Article

Moss diversity: A molecular phylogenetic analysis of genera

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Abstract

In this study we present phylogenetic and molecular phylogenetic diversity analyses of moss taxa from a total of 655 genera of mosses. Three loci were sampled: chloroplast ribosomal small protein 4, the intronic region of the mitochondrial NADH dehydogenase subunit 5, and partial sequences of the nuclear 26S ribosomal RNA. Maximum likelihood and Bayesian phylogenetic analyses were performed on individual loci and on multilocus data sets. A measure of phylogenetic diversity was calculated and constrasted among major lineages of mosses. We reveal many instances of incongruence among genomic partitions, but, overall, our analyses describe relationships largely congruent with previous studies of the major groups of mosses. Moreover, our greater sampling highlights the possible non-monophyly of many taxonomic families, particularly in the haplolepideous and pleurocarpous mosses. Comparisons of taxic and phylogenetic diversity among genera indicate that the Dicranidae (haplolepideous taxa) include about 15% of moss genera, but nearly 30% of the phylogenetic diversity. By contrast, the Hypnanae (hypnalian pleurocarps) contain about 45% of moss genera, but a lower percentage of phylogenetic diversity. Agreement between numbers of genera and phylogenetic diversity within other moss clades are remarkably consistent.

Key words: mosses, Bryopsida, phylogeny, phylogenetic diversity, Bayesian phylogenetic analysis

Introduction

Mosses (Bryophyta) are a diverse clade with over 12,700 species in more than 800 genera (Crosby *et al.* 1999) and are conspicuous floristic components in all terrestrial habitats suitable for plant growth — from the cold and barely hospitable antarctic to lush tropical rainforests. Mosses have many ecological functions, and one group, the peat mosses (*Sphagnum*), perform an important role in the global biogeochemical cycling of carbon where they represent a large reservoir of sequestrated carbon in the form of northern boreal peatlands (Wieder & Vitt 2006).

Together with the other two bryophyte lineages, the liverworts (Marchantiophyta) and hornworts (Anthocerotophyta), and the vascular plants (Tracheophyta), mosses are one of the primary lineages of land plants (embryophytes) that arose *circa* 470 MYA, after the colonisation of land by an ancestor most closely related to modern-day charophycean algae (Lewis & McCourt 2004). The transition of plants from water to land was accompanied by major morphological, developmental, and physiological innovations most markedly expressed by the intercalation of a multicellular diploid phase into the life cycle (Kenrick 1994, Bateman *et al.* 1998, Graham *et al.* 2000, Renzaglia *et al.* 2000, Hemsley & Poole 2004). Based on extant representatives, the

charophycean green algal ancestor of all embryophyte land plants is assumed to have had a haplobiontic life cycle with a single, haploid, multicellular stage, whereas the bryophytes and tracheophytes exhibit a marked alternation of generations with a diplobiontic life-cycle. However, unlike tracheophytes, in which the dominant life cycle stage is the diploid sporophyte, the three bryophyte groups are characterized by dominant, perennial gametophytic stages, with relatively small, unbranched sporophytes that remain permanently attached to the maternal gametophytes.

The description and classification of moss diversity using microscopic characters was initiated by Johann Hedwig who recognised 35 moss genera based upon characters of the sporophyte peristome and gametophyte sexuality (Hedwig 1801). Thereafter, most classifications in the eighteenth century followed the system of Bridel-Brideri (1826–27) who emphasised the position of the perichaetium (the female infloresence), and, consequently, the position of the sporophyte, as either terminal (acrocarpous) or lateral (pleurocarpous) on gametophyte shoot modules. Using this system, Bridel-Brideri described 91 acrocarpous and 31 pleurocarpous genera. Modern taxonomic treatments were initiated following the observations of peristome development and structure by Philibert (1884–1902). Classificatory systems emphasizing peristome architecture were established in the influential floras of Fleischer (1904–23) and Brotherus (1924, 1925) and remained dominant until the application of explicit evolutionary methodologies and the use of molecular phylogenies in the recent past (Buck & Goffinet 2000, Goffinet & Buck 2004, Goffinet *et al*. 2009). Today, approximately 845–866 genera of mosses are recognized (Crosby *et al*. 1999, Buck & Goffinet 2000).

In this study we describe the phylogeny and molecular diversity of mosses, focusing on genera as our operational taxonomic units. Although genera circumscribe an arbitrary degree of morphological diversity, they nevertheless provide a convenient boundary for the partitioning and comparison of morphological and molecular diversity. We have sampled 659 genera and analysed exemplar nucleotide sequences from the nuclear, plastid, and mitochondrial genomes.

TABLE 1: Numbers of orders, families, genera, and species.

	Crosby et al. 1999	*Buck & Goffinet* 2000	*Goffinet & Buck* 2004
orders	n/a	23	27
families	119	116	112
genera	845 (+56 synonyms)	866	866
species	12711	n/a	n/a

Materials and Methods

Taxon and locus sampling. A total of 657 species representing 655 genera (two species of *Takakia* (*T. lepidozioides* and *T. ceratophylla*) and two species of *Leptotheca* (*L. boliviana* and *L. gaudichaudii*) were sampled). Dried tissue was obtained mainly from the herbaria of the Missouri and New York Botanical Gardens (MO and NY, respectively). Wherever possible, material was selected from specimens determined by specialists in their respective taxonomic groups — these taxonomic assignments were not reassessed in light of the phylogenetic analyses conducted because of the large numbers of taxa sampled. Three loci were sequenced, one from each of the three genomic compartments; namely, the chloroplast small ribosomal protein 4 (*rps*4) gene, the intron and partial gene sequences of the mitochondrial NADH dehydogenase subunit 5 (*nad*5), and partial sequences of the nuclear 26S ribosomal RNA (nuc26S). Sequence data were also obtained from NCBI GenBank when available for the same species. Taxon identities, nomenclatural authorship, voucher specimen information, and NCBI GenBank accession numbers are presented in Supplemental Information (S.I.) Table S.1. The precise root node of the mosses and hence the appropriate moss outgroup to the remaining mosses is somewhat uncertain. Newton *et al*. (2000) and Cox *et al*. (2004)

both resolved *Sphagnum* plus *Takakia* as the sister-group to other mosses, though without strong support from their preferred analyses. Here, the genus *Sphagnum* was used as an outgroup to polarize relationships (see Cox *et al*. 2004), with the understanding that *Sphagnum* plus *Takakia* may in fact form the lineage sister to all other mosses.

