

Towards a natural classification of liverworts (Marchantiophyta) based on the chloroplast gene *rbcl*

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Abstract – Maximum likelihood analysis of 212 *rbcl* sequences leads to a robust phylogeny of liverworts. Our results indicate a subdivision of Marchantiophyta into three well supported classes assigned as Haplomitriopsida, Marchantiopsida and Jungermanniopsida. Haplomitriopsida (incl. Treubiopsida) are resolved as sister to the remainder of liverworts and may represent the oldest extant lineage of land plants. The proximate clade is assigned to Marchantiopsida (including *Blasia*). The robust Jungermanniopsida comprise the paraphyletic Metzgeriidae (including *Pleurozia*) and the monophyletic Jungermanniidae. The Jungermanniidae are split into two clades which correspond to the orders Jungermanniales and Porellales. The latter two groups are separated by morphological and ecological trends. Porellales are predominantly epiphytes with specialized lobules or watersacs and endosporous protonemata, while Jungermanniales are frequently terrestrial, lack watersacs, and normally develop exosporous protonemata.

Liverwort phylogeny / Haplomitriopsida / Marchantiopsida / Jungermanniopsida / Jungermanniales / Porellales / *rbcl* / maximum likelihood

INTRODUCTION

Marchantiophyta are an early diverging lineage of land plants that possibly dates back to Silurian times (Kenrick & Crane, 1997; Qiu *et al.*, 1998; Wellmann *et al.*, 2003; Graham *et al.*, 2004). The extant morphological diversity is classified in 5000-6000 species in about 350 genera, taking into account results of recent monographic work (Yano & Gradstein, 1997; Gradstein *et al.*, 2001). Liverworts share the occurrence of membrane-bound oil bodies and a non-photosynthetic sporophyte which reaches maturity within the modified archegonium (calyptra). The sporophyte is short-living upon maturity, has a fragile seta (rarely none) and unicellular elaters derived from a sporangial cell whose division usually leads to spores and elaters in a 4 : 1, 8 : 1, 16 : 1 or 32 : 1 ratio (Schuster, 1966).

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In the 19th century liverworts were roughly divided into Marchantiales and Jungermanniales (Endlicher, 1841; Schiffner, 1893). Schuster (1953, 1966) proposed a subdivision of extant liverworts in two subclasses and six orders (orders Calobryales, Jungermanniales, and Metzgeriales in subclass Jungermanniidae, orders Monocleales, Sphaerocarpaceales and Marchantiales in subclass Marchantiidae), which has been widely followed (Grolle, 1983; Schuster, 1984; Schofield, 1985; Gradstein *et al.*, 2001). Recently, recognition of “complex thalloids” (Marchantiopsida, subclasses Marchantiidae and Sphaerocarpaceae), “simple thalloids” (Jungermanniopsida, subclass Metzgeriidae), and “leafy liverworts” (Jungermanniopsida, subclass Jungermanniidae) has been proposed (Crandall-Stotler & Stotler, 2000). Relationships of and within these morphological groups are still largely unresolved although recent molecular phylogenetic studies have considerably improved our understanding of liverwort systematics at different hierarchical levels (Lewis *et al.*, 1997; Long *et al.*, 2001, Stech & Frey, 2001; Boisselier-Dubayle *et al.*, 2002; Groth & Heinrichs, 2003, 2005, He-Nygrén & Piippo, 2003; Ahonen 2004; Davis, 2004; Forrest & Crandall-Stotler, 2004; He-Nygrén *et al.*, 2004; Wilson *et al.*, 2004). These studies revealed that morphology-based classifications reflect natural relationships only to some extent. However, the available molecular investigations were usually based on a rather limited taxon sampling or were addressed to selected clades of liverworts.

The most commonly used molecular marker in land plant phylogeny above genus level is the chloroplast gene *rbcL* (Chase *et al.*, 1993; Hasebe *et al.*, 1995; Kallersjö *et al.*, 1998; Tsubota *et al.*, 2002). Within liverworts, *rbcL* has been utilized in single gene analyses (Lewis *et al.*, 1997; Wilson *et al.*, 2004; Groth & Heinrichs, 2005) or in combination with other chloroplast, nuclear or mitochondrial markers (He-Nygrén & Piippo, 2003; Ahonen *et al.*, 2003; Ahonen, 2004; Davis, 2004; Forrest & Crandall-Stotler, 2004). Currently about 300 *rbcL* sequences of liverworts have been deposited in GenBank. Here we explore the information provided by a phylogenetic analyses of 212 *rbcL* sequences which is the taxonomically densest sampling available for molecular phylogenetics of liverworts at the moment. In other lineages of land plants, the analyses of large data sets with more than 200 accessions of *rbcL* sequences generated many new insights that were largely congruent to results obtained in multigene analyses (Chase *et al.*, 1993; Hasebe *et al.*, 1995).

MATERIAL AND METHODS

Liverwort *rbcL* sequences as well as three hornwort and six moss sequences were downloaded from GenBank and aligned manually in BioEdit version 5.0.9. (Hall, 1999). Lacking parts of sequences were coded as “N” (A, C, G or T). Hornworts and mosses were designated as outgroups. Based on preliminary analyses, a subset of 212 sequences was chosen for phylogenetic analysis (Table 1). With few exceptions, only one sequence for each species was selected. Sequences obviously identified erroneously [e.g., the supposedly permuted *Lepidozia* (U87075) and *Lophocolea* (U87076) sequences] were excluded. Regions of largely incomplete data were identified and excluded from subsequent analyses, resulting in a dataset including 1028 homologous sites (alignment available upon request).

Table 1. GenBank accession numbers of the taxa investigated

<i>Acrolejeunea fertilis</i> AY684929	<i>Fossombronia pusilla</i> AF 536231
<i>Adelanthus lindenbergianus</i> AY462285	<i>Fossombronia</i> spec. AY507400
<i>Allisonia cockaynei</i> AY507389	<i>Frullania davurica</i> AY302451
<i>Anastrophyllum michauxii</i> AY507390	<i>Frullania dilatata</i> AY125929
<i>Andreaea wilsonii</i> AY312925	<i>Frullania moniliata</i> AY507401
<i>Aneura pinguis</i> AY507391	<i>Frullania monocera</i> AY302450
<i>Anthoceros laminiferus</i> AY463053	<i>Frullania muscicola</i> AY302452
<i>Aphanolejeunea gracilis</i> AY302443	<i>Frullania tamarisci</i> AY302453
<i>Archilejeunea planiuscula</i> I AY548081	<i>Frullanooides densifolia</i> AY548099
<i>Archilejeunea planiuscula</i> II AY302444	<i>Funaria hygrometrica</i> AF005513
<i>Austrofossombronia australis</i> AY507392	<i>Gackstroemia magellanica</i> AY462295
<i>Austrofossombronia peruviana</i> AY536230	<i>Goebeliella cornigera</i> AY462296
<i>Austrofossombronia</i> spec. AF536229	<i>Gottschea nuda</i> AY462297
<i>Austrolejeunea nudipes</i> AY302445	<i>Haplomitrium blumei</i> I AY507402
<i>Balantiopsis cancellata</i> AY462286	<i>Haplomitrium blumei</i> II AY608029
<i>Bazzania</i> spec. AY462288	<i>Haplomitrium gibbsiae</i> AY608030
<i>Bazzania tricrenata</i> AY699990	<i>Haplomitrium hookeri</i> U87072
<i>Blasia pusilla</i> I AF536232	<i>Haplomitrium mnioides</i> AB013678
<i>Blasia pusilla</i> II AY462288	<i>Haplomitrium</i> spec. AY462298
<i>Blepharostoma trichophyllum</i> AY462289	<i>Hattorianthus erimosus</i> AY507403
<i>Bryopteris diffusa</i> AY548084	<i>Herbertus alpinus</i> AY507404
<i>Bryopteris filicina</i> AY548087	<i>Herbertus dicranus</i> AY462300
<i>Calypogeia integristipula</i> AY462290	<i>Herbertus pensilis</i> U87073
<i>Calypogeia muelleriana</i> U87065	<i>Herbertus sakurai</i> AY608031
<i>Calicularia crispula</i> AY507396	<i>Herbertus sendtneri</i> AY699992
<i>Cavicularia densa</i> AY507396	<i>Heteroscyphus coalitus</i> AY149844
<i>Cephalozia bicuspidata</i> AY462291	<i>Heteroscyphus inflatus</i> AY149853
<i>Ceratolejeunea cornuta</i> AY548088	<i>Heteroscyphus splendens</i> AY149854
<i>Ceratolejeunea coarina</i> AY608026	<i>Heteroscyphus zollingeri</i> AY149856
<i>Ceratolejeunea filaria</i> AY548089	<i>Hygrolembidium acrocladum</i> AY462301
<i>Ceratolejeunea grandiloba</i> AJ548090	<i>Hymenophyton flabellatum</i> AY507406
<i>Chaetophyllopsis whiteleggei</i> AY462292	<i>Hymenophyton leptopodium</i> AY507405
<i>Chandonanthus</i> spec. AY462293	<i>Hypnum lindbergii</i> AF232696
<i>Cheilolejeunea imbricata</i> AY125935	<i>Isotachis humectata</i> HE AY462302
<i>Cheilolejeunea inflexa</i> AY302446	<i>Isotachis lyallii</i> AY608029
<i>Cheilolejeunea lineata</i> AY548092	<i>Isotachis multiceps</i> AY507407
<i>Cheilolejeunea trifaria</i> AY548093	<i>Jamesoniella autumnalis</i> AY462303
<i>Chiastocaulon dendroides</i> AY699991	<i>Jubula bogotensis</i> AY548100
<i>Chiloscyphus japonicus</i> AY149847	<i>Jubula hutchinsiae</i> AY548101
<i>Chiloscyphus minor</i> AY149853	<i>Jubula japonica</i> AY125938
<i>Chiloscyphus pallescens</i> AY149849	<i>Jubulopsis novae-zelandiae</i> AY608033
<i>Chiloscyphus polyanthos</i> AY149851	<i>Jungermannia leinatha</i> AY507409
<i>Cololejeunea macounii</i> AY125942	<i>Lejeunea cavifolia</i> AY548102
<i>Cololejeunea peculiaris</i> AY548095	<i>Lejeunea catanduana</i> AY125943
<i>Cololejeunea peraffinis</i> AY125941	<i>Lejeunea mimula</i> AY548104
<i>Dendromastigophora flagellifera</i> AY462294	<i>Leiomitra lanata</i> AY462305
<i>Diplasiolejeunea involuta</i> AY548096	<i>Leiosporoceros dussii</i> AY619652
<i>Diplophyllum obtusifolium</i> AY507397	<i>Lepicolea attenuata</i> AY507410
<i>Drepanolejeunea biocellata</i> AY548097	<i>Lepicolea ochroleuca</i> AY462306
<i>Drepanolejeunea erecta</i> AY125940	<i>Lepicolea pruinosa</i> AY462307
<i>Drepanolejeunea vesiculosa</i> AY302449	<i>Lepicolea scolopendra</i> AY 462308
<i>Dumortiera hirsuta</i> U87068	<i>Lepidolaena clavigera</i> AY462309
<i>Fossombronia angulosa</i> AY507398	<i>Lepidolaena taylorii</i> AY462310
<i>Fossombronia foveolata</i> AY507399	<i>Lepidolejeunea bidentula</i> AY125936
<i>Fossombronia porphyrorhiza</i> AF536234	<i>Lepidolejeunea eluta</i> AY548066



