subsp. <i>bivittatus</i> (Spruce) K.Feldberg & Heinrichs	Puerto Rico, <i>S. Schuette 203</i> (ABSH)	KF851457	KF852366
<i>Herzogobryum vermiculare</i> (Schiffn.) Grolle	Kerguelen Isls., <i>R. Ochyra 1011/06</i> (DUKE)	KF943479	KF943587
<i>Hygrobiella laxifolia</i> (Hook.) Spruce	Russia, <i>V.A. Bakalin s.n.</i> (F)	KF851422	KF852320
<i>Isotachis lyallii</i> Mitt.	New Zealand, <i>J.J. Engel 21825</i> (F)	AY608073	KF852343
<i>Jungermannia atrovirens</i> Dumort.	Switzerland, <i>Z. Hradílek s.n.</i> (DUKE)	KF851488	KF852398
<i>Jungermannia exsertifolia</i> subsp. <i>cordifolia</i> (Dumort.) Váňa	Alaska, <i>B. Shaw F951/5</i> (DUKE)	KF943484	KF943592
<i>Jungermannia pumila</i> With.	Norway, <i>P. Sova s.n.</i> (DUKE)	KF943504	KF943603
<i>Leptoscyphus jackii</i> (Steph.) Grolle	Ecuador, <i>A. Schäfer-Verwimp and M. Nebel 32769</i> (M)	KT992644	KT992571
<i>Lethocolea glossophylla</i> (Spruce) Grolle	Ecuador, <i>E.C. Davis 259</i> (DUKE)	AY608084	DQ439692
<i>Liochlaena lanceolata</i> Nees	USA: Oregon, <i>B. Shaw 6259a</i> (DUKE)	KF851471	KF852381
<i>Liochlaena subulata</i> (A.Evans) Schljakov	Czech Republic, <i>J. Košnar s.n.</i> (DUKE)	KF943498	KF943600
<i>Marsupella emarginata</i> (Ehrh.) Dumort.	Czech Republic, <i>P. Sova s.n.</i> (DUKE)	KF943507	KF943605
<i>Marsupella funckii</i> (F.Weber & D.Mohr) Dumort.	Russia, <i>N.A. Konstantinova A.N. Savchenko s.n.</i> (F)	KF943434	KF943562
<i>Mesoptychia bantriensis</i> (Hook.) L.Söderstr. & Váňa	Scotland, <i>D.G. Long 34172</i> (E)	JF513476	JF513458
<i>Mesoptychia gillmanii</i> (Austin) L.Söderstr. & Váňa	Russia, <i>N.A. Konstantinova and A.N. Savchenko 117-1-00</i> (F)	KF851410	KF852305
<i>Mesoptychia polymorpha</i> Stotler, Crand.-Stotl. & Bakalin	California, <i>W.T. Doyle 11541</i> (ABSH)	KF943468	KF943581
<i>Mesoptychia rutheana</i> (Limpr.) L.Söderstr. & Váňa	Canada, <i>B. Shaw F702</i> (DUKE)	KF943459	KF943573
<i>Mesoptychia sahlbergii</i> (Lindb. & Arnell) A.Evans	Russia, <i>V. Fedosov 107967</i> (F)	KF851415	KF852310
<i>Metacalypogeia alternifolia</i> (Nees) Grolle	Bhutan, <i>D.G. Long 28712</i> (E)	JF513474	JF513455