DNA sequencing and alignment

Total genomic DNA was extracted using the method of Edwards *et al*. (1991), or a standard CTAB procedure (Doyle & Doyle 1987), with subsequent cleaning using the Wizard DNA Clean-up Kit (Promega). Amplification and sequencing primers follow Cox *et al*. (2004) for the three DNA regions. For each taxon and sequenced DNA region, forward (5'-3') and reverse (3'-5') sequences were assembled and checked for inaccurate base calling using Sequencher (vers. 4.1, Gene Codes Corp.). Consensus sequences were aligned using ClustalW (Thompson *et al*. 1994) and manually adjusted using SeaView (vers. 3.2; Galtier, *et al.* 1996). Regions of ambiguous alignment and incomplete data (i.e., at the beginning and end of sequences) were identified and excluded from subsequent analyses.

Phylogenetic analyses

The best-fitting substitution model for the first, second, and third codon positions of the *rps*4 locus, and the *nad*5 and nuc26S regions, were assessed using Modeltest 3.06 (Posada & Crandall 1998) in conjunction with PAUP 4b10 (Swofford 2000). Two Bayesian Markov Chain Monte Carlo (MCMC) analyses were conducted using 'mpi' (parallel processing) version of MrBayes 3.0B4 (Ronquist & Heulsenbeck 2003) for each locus with parameter sampling every 500 generations for a total of 10,000,000 generations. The analyses were conducted with a single nucleotide substitution model for the nuc26S and *nad*5 loci, and a separate sub-model for each of the codon positions of the *rps*4 gene (i.e. a model data-heterogeneous analysis), with each model including the parameters as determined by the Modeltest analyses. Each MCMC analysis included four chains, one cold and three heated, and default prior distributions and proposal rates for each parameter value. The analyses were started from a user-defined starting tree (due to a limitation in the MrBayes 3.0B4 software which caused errors if random trees were used as starting trees) determined in PAUP (Swofford 2002) by the neighbor-joining algorithm with maximum likelihood distances under a GTR model. For each analysis the 'burn-in' period before the MCMC reached stationarity was determined by plotting the likelihood through time using the plotting program GNUPlot (Williams *et al*. 2004); stationarity was assumed to have been reached when the curve plateaued. Tree sets from the posterior distribution of the two independent runs for each locus were concatenated to form the sample of trees assumed to be randomly sampled from the posterior probability distribution.

Topological conflicts among loci were detected at a 95% posterior probability threshold, by comparison of the 50% majority-rule consensus trees of the trees recovered from the posterior probability distribution. The minimum number of conflicting taxa were pruned from the individual locus trees until no topological conflict remained between loci, with the locus comparison order of *rps*4 versus *nad*5, and *rps*4+*nad*5 versus nuc26S. After pruning conflicting taxa, matrices were combined to construct two concatenated matrices; namely, *rps*4/*nad*5 and *rps*4/*nad*5/nuc26s. The combined matrices, *rps*4/*nad*5 and *rps*4/*nad*5/nuc26S, were pruned of all taxa that did not have all loci present. For both combined matrices, and for each locus and codon site partition in the *rps*4 gene, the appropriate model of nucleotide substitution was re-calculated using Modeltest. Data-heterogeneous MCMC analyses were performed on each combined matrix, with the substitution model of each character partition set to that recovered from the Modeltest analyses. Further details of the combined analyses are as described for the individual locus analyses.

Maximum likelihood (ML) analyses on individual gene partitions and combined data sets were performed with PhyML (Guindon & Gascuel 2003). Ten trees were randomly selected from the 95% confidence interval of the Bayesian MCMC analyses and the branch lengths optimised in PAUP under the model and parameter values found optimal for the partition by Modeltest. The 10 trees were then used as starting-trees and analysed by PhyML with the same optimal model but with parameter values estimated during the analyses. Because

PhyML only conducts homogeneous likelihood analyses, for the *rps*4 partition and the combined data sets, Modeltest was used to find the optimal model (and parameter values for the initial branch length calculations) under a single model prior to conducting the PhyML analyses.

Phylogenetic diversity

Phylogenetic diversity (PD - Faith 1992, 1994) is a measure proportional to the length of the minimum-spanning tree connecting a group of taxa. PD was calculated on the *rps*4/*nad*5/nuc26S data set for the following taxonomic groups (*sensu* Buck & Goffinet 2000): Polytrichales, Funariales, Grimmiales, Pottiales, Bryaceae and Mniaceae, Bartramiaceae, Orthotrichales, Ptychomniales, Dicranidae (Haplolepideae), and Hypnanae (pleurocarpous mosses). PD values for each locus partition and for the combined data set for each taxonomic group were derived from the most likely tree obtained from the PhyML analyses and compared to the groups' taxic diversity in terms of numbers of genera (Buck & Goffinet 2000).