Table 1. GenBank accession numbers of the taxa investigated (suite)

<i>Lepidozia</i> spec. AY462311	<i>Plagiochilon mayebarae</i> AY699999
<i>Leptolejeunea elliptica</i> AY125939	<i>Plagiochilon oppositum</i> AY700000
<i>Lobatirricardia lobata</i> AY507421	<i>Pleurozia conchifolia</i> AY462324
<i>Lophocolea bidentata</i> (= <i>Chiloscyphus latifolius</i>) AY149842	<i>Pleurozia gigantea</i> AY462325
<i>Lophocolea cuspidata</i> (= <i>Chiloscyphus cuspidatus</i>) AY1498845	<i>Pleurozia purpurea</i> AY608037
<i>Lophocolea martiana</i> (= <i>Chiloscyphus martianus</i>) AY149848	<i>Podomitrium phyllanthus</i> AY507419
<i>Lophocolea profunda</i> (= <i>Chiloscyphus profundus</i>) AY149852	<i>Polytrichum piliferum</i> AY118263
<i>Lopholejeunea brunnea</i> AY125930	<i>Porella cordaeana</i> AY302457
<i>Lopholejeunea eulopha</i> AY548067	<i>Porella japonica</i> AY302459
<i>Lopholejeunea muelleriana</i> AY302454	<i>Porella navicularis</i> AY507420
<i>Lophozia ventricosa</i> AY69994	<i>Porella platyphylla</i> AY302458
<i>Makinoa crispata</i> U87078	<i>Preissia quadrata</i> AY312935
<i>Makinoa</i> spec. AY462313	<i>Ptilidium ciliare</i> AY608038
<i>Marchantia polymorpha</i> U87079	<i>Ptilidium pulcherrimum</i> AY302460
<i>Marchesinia brachiata</i> AY548069	<i>Ptychanthus striatus</i> AY125931
<i>Marsupidium latifolium</i> AY608034	<i>Pycnolejeunea decurviloba</i> AY548091
<i>Mastigolejeunea auriculata</i> AY548070	<i>Pycnolejeunea densistipula</i> AY548075
<i>Mastigophora woodsii</i> AY462314	<i>Radula complanata</i> AY302461
<i>Megaceros flagellaris</i> AY463040	<i>Radula kamurana</i> AY302462
<i>Metzgeria conjugata</i> AY507411	<i>Scapania nemorea</i> AY507423
<i>Metzgeria furcata</i> U87081	<i>Scapania undulata</i> AY149840
<i>Metzgeria</i> spec. AY608035	<i>Schiffneria hyalina</i> AY462327
<i>Microlejeunea ulicina</i> AY125944	<i>Schiffneriolejeunea pappeana</i> AY548076
<i>Moerkia blyttii</i> AY507412	<i>Schiffneriolejeunea tumida</i> AY548076
<i>Moerkia flotoviana</i> AY507413	<i>Schistochila appendiculata</i> I AY507424
<i>Monoclea gottschei</i> AY507412	<i>Schistochila appendiculata</i> II AY462328
<i>Myriocolea irrorata</i> AY548072	<i>Schistochila laminigera</i> AY462329
<i>Nardia assamica</i> AY462316	<i>Sphaerocarpus texanus</i> AY507425
<i>Neohodgsonia mirabilis</i> AY507415	<i>Sphagnum palustre</i> AF 231887
<i>Neotrichocolea bissetii</i> AY462317	<i>Spruceanthus semirepandus</i> AY125932
<i>Nephelolejeunea hamata</i> AY302455	<i>Spruceanthus theobromae</i> AY548078
<i>Nipponolejeunea pilifera</i> AY125937	<i>Symbiezidium transversale</i> AY548079
<i>Noteroclada confluens</i> I AY462318	<i>Symphogyna hymenophyllum</i> AY507426
<i>Noteroclada confluens</i> II AF536228	<i>Syzygiella anomala</i> AY700001
<i>Nowellia</i> spec. AY462319	<i>Syzygiella perfoliata</i> AY700002
<i>Odontolejeunea lunulata</i> AY302456	<i>Takakia lepidozoioides</i> AY312936
<i>Odontoschisma denudatum</i> AY608037	<i>Targionia hypophylla</i> AY507427
<i>Odontoschisma</i> spec. AY462320	<i>Taxilejeunea</i> spec. AY302463
<i>Omphalanthus filiformis</i> AY548074	<i>Temnoma pilosum</i> AY462330
<i>Pallavicinia lyellii</i> AY507416	<i>Thysananthus amazonicus</i> AY548080
<i>Paraschistochila tuloides</i> AY462321	<i>Treubia lacunosa</i> AY507428
<i>Pedinophyllum truncatum</i> AY149855	<i>Treubia pygmaea</i> AY507429
<i>Petalophyllum ralfsii</i> I AY507417	<i>Triandrophyllum subtrifidum</i> AY462331
<i>Petalophyllum ralfsii</i> II U87086	<i>Trichocolea tomentella</i> AY462332
<i>Phyllohallia nivicola</i> AY507418	<i>Trichocolea tomentosa</i> AY608040
<i>Plagiochila alternans</i> AY699995	<i>Trichotemnoma corrugatum</i> AY462333
<i>Plagiochila asplenioides</i> AY699996	<i>Tritomaria quinquedentata</i> AY700003
<i>Plagiochila ovata</i> AY69997	<i>Trocholejeunea sandvicensis</i> AY125934
<i>Plagiochila porelloides</i> AY699998	<i>Tylimanthus laxus</i> AY700004
	<i>Verdoornia succulenta</i> AY507430
	<i>Vetaforma dusenii</i> AY462335
	<i>Xenothallus vulcanicola</i> AY507431
	<i>Xylojejeunea grolleana</i> AY302464

DISCUSSION

Current consensus exists regarding bryophytes – mosses, liverworts, and hornworts – as the oldest extant land plants (Qiu *et al.*, 1998; Renzaglia *et al.*, 2000; Wellmann *et al.*, 2003). In contrast, relationships among and within these three groups are still subject to controversy (Nickrent *et al.*, 2000). The most recent hypotheses based on molecular data identify the three major lineages of bryophytes as monophyletic (Nishiyama *et al.*, 2004) or resolve liverworts as sister to all other land plants (Qiu *et al.*, 1998; Duff & Nickrent, 1999; Nickrent *et al.*, 2000; Kelch *et al.*, 2004; Groth-Maloney *et al.*, 2005). Many of these studies were based on a limited taxon sampling, which may potentially yield misleading results as was recently discussed for the basal angiosperm *Amborella* (Soltis & Soltis, 2004). Sampling of additional taxa or more characters are the main strategies to arrive at improved phylogenies. The conflict between these two approaches has been addressed in several recent studies (Rosenberg & Kumar, 2001; Pollock *et al.*, 2002; Zwickl & Hillis, 2002). Hillis *et al.* (2003) concluded that a broad taxonomic sampling will result in a more accurate estimate of phylogenetic relationships than a sampling of multiple genes for a smaller taxon sampling. A dense taxonomic sampling appears to be especially critical in attempts to improve existing classifications and inference of evolutionary trends. In this study, we applied the strategy of an increased taxon sampling to analyse the relationships of liverworts (Marchantiophyta) based on more than 200 accessions of the *rbcL* gene. Our results indicate a subdivision of Marchantiophyta into three well supported classes, Haplomitriopsida, Marchantiopsida and Jungermanniopsida. The latter is made up of two subclasses, paraphyletic Metzgeriidae and well supported, monophyletic Jungermanniidae. Jungermanniidae, finally, bifurcate in our analysis in two main groups, orders Jungermanniales and Porellales.