Table 1 continued

Taxon	Voucher	<i>rps4</i>	<i>rbcL</i>
<i>Mnioloma fuscum</i> (Lehm.) R.M.Schust.	Fiji, J.E. Braggins et al. 16. IV. 2008 (NSW)	KF851498	KF852404
<i>Nardia compressa</i> (Hook.) Gray	Russia, V.A. Bakalin K-74-9-04 (F)	KF943426	KF943556
<i>Nardia lescurii</i> (Austin) Underw.	North Carolina, B. Shaw 7209 (DUKE)	KF943480	KF943588
<i>Nardia succulenta</i> (Rich. ex Lehm. & Lindenb.) Spreng.	Colombia, J.C. Benavides s.n. (SIU)	KF943528	KF943622
<i>Nothogymnomitrium erosum</i> (Carrington & Pearson) R.M.Schust.	New Zealand, J.J. Engel, M. von Konrat and J.E. Braggins 24569 (F)	KF943395	KF943548
<i>Pedinophyllum interruptum</i> (Nees) Kaal.	Russia, N.A. Konstantinova and A.N. Savchenko k508/7-07 (F)	KF851405	KF852301
<i>Plagiochila asplenioides</i> (L.) Dumort.	Italy, Schäfer-Verwimp and Verwimp 35859(M)	KT992688	KT992617
<i>Prasanthus suecicus</i> (Gottsche) Lindb.	Svalbard, N.A. Konstantinova A.N. Savchenko K106/1-07 (F)	KF851408	KF852304
<i>Saccogyna viticulosa</i> (L.) Dumort.	Madeira, L. Söderström s.n. (F)	KF943437	KF943565
<i>Saccogynidium decurvum</i> (Mitt.) Grolle	Tasmania, B. Shaw 6410 (DUKE)	KF851479	KF852389
<i>Scapania nimbose</i> Taylor ex Lehm.	Scotland, D.G. Long, M. Flagmeier 37028 (DUKE)	KF851502	KF852408
<i>Schistochila laminigera</i> (Hook.f. & Taylor) A.Evans	Chile, J.J. Engel 26644 (F)	KF943439	KF943567
<i>Schistochila sciophila</i> R.M.Schust.	Chile, J. Larrain and R. Andrus 31210 (H)	KF184433	KF184407
<i>Solenostoma atrorevolutum</i> (Grolle ex Amakawa) Váňa & D.G.Long	China, D.G Long 35732 (E)	KF943461	KF943575
<i>Solenostoma crenuliforme</i> (Austin) Steph.	North Carolina, B. Shaw 7212 (DUKE)	KF943519	KF943617
<i>Solenostoma gracillimum</i> (Sm.) R.M. Schust.	Austria, P. Sova s.n. (DUKE)	KF943516	KF943614
<i>Solenostoma obovatum</i> (Nees) C. Massal.	Russia, N.A. Konstantinova A.N. Savchenko K382-3e-04 (F)	KF943422	KF943552
<i>Szyzygiella autumnalis</i> (DC.) K.Feldberg, Váňa, Hentschel & Heinrichs	Vermont, B. Shaw 6969 (DUKE)	KF851473	KF852382
<i>Trichotemnoma corrugatum</i> (Stephani) R.M.Schust.	New Zealand, Y. Qiu and J.E. Braggins NZ-03123 (AK)	KF851469	KF852379
<i>Xenochila integrifolia</i> (Mitt.) Inoue	Japan, N. Kitagawa HSE 750 (M)	KU745671	KU745670
<i>Zoopsis nitida</i> Glenny, Braggins & R.M.Schust.	New Zealand, D. Glenny 10517 (F)	KF943440	KF943568

