SYSTEMATICS AND PHYLOGENY

Micromitriaceae: A new family of highly reduced mosses

Bernard Goffinet, Jessica M. Budke & Leah C. Newman

Department of Ecology and Evolutionary Biology, University of Connecticut, 75 North Eagleville Road, Storrs, Connecticut 06269-3043, U.S.A.
Author for correspondence: *Bernard Goffinet, bernard.goffinet@uconn.edu*

Abstract Morphological complexity tends to increase at a macroevolutionary scale among land plants, but reversals or loss of previously acquired complex traits occurs across various lineages and in particular in bryophytes. In mosses reduction may pertain to the leafy gametophyte and the architecture of the sporophyte, and is often considered linked to shifts in habitats. Reduction in morphological complexity may obscure the phylogenetic affinities of the taxa, in particular when multiple derived characters are lost. Ephemeraceae comprise two genera, *Ephemerum* and *Micromitrium*, growing typically on disturbed, seasonally moist soils. The hypothesis of a shared ancestry of these genera is drawn from the similarities in their highly reduced morphologies: their ephemeral gametophytes are tiny, bearing few leaves composed of typically smooth cells, and their capsules lack a differentiated lid and are nearly sessile. The family was historically included in Funariidae, but recent phylogenetic inferences revealed that Ephemeraceae likely arose within Dicranidae and may be of polyphyletic origin. Phylogenetic inferences based on eight loci confirm that both genera belong to Dicranidae, and reconstructions of the familial relationships within this subclass corroborate the independent origins of the ephemeral life history and associated character losses, with *Ephemerum* diverging from a pottiaceous ancestor and *Micromitrium* sharing a unique common ancestor with Leucobryaceae (Dicranales). *Micromitrium* is thus excluded from Ephemeraceae and accommodated within its own newly described family, Micromitriaceae. This study strengthens the hypothesis that homoplasy is recurrent throughout the evolutionary history of bryophytes and that phylogenetic inferences from DNA sequences are essential to test the systematic concepts based on morphological characters, and particularly in cases of taxa with the most simple architectures and life histories.

Keywords bryophyte phylogeny; Ephemeraceae; *Micromitrium*; morphological reduction; neoteny

■ INTRODUCTION

Reversal, or loss of previously acquired traits, is a common major evolutionary trend among land plants and has occurred in the architecture of the vegetative body (e.g., in Lemnaceae; Les & al., 1997), floral architecture (e.g., loss of function of floral parts; Endress, 2008), reproductive modes (e.g., loss of insect-mediated pollen dispersal; Culley & al., 2002) and even trophism (e.g., loss of autotrophy in parasitic plants; Westwood & al., 2010). Although lacking the complexity of vascular plants, bryophytes also exhibit evolutionary modifications through character loss in the vegetative body (e.g., Wickett & Goffinet, 2008; Masuzaki & al., 2010) and the sporophyte (e.g., Buck & al., 2000; Shaw & al., 2000; Goffinet & al., 2004). Neoteny is one form of character reduction whereby developmental stages are skipped and maturity is reached sooner resulting in the expression of juvenile traits in a mature organism. In bryophytes, extreme forms of neoteny consist of gametophytes that are reproductively mature, but lack differentiated leaves or even leafy axes altogether, with the vegetative plant body reduced to a photosynthetic filamentous state (Gradstein & Wilson, 2008). Simplification of the plant's architecture erases the morphological or anatomical characters essential to reconstruct the phylogenetic affinities of these taxa. In bryophytes, and mosses in particular, systematic concepts are drawn from both the haploid vegetative phase and the diploid sporogenous phase, and reduction in the complexity of both generations may result in a complete lack of phylogenetically informative characters.

The moss life cycle is characterized by a free-living, photosynthetic, sex-bearing haploid phase, the gametophyte. The sporophyte arises following sexual reproduction and remains attached to the maternal gametophyte throughout its relatively short life. The sporophyte of a "typical" moss is characterized by an elongated stalk that elevates the capsule to facilitate spore dispersal. The sporangium dehisces along a subapical line defining a discrete opening that is typically lined by one or two rings of teeth (i.e., the peristome) that may control the release of spores. The capsule wall bears stomata that may regulate gas exchange during the period of extensive metabolic activity characterizing sporogenesis or be critical in the dehydration of the sporangium to facilitate spore dispersal. As advantageous as these features may be, not all members of the Bryopsida develop a long stalk (seta) or a stomatous capsule dehiscing by a lid (e.g., Ditrichaceae [Buck & Snider, 1992] and Splachnaceae [Goffinet & Shaw, 2002]). More common than these reductions in size or dehiscence is the loss of functional peristomes (Vitt, 1981). Following capsule dehiscence, hygroscopic movement of the peristome teeth controls the sporangial opening and thereby the release of spores. Ninety percent of extant mosses arose following the acquisition of an articulated peristome, suggesting that the peristome is a key innovation. Nevertheless, gymnostomous capsules (i.e., lacking peristome teeth) occur throughout the phylogenetic spectrum of mosses (Vitt, 1981),

including members of such distantly related families as Funariaceae (Fife, 1985), Orthotrichaceae (Lewinsky, 1993) and Pottiaceae (Zander, 1993). Vitt (1981) suggested that reduced morphologies were prevalent among taxa inhabiting xerophytic environments. Among Hypnalean mosses, which are characterized by monopodial stems and lateral sex organs, reductions in peristome size and complexity have occurred repeatedly following a transition to epiphytism (Buck & al., 2000).

Morphological reduction may obscure phylogenetic affinities in particular when derived character states are lost. Peristome architecture is central to much of the suprafamilial classification of mosses (Goffinet & al., 2009), and assessing the affinities of gymnostomous taxa can be problematic. Combinations of derived vegetative character-states, such as incrassate, papillose, or dimorphic laminal cells may diagnose families or genera but lax, smooth laminal cells may not necessarily be plesiomorphic. Mishler (1988) showed that the heteroblastic series leading to architecturally complex leaves in the Pottiaceae includes juvenile and immature leaves that exhibit plesiomorphic states unlike the highly differentiated and characteristic mature leaves. Simple vegetative morphologies may thus represent truly plesiomorphic architectures or be acquired through paedomorphic development. Similarly, aperistomate sessile capsules may be neotenous sporophytes that skipped seta elongation and peristome development to proceed directly to sporogenesis (Shaw & al., 2000). Perhaps the best-known example of reverse evolution through loss of symplesiomorphies is *Physcomitrella patens* (Hedw.) Bruch & Schimp. (Funariaceae), a species characterized by short stems, bearing few leaves surrounding a nearly sessile capsule that lacks a differentiated mode of dehiscence and a peristome. *Physcomitrella patens* likely represents the ultimate product of a series of reductionary events from a *Funaria*-type ancestor with long setae terminated by an arcuate sporangium with an apical mouth lined by two well-developed rings of teeth (Fife, 1985).

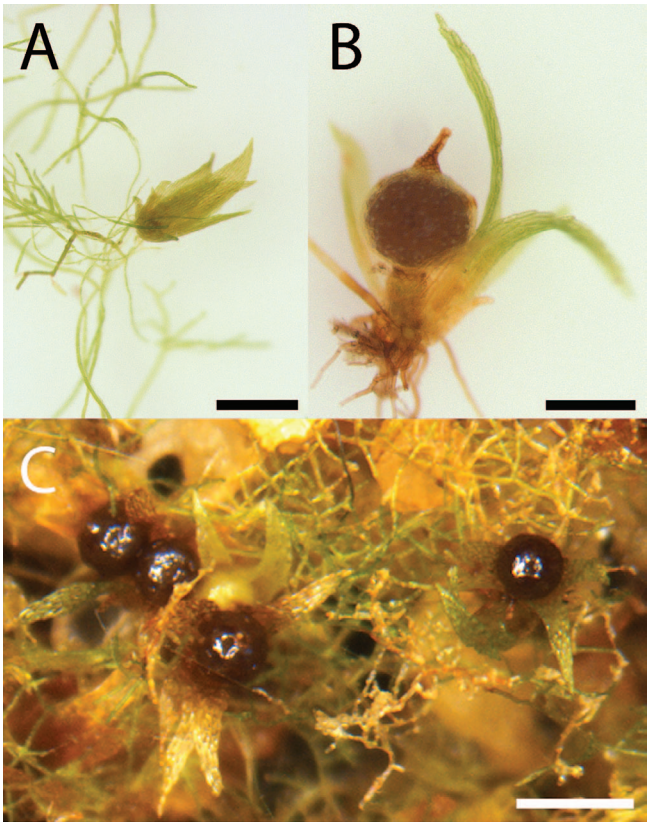

Fig. 1. Habit of *Micromitrium megalosporum* Austin. **A,** Leafy gametophyte developing from protonema; **B,** immersed capsule, covered by the calyptra; **C,** population of persistent protonema with developing and mature sporophytes. Scale bars = 1 mm.