Class 1. Haplomitriopsida (incl. Treubiopsida)

As in the multigene analysis of Forrest & Crandall-Stotler (2004), *Haplomitrium* and *Treubia* constitute a monophyletic lineage which is sister to all other investigated liverworts. Both the sister relationship of *Treubia* and *Haplomitrium* and that of this clade to the remainder of liverworts are robust, indicating that presumably the earliest diverging branch of extant hepatics is at hand. *Haplomitrium* is characterized by lack of rhizoids, triradial gametophytes with unlobed leaves in three equal rows (or the dorsal row somewhat reduced in size), scattered, exposed gametangia arranged along the leafy branches, and a largely unistratose capsule wall opening by 1-4 slits (Schuster, 1984, 2000). The genus may be close to a hypothetical intermediate of liverworts and mosses and shows some morphological affinity to the “basal” moss *Takakia* (Cox *et al.*, 2004; Shaw & Renzaglia, 2004), which also lacks rhizoids, has antheridia in the axils of all three rows of leaves, and a capsule opening with one slit (Schuster, 1997, 2000; Renzaglia *et al.*, 1997). Until the discovery of the male plants and sporophyte (Smith & Davidson, 1993), *Takakia* was usually classified with the liverworts, near to *Haplomitrium*, by its 3-ranked, 4-fid leaves and other gametophytic features (e.g., Hattori & Mizutani, 1958; Schuster, 1966; Schofield, 1985). Dombrowska & Qiu (2004) reported strong RNA editing in the *nadI* gene of *Haplomitrium* and *Takakia*.

Treubia has two lateral and two dorsal rows of polystratose leaf-like appendages, opaque cells with single oil bodies as well as chlorophyllose cells lacking oil bodies, and unprotected archegonia (Schuster, 2000). Carafa *et al.* (2003)

A phylogenetic tree was inferred using maximum likelihood (ML) criteria, as implemented in PAUP* version 4.0b10 (Swofford, 2000). To choose the nucleotide substitution model with the smallest number of parameters that best fits the data, the program Modeltest 3.06 (Posada & Crandall, 1998) was used. Based on the results of the hierarchical likelihood ratio test, the TrN model (Tamura & Nei, 1993) was chosen with gamma shape parameter (G) for among site variation and proportion of invariable sites (I) (TrN + I + G). A ML analysis was implemented as a heuristic search using a neighbor joining tree as starting tree. Branching confidence was assessed using Bayesian inference as implemented in the program MrBayes (version 3.0b4; Huelsenbeck & Ronquist, 2001). Modeltest 3.06 output values were applied to the command block of MrBayes. Fifteen million generations were calculated of which every 1000th was saved, resulting in 15,000 saved trees. Trees collected before the likelihood score had stabilized were deleted. The remaining 9000 trees with a stable likelihood score were condensed to a majority rule consensus tree. Branch support of the consensus tree, referred to as posterior probabilities, was regarded as significant when exceeding 0.95 (Larget & Simon, 1999).

RESULTS

The maximum likelihood analysis resulted in a single optimal topology (-ln = 29464,31194) which is presented in Figure 1. Accessions of *Haplomitrium* and *Treubia* are in a robust sister relationship and sister to the remainder of liverworts with good support. The proximate robust clade is made up of complex thalloid genera and *Blasia* in a well supported sister relationship. Simple thalloid and leafy liverworts form a robust monophyletic lineage with *Cavicularia* as sister to the remainder of this group, albeit without support. The rest of the simple thalloids is resolved in two main clades with unclear relationships, one of these including a.o. several accessions of *Pleurozia*. The robust crown group is made up of the leafy liverworts (excluding *Pleurozia*) in two well resolved main clades. One of the main clades is well supported with Schistochilaceae placed sister to the remainder of the clade. The remainder bifurcates in two unsupported clades. One polytomous clade includes the well supported Geocalyceae and Plagiochilaceae, the well supported Herbertaceae and Lepidoziaceae in robust sister relationships, as well as a robust clade made up of Lepicoleaceae and Vetaformaceae. The other clade is split into two well supported clades. One of these is made up of the robust Balantiopsidaceae in a well supported sister relationship with the robust Acrobolaceae, Calypogeiaceae, and Jungermanniaceae. The other clade includes Cephaloziaceae and Scapaniaceae in a well supported sister relationship. A well supported clade made up of *Syzygiella*, *Jamesoniella*, *Adelanthus* and *Pedinophyllum truncatum* is placed sister to the Scapaniaceae/Cephaloziaceae clade in a robust sister relationship.

The unsupported second main clade is made up of a paraphyletic grade with a robust *Neotrichocolea/Ptilidium* clade at the base, followed by the robust Porellaceae in an unsupported sister relationship with a robust clade made up of Jubulopsidaceae and Lepidolaenaceae. The proximate clade includes *Goebeliella* and *Radula* in an unsupported sister relationship. The robust Frullaniaceae are sister to the robust Jubulaceae and Lejeuneaceae, which are resolved in a well supported sister relationship.

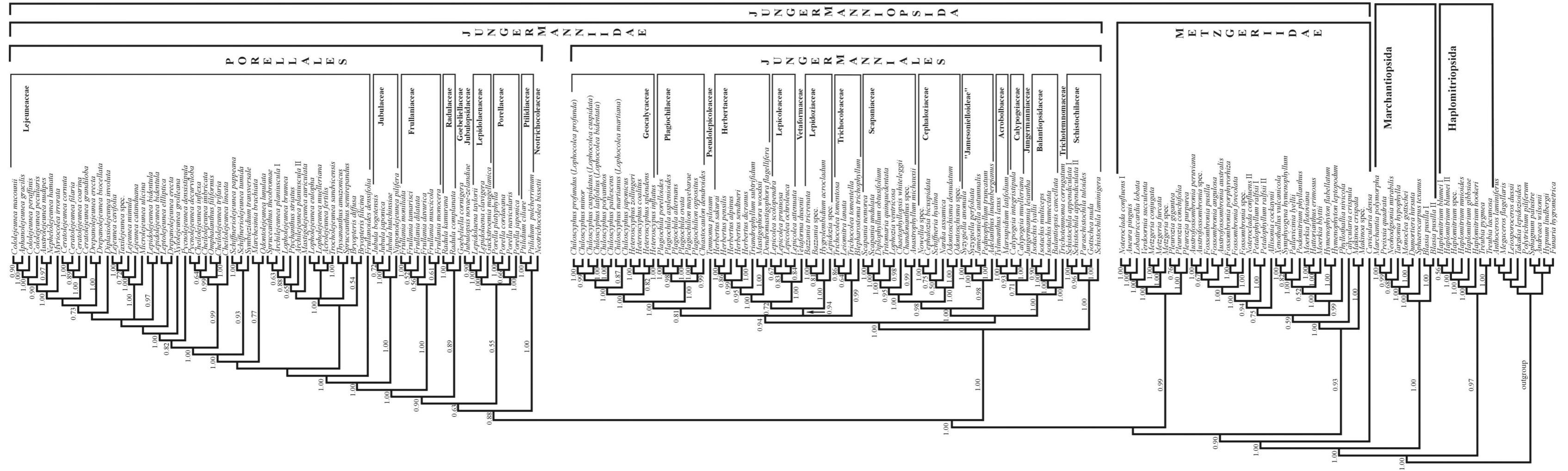


Fig. 1. Molecular phylogeny of liverworts based on *rbcL* sequence comparisons using 1028 aligned positions. The single most likely tree (-ln = 29464,31194) resulted from a maximum likelihood analysis of 212 sequences using the model of Tamura & Nei (1993) with estimated gamma shape (G = 0,857) and proportion of invariable sites (I = 0,399). Bayesian support at branches.

highlighted similarities of *Treubia* to leafy liverworts in the leaflike appendages and to complex thalloids in the oil bodies confined to specialized idioblasts, supporting the idea of *Treubia* being ancestral to more derived lineages of liverworts.