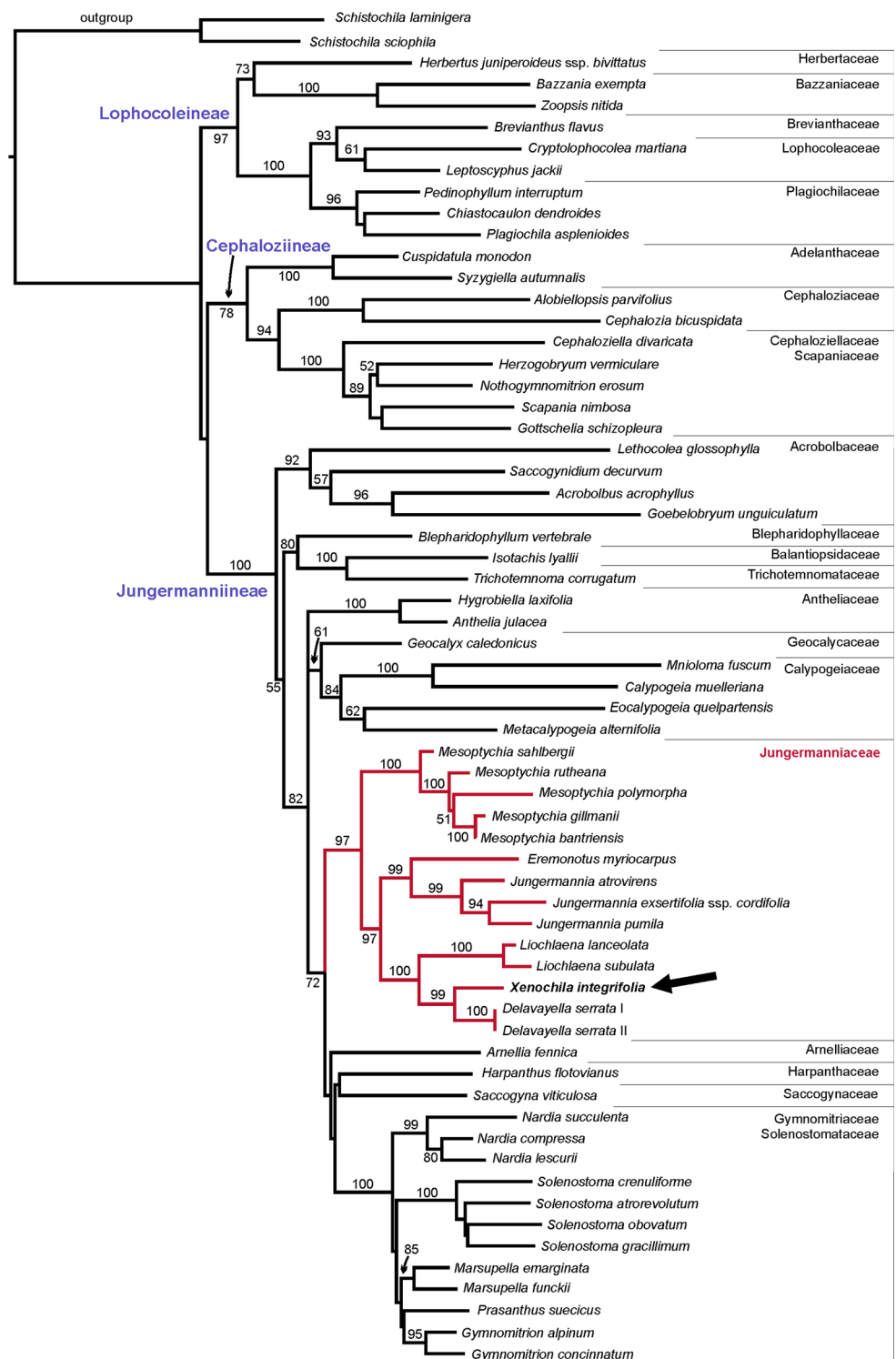
Discussion

Plagiochilaceae (Lophocoleineae) typically have reduced underleaves, scattered rhizoids, undivided leaves and laterally compressed, bilabiate perianths with a truncate mouth (Crandall-Stotler et al. 2009). Their classification is still in flux and several important changes have been proposed since the reviews of Söderström et al. (2015, 2016). These include the synonymy of the genera *Acrochila* R.M.Schust. and *Plagiochilion* S.Hatt. with *Chiastocaulon* Carl and the establishment of the genus *Cryptoplagiochila* S.Patzak, M.A.M.Renner & Heinrichs (Patzak et al. 2016) as well as a recircumscription of *Dinckleria* Trevis. (Renner et al. 2016). Our phylogeny (Fig. 1) leads to another change and provides convincing evidence that *Xenochila* is not related to Plagiochilaceae. It is a member of Jungermanniineae rather than Lophocoleineae and placed in the *Delavayella*-clade of Jungermanniaceae.