Ephemeraceae Schimp. comprise species with ephemeral gametophytes, persistent protonema bearing tiny stems, less than 2 mm tall, with few leaves surrounding terminal sex organs (Bryan, 1957; Fig. 1). The sporophyte is always subtended by a very short seta, so that the sporogenous capsule is immersed among the vegetative leaves. The columella ultimately disintegrates and is absent in mature capsules. The capsule either lacks a differentiated line of dehiscence (cleistocarpy) or dehisces along a more or less median line, defining a rudimentary operculum (Bryan, 2007). The family comprises two genera, *Ephemerum* Hampe and *Micromitrium* Austin that are best differentiated by the size of the calyptrae, as states of no other trait consistently segregate between them (Bryan, 2007).

Ephemeraceae are thus characterized by highly simplified vegetative and sporogenous bodies, a combination that is unique among mosses. In the absence of complex traits, the affinities of the family remained ambiguous. Bruch & al. (1836), Müller (1849) and Lorch (1923) among others placed *Ephemerum* with other cleistocarpous mosses. Although such a concept was questioned as early as 1823 by Nees von Esenbeck & al., a consensus on the affinities of Ephemeraceae with Funariaceae only emerged in the early 1900s (e.g., Brotherus, 1909), and has prevailed since (see historical review in Bryan, 1957; Crosby, 1980; Vitt, 1984). Support for this hypothesis was further seen in cytological data, but the evidence was rather circumstantial given the chromosome numbers of $n = 11$ and 22 in *Micromitrium*, $n = 27$ in *Ephemerum*, and $n = 10$, 14, 26, 28, 56, or even 72 in Funariaceae (Bryan, 1957). Inferences from sequence data, however, revealed that the family (1) is only distantly related to Funariidae (Goffinet & Cox, 2000; Werner & al., 2007) sharing a common ancestor with members of a distinct subclass, Dicranidae (i.e., the haplolepideous mosses sensu Goffinet & al., 2009), and (2) may not be monophyletic, although the closest relative of *Micromitrium* remained ambiguous (Hedderson & al., 2004). A polyphyletic origin of Ephemeraceae would underline the extreme morphological convergence resulting from the architectural simplification of the vegetative and sporogenous body associated with a shift to transient habitats, a pattern also seen in liverworts (Gradstein & al., 2006).

We sought to further test the hypothesis of a haplolepideous origin of Ephemeraceae and assess the affinities of *Micromitrium*. We inferred their relationships with the backbone phylogeny of mosses based on inferences from variation in multiple loci (Cox & al., 2004), and then within Dicranidae based on *rps4* (cpDNA), the *nad5*-intron (mtDNA) and partial sequences of the large subunit (26S) of the nuclear ribosomal RNA retrieved from Cox & al. (2010).

■ MATERIALS AND METHODS

Taxon sampling and DNA sequencing. — Two or three undehisced capsules were removed from herbarium collections of *Micromitrium austinii* Sull. (U.S.A., Florida, Madison Co., *Buck 24917*, DUKE), *M. megalosporum* Austin (U.S.A., Georgia, Long Co., *B. Shaw 5348*, DUKE), *M. synoicum* Austin (U.S.A., Oregon, Linn Co., *Christy 8505-1*, DUKE), *M. tenerum* (Bruch & Schimp.) Crosby (U.S.A., North Carolina, Orange Co., *Majestyk & Cortez V. 7728*, DUKE), and *Ephemerum serratum* (Schreb. ex. Hedw.) Hampe (U.S.A., North Carolina, Durham Co., *Goffinet 4524*, CONN). DNA was extracted using a modification of the CTAB method (Doyle & Doyle, 1984) as described in Goffinet & al. (1998). Each extracted DNA sample was suspended in 50 µl of room temperature TE buffer.

Eight regions were targeted for amplification: *rps4*, *trnL-F*, *psbA*, *rbcL* (all from the chloroplast genome), *nad5*-intron, *nad7* (both mitochondrial), 26S and 18S (both in the nuclear genome). PCR reactions were performed in 25 µl comprising 1.0–2.0 µl of DNA, 1.0–1.5 µl of 10 mM dNTPs, 2.5 µl Hot Master Taq Buffer (5 Prime, Gaithersburg, Maryland, U.S.A.), 1.0–1.5 µl 10 mM primers, and 0.15 µl of Hot Master Taq. To amplify the 18S and *rps4* loci we used primers reported in Cox & al. (2000), for 26S those of Shaw (2000), for *trnL-F* those of Taberlet & al. (1991), for *nad5* those of Beckert & al. (1999). For the *nad5* gene region a nested PCR was used, with primers N5Ki and N5Li used on a template generated using primers NadF4 and NadR3.

The amplification protocol consisted of an initial denaturation at 94°C for 1 min 30 s, followed by 30 cycles, each composed of a denaturation step of 95°C for 20 s, annealing for 45 s at 48° or 52°C depending on locus, 68°C for 1 min, followed by a final extension at 68°C for 7 min. PCR products were then purified using the NucleoSpin Purification Kit (Takara Biotechnology, Otsu, Shiga, Japan) and eluded in 20 µl. Amplicons were sequenced using Big Dye v.1.1 (Applied Biosystems, Foster City, California, U.S.A.), in a 10 µl volume containing 2.0 µl Big Dye Buffer, 1.0 µl Big Dye, 0.33 µl primer, and 1.0 µl purified PCR product. Sequence products were purified using Sephadex columns and run on a 3100 ABI PRISM automated sequencer. Resulting chromatograms were edited using Sequencher v.4.9 (Gene Codes Corporation, Ann Arbor, Michigan, U.S.A.). Alignments were generated using Mafft v.6 (http://align.bmr.kyushu-u.ac.jp/mafft/online/server/) and manually corrected when needed, in MacClade v.4.08 (Maddison & Maddison, Sinauer Associates).

Phylogenetic analyses. — Sequences for the eight loci were added to the matrix used by Cox & al. (2004) to reconstruct the backbone phylogeny of mosses, restricting the taxon sample to the Bryopsida sensu Goffinet & al. (2009) (see Appendix 1 for list of species and GenBank accession numbers). Similarly, the *rps4*, *nad5*, and 26S sequences obtained for species of *Micromitrium* and one *Ephemerum* were added to a matrix composed of 97 Haplolepideae sampled by Cox & al. (2010) (see Appendix 2 for list of species and GenBank accession numbers). Matrices and trees are deposited into TreeBase, under accession number S11083.

Unweighted maximum parsimony analyses were performed on both matrices as follows using PAUP* v.4.0b10 (Swofford, 2002): An initial run performed by using the "tree bisection reconnection" (TBR) branch swapping algorithm, with the steepest descent option on, and only 10 trees saved for each of the 200 random addition replicates, was followed by a second analysis whereby all saved trees were swapped to completion with no limit to the number of trees saved. All other parameters were set to the default options (e.g., gaps were treated as missing data). Support for the branches was estimated using the bootstrap approach with a heuristic search algorithm on 500 pseudoreplicates each analyzed two times by randomly adding sequences; a limit of 1000 trees saved per pseudoreplicate was imposed. Bootstrap frequencies (MPB) were considered significant if higher than 70% (Hillis & Bull, 1993; Reeb & al., 2004).

For each of the eight gene regions and the combined dataset, jModelTest v.0.1.1 (Guindon & Gascuel, 2003; Posada, 2008) was used to identify the model with the lowest Bayesian Information Criterion (BIC). Based on a likelihood ratio test, each dataset was partitioned into single-locus character sets. For the eight loci analyzed for the backbone phylogeny, the following models were applied: HKY+Γ (*nad7*), HKY+I+Γ (*trnL-F*), GTR+Γ (*nad5*), and GTR+I+Γ (18S, 26S, *psbA*, *rbcL*, *rps4*). The three-loci matrix was divided into five partitions, and the following models implemented in the maximum likelihood and Bayesian analyses: HKY+Γ (*rps4*-1st), GTR+Γ (*rps4*-2nd, *nad5*-intron), GTR+I+Γ (*rps4*-3rd), and HKY+I+Γ (26S). In each analysis, the substitution model applied to each locus was allowed to vary independently.