Already Goebel (1898-1901) suggested a relationship of *Haplomitrium* and *Treubia* based on the shared occurrence of basally polystratose leaves with slime papillae, tetrahedral apical cells, and lack of perichaetial scales. Similarities in placenta morphology, size differences of mitochondria in gametophyte and sporophyte, and blepharoplast morphology are putative synapomorphic character states of a clade comprising these two genera (Carothers & Rushing, 1990; Ligrone *et al.*, 1993; Carafa *et al.*, 2003). Sister group relationships were also found in characters related to male gametogenesis (Garbary *et al.*, 1993). The two groups were placed in Jungermanniopsida, in separate orders, by Crandall-Stotler & Stotler (2000). Earlier these authors (Stotler & Crandall-Stotler, 1977) had established a separate class Haplomitriopsida for *Haplomitrium* whereas Stech *et al.* (2000, 2002) proposed the class Treubiopsida to accommodate *Treubia* and *Apotreubia*. In the light of the morphological similarities outlined above and the *rbcL* based topology, we propose to combine the two classes in Haplomitriopsida. In view of a presumed old age for Haplomitriopsida, with an extant diversity of less than 20 species, it is not surprising that gametophytes of *Haplomitrium* and *Treubia* show only limited overlap in macroscopic characters. The ultrastructural similarities shared by the two taxa underline the importance of evidence from ultrastructure for high level taxonomy of liverworts (Miller, 1988; Garbary & Renzaglia, 1998). Ultrastructural data derived from a large taxon set of liverworts as well as the other two lineages of bryophytes are necessary to further corroborate the assumption of Haplomitriopsida as being the oldest extant clade of the land plants.

Class 2. Marchantiopsida

The well supported Marchantiopsida are resolved as the proximate clade. They include not only complex thalloids such as *Marchantia* or *Preissia* but also the more simple thalloid *Monoclea* as was shown earlier by Wheeler (2000) and Boisselier-Dubayle *et al.* (2002) based on nuclear LSU rDNA data, as well as by Davis (2004), Forrest & Crandall-Stotler (2004) and He-Nygrén *et al.* (2004) based on analyses of various chloroplast, nuclear or mitochondrial markers. Within Marchantiopsida, *Monoclea* stands somewhat isolated by the absence of air chambers and specialized archegoniophores, as well as by the capsules opening with only one longitudinal slit (Gradstein *et al.*, 1992). Its position in the complex thalloid liverworts is supported by the development of antheridia in receptacles, the (rare) occurrence of pegged rhizoids, the elaboration of glucuronide and galacturonide flavone glycosides, and the typically marchantialean placenta with transfer cells both in the gametophyte and the sporophyte (Gradstein *et al.*, 1993; Carafa *et al.*, 2003). The latter type of placenta also occurs in *Blasia* (Ligrone *et al.*, 1992), which is resolved sister to the remainder of Marchantiopsida (see also Forrest & Crandall-Stotler, 2004). *Blasia* was placed in Jungermanniopsida by Crandall-Stotler & Stotler (2000), in a separate class Blasiopsida by Stech & Frey (2001), or was resolved sister to the remainder of liverworts by He-Nygrén *et al.* (2004). Our results suggest inclusion of *Blasia* in Marchantiopsida. This classification is morphologically supported by its monoplastidic meiosis – occurring otherwise in *Monoclea* and *Haplomitrium* (Carafa *et al.*, 2003) –, a perichaetium identical to that of *Monoclea* (Forrest & Crandall-Stotler, 2004), and similarities of the blepharoplast (Carothers, 1973). The monophyly of Marchantiidae plus

Blasia was earlier suggested by Pass & Renzaglia (1995) based on blepharoplast similarities. The assumed sister genus of *Blasia*, *Cavicularia* (Pass & Renzaglia, 1995), is resolved here as the first diverging branch of Jungermanniopsida. As the *Cavicularia* sequence is very different from those of all other investigated taxa of Jungermanniopsida, the phylogenetic relationships of this genus require verification with additional sequences from other specimens.

Class 3. Jungermanniopsida

Subclass Metzgeriidae — As in previous phylogenetic studies (Forrest & Crandall-Stotler, 2004; Davis, 2004; He-Nygrén *et al.*, 2004), the Metzgeriidae are resolved as paraphyletic and consisting of two main clades: an unsupported clade including Fossombroniales as well as many genera currently included in Metzgeriales (Crandall-Stotler & Stotler, 2000), and a strongly supported Metzgeriales *s.str.* clade. *Austrofossombronia* is nested within *Fossombronia*. The two sequences of *Noterochlada* are resolved in the Fossombroniales clade and the Metzgeriales *s.str.* clade respectively; the taxonomic identity of the voucher specimens requires confirmation.

As already shown by Davis (2004) and He-Nygrén *et al.* (2004), the leafy liverwort *Pleurozia* is placed sister to Metzgeriales *s.str.* in a robust sister relationship, leading to the exclusion of this genus from Jungermanniidae and synonymy of *Pleuroziales* with Metzgeriales. *Pleurozia* has often been regarded as an isolated genus of Jungermanniales because of its unique leaves with dorsal saccate lobules (Crandall-Stotler, 1976) and lenticular apical cell with only two cutting faces (Evans, 1912; Schuster, 1966; Crandall-Stotler, 1976; Thiers, 1993). Jungermanniidae otherwise have a tetrahedral apical cell with three cutting faces. The two-sided apical cell links *Pleurozia* with Metzgeriidae. Ultrastructural data of *Pleurozia* are needed to clarify the phylogenetic position of this enigmatic genus. Carafa *et al.* (2003) reported the presence of one single type of placenta in Jungermanniidae, characterized by transfer cells occurring only in the sporophyte, and three different types in Metzgeriidae. Based on the existing data, it seems a worthwhile undertaking to investigate the sporophyte-gametophyte junction of *Pleurozia*.

Subclass Jungermanniidae — In our analysis, the accessions of Jungermanniidae are divided into two major clades. Based on this topology we propose to subdivide this subclass into two orders, Porellales (including Radulales and *Ptilidium*) and Jungermanniales (including Lepicoleales *p.p.maj.*). The monophyly of the Porellales is unsupported at basal nodes although the genera assigned to this order share several morphological characters, e.g., lack of ventral branching, rhizoids in bundles, and incubous bifid or trifid leaves (Schuster, 1979, 1984; Gradstein *et al.*, 2001). In Jungermanniales, as defined here, ventral branching, succubous leaves, and non-fasciculate rhizoids may be produced. In addition, Porellales stand out by the development of leaves by means of three initial cells (normally two in Jungermanniales), by development of lobules or watersacs on the ventral side of the leaf, and by their typically multicellular spores with endosporous protonemata (Gradstein *et al.*, 2001). The development of watersacs and endosporous protonemata of the Porellales are possibly associated with the epiphytic habitat, which is characteristic of almost all Porellales. In contrast, many Jungermanniales are terrestrial taxa; when epiphytic they lack clear morphological adaptations to the epiphytic habitat.

The basal position in Porellales is taken by *Ptilidium* and *Neotrichocolea*. The systematic position of *Ptilidium* has long been controversial. Crandall-Stotler

& Stotler (2000) placed the genus close to *Mastigophora*, which is not supported by our analysis. Instead, the *rbcL* phylogeny supports a position close to Porellaceae, suggested earlier by Schljakov (1972), Gradstein & van Melick (1996), and Ahonen (2004). Schuster (1980) speculated that the 2-3-lobed Porellales leaf may have developed from the trifid leaf of *Ptilidium*. Support for the position of *Ptilidium* in Porellales comes from its *Frullania* type branching pattern, shared with Porellaceae and Lepidolaenaceae, and chemical evidence (Asakawa, 1995; Gradstein & van Melick, 1996). Within Porellales, pinguisane-type sesquiterpenes are known from Neotrichocoleaceae, Ptilidiaceae, Porellaceae, Lepidolaenaceae, and Lejeuneaceae (Asakawa, 1995, 2004). However, pinguisanes are also known from some representatives of *Plagiochila* sect. *Alternantes* (Asakawa, 2004) in the Jungermanniales clade. Radulaceae are morphologically and chemically rather isolated among Porellales (Schuster, 1984; Asakawa, 2004) but their position in this order is confirmed by other molecular studies (Ahonen, 2004; Davis, 2004).

The crown group of Porellales is made up of Jubulaceae and Lejeuneaceae in a robust sister relationship. *Frullania* is placed sister to this crown group with good support. The topology is in favor of a separate family Frullaniaceae (Ahonen, 2004) and not of fusion of Frullaniaceae with Jubulaceae as proposed on morphological grounds (Guerke, 1978; Schuster, 1992).

Representatives of the Frullaniaceae-Jubulaceae-Lejeuneaceae clade are separated from the remainder of Porellales by spores with rosettes (van Slageren, 1995; Weis, 2001), sporophyte development entirely within the calyptra, a reduced foot, and fixed elaters (Spruce, 1884-1885). Jubulaceae include only *Nipponolejeunea* and *Jubula*. Based on morphology *Nipponolejeunea* has been assigned to Lejeuneaceae by all authors (e.g., Schuster, 1979; Grolle, 1983; Gradstein *et al.*, 2003) except Mizutani (1961) who placed the genus in Jubulaceae. Based on molecular data, *Nipponolejeunea* is unequivocally resolved sister to *Jubula* (Ahonen *et al.*, 2003; Ahonen, 2004; Wilson *et al.*, 2004).