Numerous Jungermanniaceae concepts have been proposed since the initial description of this family by Reichenbach (1828). Jungermanniaceae were often

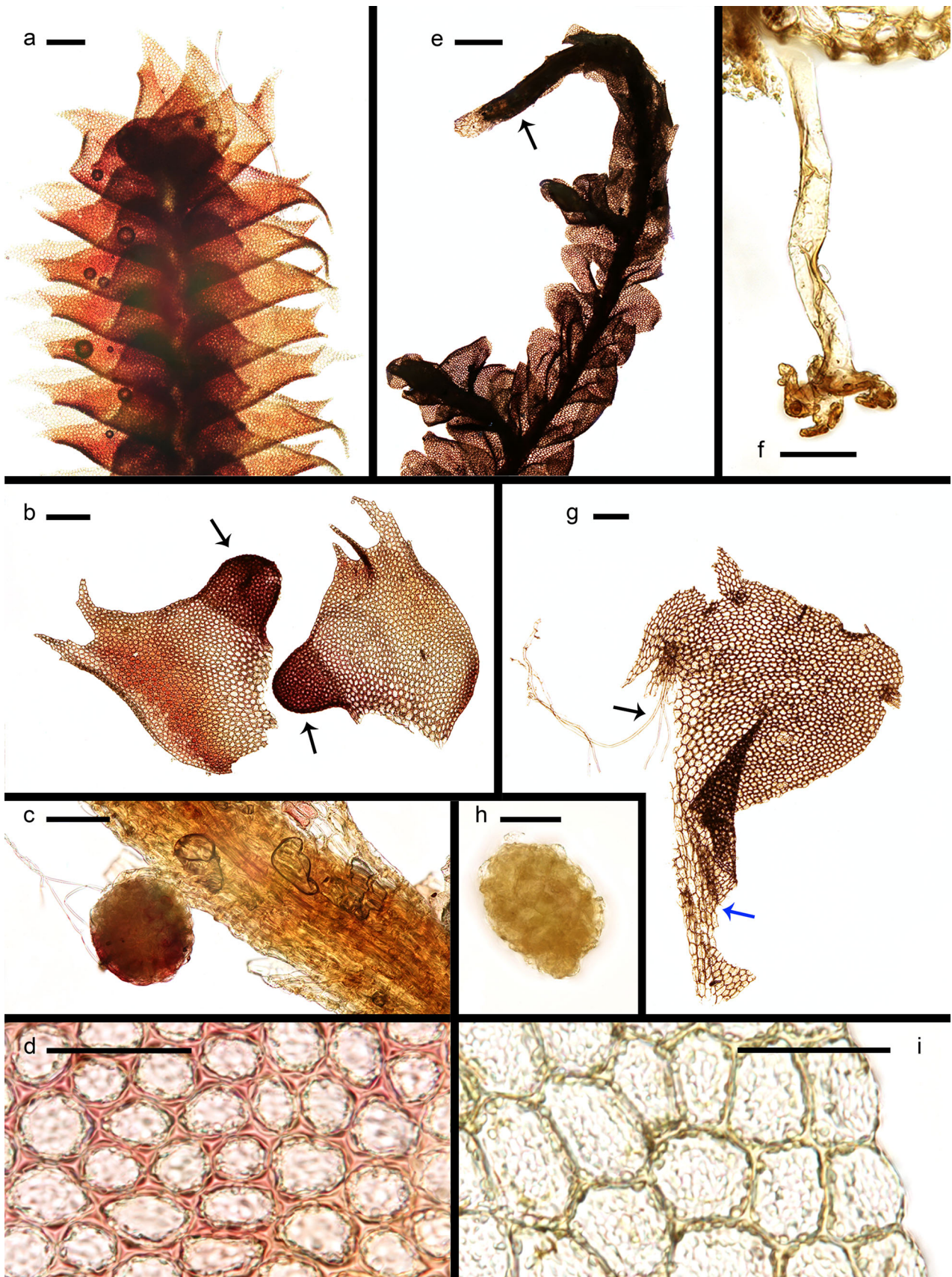
considered to include nearly exclusively species with unlobed leaves (Amakawa 1959, 1960; Müller 1951–1958) yet Schuster (1970) extended the family to include former elements of Lophoziaceae, i.e., species with lobed leaves. This treatment rendered Jungermanniaceae one of the most speciose families of liverworts, but was not confirmed by molecular studies (Yatsentyuk et al. 2004; Heinrichs et al. 2005; He-Nygrén 2007). Schill et al. (2004) and He-Nygrén et al. (2006) were the first to demonstrate a close relationship of *Jungermannia* and *Delavayella* of the monogeneric Delavayellaceae. Based on phylogenetic analyses of *rbcL* sequences, Hentschel et al. (2007) excluded *Liochlaena* Nees and *Solenostoma* Mitt. from the synonymy of *Jungermannia* s.l. (Váňa 1973) and proposed to include *Delavayella*, *Eremonotus* Pearson, *Jungermannia* s.s., *Leiocolea* (Müll.Frib.) Buch, and *Liochlaena* in Jungermanniaceae. De Roo et al. (2007) showed that *Mesoptychia* is nested in *Leiocolea* and Váňa et al. (2012) consequently treated *Leiocolea* as a synonym of *Mesoptychia*. The above genera have never been considered to form a monophyletic lineage representing Jungermanniaceae,