Maximum likelihood (ML) searches were performed using GARLi v.0.951 (Zwickl, 2006). Bootstrap frequencies (MLB) were obtained from the majority-rule consensus tree of 100 trees obtained from 100 pseudoreplicate runs. MrBayes v.3.1.2 (Ronquist & Huelsenbeck, 2003) was used to carry out the Bayesian phylogenetic analyses. Each dataset was analyzed during four separate runs of 20,000,000 generations each. The only prior modified from the default was shapepr = exp (1.0) (Brown & al., 2010; Marshall, 2010). Trees were sampled every 1000 generations. The log-likelihood values were visually assessed using Tracer v.1.5 (Rambaut & Drummond, 2009) and found to converge at generation 30,000 for the eight loci and at 80,000 for the three loci analyses. All trees prior to convergence were excluded with all other trees combined using PAUP* to form a 50% majority-rule consensus tree with posterior probability (BPP) representing the frequency of particular clades across the sample of trees.

■ RESULTS

Sequences and aligned matrices. — Of the eight loci surveyed (*rps4*, *trnL-F*, *psbA*, *rbcL*, *nad5*, *nad7*, 26S, 18S), novel sequences were generated for three of the four *Micromitrium* and one *Ephemerum* species. Sequences of *psbA* and *rbcL* were not obtained for any of the Ephemeraceae surveyed. No additional sequences were generated for *M. austinii* with those previously published for *rps4* and *nad5* used in the subsequent analyses

(Appendix 2). Similarly we were unable to obtain 18S for *M. megalosporum*, *nad7*, *rps4*, 26S and 18S for *M. synoicum* and *trnL-F* for *M. tenerum*. The sequences obtained are deposited in GenBank with accession numbers as follows (18S/26S/*rps4*/*trnL-F*/*nad5*/*nad7*): *M. megalosporum* –/GU252050/GU252053/GU252056/GU252043/GU252046; *Micromitrium synoicum* –/–/–/GU252054/GU252041/–; *M. tenerum* GU252047/GU252049/GU252052/–/GU252042/GU252044; *Ephemerum serratum* GU252048/ GU252051/–/GU252055/–/GU252045. The *nad5*-introns of *M. tenerum* and *M. austinii* differed by a 512 base pair deletion in the former. The eight-locus matrix comprised 24 taxa and 10,053 characters of which 8392 were included in the analyses. The 26S, *nad5*, and *rps4* matrix sampled for the 101 species, with an emphasis on Dicranidae, contained after exclusion of regions of ambiguous alignment (i.e., 1696 sites) 2530 characters, of which nearly 35% were variable and 21% potentially parsimony informative. The distribution of variable sites across the partitions is presented in Table 1.

Topologies of the optimal trees or sets of trees were compared across the three analyses and found to be congruent at 75% bootstrap and 0.95 posterior probability support levels. Overall, the eight-loci phylogeny is congruent with that of Cox & al. (2004): Funariidae and Timmiidae are sister, and together compose the sister-group to Dicranidae and Bryidae, which are each monophyletic. Within the backbone phylogeny reconstructed from eight loci, Ephemeraceae are unambiguously resolved within Dicranidae (Fig. 2). Within Dicranidae, Ephemeraceae compose a polyphyletic group, with *Ephemerum* nested well within Pottiaceae, and *Micromitrium* composing the sister-group to Leucobryaceae (Fig. 3). The monophyly of *Micromitrium* is supported only by a high posterior probability (0.99) as bootstrap frequencies were low under MP (64%) and ML (59%).

■ DISCUSSION

Ephemerum and *Micromitrium* share highly reduced gametophytes characterized by a short leafy stem borne on a persistent protonema and immersed to emergent, aperistomate

Table 1. Distribution of variable sites across the five partitions of the 26S+*rps4*+*nad5*-intron matrix among all 101 accessions of Dicranidae.

	26S	*rps4*-1st	*rps4*-2nd	*rps4*-3rd	*nad5*	Total
Number of sites	918	188	189	189	1046	2530
Number of variable sites	140	95	64	150	426	874
Number of non-apomorphic sites	86	62	37	107	251	543

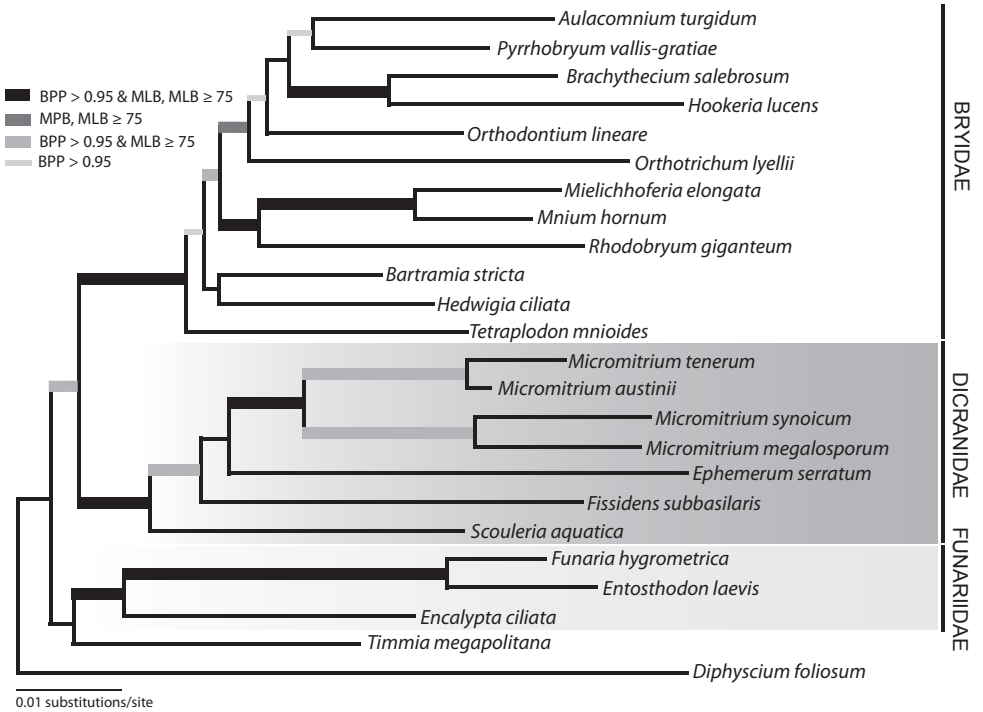

Fig. 2. Phylogram of most likely tree (−ln likelihood = 32,989.67) obtained for the relationships of major lineages of Bryopsida based on inferences from eight loci using a partitioned likelihood approach. Degree of support for branches in terms of parsimony and likelihood bootstrap frequencies and Bayesian posteriors is marked by thickness of branches.