Analyses of *rbcL* gene sequences of Lejeuneaceae by Wilson *et al.* (2004) and the present one do not yet lead to a robust phylogeny. Noteworthy new relationships in our topology are the positions of *Aphanolejeunea* nested in the robust *Cololejeunea* clade and of *Taxilejeunea* in the robust *Lejeunea* clade. The synonymy of *Taxilejeunea* in *Lejeunea* has frequently been suggested on morphological grounds (e.g., Gradstein & Costa, 2003), that of *Aphanolejeunea* in *Cololejeunea* is new. The paraphyly of *Drepanolejeunea* in the present analysis is unexpected and necessitates verification of the position of *D. erecta* and other species of this genus by further sequences.

Sister relationships are better resolved within the robust Jungermanniales than within Porellales. Jungermanniales differ from Porellales by the occurrence of ventral branches, succubous leaves, and non-fasciculate rhizoids (Schuster, 1984). Schistochilaceae are placed sister to the remainder of Jungermanniales, with *Gottschea* and *Paraschistochila* nested within *Schistochila*. This position is also supported by morphology, Schistochilaceae being linked to Porellales by dorsiventrally compressed gametophytes with exclusively lateral branching, broad ventral merophytes, and incubous leaves (Schuster, 1984; He-Nygrén *et al.*, 2004).

Geocalycaceae are placed sister to Plagiochilaceae in a well supported sister relationship which has already been suggested earlier based on morphological similarities. According to Schuster & Engel (1982) the two families grade into each other morphologically, with family affiliation of several genera of this group being unclear. Plagiochilaceae typically have a bilabiate perianth whereas the perianth of Geocalycaceae is 0-3 keeled or lacking; in the latter case a marsupium is

present instead. Critical are the Geocalycaceae subfam. Leptoscyphoideae whose representatives share with Plagiochilaceae the bilabiate perianth and scattered rhizoids. Difficulties in assignment of genera are evident from *Pedinophyllopsis*, which was placed in Geocalycaceae by Schuster & Engel (1982) but transferred to Plagiochilaceae based on molecular data (He-Nygrén & Piippo, 2003). A broader taxon sampling is necessary to determine the status of the two families.

The molecular data allow to decide on the long lasting controversy regarding the separation of *Chiloscyphus* and *Lophocolea* (see Grolle, 1995). As already demonstrated by He-Nygrén & Piippo (2003), *Chiloscyphus* is nested within *Lophocolea*, supporting the broad genus concept of *Chiloscyphus* (with *Lophocolea* as a synonym) advocated by Engel & Schuster (1984). The monophyly and nomenclature of Geocalycaceae should be revised using a broad taxon set and including representatives of subfamily Geocalycoideae, characterized by the presence of a marsupium. The single genus of Geocalycoideae studied at the molecular level to date, *Harpanthus*, is placed in a clade with Jungermanniaceae and Calypogeiaceae rather than Geocalycaceae (Davis, 2004). Unfortunately, *rbcL* sequences of Geocalycoideae are not available. If *Geocalyx* would also be resolved outside the remainder of the family, reinstatement of Lophocoleaceae would become necessary.

The Plagiochilaceae genera *Plagiochilion* and *Chiastocaulon* are separated from *Plagiochila* by the frequent occurrence of ventral-intercalary branches and form a clade sister to the latter genus (see also Groth & Heinrichs, 2003, 2005). *Pedinophyllum truncatum* is resolved sister to *Jamesoniella*, throwing doubt on the monophyly of Plagiochilaceae. In contrast, the type species *Pedinophyllum interruptum* forms a monophyletic lineage with *Chiastocaulon* and *Plagiochilion* in trees derived from *rps4* and nrITS sequence alignments (Groth & Heinrichs, 2003).

Mastigophora in its own family Mastigophoraceae has been placed close to Ptilidiaceae based on the collenchymatous cells, incubous leaves, and terminal pinnate branching (Schuster, 1972). The genus is identified here as a member of the Jungermanniales clade. Based on the *rbcL* tree we propose to include *Mastigophora* and *Dendromastigophora* in Herbertaceae (*Herbertus*, *Triandrophyllum*), which is also supported by the shared occurrence of herbertane-type sesquiterpenes (Harinantenaina & Asakawa, 2004) and basically incubous leaves. Close relationships of Herbertaceae, Lepicoleaceae, and Lepidoziaceae have been proposed by Grolle (1983) and Schuster (2000) based on their usually large underleaves, isophyllous gynoecia and other characters. Calypogeiaceae were usually placed near Lepidoziaceae by previous authors (e.g., Schuster, 1979; Grolle, 1983; Crandall-Stotler & Stotler, 2000), from which they differ by the reduced female bracts and bracteoles, lack of perianth (having a marsupium instead), spirally twisted capsule valves, and presence of vegetative reproduction by gemmae. A position of Calypogeiaceae near Lepidoziaceae is not confirmed by our topology, which shows a robust sister relationship of Acrobolbaceae and Calypogeiaceae, two families sharing pendent marsupia.

Different definitions of Jungermanniaceae and Lophoziaceae have been proposed based on morphology (Inoue, 1966, Crandall-Stotler & Stotler, 2000; Yatsentyuk *et al.*, 2004). Neither of these concepts is reflected in the molecular topology. As demonstrated earlier by Schill *et al.* (2004), Yatsentyuk *et al.* (2004), and Groth & Heinrichs (2005), *Scapania* is nested within Lophoziaceae. A derivation of *Scapania* from *Lophozia*-like ancestors has repeatedly been suggested based on morphological grounds (e.g., Buch, 1928; Schuster, 1951). Based on the morphological and molecular evidence, we propose to merge Lophoziaceae into

Scapaniaceae. Inoue (1966) established a subfamily Jamesonielloideae of Lophoziaceae to accommodate for *Jamesoniella* and *Syzygiella*. The close relationship of the latter two genera is confirmed in our study. However, their inclusion in Scapaniaceae (s.l., incl. Lophoziaceae) would lead to synonymy of Scapaniaceae with Cephaloziaceae according to our topology. Cephaloziaceae are morphologically very different from Scapaniaceae by the very thin seta, the ellipsoid, thin-walled capsule, the isophyllous gynoecia, the usually large, pellucid cells, and by the lateral insertion of the leaves, which do not extend to the dorsal midline of the stem.

A wide definition of Jungermanniaceae advocated by Crandall-Stotler & Stotler (2000), is not supported by our topology. Such a wide circumscription would lead to synonymy of Jungermanniaceae with highly disparate groups such as Balantiopsidaceae, Acrobolbaceae, Calypogeaceae, and Cephaloziaceae. Our topology indicates that a broader taxon sampling is necessary to arrive at a natural concept of the family Jungermanniaceae (see also Schuster, 1970).

GENERAL TRENDS AND FUTURE PROSPECTS

The *rbcL* topology supports a classification of Marchantiophyta in three monophyla assigned as Haplomitriopsida, Marchantiopsida, and Jungermanniopsida. This hypothesis requires confirmation in phylogenetic analyses based on other molecular markers as well as biochemical and morphological evidence. The potential to address these questions has been shown especially for the conservative mitochondrial markers (Knoop, 2004) and genomic characters (Kelch *et al.*, 2004). Jungermanniidae are subdivided in two orders Jungermanniales and Porellales. The latter two groups are separated by clear morphological and ecological trends, Porellales being predominantly epiphytes with specialized watersacs, leaves developing by three instead of two initial cells, and endosporous protonemata, while Jungermanniales are frequently terrestrial, have leaves developing with two initial cells, lack watersacs, and normally develop exosporous protonemata. Additional phylogenetically relevant information comes from recent studies on liverwort – endophytic fungi associations (Read *et al.*, 2000; Kottke *et al.*, 2003; Nebel *et al.*, 2004; Russel & Bulman, 2005). It appears that early diverging lineages of liverworts (Haplomitriopsida, Marchantiopsida, metzgerialean Jungermanniopsida associated to the Fossombroniales *s.l.* clade) are usually associated with Glomeromycota, the typical mycorrhiza fungi, whereas derived lineages (jungermannialean liverworts and Metzgeriales *s.str.*) are associated with Asco- and Basidiomycota, when symbiotic association with fungi is present. A trend towards lack of symbiotic association with fungi is observed in Porellales; association with Basidiomycota is observed only in the basal Ptilidiaceae and in none of the other groups. This trend may again be interpreted as an adaptation to epiphytism, with lack of development of symbiotic relationships in the epiphytic habitat. The same trend is found in epiphytic ferns (Schmid *et al.*, 1995; Moteeteete *et al.*, 1996). These new findings are to be confirmed by an extended sampling, with inclusion of both molecular and morphological evidence.

The present topology does not allow to decide on the old controversy concerning the ancestry of the liverworts. Basically there are two contrasting opinions 1) the ancestor was more or less radially symmetric (see Schuster, 1966, 1979),

2) the ancestor was dorsiventrally symmetric (“planate”) (Leitgeb, 1874-1881; Grolle, 1968). The basal position in our topology of the radially symmetric *Haplomitrium* and the dorsiventrally symmetric *Treubia* indicates that both opinions could be correct and possibly several morphotypes occurred in early stages of liverwort evolution. The integration of the fossil record (Krassilov & Schuster, 1984; Oostendorp, 1987; Edwards, 2000) into a phylogenetic framework may shed some light on the ancestor of liverworts.