Fig. 1 Most likely phylogram resulting from maximum likelihood analysis of the *rps4-rbcL* dataset. Bootstrap percentage values >50 are indicated at branches



however, Hentschel et al. (2007: 155) noted that they “share the exclusive presence of perianths, whereas related Jungermanniiineae clades comprise genera with female involucre that are at least partly formed by stem tissue”. Shaw et al. (2015) considerably extended the taxon and marker sampling of Hentschel et al. (2007) and confirmed

their treatment of Jungermanniaceae. They added further morphological characters supporting the recognition of the newly circumscribed Jungermanniaceae including “branching only from lateral merophytes, gynoecia and androecia only on leading stems, long emergent perianths that are contracted at the mouth, bistratose capsule walls,



◀ **Fig. 2** Photomicrographs of *Delavayella serrata* (a–d) and *Xenochila integrifolia* (e–i). **a** Upper portion of gametophyte in dorsal view. **b** Leaves. *Arrows* point to water-sacs. **c** Portion of small-leaved gemmiparous shoot with a multicellular propagule. **d, i** Papillose leaf cells in surface view. **e** Upper portion of gametophyte with two lateral branches. *Arrow* points to gemmiparous shoot (propagules already detached). **f** Rhizoid covered with fungal hyphae. **g** Leaf and portion of stem tissue. *Blue arrow* points to insertion of dorsal leaf base; the vertically oriented cells derive from the outermost stem layer. *Black arrow* points to rhizoid bundle initiating from the ventral leaf base. **h** detached multicellular propagule (a–d from Nepal, Thodung, Poelt H22 (M); e, f from Hepaticae Japonicae Exsiccatae 750, Kitagawa Oct. 4, 1965 (M); g, h, i from Hepaticae Japonicae Exsiccatae 1100, Tatebe June 5, 1951 (M); *scale bars* a, b, g = 200 µm; c = 100 µm; d, f, h, i = 50 µm; e = 500 µm)

and in all taxa except *M. sahlbergii* (Lindb. & Arnell) A. Evans, calyptral development with little or no shoot involvement, no perigynial development, and no geocauly” (Shaw et al. 2015: 35). As a consequence of the new arrangement of Jungermanniaceae, this family includes species with either rounded or bifid leaves.