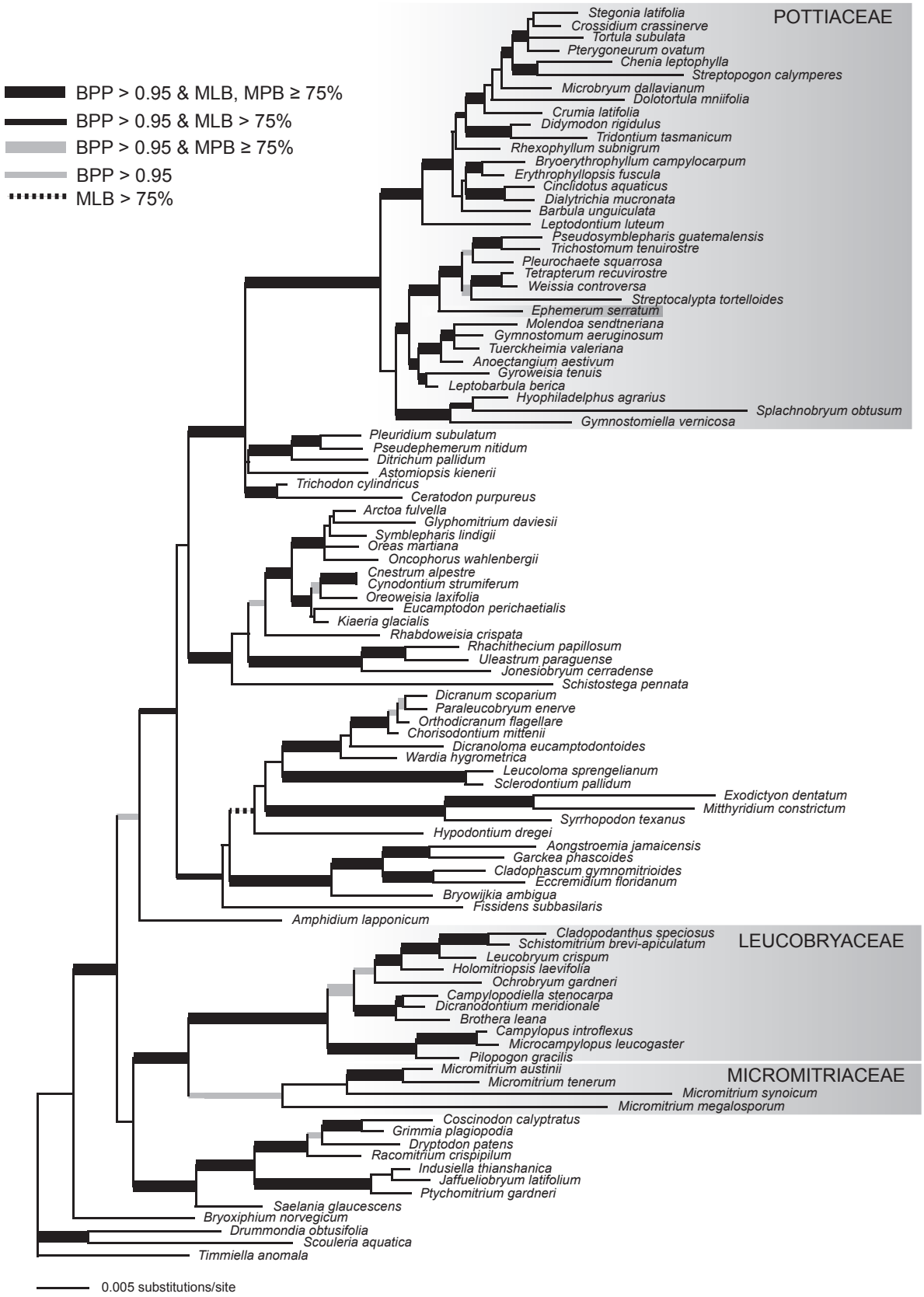

Fig. 3. Phylogram of most likely tree (−ln likelihood = 17,871.53) obtained for the relationships of major lineages of Dicranidae based on inferences from three loci using a partitioned likelihood approach. Degree of support for branches in terms of parsimony and likelihood bootstrap frequencies and Bayesian posteriors is marked by thickness of branches.

capsules (Bryan, 1957). The similarity in growth form and architecture composed the basis for uniting these genera within a single family, Ephemeraceae Schimp. The family has been characterized by its lax and smooth to papillose laminal cells (Vitt, 1982), which are somewhat reminiscent of those of Funariaceae. Inferences from gene sequences led Goffinet & Cox (2000) to suggest that *Ephemerum* may belong to Dicranidae, a hypothesis later endorsed by Werner & al. (2007), and extended to *Micromitrium* by Hedderson & al. (2004). Such haplolepideous ancestry of Ephemeraceae is here unequivocally confirmed based on a broader sampling of loci (Fig. 2).

Ephemerum and *Micromitrium* were thought to be closely related solely on the basis of the minute gametophytes, similar (yet distinct) leaf areolations, growth form, an ephemeral habit with a persistent protonema and also the lysis of the columella during the final stages of sporangial maturation (Bryan, 1957). Unambiguous morphological differentiation between the genera is lacking, except for the consistently smaller calyptrae in *Micromitrium* (Bryan, 1957, 2007). Indeed, each genus could be defined by a suite of typical characters but exceptions weaken the systematic significance of these traits. The two genera are, however, characterized by distinct chromosome numbers, namely $n = 27$ in *Ephemerum* and $n = 11$ or 22 in *Micromitrium* (Bryan, 1957). Duckett & al. (2004) and Pressel & al. (2005) also noted a difference in sporeling ontogeny, with *Ephemerum* following a pathway similar to that seen in Pottiaceae and Dicranales, whereas germination of *Micromitrium* spores resembled that of Funariaceae. Such distinct phyletic affinities were previously proposed by Lindberg (1879) and Braithwaite (1887), who included *Ephemerum* in Pottiaceae (as Tortulaceae) and *Micromitrium* in Funariaceae. The possibility that Ephemeraceae do not share a unique common ancestor was also raised by Hedderson & al. (2004) based on phylogenetic inferences from variation in the *rps4* locus. Within the backbone phylogeny of peristomate mosses, both genera of Ephemeraceae are unambiguously resolved as members of Dicranidae (Fig. 2), but within this diverse lineage they arose from distinct ancestors: *Ephemerum* is nested within Pottiaceae and *Micromitrium* composes the sister group to Leucobryaceae (Fig. 3). Such relationships may be consistent with patterns in chromosomes number. In *Micromitrium* $n = 11$ or 22 and in Leucobryaceae sensu Goffinet & al. (2009), several species of *Campylopus* Brid., *Dicranodontium* Bruch & Schimp., and *Leucobryum* Hampe have $n = 11$ although overall the number varies from 6 to 15 (Fritsch, 1982). *Ephemerum* with $n = 27$ may be a diploid based on $n = 12$ or 13 (plus one minute autosome) as is common in closely taxa related to it based on Cox & al. (2010), namely *Pleurochaete* Lindb. ($n = 13$), *Trichostomum* Hedw. ($n = 12$), or *Weissia* Hedw. (n = mostly 13; Fritsch, 1982).

Micromitrium shares a unique ancestry with Leucobryaceae, and could hence be included in this family. However, Leucobryaceae sensu Goffinet & al. (2009) are here resolved as a robust monophyletic group that is phylogenetically well differentiated from *Micromitrium*. Leucobryaceae typically comprise taxa exhibiting a unique leaf architecture, wherein the costa is broadened, and composed of a median layer of chlorophyllose cells sandwiched by two or more layers of hyaline cells (Vitt, 1982). Its circumscription with only four genera (Buck & Goffinet, 2000) was broadened following phylogenetic inferences within Dicranales (LaFarge & al., 2000) to include also members of Dicranaceae characterized by a broad costa, namely Campylopodioideae sensu Stech (1999). The gametophyte of Leucobryaceae is well developed, with densely foliated stems, leaves with a complex costal anatomy and long exserted capsules with a well-developed peristome. In comparison, *Micromitrium* exhibits neotenous development of both generations, resulting in a persistent protonemata, with tiny stems bearing few leaves, and sessile capsules, lacking in part a differentiated mode of dehiscence. Considering the broad morphological differences spanning the extremely simple body form in *Micromitrium* and complex gametophytic and sporophytic architectures in Leucobryaceae we propose to treat these two sister lineages as distinct families. The name Micromitriaceae was first introduced by Smyth (1913) in his "Provisional catalogue of the flora of Kansas", without a description or even circumscription, and in fact only in his classification. The entry follows Jungermanniaceae (liverworts) and precedes Archidiaceae and Phascaceae. We assume that the name was applied to mosses and that Smyth introduced it to accommodate *Ephemerum* and *Micromitrium*, ignoring the previously established Ephemeraceae Schimp. (1856).

Micromitriaceae Smyth ex Goffinet & Budke, **fam. nov.** ("Micromitriaceae" Smyth in Trans. Kansas Acad. Sci. 25: 73. 1913, nom. nud.) – Type: *Micromitrium* Austin, Musci Appalach.: 10. 1870.

Plantae ephemerae protonematibus abundis et caulibus minutis foliis paucis, cellulis folii laevibus, costa debilibus vel destituta; capsula globosa sessilis indehiscens vel dehiscens prope aequatorem, peristomio nullo.

Plants ephemeral, with extensive protonema and tiny stems bearing few leaves, composed on smooth laminal cells; costa weak or absent. Capsule globose, sessile, indehiscent or dehiscent near equator, peristome lacking.

The monophyly of *Micromitrium* is only supported by posterior probabilities (Fig. 3). This weakness may be an artifact resulting from the extremely long branch defining both *M. megalosporum* and *M. synoicum*. Such marked differentiation is also reflected in the distinct morphology of *M. megalosporum*: a two-layered stomatose capsule wall, spores larger than 40 µm (Bryan & Anderson, 1957), a diploid chromosome number (i.e., $n = 22$), and the lack of minute autosomes, expected to be present should this species be a diploid (Bryan, 1957). Support for the monophyly of *Micromitrium* may be strengthened by sampling more of the potentially nine species composing the genus (Crosby & al., 1999).

Micromitrium austinii and *M. tenerum* are distinguished by the serrulation toward the apex of the leaf. Bryan (1999) considers this feature to be variable within samples, and hence unreliable for separating taxonomic entities, whereas Kiguchi & al.