One hundred and eighteen or approximately one third of the genera of hepatics have been included in the present study. Although sampling included representatives of most major groups we would like to point out some gaps in our sampling, especially in some of the major families of Jungermanniopsida such as Jungermanniaceae *s.l.*, Pseudolepicoleaceae, and Gymnomitriaceae. Other groups that need inclusion in future analyses are the families Arnelliaceae, Cephalozellaceae, and the odd Perssonelliaceae. Previous systems based on morphology are reflected only to some extent. The conflict between morphologically-based classifications and molecular topologies is still hampered by appropriate taxon sampling. Interestingly many relationships indicated by the *rbcL* phylogeny are supported by ultrastructural data. Disagreements between traditional classifications and the presented *rbcL* based phylogeny may have been at least partly the result of overweighting of homoplastic characters in the gross morphology of liverwort gametophytes. Ultrastructural characters, such as placenta structure and spermatozoid morphology, and secondary chemical components are major sources of information concerning the phylogenetic relationships of these plants and require much more work. Ultrastructural characters may also allow us to identify unequivocally the oldest fossils of major liverwort lineages, which are critical in our efforts to understand the evolution of these plants in time. Our topology and classification may serve as a basis for future phylogenetic investigations at the genus and/or family level and may inspire other researchers to explore the evolution of liverworts by integrating evidence from morphology as well as DNA sequencing.

Acknowledgements. We dedicate this paper to the memory of Riclef Grolle, who was one of the most influential and inspiring experts in liverwort systematics of our times. A few weeks before his death, the first author had the pleasure to discuss the classification of the Porellales and the relevance of amber fossils to our understanding of the diversification of derived liverwort groups with him. This work is a first step towards a scientific framework for exploring liverwort evolution, in the footsteps of the late Riclef Grolle. This study was supported by the Deutsche Forschungsgemeinschaft (grants HE 3584/1, GR 1588/9).

REFERENCES

- AHONEN I., 2004 — Molecular phylogeny of the liverwort order Porellales (Marchantiophyta, Jungermanniopsida). *Monographs in Systematic Botany from the Missouri Botanical Garden* 98: 168-188.
- AHONEN I., MUONA M. & PIIPPO S., 2003 — Inferring the phylogeny of the Lejeuneaceae (Jungermanniopsida): A first appraisal of molecular data. *The Bryologist* 106: 297-308.
- ASAKAWA Y., 1995 — Chemical constituents of the bryophytes. In: HERZ W., KIRBY G.W., MOORE R.E., STEGLICH W., & TAMM C. (Eds.), *Progress in the chemistry of organic natural products* 65. Wien: Springer.

- ASAKAWA Y., 2004 — Chemosystematics of the Hepaticae. *Phytochemistry* 65: 623-669.
- BOISSELIER-DUBAYLE M.-C., LAMBOURDIÈRE J. & BISCHLER H., 2002 — Molecular phylogenies support multiple morphological reductions in the liverwort subclass Marchantiidae (Bryophyta). *Molecular Phylogenetics and Evolution* 24: 66-77.
- BUCH H., 1928 — Die Scapanien Nordeuropas und Sibiriens. II, Systematischer Teil. *Societas Scientiarum Fennici, Commentationes Biologicae* 3, 1: 1-173.
- CARAFA A., DUCKETT J.G. & LIGRONE R., 2003 — The placenta in *Monoclea forsteri* Hook. and *Treubia lacunosa* (Col.) Prosk.: Insights into placental evolution in liverworts. *Annals of Botany* 92: 299-307.
- CAROTHERS Z.B., 1973 — Studies of spermatogenesis in the Hepaticae. IV. On the blepharoblast of *Blasia*. *American Journal of Botany* 60: 819-828.
- CAROTHERS Z.B. & RUSHING A.E., 1990 — Blepharoblast morphology in *Treubia tasmanica* (Hepaticae: Treubiales). *The Bryologist* 93: 409-416.
- CHASE M.W., SOLTIS D.E., OLMSTEAD R.G., MORGAN D., LES D.H., MISHLER B.D., DUVAL M.R., PRICE R.A., HILLS H.G., QIU Y.L., KRON K.A., RETTIG J.H., CONTI E., PALMER J.D., MANHART J.R., SYTSMA K.J., MICHAELS H.J., KRESS W.J., KAROL K.G., CLARK W.D., HEDREN M., GAUT B.S., JANSEN R.K., KIM K.J., WIMPEE C.F., SMITH J.F., FURNIER G.R., STRAUSS S.H., XIANG Q.Y., PLUNKETT G.M., SOLTIS P.S., SWENSEN S.M., WILLIAMS S.E., GADEK P.A., QUINN C.J., EGUIARTE L.E., GOLENBERG E., LEARN G.H., GRAHAM S.W., BARRETT S.C.H., DAYANANDAN S. & ALBERT V.A., 1993 — Phylogenetics of seed plants — an analysis of nucleotide-sequences from the plastid gene *rbcL*. *Annals of the Missouri Botanical Garden* 80: 528-580.
- COX C.J., GOFFINET B., SHAW A.J. & BOLES S.B., 2004 — Phylogenetic relationships among the mosses based on heterogeneous Bayesian analysis of multiple genes from multiple genomic compartments. *Systematic Botany* 29: 234-250.
- CRANDALL-STOTLER B., 1976 — The apical cell and early development of *Pleurozia purpurea* Lindb. *Lindbergia* 3: 197-208.
- CRANDALL-STOTLER B. & STOTLER R.E., 2000 — Morphology and classification of the Marchantiophyta. In: Shaw A.J. & Goffinet B. (Eds.), *Bryophyte Biology*. Cambridge: Cambridge University Press, pp. 21-70.
- DAVIS E.C., 2004 — A molecular phylogeny of leafy liverworts (Jungermanniidae: Marchantiophyta). *Monographs in Systematic Botany from the Missouri Botanical Garden* 98: 87-118.
- DOMBROWSKA O. & QIU Y.L., 2004 — Distribution of introns in the mitochondrial gene *nad1* in land plants: phylogenetic and molecular evolutionary implications. *Molecular Phylogenetics and Evolution* 32: 246-263.
- DUFF R.J. & NICKRENT D.L., 1999 — Phylogenetic relationships of land plants using mitochondrial small-subunit rDNA sequences. *American Journal of Botany* 86: 372-386.
- EDWARDS D., 2000 — The role of Mid-Palaeozoic mesofossils in the detection of early bryophytes. *Philosophical Transactions of the Royal Society of London Series B — Biological Sciences* 355: 733-755.
- ENDLICHER S., 1841 — *Enchiridion botanicum*. Leipzig: Engelmann.
- ENGEL J.J. & SCHUSTER R.M., 1984 — An overview and evaluation of the genera of Geocalyceaceae subfamily Lophocoleoideae (Hepaticae). *Nova Hedwigia* 39: 385-463.
- EVANS A.W., 1912 — Branching in the leafy Hepaticae. *Annals of Botany* 26: 1-37.
- FORREST L.L. & CRANDALL-STOTLER B.J., 2004 — A phylogeny of the simple thaloid liverworts (Jungermanniospida, Metzgeriidae) as inferred from five chloroplast genes. *Monographs in Systematic Botany from the Missouri Botanical Garden* 98: 119-140.
- GARBARY D.J., RENZAGLIA K.S. & DUCKETT J.G., 1993 — The phylogeny of land plants: A cladistic analysis based on male gametogenesis. *Plant Systematics and Evolution* 188: 237-269.