Results

Single locus data sets

A total of 649, 634, and 578 sequences of the chloroplast *rps*4, mitochondrial *nad*5, and nuc26S loci, respectively, were newly generated during this project or were obtained from NCBI GenBank. Data characteristics of the sequences are given in Table 2. All data partitions, including individual loci, were found to be most appropriately described by a general time-reversible model of nucleotide substitution with gamma distributed rates of substitution among sites and a proportion of invariant characters (GTR+I+Γ: Rodriguez *et al*. 1990)(Supplementary Information Table S.2.1). MCMC analyses of the *rps*4, *nad*5, and nuc26S data sets each resulted in 30,415 unique topologies within the 95% posterior distributions after removal of 4,000 trees as the burn-in phase of each run and concatenation of the trees from both runs of each data set. The similarity in the number of unique topologies recovered by each analysis is because in each analysis no single topology was ever sampled twice, and the same numbers of topologies were discarded as burnin. The 50% majority-rule consensus trees of the combined tree set (assumed to be sampled from the posterior probability distribution) of each of the *rps*4, *nad*5, and nuc26S analyses is given in S.I. Figures S.2.1, S.2.2, and S.2.3, respectively. The optimal maximum likelihood tree found by PhyML of the *rps*4, *nad*5, and nuc26S data sets is presented in S.I. Figures S.2.4, S.2.5, and S.2.6, respectively.

Topological incongruence

A total of 284 topological conflicts were observed, at the threshold of 95% posterior probability support for node incongruence, between the *rps*4 and *nad*5 MCMC analyses. A total of 25 taxa were removed to eliminate all conflicts: these taxa are indicated in the S.I. (Materials and Methods). Of particular interest are a group of eight taxa, namely, *Bruchia drummondii* (Bruchiaceae), *Calymperes lonchophyllum* (Calymperaceae), *Bellibarbula recurva* (Pottiaceae), *Campylopodium euphorocladium* (Dicranaceae), *Bryohumbertia filifolia* (Dicranaceae), *Bryomanginia saintpierrei* (Ditrichaceae), *Reimersia inconspicua* (Pottiaceae), and *Timmia megapolitana* (Timmiaceae). In the *rps*4 phylogeny, the haplolepideous taxa (all the above taxa with the exception of *Timmia*) fall in well-supported groups close to members of their respective taxonomic families. *Timmia* is placed outside of the haplolepideae and close to the Encalyptaceae. However, in the *nad*5 tree all eight taxa form a monophyletic group with 100% posterior probability either to the exclusion of all other haplolepideous taxa (S.I. Figure S.2.2) or toward the base of haplolepideae (S.I. Figure S.2.5) but in neither case close to the Funariaceae. Moreover, the nucleotide sequences are remarkably similar with most sequences sharing similar polymorphic sites. These data characteristics suggest that these haplolepideous *nad*5 sequences are the result of human error in collecting the data: however, there are unique differences among the sequences and this has not been tested with additional sequencing.

TABLE 2: Data characteristics for sites included in the analyses.

	*rps*4	*nad*5	nuc26S
Nº of sequences	649	634	578
Nº of sites excluded	668	1437	531
Nº of sites included	566	1047	918
Variable sites	444	767	306
Parsimony informative sites	400	616	208
Sequence lengths (mean)	654	1128	1072
Sequence lengths (max)	919	1935	1174
Sequence lengths (min)	516	622	538
*Mean GC content (%)	26	39	55
*Max GC content (%)	34	43	62
*Min GC content (%)	24	32	46

Between the *rps*4/*nad*5 and nuc26S MCMC analyses, a total of 102 node conflicts were observed, at the threshold of 95% posterior probability support for topological incongruence. A total of 62 taxa were removed to eliminate conflicts among the two partitions (S.I.: Materials and Methods). Of particular note was the conflict between the positions of the Ptychomniales in the topologies. In the *rps*4/*nad*5 tree the Ptychomniales were the sister-group to all other pleurocarpous mosses (Buck *et al*. 2004), whereas in the nuc26S tree they were allied with Aulacomniaceae, Phyllodrepaniaceae, Bryaceae, Mniaceae, and a few members of the Rhizogoniaceae (all non-pleurocarpous families). The Ptychomniales were removed from the combined *rps*4/*nad*5/nuc26S data set. In addition, the acrocarpous order Splachnales was removed as the order was resolved as a monophyletic unit among the pleurocarpous mosses in the nuc26S data set in contrast to the traditional placement among the diplolepideous-alternate peristomate mosses leading in a grade to the pleurocarpous mosses, as was resolved by the *rps*4/*nad*5 data set.

Multilocus data sets

The combined *rps*4/*nad*5 data set consisted of 603 taxa after removal of 25 taxa in conflict between the partitions and a further 31 taxa for which only one of the loci was available. The aligned matrix included 3718 sites of which 2105 sites were excluded due to ambiguous alignment or missing data at the ends of the sequences. The remaining 1613 sites included 1002 parsimony informative sites. The combined *rps*4/*nad*5/nuc26s data set consisted of 477 taxa after removal of 62 taxa in conflict between the data sets and 103 taxa that had missing data. Both combined data sets were most-appropriately described by a GTR+I+Γ substitution model (S.I. Table S.2.1). Heterogeneous MCMC analysis of the *rps*4/*nad*5 data set resulted in 30,145 unique topologies within the 95% confidence interval of the posterior probability distribution, while similar analyses for the *rps*4/*nad*5/nuc26s data set resulted in 22,797 unique trees. The 50% majority-rule consensus trees of the topologies from the posterior probability distribution are presented in S.I. Figure S.2.7 (*rps*4/*nad*5) and Figure S.2.8 (*rps*4/*nad*5/nuc26s). The optimal maximum likelihood tree found by PhyML of the *rps*4/*nad*5 and *rps*4/*nad*5/nuc26S data sets is presented in S.I. Figures S.2.9 & S.2.10, respectively.