The position of *Xenochila* within Jungermanniaceae and its sister relationship with *Delavayella* have never seriously been considered although Inoue (1963) mentioned similarities with some papillose *Jungermannia* species. Schuster (1999) pointed to similarities in the method of asexual reproduction of *Delavayella* and *Xenochila* yet aimed at demonstrating morphological differences between Scapaniaceae and Delavayellaceae. *Delavayella* and *Xenochila* share the presence of multicellular parenchymatous green to red propagules forming singly from leaf margins on specialized gemmiparous shoots (Fig. 2c, e, h), a strongly papillose gametophyte surface (Fig. 2d, i), undifferentiated or weakly differentiated stem cells, absence of underleaves and bracteoles, weakly to distinctly bifid female bracts that are slightly larger than the leaves, and long cylindrical perianths with a dentate mouth (Inoue 1963; Schuster 2002; Singh Deo and Singh 2013). Small-leaved gemmiparous shoots are also known from the Jungermanniaceae elements *Mesoptychia heterocolpos* (Thed. ex Hartm.) L.Söderstr. & Váňa and *Liochlaena subulata* (A.Evans) Schljakov but these species produce only 1-2-celled gemmae on slightly bilobed, spoon-shaped (*Mesoptychia*) or unlobed (*Liochlaena*) leaves (Paton 1999). *Xenochila* also matches the other characters of Jungermanniaceae listed by Shaw et al. (2015) yet stands out by the presence of a somewhat plagiocbiloid, laterally compressed perianth. Its sporophyte has not yet been described, but considering its expression in other Jungermanniaceae species it may comprise capsules with bis-tratose walls. The robust sister relationship and the morphological similarities brings into question the generic separation of *Xenochila* and *Delavayella*. We are aware

that monospecific genera have limited information on the relationships of their members; however, need to consider important morphological differences of *Xenochila* and *Delavayella*. In particular and in contrast to all other Jungermanniaceae and nearly all other members of Jungermanniales, *Delavayella* has a ventral leaf margin forming a large water-sac (Fig. 2b). Its frontally rather than laterally compressed perianths (Schuster 1999) are also untypical for Jungermanniaceae. *Delavayella* is the only member of Jungermanniaceae with serrulate leaf margins, much more robust (Fig. 2a) than the “lax” *Xenochila* (Fig. 2e), and provided with diffusely distributed rather than fascicled rhizoids (Fig. 2g). In light of these differences we keep both genera.

Schuster (1959) based *Xenochila* on its supposedly septate rhizoids; however, this description was based on Degenkolbe (1937) who mentioned the presence of septae in rhizoids of germinating propagules. Presence of septate rhizoids in *Xenochila* could not be confirmed by Inoue (1963), Yang and Hsu (1967), and Singh Deo and Singh (2013) and was also not observed by us. Septate rhizoids occur only in some members of the liverwort families Schistochilaceae, Lepidoziaceae and Lophocoleaceae (Heinrichs et al. 2015) and are—at least in part—induced by mycorrhizal fungi (Pressel et al. 2010). It is thinkable that fungal hyphae on the surface of *Xenochila* rhizoids (Fig. 2f) have misleadingly appeared to indicate septae.

Perspectives

Our study provided evidence for the affiliation of *Xenochila* to Jungermanniaceae rather than Plagiocbilaceae, and indeed a robust sister relationship to *Delavayella*. Presence of rhizoid fascicles in the leaf axils of *Xenochila* and *Syzygiella* (Patzak et al. 2016) is the result of convergent evolution which is a major obstacle in reconstructing evolutionary relationships of liverworts based on morphology (Crandall-Stotler et al. 2005; Renner 2015). Only a single “plagiocbiloid” genus (Söderström et al. 2016) has not yet been included in molecular phylogenies, *Plagiocbilidium* Herzog. This genus includes only a single species and close relationships to either *Syzygiella* (Herzog 1931) or *Xenochila* (Inoue 1963) have been proposed. However, a more definite statement should be based on molecular phylogenetic evidence.

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Compliance with ethical standards

Conflict of interest The authors declare they have no conflict of interest.

Information on Electronic Supplementary Material

Online Resource 1. Alignment of the concatenated *rbcL* and *rps4* sequences.

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