- GARBARY D.J. & RENZAGLIA K.S., 1998 — Bryophyte phylogeny and the evolution of land plants: evidence from development and ultrastructure in Bates J.W., Ashton N.W. & Duckett J.G. (Eds.), *Bryology for the Twenty-first Century*. Leeds: Maney Publishing and the British Bryological Society, pp. 45-63.
- GOEBEL K., 1898-1901 — *Organographie der Pflanzen*. Jena, G. Fischer.
- GRADSTEIN S.R., KLEIN R., KRAUT L., MUES R., SPÖRLE J., BECKER H., 1992 — Phytochemical and morphological support for the existence of two species in *Monoclea* (Hepaticae). *Plant Systematics and Evolution* 180: 115-135.
- GRADSTEIN S.R. & VAN MELICK H.M.H., 1996 — *De Nederlandse Levermossen en Haauwmossen*. Utrecht: Dutch Bryological and Lichenological Society and the Royal Dutch Natural History Society, 366 p.
- GRADSTEIN S.R., CHURCHILL S.P. & SALAZAR-ALLEN, 2001 — A guide to the bryophytes of tropical America. *Memoirs of the New York Botanical Garden* 86: 1-577.
- GRADSTEIN S.R. & COSTA D.P., 2003 — The Hepaticae and Anthocerotae of Brazil. *Memoirs of the New York Botanical Garden* 87: 1-318.
- GRADSTEIN S.R., REINER-DREHWALD M.E. & SCHNEIDER H., 2003 — A phylogenetic analysis of the genera of Lejeuneaceae. *Botanical Journal of the Linnean Society* 143: 391-410.
- GRAHAM L.E., WILCOX L.W., COOK M.E. & GENSEL, P.G., 2004 — Resistant tissues of modern marchantioid liverworts resemble enigmatic Early Paleozoic microfossils. *Proceedings of the National Academy of Sciences U.S.A.* 101: 11025-11029.
- GROLLE R., 1968 — Review of SCHUSTER R.M., *The Hepaticae and Anthocerotae of North America*. *Nova Hedwigia* 16: 539-542.
- GROLLE R., 1983 — Nomina generica Hepaticarum: references, types and synonymies. *Acta Botanica Fennici* 121: 1-62.
- GROLLE R., 1995 — The Hepaticae and Anthocerotae of the East African Islands, an annotated catalogue. *Bryophytorum Bibliotheca* 48: 1-178.
- GROTH H. & HEINRICHS J., 2003 — Reinstatement of *Chiastocaulon* Carl (Plagiogchilaceae), based on evidence from nuclear ribosomal ITS and chloroplast gene *rps4* sequences. *Plant Biology* 5: 615-622.
- GROTH H. & HEINRICHS J., 2005 — Maximum likelihood analyses of chloroplast gene *rbcL* sequences indicate relationships of *Syzygiella* (Jungermanniopsida) with Lophoziaaceae rather than Plagiogchilaceae. *Cryptogamie, Bryologie* 26: 49-57.
- GROTH-MALONEK M., PRUCHNER M., GREWE F. & KNOOP V., 2005 — Ancestors of trans-splicing mitochondrial introns support serial sister group relationships of hornworts and mosses with vascular plants. *Molecular Biology and Evolution* 22: 117-125.
- GUERKE W., 1978 — A monograph of the genus *Jubula* Dumortier. *Bryophytorum Bibliotheca* 17: 1-118.
- HARINANTENAINA L. & ASAKAWA Y., 2004 — Chemical constituents of Malagasy liverworts, Part II: Mastigophoric acid methyl ester of biogenetic interest from Mastigophora diclados (Lepicoleaceae subf. Mastigophoroideae). *Chemical & Pharmaceutical Bulletin* 52: 1382-1384.
- HASEBE M., WOLF P.G., PRYER K.M., UEDA K., ITO M., SANO R., GASTONY G.J., YOKOYAMA J., MANHART J.R., MURAKAMI N., CRANE E.H., HAUFLE C.H. & HAUKE W.D., 1995 — Fern phylogeny based on *rbcL* nucleotide sequences. *American Fern Journal* 85: 134-181.
- HATTORI S. & MIZUTANI M., 1958 — What is *Takakia lepidiozoides*? *Journal of the Hattori Botanical Laboratory* 20: 295-303.
- HALL T.A., 1999 — BIOEDIT: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposia Series* 41: 95-98.
- HE-NYGRÉN X. & PIIPPO S., 2003 — Phylogenetic relationships of the generic complex *Chiloscyphus-Lophocolea-Heteroscyphus* (Geocalycaceae, Hepaticae): Insights from three chloroplast genes and morphology. *Annales Botanici Fennici* 40: 317-329.

- HE-NYGRÉN X., AHONEN I., JUSLÉN A., GLENNY D. & PIIPPO S., 2004 — Phylogeny of liverworts – beyond a leaf and a thallus. *Monographs in Systematic Botany from the Missouri Botanical Garden* 98: 87-118.
- HILLIS, D.M., PÖLLOCK, D.D., MCGUIRE, J.A., ZWICKL, D.J., 2003 — Is sparse taxon sampling a problem for phylogenetic inference? *Systematic Biology* 52: 124-126.
- HUELSENBECK J.P. & RONQUIST F., 2001 — MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17: 754-755.
- INOUE H., 1966 — A monograph of the hepatic genus *Syzygiella* Spruce. *Journal of the Hattori Botanical Laboratory* 29: 171-213.
- KALLERSJÖ M., FARRIS J.S., CHASE M.W., BREMER B., FAY M.F., HUMPHRIES C.J., PETERSEN G., SEBERG O., BREMER K., 1998 — Simultaneous parsimony jackknife analysis of 2538 *rbcL* DNA sequences reveals support for major clades of green plants, land plants, seed plants and flowering plants. *Plant Systematics and Evolution* 213: 259-287.
- KELCH D.G., DRISKELL A., MISHLER B.D., 2004 — Inferring phylogeny using genomic characters: a case study using land plant plastomes. *Monographs in Systematic Botany from the Missouri Botanical Garden* 98: 3-12.
- KENRICK P. & CRANE P.R., 1997 — The origin and early evolution of plants on land. *Nature* 389: 33-39.
- KNOOP V., 2004 — The mitochondrial DNA of land plants: peculiarities in phylogenetic perspective. *Current Genetics* 46: 123-139.
- KOTTKE I., BEITER A., WEISS M., HAUG I., OBERWINKLER F. & NEBEL M., 2003 — Heterobasidiomycetes form symbiotic associations with hepatics: Jungermanniales have sebacinoid mycobionts while *Aneura pinguis* (Metzgeriales) is associated with a *Tulasnella* species. *Mycological Research* 107: 957-968.
- KRASSILOV V.A. & SCHUSTER R.M., 1984 — Paleozoic and mesozoic fossils. In: Schuster, R.M. (Ed.), *New Manual of Bryology*. Nichinan: The Hattori Botanical Laboratory, pp. 1172-1193.
- LARGET B. & SIMON D.L., 1999 — Markov chain Monte Carlo algorithms for the Bayesian analysis of phylogenetic trees. *Molecular Biology and Evolution* 16: 750-759.
- LEITGEB H., 1874-1881 — *Untersuchungen über die Lebermoose. I-VI*. Jena: Deistung's Buchhandlung.
- LEWIS L.A., MISHLER B.D. & VILGALYS R., 1997 — Phylogenetic relationships of the liverworts (Hepaticae), a basal embryophyte lineage, inferred from nucleotide sequence data of the chloroplast gene *rbcL*. *Molecular Phylogenetics and Evolution* 7: 377-393.
- LIGRONE R., DUCKETT J.G. & RENZAGLIA, K.S., 1993 — The gametophyte-sporophyte junction in land plants. *Advances in Botanical Research* 19: 231-317.
- LONG D.G., MÖLLER M. & PRESTON J., 2001 — Phylogenetic relationships of *Asterella* (Aytoniaceae, Marchantiopsida) inferred from chloroplast DNA sequences. *The Bryologist* 103: 625-644.
- MILLER N. (Ed.), 1988 — Bryophyte Ultrastructure. *Advances in Bryology* 3: 1-281.
- MIZUTANI M., 1961 — A revision of Japanese Lejeuneaceae. *Journal of the Hattori Botanical Laboratory* 24: 115-302.
- MOTEETEE A., DUCKETT J.G. & RUSSELL A.J., 1996 — Mycorrhizas in the ferns of Lesotho. In: CAMUS J.M., GIBBY M. & JOHNS R.J. (Eds.), *Pteridology in Perspective*. Kew: Royal Botanic Gardens, pp. 621-631.
- NEBEL M., KREIER H.-P., PREUSSING M., WEISS M. & KOTTKE I., 2004 — Symbiotal fungal associations of liverworts are the possible ancestors of mycorrhizae. In: AGERER R., PIEPENBRING M. & BLANZ, P. (Eds.), *Frontiers in Basidiomycote Mycology*. Eching: IWH-Publisher, pp. 339-360.
- NICKRENT D.L., PARKINSON C.L., PALMER J.D., & DUFF R.J., 2000 — Multigene phylogeny of land plants with special reference to bryophytes and the earliest land plants. *Molecular Biology and Evolution* 17: 1885-1895.
- NISHIYAMA T., WOLF P.G., KUGITA M., SINCLAIR R.B., SUGITA M., SUGIURA C., WAKASUGI T., YAMADA K., YOSHINAGA K., YAMAGUCHI K., UEDA K.