Phylogenetic relationships

Overall, relationships indicated by the three individual loci are remarkably similar, with the nematodontous mosses forming a grade at the base of the trees leading to a monophyletic arthrodontous moss clade. Within the arthrodontous mosses, the Encalyptales and Funariales tend to be sister to a haplolepideous/diplolepideous-alternate peristomate moss split (*rps*4) or of unresolved affinities (*nad*5, nuc26S). The haplolepideae are monophyletic (*nad*5 – minus the "*Timmia*" clade, see above) or lacking sufficient support to

contradict their monophyly (*rps*4, nuc26S). The monophyly of the diplolepideous-alternate moss clade is strongly supported by all loci, with, typically, a grade of acrocarpous familes and orders leading to a monophyletic pleurocarpous mosses. With the exception of the nuc26S locus, the monophyly of the pleurocarpous mosses is strongly supported.

Although the trees presented here are informally rooted (outgroup rooting) with *Sphagnum*, we note that the *rps*4 and *nad*5 loci do not contradict previous studies that placed both *Sphagnum* and *Takakia* as the sister group to the remaining mosses (Newton *et al.* 2000, Cox *et al.* 2004), while nuc26S does not provide strong conflicting evidence. The placement of Andreaeales as a clade sister to (*nad*5), or a grade leading to (*rps*4), other mosses is again in agreement with these previous studies. The relationships concerning the Polytrichales, Buxbaumiales, Oedipodiales, Tetraphidales, and arthrodontous mosses are problematic. The *rps*4 data set has strong support (100% posterior probability (p.p.)) for the sister relationship of the Tetraphidales to a clade consisting of the other four groups, with some support (90% p.p.) for *Oedipodium*, and *Buxbaumia* (94% p.p.) as forming the two sister groups to the Polytrichales. In contrast, *nad*5 places, without support, *Oedipodium* as sister to the Tetraphidales, and *Buxbaumia* as sister to the arthrodontous clade with moderate support (92% p.p.). When the *rps*4 and *nad*5 loci are combined, there is good support for the basal-most position of the Tetraphidales (99% p.p.), with Oedipodiales as the next branching lineage (86% p.p.), and moderate support for *Buxbaumia* as sister to arthrodontous clade (89% p.p.). However, support for these relationships is further eroded when combined with the nuc26S data set (61% p.p., <50% p.p., and 84% p.p., respectively). The nuc26S data set itself provides little support for relationships within this basal-most portion of the tree, but contains enough contradictory signal to erode the support provided by the other partitions.

Placement of the order Buxbaumiales as the sister-group to the arthrodontous mosses is resolved only in the *nad*5 and combined analyses; although the relationship does not receive significant support from the analyses. The sister-group relationship of Diphysciales to other arthrodontous mosses is well supported by both the *rps*4 and *nad*5 loci, and the combined analyses.

The relationship of the Funariales to the Encalyptales and Timmiales, to the exclusion of the Gigaspermaceae, is supported by the *rps*4 locus alone, with strong contradictory evidence provided by the nuc26S locus, which places *Timmia* in a clade with the haplolepideous taxa, *Timmiella* (Pottiaceae) and *Distichium* (Ditrichaceae). The combined *rps*4/*nad*5 (i.e. without *Timmia*) data set strongly supports the clade uniting Funariales and Encalyptales (100% p.p.), with Gigaspermaceae as the immediate sister-group (75% p.p.). However, the support for these relationships is much reduced by the addition of the nuc26S data; 82% p.p. and 60% p.p. respectively. In the *rps*4/*nad*5 combined analyses the Funariales/Encalyptales clade is placed as the sister group to the haplolepideae, whereas in the *rps*4/*nad*5/nuc26S analyses the clade is resolved as sister to the haplolepideae plus Bryineae, however, in neither case is the relationship significantly supported.

The monophyly of the haplolepideous mosses is only supported by *nad*5 among the single-locus analysis (excepting the "*Timmia*" clade), but is maximally supported in combined analyses. In general, the relationships among haplolepideous taxa are well resolved and supported, although few traditional groupings are evident from the analyses. The enigmatic taxon, *Catoscopium nigritum*, classified in the Splachnales by Buck & Goffinet (2000), is resolved as the first-branching taxon in the haplolepideae clade of the *rps*4/*nad*5 tree, with a clade consisting of *Chrysoblastella* (Ditrichaceae), *Luisierella* (Pottiaceae), *Distichium* (Ditrichaceae), and *Timmiella* (Pottiaceae) also forming an early branching lineage separated from the remaining haplolepideae. A clade consisting of *Scouleria* (Scouleriaceae), *Dicranoweisia* (Rhabdoweisiaceae), and *Drummondia* (Drummondiaceae) form the next branching lineage, followed by separation of *Bryoxiphium* (Bryoxiphiaceae) in the *rps*4/*nad*5 tree. However, in the three gene combined analysis *Bryoxiphium* forms a clade with *Drummondia* and *Scouleria* although the relationship is not well-supported. A well-supported clade is formed between members of the Grimmiaceae and Ptychomitriaceae (100% p.p.), although the latter appears to be paraphyletic with the grimmiaceous genera *Jaffueliobryum* and *Indusiella* derived from the Ptychomitriaceae. Sister to this clade are *Saelania* (Ditrichaceae) and *Blindia*

(Seligeriaceae) respectively. The gymnostomous (lacking a peristome) taxon, *Pseudobraunia*, typically classified with the Hedwigiales, is also recognised as a member of the Grimmiales by each of the loci, with strong support (99% p.p.) for its sister relationship to *Dryptodon* in the *rps*4/*nad*5 analyses.