(2006) maintain the distinction of the species. The two North American samples included here differ in their sequences, most notably by the presence of a unique 512-nucleotide deletion in the *nad5*-intron of *M. tenerum*. We have not seen the material upon which the sequences provided by Cox & al. (2010) are based. The taxonomic significance of the sequence divergence should be assessed based on a broader geographic sampling and integrated with a detailed morphometric analysis. *Micromitrium* species have been grouped together based on their similar spore size, lack of peristome, small calyptra and overall reduced stature (Bryan & Anderson, 1957). Although none of these features, nor even their combination is unique to *Micromitrium* we prefer to maintain the current circumscription of the genus.

Phylogenetic inferences from DNA sequence data have confirmed and strengthened the hypothesis that homoplasy via reduction is a recurrent process in the evolutionary history of bryophytes (e.g., Buck & al., 2000; Sotiaux & al., 2009; Masuzaki & al., 2010). Parallel transformations resulting in highly similar morphologies may be correlated to independent shifts to similar habitats, such as to xeric (Vitt, 1981) or aquatic (Olsson & al., 2009) environments. However, the direction of the transformations is not always evident. In the *Rhynchostegium* complex, aquatic species with elaborate multistratose leaves compose a derived polyphyletic entity that may have resulted from convergence from a terrestrial ancestor or by contrast represent a plesiomorphic grade of aquatic species that independently gave rise to terrestrial or epiphytic taxa (Huttunen & Ignatov, 2010). Characters associated with highly specialized insect-mediated spore dispersal (Marino & al., 2009) are lacking in approximately half Splachnaceae, defining a polyphyletic entity within the genus *Tayloria* Hook. Whether the taxa exhibiting simple sporophytes represent a plesiomorphic morphology or whether they evolved through sporophyte reduction following a return to a generalist habitat, remains ambiguous (Goffinet & al., 2004). The shared ancestry of Micromitriaceae and Leucobryaceae alone does not suffice to assess the origin of the reduced morphological complexity of the former. In the absence of a known sister group to this combined lineage, the evolutionary significance of Micromitriaceae is obscure. Taxa with reduced morphologies are intuitively considered evolutionary dead-ends, yet considering that the genetic networks underlying the development of complex architecture are not yet elucidated, the hypothesis that reduction can be reversed can not and should not be dismissed (Zander, 2006).

■ ACKNOWLEDGMENTS

This study was funded by a grant from the National Science Foundation to B.G. (DEB-0919284) and a Summer Undergraduate Research Fellowship from the University of Connecticut to L.C.N. We gratefully acknowledge the loan of specimens and permission to sample DNA from the curators at DUKE and NY. Dr. William R. Buck (NY) kindly provided a Latin diagnosis. We thank Dr. Alain Vanderpoorten and an anonymous reviewer for their constructive review of the manuscript.

■ LITERATURE CITED

Beckert, S., Steinhauser, S., Muhle, H. & Knoop, V. 1999. A molecular phylogeny of bryophytes based on nucleotide sequences of the mitochondrial *nad5* gene. *Pl. Syst. Evol.* 218: 179–192.

Braithwaite, R. 1887. *The British moss flora*, vol. 1, *Families of acrocarpous mosses*. London: Pewtress & Co.

Brotherus, F. 1909. Unterklasse Bryales. Pp. 1173–1239 in: Engler, A. & Prantl, K. (eds.), *Die natürlichen Pflanzenfamilien*, vol. 1(3). Leipzig: Engelmann.

Brown, J.M., Hedke, S.M., Lemmon, A.R. & Lemmon, E.M. 2010. When trees grow too long: Investigating the causes of highly inaccurate Bayesian branch-length estimates. *Syst. Biol.* 59: 145–161.

Bruch, P., Schimper, W.P. & Gümbel, T. 1836. *Bryologia europea*, vol. 1. Stuttgart: Schweizerbart.

Bryan, V.S. 1957. Cytotaxonomic studies in the Ephemeraceae and Funariaceae. *Bryologist* 60: 103–126.

Bryan, V.S. 1999. Synonymy in *Micromitrium*. *Bryologist* 102: 409–411.

Bryan, V.S. 2007. *Ephemeraceae* Schimper. Pp. 646–653 in: Flora of North America Editorial Committee (eds.), *Flora of North America north of Mexico*, vol. 27, *Bryophyta*, part 1. Oxford: Oxford University Press.

Bryan, V.S. & Anderson, L.E. 1957. The Ephemeraceae in North America. *Bryologist* 60: 67–102.

Buck, W.R. & Goffinet, B. 2000. *Morphology and classification of mosses*. Pp. 124–149 in: Shaw, A.J. & Goffinet, B. (eds.), *Bryophyte biology*. Cambridge: Cambridge University Press.

Buck, W.R. & Snider, J.A. 1992. *Crumuscus vitalis* gen. et sp. nov. (Ditrichaceae). *Contr. Univ. Michigan Herb.* 18: 39–41.

Buck, W.R., Goffinet, B. & Shaw, A.J. 2000. Testing morphological concepts of orders of pleurocarpous mosses (Bryophyta) using phylogenetic reconstructions based on *trnL-trnF* and *rps4* sequences. *Molec. Phylog. Evol.* 16: 180–198.

Cox, C.J., Goffinet, B., Newton, A.E., Shaw, A.J. & Hedderson, T.A.J. 2000. Phylogenetic relationships among the diplolepideous-alternate mosses (Bryidae) inferred from nuclear and chloroplast DNA sequences. *Bryologist* 103: 224–241.

Cox, C.J., Goffinet, B., Shaw, A.J. & Boles, S.B. 2004. Phylogenic relationships among the mosses based on heterogeneous Bayesian analysis of multiple genes from multiple genomic compartments. *Syst. Bot.* 29: 234–250.

Cox, C.J., Goffinet, B., Wickett, N.J., Boles, S.B. & Shaw, A.J. 2010. Moss diversity: A molecular phylogenetic analysis of genera. *Phytotaxa* 9: 175–195.

Crosby, M.R. 1980. The diversity and relationships of mosses. Pp. 115–129 in: Taylor, R.J. & Leviton, A.E. (eds.), *The mosses of North America*. San Francisco: California Academy of Sciences.

Crosby, M.R., Magill, R.E., Allen, B. & He, S. 1999. *Checklist of mosses*. St. Louis: Missouri Botanical Garden.

Culley, T.M., Weller, S.G. & Sakai, A.K. 2002. The evolution of wind pollination in angiosperms. *Trends Ecol. Evol.* 17: 361–369.

Doyle, J.J. & Doyle, J.L. 1984. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Focus* 12: 13–15.

Duckett, J.G., Burch, J., Fletcher, P.W., Matcham, H.W., Read, D.J., Russel, A.J. & Pressel, S. 2004. In vitro cultivation of bryophytes: A review of practicalities, problems, progress and promise. *J. Bryol.* 26: 3–20.

Endress, P.K. 2008. The whole and the parts: Relationships between floral architecture and floral organ shape, and their repercussions on the interpretation of the fragmentary floral fossils. *Ann. Missouri Bot. Gard.* 95: 101–120.

Fife, A.J. 1985. A generic revision of the Funariaceae (Bryophyta: Musci). *J. Hattori Bot. Lab.* 58: 149–196.

Fritsch, R. 1982. *Index to plant chromosome numbers—Bryophyta*. Regnum Vegetabile 108. Utrecht: Bohn, Scheltema & Holkema.

Goffinet, B. & Cox, C.J. 2000. Phylogenetic relationships among

basal-most arthrodontous mosses with special emphasis on the evolutionary significance of the Funariineae. *Bryologist* 103: 212–223.

Goffinet, B. & Shaw, A.J. 2002. Independent origins of cleistocarpy in the Splachnaceae: Analyses of cpDNA sequences and polyphyly of the Voitioideae (Bryophyta). *Syst. Bot.* 27: 203–208.

Goffinet, B., Bayer, R.J. & Vitt, D.H. 1998. Circumscription and phylogeny of the Orthotrichales (Bryopsida) inferred from *rbcL* sequence analysis. *Amer. J. Bot.* 85: 1324–1337.

Goffinet, B., Buck, W.R. & Shaw, A.J. 2009. Morphology, anatomy, and classification of the Bryophyta. Pp. 55–138 in: Goffinet, B. & Shaw, A.J. (eds.), *Bryophyte biology*, 2nd ed. Cambridge: Cambridge University Press.

Goffinet, B., Shaw, A.J. & Cox, C.J. 2004. Phylogenetic inferences in the dung-moss family Splachnaceae from analyses of cpDNA sequence data and implications for the evolution of entomophily. *Amer. J. Bot.* 91: 748–759.