- & HASEBE M., 2004 — Chloroplast phylogeny indicates that bryophytes are monophyletic. *Molecular Biology and Evolution* 21: 1813-1819.
- OOSTENDORP C., 1987 — The bryophytes of the Paleozoic and Mesozoic. *Bryophytorum Bibliotheca* 34: 5-112, Plates I-XLIX.
- PASS J.M. & RENZAGLIA K.S., 1995 — Comparative microanatomy of the locomotory apparatus of *Conocephalum conicum* (Hepaticae, Conocephalaceae). *Fragmenta Floristica et Geobotanica* 40: 365-377.
- POLLOK D.D., ZWICKL D.J., MCGUIRE, J.A. & HILLIS D.M., 2002 — Increased taxon sampling is advantageous for phylogenetic inference. *Systematic Biology* 51: 664-671.
- POSADA D. & CRANDALL K.A., 1998 — Modeltest: testing the model of DNA substitution. *Bioinformatics* 14: 817-818.
- QIU Y.L., CHO Y.R., COX J.C. & PALMER J.D., 1998 — The gain of three mitochondrial introns identifies liverworts as the earliest land plants. *Nature* 394: 671-674.
- READ D.J., DUCKETT J.G., FRANCIS R., LIGRONE R. & RUSSELL A., 2000 — Symbiotic fungal associations in “lower” land plants. *Philosophical Transactions of the Royal Society of London, Series B – Biological Sciences* 355: 815-831.
- RENZAGLIA K.S., DUFF R.J., NICKRENT D.L. & GARBARY D.J., 2000 — Vegetative and reproductive innovations of early land plants: implications for a unified phylogeny. *Philosophical Transactions of the Royal Society of London, Series B – Biological Sciences* 355: 769-793.
- RENZAGLIA K.S., MCFARLAND K.D. & SMITH D.K., 1997 — Anatomy and ultrastructure of the sporophyte of *Takakia ceratophylla* (Bryophyta). *American Journal of Botany* 84: 1337-1350.
- ROSENBERG M.S. & KUMAR S., 2001 — Incomplete taxon sampling is not a problem for phylogenetic inference. *Proceedings of the National Academy of Science, U.S.A.* 98: 10751-10756.
- RUSSEL J. & BULMAN S., 2005 — The liverwort *Marchantia foliacea* forms a specialized symbiosis with arbuscular mycorrhizal fungi in the genus *Glomus*. *New Phytologist* 165: 567-579.
- SCHIFFNER V., 1893 — Hepaticae. In: Engler A. & Prantl K. (Eds.), *Die natürlichen Pflanzenfamilien* 1, 3. Leipzig, W. Engelmann, pp. 3-141.
- SCHILL D.B., LONG D.G., MOELLER M. & SQUIRRELL J., 2004 — Phylogenetic relationships between Lophoziaceae and Scapaniaceae based on chloroplast sequences. *Monographs in Systematic Botany from the Missouri Botanical Garden* 98: 140-149.
- SCHLJAKOV R.N., 1972 — On the higher taxa of liverworts (Hepaticae s.str.). *Botaničeskij Žurnal SSSR* 57: 496-508.
- SCHMID E., OBERWINKLER F. & GOMÉZ L.D., 1995 — Light and electron microscopy of a host-endemophyte interaction in the roots of some epiphytic ferns from Costa Rica. *Canadian Journal of Botany* 73: 991-996.
- SCHOFIELD W.B., 1985 — *Introduction to Bryology*. New York, Macmillan Publ. Co.
- SCHUSTER, M., 1951 — Notes on Nearctic Hepaticae. III. A conspectus of the family Lophoziaceae, with a revision of the genera and subgenera. *The American Midland Naturalist* 45: 1-117.
- SCHUSTER R.M., 1953 — Boreal Hepaticae. *The American Midland Naturalist* 49: 257-684 (reprinted 1977 as *Bryophytorum Bibliotheca* 11: 1-332).
- SCHUSTER R.M., 1965 — Studies on Hepaticae. XVI. The morphology and systematic position of the suborder Pleuroziinae. *Transactions of the British Bryological Society* 4: 794-800.
- SCHUSTER R.M., 1966 — *The Hepaticae and Anthocerotae of North America*, Vol. I. New York, Columbia University Press, 812 p.
- SCHUSTER R.M., 1970 — Studies on Hepaticae XVIII. The family Jungermanniaceae, s.lat.: a reclassification. *Transactions of the British Bryological Society* 6: 86-107.
- SCHUSTER R.M., 1972 — Phylogenetic and taxonomic studies on Jungermanniidae. *Journal of the Hattori Botanical Laboratory* 36: 321-405.

- SCHUSTER R.M., 1979 — The phylogeny of the Hepaticae. In: CLARKE G.C.S. & DUKETT J.G. (Eds.), *Bryophyte Systematics*. The Systematics Association Special Volume 14. New York: Academic Press, pp. 41-82.
- SCHUSTER R.M., 1980 — *The Hepaticae and Anthocerotae of North America*, Vol. IV. New York: Columbia University Press, 1334 p.
- SCHUSTER R.M. & ENGEL J.J., 1982 — Austral Hepaticae XVI. Gondwanalandic Leptoscyphoideae (Geocalycaceae). *Lindbergia* 8: 65-74.
- SCHUSTER R.M., 1984 — Evolution, Phylogeny and Classification of the Hepaticae. Marchantiophyta. In: Schuster, R.M. (Ed.), *New Manual of Bryology*. Nichinan: The Hattori Botanical Laboratory, pp. 21-70.
- SCHUSTER R.M., 1992 — *The Hepaticae and Anthocerotae of North America*, Vol. V. New York: Columbia University Press, 854 p.
- SCHUSTER R.M., 1997 — On *Takakia* and the phylogenetic relationships of the Takakiales. *Nova Hedwigia* 64: 281-310.
- SCHUSTER R.M., 2000 — Austral Hepaticae, Part I. *Nova Hedwigia Beiheft* 118: III-VII, 1-524.
- SHAW J. & RENZAGLIA K., 2004 — Phylogeny and diversification of bryophytes. *American Journal of Botany* 91: 1557-1581.
- SLAGEREN M.W. VAN, 1985 — A taxonomic monograph of the genera *Brachiolejeunea* and *Frullanoides* (Hepaticae), with a SEM analysis of the sporophyte in the Ptychanthoideae. *Mededelingen van het Botanisch Museum en Herbarium van de Rijksuniversiteit te Utrecht* 544: 243-302.
- SMITH D.K. & DAVIDSON P.G. 1993 — Antheridia and sporophytes in *Takakia ceratophylla* (Mitt.) Grolle: evidence for reclassification among the mosses. *Journal of the Hattori Botanical Laboratory* 73: 263-271.
- SOLTIS D.E. & SOLTIS P., 2004 — *Amborella* not a “basal angiosperm”? Not so fast. *American Journal of Botany* 91: 997-1001.
- SPRUCE R., 1884-1885 — Hepaticae of the Amazon and the Andes of Peru and Ecuador. *Transactions and Proceedings of the Botanical Society of Edinburgh* 15: 1-589, plates i-xiii.
- STECH M., FRAHM J.-P., HILGER H.H. & FREY W., 2000 — Molecular relationship of *Treubia* Goebel (Treubiaceae, Treubiopsida) and high taxonomic level classification of the Hepaticophytina. *Nova Hedwigia* 71: 195-208.
- STECH M. & FREY W., 2001 — CpDNA-relationship and classification of the liverworts (Hepaticophytina, Bryophyta). *Nova Hedwigia* 72: 45-58.
- STECH M., KONSTANTINÓVA N.A. & FREY W., 2002 — Molecular divergence between *Treubia* Goebel and *Apotreubia* S. Hatt. & Mizut., the two genera of the archaic liverwort class Treubiopsida (Hepaticophytina). *Nova Hedwigia* 75: 91-100.
- STOTLER R. & CRANDALL-STOTLER B., 1977 — A checklist of the liverworts and hornworts of North America. *The Bryologist* 80: 405-428.
- SWOFFORD D.L., 2000 — PAUP*, phylogenetical analyses using parsimony (*and other methods). Version 4.0b10. Sinauer Associates, Sunderland, Massachusetts.
- TAMURA K. & NEI M., 1993 — Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution* 10: 512-526.
- THIERS B.M., 1993 — A monograph of *Pleurozia* (Hepaticae; Pleuroziaceae). *The Bryologist* 96: 517-554.
- TSUBOTA H., ARIKAWA T., AKIYAMA H., DE LUNA E., GONZALEZ D., HIGUCHI M. & DEGUCHI, H., 2002 — Molecular phylogeny of hypnobryalean mosses as inferred from a large-scale dataset of chloroplast *rbcL*, with special reference to the Hypnaceae and possibly related families. *Hikobia* 13: 645-665.
- WEIS G., 2001 — Morphologische und anatomische Untersuchungen der Sporophyten bei den Jubulaceae Klinggr. und Lejeuneaceae Casares-Gil (Hepaticae) und deren systematische Bedeutung. *Bryophytorum Bibliotheca* 57: 5-302.
- WELLMAN C.H., OSTERLOFF P.L. & MOHIUDDIN, U., 2003 — Fragments of the earliest land plants. *Nature* 425: 282-285.

- WHEELER J.A., 2000 — Molecular phylogenetic reconstructions of the marchantioid liverwort radiation. *The Bryologist* 103: 314-333.
- WILSON R., GRADSTEIN S.R., HEINRICHS J., GROTH H., ILKIU BORGES A.L. & HARTMANN F.A., 2004 — Phylogeny of Lejeuneaceae: A cladistic analysis of chloroplast gene *rbcL* sequences and morphology with preliminary comments on the mitochondrial *nad4-2* spacer region. *Monographs in Systematic Botany from the Missouri Botanical Garden* 98: 189-202.
- YANO O. & GRADSTEIN S.R., 1997 — *Genera of Hepatics*. Göttingen: Systematisch-Geobotanisches Institut, 29 p.
- YATSENYUK S.P., KONSTANTINOVA N.A., IGNATOV M.S., HYVÖNEN J. AND TROITSKY A.V., 2004 — On phylogeny of Lophoziaceae and related families (Hepaticae, Jungermanniales) based on *trnL-trnF* intron-spacer sequences of chloroplast DNA. *Monographs in Systematic Botany from the Missouri Botanical Garden* 98: 150-167.
- ZWICKL D.J. & HILLIS D.M. 2002 — Increased taxon sampling greatly reduces phylogenetic error. *Systematic Biology* 51: 588-598.