The Grimmiaceae/Ptychomitriaceae clade is resolved (75% p.p.) as sister to a clade consisting of taxa from the Dicranaceae, Leucobryaceae, Ephemeraceae (*Micromitrium* only), and Archidiaceae. The remaining haplolepideae form a well-supported (100% p.p.) clade in the *rps*4/*nad*5 data set, which is considerably weakened (89% p.p.) by the addition of the nuc26S data. Within this latter group, *Amphidium* (Rhabdoweisiaceae) forms the sister taxon to the remaing taxa, which themselves are divided into two well-supported lineages. Both of these latter clades are very heterogeneous in taxon composition, the first consisting of members of the Rhabdoweisiaceae, Fissidentaceae, Erpodiaceae, Bruchiaceae, Ditrichaceae, Pottiaceae, Serportortellaceae, Calymperaceae, and Dicranaceae. The second clade consists of the majority of members of the Pottiaceae (100% p.p.), plus the remainder of the Dicranaceae, Dicnemonaceae, Rhabdoweisiaceae, Ditrichaceae, Rhachitheciaceae, Erpodiaceae, Bruchiaceae, and the monogeneric Wardiaceae.

The diplolepideous-alternate peristomate mosses (Bryidae) are maximally supported as a monophyletic group in all individual and combined analyses. The Bartramiaceae, Hedwigiales, Mniaceae, Bryaceae, and Splachnales, all form well-supported groups in the *rps*4/*nad*5 tree. In the *rps*4/*nad*5/nuc26S analyses, the Hedwigiales or Bartramiaceae are identified as the two first-branching lineages, with neither group supported as the earliest branching lineage. However, the removal of the Splachnales from the three gene combined analyses was necessary due to its positioning within the pleurocarpous taxa in the tree inferred from nuc26S, nevertheless, the *rps*4 and *nad*5 loci suggest the group may also be one of the earliest diverging lineages of the Bryidae. The Bryaceae plus Leptostomataceae and the Phyllodrepaniaceae plus Mniaceae both form a maximally supported monophyletic group, and together these four families form a clade (100% p.p.) sister to the remaining Bryidae. Of note is the inclusion of the genus *Pulchrinodus* in this clade, with good support (97% p.p.) for its sister relationship to the Leptostomataceae plus Bryaceae in the *rps*4/*nad*5 trees (c.f. Stech *et al*. 2003).

The Orthotrichales, which themselves are a well-supported lineage (100% p.p.), are supported (97% p.p.) as the sister lineage to the Rhizogoniales and pleurocarpous mosses by the *rps*4/*nad*5 analyses. The support is, however, significantly lessened by the addition of nuc26S data (80% p.p.).

The Rhizogoniales, as traditionally circumscribed, form a grade of taxa leading to the pleurocarpous mosses (Hypnanae); three well-supported clades of rhizogoniaceaeous taxa are resolved with the individual *rps*4 and *nad*5, and the combined *rps*4/*nad*5 analyses. The first indicates that the Orthodontiaceae (Bryales) are more closely related to *Leptotheca* and *Hymenodon* (Rhizogoniaceae) than to other member of the Bryales. The second clade includes *Rhizogonium*, *Goniobryum*, *Pyrrhobryum*, *Cyrtopodium*, all classified in the Rhizogoniaceae, plus *Calomnion* from the Calomniaceae – *Calomnion* and *Cyrtopodium* are maximally supported as sister groups. The third clade includes *Hypnodendron* (Hypnodendraceae), *Spiridens* (Spiridentaceae), *Cyrtopus* (Cyrtopodaceae), *Cyrtopodendron* (Cyrtopodaceae), *Pterobryella* (Pterobryellaceae), *Racopilum* (Racopilaceae), and *Braithwaitea* (Trachylomataceae). Furthermore, the Bryalean family Aulacomniaceae is also part of this rhizogoniaceaous grade leading to the pleurocarps. Only the *nad*5 locus provides strong evidence for the resolution order of these clades, while the *rps*4 locus does not conflict with this set of relationships, and the nuc26S provides strikingly contradictory evidence, although of dubious validity (e.g. part of the Hypnodendraeae are nested within the Orthotrichales). In the *nad*5 tree, the Orthodontiaceae are the first branching lineage followed by the branching of the Rhizogoniaceae, then the Aulacomniaceae, and finally the Hypnodendraceae which is the sister-group to the pleurocarpous mosses (taxonomy *sensu* Bell *et al*. 2007). In the *nad*5 analyses, only the position of the Aulacomniaceae is uncertain, yet all relationships are significantly weakened by the addition of nuc26S data, excepting the placement of the Hypnodendraceae.

In the individual *rps*4 and *nad*5 analyses and the *rps*4/*nad*5 combined analysis, the Ptychomniales (*sensu* Buck *et al*. 2004) are significantly supported (96% p.p. and 100% p.p., respectively) as the sister-group to all

other pleurocarpous mosses (c.f. Cox *et al.* 2004). In contrast, the nuc26S data place the order with the Aulacomniaceae and Phyllodrepaniaceae, and closely related to the Mniaceae and Bryaceae.

In none of the *rps*4, *nad*5, or combined *rps*4/*nad*5 analyses are the Hookeriales resolved as a monophyletic group, but rather they form two strongly supported and distinct groups. One consists of the the the Hypopterygiaceae, plus *Cyathophorum* and *Cyathophorella* of the Hookeriaceae, while the second grouping includes all other members of the Hookeriales. In the combined *rps*4/*nad*5 tree, there is moderate, but insignificant, support for the closer relationship of the latter clade to the Hypnales, whereas in the combined 3 locus analyses there is likewise insignificant support for the former clades' closer relationship to the Hypnales. The Hypnalean pleurocarpous mosses are unresolved as a monophyletic group by the *rps*4 locus, resolved as a clade by the *nad*5 locus (86% p.p.), and supported as polyphyletic by the nuc26S locus (but the Splachnales are strongly supported as derived from the hypnalean clade). The combined *rps*4/*nad*5 analyses provide maximum support for Hypnalean monophyly. However, addition of the nuc26S data resolves a paraphyletic (with Hypopterygiaceae plus *Cyathophorella* nested within) Hypnales, but without significant support.