Gradstein, S.R. & Wilson, R. 2008. Protonemal neoteny in Bryophytes. Pp. 1–11 in: Mohamed, H., Baki, B.B. Nasrulhaq-Boyce, A. & Lee, P.K.Y. (eds.), *Bryology in the New Millenium*. Kuala Lumpur: University of Malaya.

Gradstein, R.R., Wilson, R., Ilkiu-Borges, A.L. & Heinrichs, J. 2006. Phylogenetic relationships and neotenic evolution of *Metzgeriopsis* (Lejeuneaceae) based on chloroplast DNA sequences and morphology. *Bot. J. Linn. Soc.* 151: 293–308.

Guindon, S. & Gascuel, O. 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Syst. Biol.* 52: 696–704.

Hedderson, T.A., Murray, D.J., Cox, C.J. & Nowell, T.L. 2004. Phylogenetic relationships of haplolepideous mosses (Dicranidae) inferred from *rps4* gene sequences. *Syst. Bot.* 29: 29–41.

Hillis, D.M. & Bull, J.J. 1993. An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Syst. Biol.* 42: 182–192.

Huttunen, S. & Ignatov, M.S. 2010. Evolution and taxonomy of aquatic species in the genus *Rhynchostegium* (Brachytheciaceae, Bryophyta). *Taxon* 59: 791–808.

Kiguchi, H., Iwatsuki, Z. & Suzuki, T. 2006. *Micromitrium austinii* (Sull.) Lindb. newly found in Japan. *Bryol. Res.* 9: 103–106.

LaFarge, C., Mishler, B.D., Wheeler, J.A., Wall, D.P., Johannes, K., Schaffer, S. & Shaw, A.J. 2000. Phylogenetic relationships within the haplolepideous mosses. *Bryologist* 103: 257—276.

Les, D.H., Landolt, E. & Crawford, D.J. 1997. Systematics of the Lemnaceae (duckweeds): Inferences from micromolecular and morphological data. *Pl. Syst. Evol.* 204: 161–177.

Lewinsky, J. 1993. A synopsis of the genus *Orthotrichum* Hedw. (Musci, Orthotrichaceae). *Bryobrothera* 2: 1–59.

Lindberg, S.O. 1879. *Musci scandinavici in systemate novo naturali dispositi*. Upsala: ex officina Jesaiae Edquisi.

Lorch, W. 1923. *Die Laubmoose*. Berlin: Springer.

Marino, P., Raguso, R. & Goffinet, B. 2009. The ecology and evolution of fly dispersed dung mosses (Family Splachnaceae): Manipulating insect behaviour through odour and visual cues. *Symbiosis* 47: 61–76.

Marshall, D.C. 2010. Cryptic failure of partitioned Bayesian phylogenetic analyses: Lost in the land of long trees. *Syst. Biol.* 59: 108–117.

Masuzaki, H., Shimamura, M., Furuki, T., Tsubota, H., Yamaguchi, T., Haji Mohamed Abdul Majid & Deguchi, H. 2010. Systematic position of the enigmatic liverwort *Mizutania* (Mizutaniaceae, Marchantiophyta) inferred from molecular phylogenetic analyses. *Taxon* 59: 448–458.

Mishler, B.D. 1988. Relationships between ontogeny and phylogeny, with reference to bryophytes. Pp. 117–136 in: Humphries, C.J. (ed.), *Ontogeny and systematics*. New York: Columbia University Press.

Müller, K. 1849. *Synopsis muscorum frondosorum omnium hucusque cognitorum*, vol. 1, *Musci vegetationis acrocarpiae*. Berlin: Foerstner.

Nees von Esenbeck, C.G., Hornschuch, F. & Sturm, J. 1823–31. *Bryologia Germanica, oder Beschreibung der in Deutschland und in der Schweiz wachsenden Laubmoose*, Theil 1. New York: G.E. Stechert & Co.

Olsson, S., Rumsey, F., Grundmann, M., Russell, S., Enroth, J. & Quandt, D. 2009. The origin of the British and Macaronesian endemic *Thamnobryum* species (Neckeraceae). *J. Bryol.* 31: 1–10.

Posada, D. 2008. jModelTest: Phylogenetic model averaging. *Molec. Biol. Evol.* 25: 1253–1256.

Pressel, S., Matcham, H. & Duckett, J.G. 2005. Studies of protonemal morphogenesis in mosses. X. Ephemeraceae revisited; New dimensions underground. *J. Bryol.* 27: 311–318.

Rambaut, A. & Drummond, A.J. 2009. Tracer, version 1.5. http://tree.bio.ed.ac.uk/software/tracer/.

Reeb, V., Lutzoni, F. & Roux, C. 2004. Contribution of *RPB2* to multilocus phylogenetic studies of the Pezizomycotina (euascomycetes, Fungi) with special emphasis on the lichen-forming Acarosporaceae and evolution of polyspory. *Molec. Phylog. Evol.* 32: 1036–1060.

Ronquist, F. & Huelsenbeck, J.P. 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574.

Schimper, W.P. 1856. *Corollarium bryologiae europaeae, conspectum diagnosticum familiarum, generum et specierum, adnotationes novas atque emendations complectens.* Stuttgart: sumptibus Librariae E. Schweizerbart.

Shaw, A.J. 2000. Phylogeny of the Sphagnopsida based on chloroplast and nuclear DNA sequences. *Bryologist* 103: 277–306.

Shaw, A.J., Anderson, L.E. & Mishler, B.D. 2000. Paedomorphic sporophyte development in *Bruchia flexuosa* (Bruchiaceae). *Bryologist* 103: 147–155.

Smyth, B.B. 1913. Provisional catalogue of the flora of Kansas. Part II. Gymnosperms and monocotyls. *Trans. Kansas Acad. Sci.* 25: 63–128.

Sotiaux, A., Enroth, J., Olsson, S., Quandt, D. & Vanderpoorten, A. 2009. When morphology and molecules tell us different stories: A case-in point with *Leptodon corsicus*, a new and unique endemic moss species from Corsica. *J. Bryol.* 31: 186–196.

Stech, M. 1999. A reclassification of Dicranaceae (Bryopsida) based on non-coding cpDNA sequence data. *J. Hattori Bot. Lab.* 86: 137–159.

Swofford, D.L. 2002. PAUP*: Phylogenetic Analysis Using Parsimony (*and other methods), version 4.0. Sunderland, Massachusetts: Sinauer.

Taberlet, P., Gielly, L., Pautou, G. & Bouvet, J. 1991. Universal primers for amplification for three non-coding regions of chloroplast DNA. *Annual Rev. Pl. Physiol. Pl. Molec. Biol.* 17: 1105–1109.

Vitt, D.H. 1981. Adaptive modes of the moss sporophyte. *Bryologist* 84: 166–186.

Vitt, D.H. 1982. Sphagnopsida and Bryopsida, Pp. 305, 307–336 in: Parker, S.P. (ed.), *Synopsis and classification of living organisms.* New York: McGraw-Hill.

Vitt, D.H. 1984. Classification of the Bryopsida. Pp. 696–759 in: Schuster, R.M. (ed.), *New manual of bryology*, vol. 2. Nichinan: Hattori Botanical Laboratory.

Werner, O., Ros, R.M. & Goffinet, B. 2007. A reconsideration of the systematic position of *Goniomitrium* (Funariaceae) based on chloroplast sequence markers. *Bryologist* 110: 108–114.

Westwood, J.H., Yoder, J.I., Timko, M.P. & dePamphilis, C.W. 2010. The evolution of parasitism in plants. *Trends Pl. Sci.* 15: 227–235.

Wickett, N.J. & Goffinet, B. 2008. The origin and relationships of the myco-heterotrophic liverwort *Cryptothallus mirabilis* Malmb. (Metzgeriales, Marchantiophyta). *Bot. J. Linn. Soc.* 156: 1–12.

Zander, R.H. 1993. Genera of the Pottiaceae: Mosses of harsh environments. *Bull. Buffalo Soc. Nat. Sci.* 32: i–vi, 1–378.

Zander, R.H. 2006. The Pottiaceae s. str. as an evolutionary Lazarus taxon. *J. Hattori Bot. Lab.* 100: 581–602.

Zwickl, D. 2006. *Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion*. Dissertation, University of Texas, Austin.

Appendix 1. GenBank accession numbers for taxa included in the eight loci phylogenetic reconstruction of Bryopsida derived from Cox & al. (2004). Number order follows: 18S/26S/*nad5*/*nad7*/*trnL-F*/*rps4*/*rbcL*/*psbA*. Missing data: –.