Overall, support for lineages in the Hypnales was poor, but the few sizable clades that were resolved indicate that all the large Hypnalean families are non-monophyletic. In the *rps*4/*nad*5 combined analyses, a large brachytheciaceous clade is resolved (*Bryoandersonia – Schwetschkea* in S.I. Figure S.2.7) but also contains representatives from Hyocomiaceae (*Neodolichomitra*), Stereophyllaceae (*Entodontopsis*), Campyliaceae (*Hygrohypnum*), Leskeaceae (*Okamuraea, Schwetschkea*), and Myriniaceae (*Helicodontium*). Similarly, the clade *Sanionia – Pseudocalliergon* (99% p.p.) consists of the majority of Campyliaceae, but also includes members of the Hypnales (*Campylophyllum, Taxiphyllopsis*), Amblystegiacae (*Amblystegium*, *Hygroamblystegium, Orthotheciella, Vittia, Gradsteinia*), Cratoneuronaceae (*Cratoneuron*), Helodiaceae (*Palustriella*), and *Eriodon* (Brachytheciaceae). Many other examples could be given of similar conflict with traditional taxonomy.

Some small families of the Hypnales are placed with support on the *rps*4/*nad*5 tree. The small family Rutenbergiaceae (*Neorutenbergia, Rutenbergia*, and *Pseudocryphaea*) is monophyletic (98% p.p.) and forms a clade (95% p.p.) with *Trachyloma*, the sole genus in the family Trachylomataceae (excepting *Braithwaitea*), and *Rhizofabronia* (Fabroniaceae). The two monogeneric families Rhytidiaceae (*Rhytidium*) and Regmatodontaceae (*Regmatodon*) are well-supported (99% p.p.) as belonging to a clade that includes members of the Leskeaceae (*Linbergia, Pseudoleskeella, Leskeadelphus, Pseudoleskeopsis*) and Fabroniaceae (*Dimerodontium*). The monogeneric Rigodiaceae (*Rigodium*) are placed in a well-supported clade (99% p.p.) with *Camptochaete, Lembophyllum*, and *Weymouthia*, all members of the Lembophyllaceae. *Isopterygiopsis* and *Mahua*, both members of the Hynaceae, along with *Struckia* from the *Amblystegiacae*, form a maximally supported clade with the monogeneric family Plagiotheciaceae (*Plagiothecium*). The Fontinalaceae (*Fontinalis, Brachylema*, and *Dichelyma*) are monophyletic (100% p.p.) and forms a sister-group to *Habrodon* (Pterigynandraceae). The two monogeneric families Climaciaceae (*Climacium*) and Pleuroziopsaceae (*Pleuroziopsis*) form a monophyletic group (100% p.p.). The Symphyodontaceae (*Symphyodon*, *Dimorphocladon, Chaetomitriopsis* and *Chaetomitrium*) form a clade with *Phyllodon* and *Bryocrumia*, both members of the Hypnaceae. Finally, the shared ancestry between the monogeneric Theliaceae (*Thelia*) and *Leptopterigynandrum* (Leskeaceae), *Schwetschkeopsis* (Anomodontaceae), and *Taxiphyllum* (Hypnaceae) is maximally supported.

Taxic and phylogenetic diversity

Numbers of genera (taxic diversity) and phylogenetic diversity (PD) are compared for selected groups within the mosses in Table 3. We limit these comparisons to monophyletic groups. Phylogenetic diversity estimated from each of the three loci, and taxic diversity, are all highly correlated; correlation coefficients among all pairwise combinations of estimates are greater than 0.90 (statistics not shown). Thus, diversity estimates based on at least broad groupings recognized in the widely accepted classification scheme captures phylogenetic structure in the mosses.

Within that broad agreement, some consistent patterns of relationship between phylogenetic and taxic diversity estimates emerge. The Dicranidae (haplolepideous taxa), for example, include about 15% of moss genera, but nearly 30% of the phylogenetic diversity. The hypnalian pleurocarps (Hypnanae in Table 3), in contrast, contain about 45% of moss genera, but a lower percentage of phylogenetic diversity. One way to interpret this pattern is that genera within the Dicranidae are, on average, separated by a larger phylogenetic distance than are hypnalean genera. Agreement between numbers of genera and phylogenetic diversity within other moss clades are remarkably consistent.

TABLE 3: Phylogenetic and taxic diveristy based on the best ML tree obtained from the PhyML analysis of the *rps*4/*nad*5/nuc26s combined data set.

Taxon[1]	Genera[2]	*rps*4 (10.247)[3]	*nad*5 (4.059)	nuc26s (5.163)	*rps*4/*nad*5/nuc26S (7.539)
Polytrichales (11: 1.6%)	23	3.1% (0.320)	3.4% (0.136)	4.0% (0.206)	3.3% (0.250)
Funariales[4] (9: 1.4%)	24	3.3% (0.339)	3.5% (0.143)	6.4% (0.330)	4.1% (0.305)
Grimmiaceae (7: 1.1%)	10	1.5% (0.157)	1.2% (0.048)	2.0% (0.104)	1.5% (0.113)
Pottiaceae (34: 5.2%)	82	7.2% (0.741)	8.2% (0.332)	5.3% (0.275)	7.1% (0.536)
"Bryum/Mnium"[5] (21: 3.2%)	28	5.2% (0.530)	2.9% (0.116)	6.1% (0.313)	4.6% (0.347)
Bartramiaceae (8: 1.2%)	10	1.3% (0.132)	2.8% (0.112)	2.7% (0.138)	2.2% (0.162)
Orthotrichales (13: 2.0%)	22	4.3% (0.443)	3.4% (0.137)	5.8% (0.299)	4.1% (0.314)
Dicranidae (101: 15.3%)	234	28.7% (2.941)	28.0% (1.137)	27.1% (1.400)	28.1% (2.120)
Bryidae (348: 52.9%)	91	55.2% (5.653)	47.3% (1.919)	56.9% (2.937)	53.1% (4.006)
Hypnanae (294: 44.6%)	465	40.0% (4.099)	33.8% (1.370)	36.25 (1.869)	37.4% (2.819)

1. Taxonomic group and number of taxa (with percentages) included in the analysis (*rps*4/*nad*5/nuc26s data set).

2. Numbers of genera follow Buck & Goffinet (2000).

3. Total tree length is indicated with the locus in each column.

4. Includes the Encalyptales, represented by *Encalypta ciliata* and the Gigaspermales *sensu* Goffinet *et al*. (2007).

5. Includes the Bryaceae, Mniaceae, and Leptostomataceae.

Discussion

The broad pattern of phylogenetic relationships among the mosses we present here is in general agreement with our current understanding of evolutionary relationships based on molecular data (Newton *et al*. 2000, Cox *et al*. 2004, Bell *et al*. 2007, Wahrmund *et al*. 2010). Our combined analysis (Figure 1A) depicts the Andreaeopsida as the sister-group of the peristomate mosses (plus *Oedipodium*, which is gymnostomous) with the nematodontous mosses forming a grade of taxa leading to the arthrodontous mosses. Within the latter, the haplolepideae are derived from mosses with a diplolepideous peristome and pleurocarpous mosses are derived from acrocarpous diplolepideous mosses. Among the primary phylogenetic divisions, no divergences gain support here that have not previously been firmly established, and some are without support where previously support was evident (cf. Cox *et al*. 2004). These differences in levels of support may reflect the relative paucity of data per taxon in our current analyses where some earlier analyses have focused on obtaining greater amounts of data per taxon at the expense of taxon sampling density. On the other hand, the increased taxon sampling here may have reduced support where in previous analyses it was erroneously present due to sparse taxon sampling. The relative importance of dense taxon sampling versus obtaining larger amounts of data per taxon is a long debated topic in phylogenetics (e.g. Graybeal 1998, Rosenberg & Kumar 2001, Zwickl & Hillis 2002), but is often discussed with regard to phylogenetic analyses as a whole. However,

Takakiaceae — *Takakia lepidozioides*
Sphagnaceae — *Sphagnum palustre*
Andreaeaceae — *Acroschisma wilsonii* / *Andreaea rothii*
Tetraphidaceae — *Tetraphis pellucida*
Oedipodiaceae — *Oedipodium griffithianum*
Polytrichaceae — *Alophosia azorica* / *Bartramiopsis lescurii* / *Lyellia crispa* / *Polytrichadelphus purpureus* / *Oligotrichum parallelum* / *Meiotrichum lyellii* / *Polytrichastrum alpinum* / *Dawsonia superba* / *Polytrichum pallidisetum* / *Atrichum undulatum* / *Psilopilum cavifolium*
Buxbaumiaceae — *Buxbaumia aphylla*
Diphysciaceae — *Diphyscium foliosum*
Gigaspermaceae — *Chamaeobryum pottioides* / *Gigaspermum repens*
Encalyptaceae — *Encalypta ciliata*
Disceliaceae — *Discelium nudum*
Funariaceae — *Funaria hygrometrica* / *Entosthodon laevus* / *Quathlamba delicostata* / *Aphanorrhegma serratum* / *Physcomitrella patens*

Dicranidae

0.01 substitutions per site ⎯

Rhacocarpaceae — *Rhacocarpus purpurascens*
Hedwigiaceae — *Hedwigia ciliata* / *Braunia secunda* / *Hedwigidium integrifolium*
Bartramiaceae — *Plagiopus oederi* / *Anacolia webbii* / *Bartramia stricta* / *Leiomela bartramioides* / *Conostomum tetragonum* / *Philonotis fontana* / *Breutelia scoparia* / *Fleischerobryum longicolle*
Bryaceae — *Leptostomum indicans* / *Rhodobryum giganteum* / *Brachymenium nepalense* / *Plagiobryum zierii* / *Acidodontium sprucei* / *Anomobryum julaceum* / *Bryum argenteum* / *Rosulabryum albolimbatum*
Mniaceae — *Schizymenium bryoides* / *Mielichhoferia elongata* / *Pohlia nutans* / *Epipterygium wrightii*
Bryaceae in part — *Roellia roellii*
Cinclidium stygium / *Rhizomnium gracile* / *Cyrtomnium hymenophyllum* / *Mnium hornum* / *Pseudobryum cinclioides* / *Leucolepis acanthoneuron* / *Orthomnion elimbatum* / *Plagiomnium cuspidatum*
Orthotrichaceae — *Bryomaltaea obtusifolia* / *Schlotheimia torquata* / *Desmotheca apiculata* / *Leiomitrium plicatum* / *Macrocoma tenue* / *Matteria gracillima* / *Ulota phyllantha* / *Orthomitrium tuberculatum* / *Orthotrichum anomalum* / *Leptodontiopsis fragilifolia* / *Zygodon bartramioides* / *Pentastichella pentastichum* / *Pleurorthotrichum chilense*
Orthodontiaceae — *Hymenodon pilifer* / *Leptotheca gaudichaudii* / *Orthodontium lineare* / *Orthodontiopsis bardunowii*
Rhizogoniaceae — *Cryptopodium bartramioides* / *Rhizogonium lindigii* / *Goniobryum subbasilare*