Aulacomnium turgidum (Wahlenb.) Schwägr.: AF023687/AY330427/AY312869/AY330455/AF023728/AF023809/AJ275180/AY312894; ***Bartramia stricta*** Brid.: AF023698/AY330428/AY312870/AY330456/AF023756/AF023799/AY312926/AY312895; ***Brachythecium salebrosum*** (G.F. Hoffmann ex Weber & D. Mohr) Schimp. in B.S.G.: AY330417/AY330429/AY312871/AY330457/AF161120/AF143027/AY312927/AY312896; ***Diphyscium foliosum*** (Hedw.) D. Mohr: Y17765/AY330432/AY312874/AY330459/AF229891/AJ251065/AY312928/AY312899; ***Encalypta ciliata*** Hedw.: AF223011/AY330433/AY312875/AY330460/AF229897/AF223040/AY312929/AY312900; ***Entosthodon laevis*** (Mitten) Fife: AY330419/AY330434/AY312876/AY330461/AY312941/AY330478/AY312930/AY312901; ***Fissidens subbasilaris*** Hedw.: AF223027/AY330435/AY312877/AY330462/AF229913/AF223056/AF231304/AY312902; ***Funaria hygrometrica*** Hedw.: X74114/AY330436/Z98959/AY330463/AF231175/AF023776/AF005513/AY312903; ***Hedwigia ciliata*** (Hedw.) P. De Beauv.: AJ275010/AY330438/Z98966/AY330464/AF233587/AJ251309/AF231073/AY312905; ***Hookeria lucens*** (Hedw.) Smith: AJ243168/AY330439/Z98969/AY330465/AF215906/AJ251316/AY312931/AY312906; ***Mielichhoferia elongata*** (Hoppe & Hornschuch in W.J. Hooker) Nees & Hornschuch: AF023708/AY330440/AY312878/–/AF023766/AF023793/AF232693/AY312907; ***Mnium hornum*** Hedw.: X80985/AY330441/AY312879/–/AF182360/AF023796/AF226820/AY312908; ***Orthodontium lineare*** Schwägr.: AF023697/AY330443/AY312881/AY330467/AF023768/AF023800/AJ275174/AY312910; ***Orthotrichum lyellii*** W.J. Hooker & Taylor: AF025291/AY330444/AY312882/AY330468/AF023727/AF023814/AF005536/AY312911; ***Pyrrhobryum vallis-gratiae*** (Hampe ex C. Müller) Manuel: AF023695/AY330448/AY312885/AY330471/AF023754/AF023825/AJ275179/AY312917; ***Rhodobryum giganteum*** (Schwägr.) Paris: AF02369/AY330449/AY312886/–/AF023737/AF023789/AJ275176/AY312918; ***Scouleria aquatica*** W.J. Hooker in Drummond: AF023684/AY330450/AY312887/AY330472/AF023723/AF023780/AF226822/AY312919; ***Tetraplodon mnioides*** (Swartz ex Hedw.) Bruch & Schimp. in B.S.G.: AF023691/–/–/AY330474/AF023730/AF023804/AY312937/AY312922; ***Timmia megapolitana*** Hedw.: AY330423/AY330452/AY312890/AY330475/AY312948/AF222902/AY312938/AY312923.

Appendix 2. Taxa, voucher information and associated GenBank number for the *rps4*, *nad5,* and 26S loci sampled from Cox & al. (2010) for inferring the relationships of Ephemeraceae within Dicranidae.

Amphidium lapponicum (Hedw.) Schimp., *Schofield 98089* (DUKE): AF222896/AY908962/HM751586; ***Anoectangium aestivum*** (Hedw.) Mitten, *Schofield 104414* (MO): AY908049/AY908832/HM751554; ***Aongstroemia jamaicensis*** C. Müll., *Allen 6403* (DUKE): AY908094/AY908869/HM751612; ***Arctoa fulvella*** (Dickson) Bruch & Schimp. in B.S.G., *Schofield 102571* (DUKE): AY908075/AY908894/HM751635; ***Astomiopsis kienerii*** (Bartr.) Delgadillo & Cardenas, *Cardenas 3953* (DUKE): AY908072/AY908857/HM751549; ***Barbula unguiculata*** Hedw., *Zander 1975* (BUF): AF306986/AY908844/HM751536; ***Brothera leana*** (Sull.) C. Müll., *Long 21998* (DUKE): AY908129/AY908911/HM751587; ***Bryoerythrophyllum campylocarpum*** (C. Müll.) H. Crum, *Churchill 19042* (BUF): AY908027/AY908845/–; ***Bryoxiphium norvegicum*** (Brid.) Mitt., *Norris 81646* (UC): AY908092/AY908957/HM751720; ***Bryowijkia ambigua*** (W.J. Hooker) Noguchi, *Ellis 901* (BM): AY908100/AY908873/HM751613; ***Campylopodiella stenocarpa*** (Wilson in Seemann) P. Müller & Frahm, *Delgadillo 5002* (DUKE): AY908131/AY908909/HM751589; ***Campylopus introflexus*** (Hedw.) Brid., *Shaw 10490* (DUKE): AY908128/AY908906/HM751595; ***Ceratodon purpureus*** (Hedw.) Brid., *Arts REU 44/15* (DUKE): AY908123/AY908862/HM751561; ***Chenia leptophylla*** (C. Müll.) Zander, *Schäfer-Verwimp 14361* (MO): AY908042/AY908815/HM751532; ***Chorisodontium mittenii*** (C. Müll.) Broth., *Churchill & al. 19750* (MO): AY908107/AY908885/HM751624; ***Cinclidotus aquaticus*** (Hedw.) Bruch ex Schimp., *Boscher & al. s.n.* (CONN): AY908029/AY908843/HM751712; ***Cladophascum gymnomitrioides*** (Dixon) Dixon, *Perold 2475* (MO): AY908097/AY908871/HM751615; ***Cladopodanthus speciosus*** (Dozy & Molkenboer) Fleischer, *Tan s.n.*, 1991 (NY): AY908132/AY908912/HM751590; ***Cnestrum alpestre*** (Wahlen.) Nyholm, *Buck 36198* (NY): AY908077/AY908896/HM751631; ***Coscinodon calyptratus*** (Drummond) C.E.O. Jensen, *Schofield 109633* (DUKE): AJ553978/AY908918/HM751577; ***Crossidium crassinerve*** (De Not.) Juratzka, *Ros s.n.* 26/2/2002 (MU): AY908037/AY908823/HM751711; ***Crumia latifolia*** (Kindb.) W.B. Schofield, *Buck 30338* (NY): AY908031/AY908821/HM751534; ***Cynodontium strumiferum*** (Hedw.) Lindb., *Allen s.n.*, 2000 (MO): AY908078/AY908897/HM751632; ***Dialytrichia mucronata*** (Brid.) Broth., *DeSloover 45.173* (MO): AY908030/AY908830/HM751540; ***Dicranodontium meridionale*** Bartr., *Lyon 1992* (MO): AY908130/AY908910/HM751588; ***Dicranoloma eucamptodontoides*** (Broth. Ex Geheeb) Paris, *Newton & Bell 5757* (herb. Newton): AY908103/AY908887/HM751628; ***Dicranum scoparium*** Hedw., *Rumsey s.n.* (herb. Rumsey): AF234158/AY908884/HM751626; ***Didymodon rigidulus*** Hedw., *Allred & Allred 6443* (MO): AY908047/AY908828/HM751543; ***Ditrichum pallidum*** (Hedw.) Hampe, *Nelson 13749* (DUKE): AF306979/AY908934/HM751562; ***Dolotortula mniifolia*** (Sull.) Zander, *Djan-Chekan 94-71* (NY): AY908036/AY908824/HM751535; ***Drummondia obtusifolia***, C. Müll., *Goffinet 5586* (DUKE): AF223038/AY908926/HM751717; ***Dryptodon patens*** (Hedw.) Brid., *Shevock 20102* (MO): AY908142/AY908921/HM751604; ***Eccremidium floridanum*** H. Crum, *Allen 7505* (DUKE): AY908098/AY908872/HM751614; ***Ephemerum serratum*** (Schreber ex Hedwig) Hampe, *Goffinet 4524* (CONN): AY908061/AY908848/HM751716; ***Erythrophyllopsis fuscula*** (C. Müll.) Hilpert, *Churchill & al. 19928* (MO): AY908028/AY908831/HM751539; ***Eucamptodon perichaetialis*** (Montagne) Montagne, *Holz & Franzaring CH00-119* (MO): AY908081/AY908899/HM751641; ***Exodictyon dentatum*** (Mitt.) Cardot, *Newton & Bell 5305* (BM): AY908149/AY908875/HM751619; ***Fissidens subbasilaris*** Hedw., *Goffinet 5263* (CONN): AF223056/AY312877/AY330435; ***Garckea phascoides*** (Hook.) C. Müll., *Magill & Pocs 11583* (MO): AY908096/AY908870/HM751611; ***Glyphomitrium daviesii*** (Dicks. ex With.) Brid., *Buck 14830* (NY): AY908082/AY908895/HM751639; ***Grimmia plagiopodia*** Hedw., *Buck 39823* (NY): AY908144/AY908919/HM751606; ***Gymnostomiella vernicosa*** (W.J. Hooker) Fleischer, *Long 28119* (DUKE): AY908066/AY908837/HM751572; ***Gymnostomum aeruginosum*** Smith, *Zander 4218* (BUF): AY908050/AY908847/HM751550; ***Gyroweisia tenuis*** (Schrader ex Hedwig) Schimp., *Long 16061* (DUKE): AY908062/AY908834/HM751556; ***Holomitriopsis laevifolia*** (Broth.) H. Robins., *Leisner 23093* (DUKE): AY908135/AY908915/HM751591; ***Homaliadelphus targionianus*** (Mitt.) Dixon & P. de la Varde, *Allen 6752* (MO): AY908552/AY908449/HM751428; ***Hypodontium dregei*** (Hornsch.) C. Müll., *Arts 105/05* (DUKE): AY908112/AY908877/HM751608; ***Indusiella thianshanica*** (Broth. & C. Müll., *Long 26986* (DUKE): AY908139/AY908923/HM751601; ***Jaffueliobryum latifolium*** Thér., *Long 23992* (DUKE): AY908617/AY908950/HM751699; ***Jonesiobryum cerradense*** Vital ex. B.H. Allen & Pursell, *Yano 4677* (NY): AY908120/AY908901/HM751644; ***Kiaeria glacialis*** (Hedw.) I. Hagen, *Long 30073* (E): AY908085/AY908900/HM751633; ***Leptobarbula berica*** (De Notaris) Schimp., *Long 15819* (DUKE): AY908063/AY908835/HM751557; ***Leptodontium luteum*** (Tayl.) Mitten, *Churchill 19048* (BUF): AY908045/AY908841/HM751545; ***Leucobryum crispum*** C. Müll., *Buck 39451* (DUKE): AY908134/AY908914/HM751592; ***Leucoloma sprengelianum*** (C. Müll.) Jaeger, *Arts RSA 104/09* (DUKE): AY908110/AY908889/HM751650; ***Microbryum davallianum*** (Sm.) R.H. Zander, *Eckel & Zander 9104014* (NY): AY908033/AY908825/HM751710; ***Microcampylopus leucogaster*** (C. Müll.) B.H. Allen, *Lyon 137A* (MO): AY908136/AY908908/HM751594; ***Micromitrium austinii*** Sull., *Buck 24917* (DUKE): AY908093/AY908917/–; ***Mitthyridium constrictum*** (Sull.) H. Robins., *Withey 560* (DUKE/AF306987/AY908976/HM751620; ***Molendoa sendtneriana*** (Bruch & Schimp. in B.S.G.) Limpricht, *Dall'Aglio 428* (BUF): AY908053/AY908846/HM751552; ***Ochrobryum gardneri*** (C. Müll.) Mitten, *Allen 13706* (MO): AY908138/AY908916/HM751609; ***Oncophorus wahlenbergii*** Brid., *Schofield 112320* (DUKE): AY908083/AY908891/HM751640; ***Oreas martiana*** (Hoppe & Hornschuch) Bridel, *Long 20863* (DUKE): AY908084/AY908892/HM751636; ***Oreoweisia laxifolia*** (J.D. Hooker) Kindb., *Shevock 19097* (MO): AY908080/AY908898/HM751634; ***Orthodicranum flagellare*** (Hedw.) Loeske, *Churchill 19600* (NY): AY908108/AY908882/HM751627; ***Paraleucobryum enerve*** (Thedenius) Loeske, *Long 16815* (DUKE): AY908106/AY908883/HM751625; ***Pilopogon gracilis*** (Hook.) Brid., *Breedlove 66830* (MO): AY908137/AY908907/HM751596; ***Pleuridium subulatum*** (Hedw.) Rabenh., *Anderson 27634* (DUKE): AF306980/AY908952/HM751563; ***Pleurochaete squarrosa*** (Brid.) Lindb., *Goffinet 6453* (CONN): AY908058/AY908854/HM751714; ***Pseudephemerum nitidum*** (Hedw.) Loeske, *Soldan s.n.* (DUKE): AY908074/AY908856/HM751546; ***Pseudosymblepharis guatemalensis*** (E.B. Bart.) B.H. Allen, coll. unknown, (MO): AY908056/AY908850/–; ***Pterygoneurum ovatum*** (Hedw.) Dixon, *Shevock 15251* (MO): AY908038/AY908818/HM751573; ***Ptychomitrium gardneri*** Lesq., *Ireland 7038* (PMAE): AY908616/AY908951/HM751600; ***Racomitrium crispipilum*** (Tayl.) A. Jaeger, *Buck 39718* (DUKE): AY908146/AY908922/HM751602; ***Rhabdoweisia crispata*** (Dicks. ex With.) Lindb., *Goffinet 4553* (CONN): AF222899/AY908966/HM751638; ***Rhachithecium papillosum*** (R.S. Williams) Wijk & Margad., *Pocs & Lye 97123A* (CONN): AF306978/AY908963/HM751642; ***Rhexophyllum subnigrum***

Appendix 2. Continued.

(Mitten) Hilp., *Churchill 19804* (MO): AY908035/AY908817/HM751538; ***Saelania glaucescens*** (Hedw.) Broth., *Hedderson 8339* (NY): AY908148/AY908924/HM751603; ***Schistomitrium brevi*-apiculatum** Broth., *Koponen 35844* (NY): AY908133/AY908913/–; ***Schistostega pennata*** (Hedw.) F. Weber & D. Mohr [*rps4* & 26S: *Hedderson s.n.* (RNG); *nad5*: unknown]: AF265359/AJ224856/HM751646; ***Sclerodontium pallidum*** (Hook.) Schwägr., *Streimann 61222* (MO): AY908111/AY908890/HM751651; ***Scouleria aquatica*** Hook., *Hedderson 5811* (RNG): AF023780/AY312887/AY330450; ***Splachnobryum obtusum*** (Brid.) C. Müll., *Buck 29822* (NY): AF223058/AY908855/HM751565; ***Stegonia latifolia*** (Schwägr.) Venturi ex Broth., LaFarge 10 Aug. 1990 (ALTA): AY908039/AY908826/HM751529; ***Streptocalypta tortelloides*** (Cardot) R.H. Zander, *Breedlove & Bourel 67446* (MO): AY908055/AY908839/HM751571; ***Streptopogon calymperes*** C. Müll., *Price 1733* (MO): AY908044/AY908813/HM751578; ***Symblepharis lindigii*** Hampe, *Price 1467* (MO): AY908076/AY908893/HM751637; ***Syrrhopodon texanus*** Sull., *Zartman 1375* (DUKE): AY908153/AY908876/HM751622; ***Tetrapterum recuvirostre*** (C. Müll.) Broth., *Vital & Buck 12121* (NY): AY908059/AY908853/HM751567; ***Timmiella anomala*** (Bruch & Schimp.) Limpr., *Weber 1978* (BUF): AY908163/AY908958/HM751585; ***Tortula subulata*** Hedw., *O'Shea s.n.* (DUKE): AY908040/AY908814/HM751531; ***Trichodon cylindricus*** (Hedw.) Schimp., *Vitt 35814* (NY): AY908125/AY908863/HM751564; ***Trichostomum tenuirostre*** (Hook. & Tayl.) Lindb., *Zander & Eckel s.n.*, 1996 (BUF): AY908057/AY908852/HM751569; ***Tridontium tasmanicum*** Hook. f., *Streimann 51280* (MO): AY908048/AY908829/HM751544; ***Tuerckheimia valeriana*** (E.B. Bartr.) R.H. Zander, *Holz & Schafer-Verwimp 99-1178* (MO): AY908052/AY908833/HM751553; ***Uleastrum paraguense*** (Besch.) W.R. Buck, *Zardini & Aquino 32310* (DUKE): AY908118/AY908965/HM751643; ***Wardia hygrometrica*** Harv. & Hook., *Hedderson 11709* (RNG): AF023782/AY908880/HM751630; ***Weissia controversa*** Hedw., *Bovers 15234* (BUF): AY908060/AY908849/HM751566.