Hypnanae Fig. 1b

Chrysoblastella chilense — Ditrichaceae in part
Distichium capillaceum
Timmiella anomala — Pottiaceae in part
Bryoxiphium norvegicum — Bryoxiphiaceae
Drummondia obtusifolia — Drummondiaceae
Scouleria aquatica — Scouleriaceae
Saelania glaucescens — Ditrichaceae in part
Ptychomitrium gardneri — Ptychomitriaceae
Indusiella thianshanica / *Jaffueliobryum latifolium*
Racomitrium crispulum — Grimmiaceae
Dryptodon patens / *Coscinodon calyptratus* / *Grimmia plagiopodia*
Piloppogon gracilis — Leucobryaceae
Campylopus introflexus / *Microcampylopus leucogaster* / *Brothera leana* / *Campylopodiella stenocarpa* / *Dicranodontium meridionale* / *Ochrobryum gardneri* / *Holomitriopsis laevifolia* / *Leucobryum crispum* / *Cladopodanthus speciosus* / *Schistomitrium breviapiculatum*
Amphidium lapponicum — Rhabdoweisiaceae in part
Fissidens subbasilaris — Fissidentaceae
Bryowijkia ambigua — Hedwigiaceae in part
Aongstroemia jamaicensis — Dicranaceae in part
Garkea phascoides — Ditrichaceae in part
Cladophascum gymnomitrioides — Bruchiaceae in part
Eccremidium floridanum — Ditrichaceae in part
Hypodontium dregei — Hypodontiaceae
Syrrhopodon texanus — Calymperaceae
Exodictyon diatatum / *Mitthyridium constrictum*
Leucoloma sprengelianum — Dicranaceae
Sclerodontium pallidum / *Wardia hygrometrica* / *Dicranoloma eucamptodontioides* / *Chorisodontium mittenii* / *Orthodicranum flagellare* / *Dicranum scoparium* / *Paraleucobryum enerve*
Schistostega pennata — Schistostegaceae
Jonesiobryum cerradense — Rhachitheciaceae
Rhachithecium papillosum / *Uleastrum paraguensis*
Rhabdoweisia crispata — Rhabdoweisiaceae in part
Oreas martiana / *Oncophorus wahlenbergii* / *Symblepharis lindigii* / *Arctoa fulvella* / *Glyphomitrium daviesii*
Eucamptodon perichaetialis — Dicranaceae in part
Kiaeria gracilis / *Oroweisia laxifolia*
Cnestrum alpestre — Dicranaceae in part
Cynodontium strumiferum
Trichodon cylindricus — Ditrichaceae in part
Ceratodon purpureus / *Ceratodon purpureus** / *Ceratodon purpureus**
Astomiopsis kienerii — Ditrichaceae in part
Ditrichum pallidum / *Pleuridium subulatum*
Pseudephemerum nitidum — Dicranaceae in part
Gymnostomiella vernicosa — Pottiaceae
Hyophiladelphus agrarius / *Splachnobryum obtusum* / *Gyroweisia tenuis* / *Leptobarbula berica* / *Anoetangium aestivum* / *Molendoa sendtneriana* / *Gymnostomum aeruginosum* / *Tuerckheimia valeriana* / *Ephemerum serratum* / *Pleurochaete squarrosa* / *Pseudosymblepharis guatemalensis* / *Trichostomum tenuirostre* / *Streptocalypta tortelloides* / *Tetrapterum recuvirostrum* / *Weissia controversa* / *Leptodontium luteum* / *Barbula unguiculata* / *Bryoerythrophyllum campylocarpum* / *Erythrophyllopsis fuscula* / *Cinclidotus aquaticus* / *Dialytrichia mucronata* / *Rhexophyllum subnigrum* / *Didymodon rigidulus* / *Tridontium tasmanicum* / *Crumia latifolia* / *Dolotortula mniifolia* / *Microbryum dallavianum* / *Chenia leptophylla* / *Streptopogon calymperes* / *Stegonia latifolia* / *Tortula latifolia* / *Pterygoneuron ovatum* / *Crossidium crassinerve*

Ⓐ

Left column

Braithwaitiaceae — *Braithwaitea sulcata*

Saulomataceae — *Ancistrodes genuflexa*, *Sauloma tenella*

Daltoniaceae — *Calpytrochaeta brownii*

Schimperobryaceae — *Schimperobryum splendidissimum*

Hookeriaceae — *Crossomitrium epiphyllum*

Leucomiaceae — *Rhynchostegiopsis flexuosa*, *Leucomium strumosum*, *Tetrastichium fontanum*

Pilotrichaceae —
Pilotrichum bipennatum
Callicostellopsis meridensis
Brymela tutezona
Trachyxiphium drepanophyllum
Thamniopsis pendula
Pilotrichidium callicostatum
Hemiragis aurea
Hypnella diversifolia
Lepidopilidium portoricense
Lepidopilum scabrisetum
Stenodictyon wrightii
Philophyllum tenuifolium
Actinodontium sprucei
Diploneuron connivens
Cyclodictyon albicans
Callicostella pallida
Hookeriopsis cuspidata

Stereophyllaceae in part — *Catagoniopsis berteroana*

Fabroniaceae in part — *Fabronia pusilla*, *Ischynodon leptura*

Hypnaceae in part — *Pseudotaxiphyllum elegans*

Pterigynandraceae in part — *Myurella tenerrima*, *Orthothecium chryseum*

Isopterygiopsis muelleriana

Plagiotheciaceae — *Plagiothecium cavifolium*

Hypnaceae in part — *Mahua enervis*

Struckia argentata

Acrocladiaceae — *Acrocladium auriculatum*

Lepyrodontaceae — *Lepyrodon tomentosum*

Stereophyllaceae in part — *Eulacophyllum cultelliforme*

Brachytheciaceae in part — *Juratzkaella sinensis*

Stereophyllaceae in part — *Pilosium chlorophyllum*

Catagoniaceae — *Catagonium nitens*

Rutenbergiaceae — *Neorutenbergia usugare*, *Pseudocryphaea domingensis*

Fabroniaceae in part — *Rhizofabronia persoonii*

Trachylomataceae — *Trachyloma planifolium*

Hypopterygiaceae —
Cyathophorella hookeriana
Hypopterygium tamarasci
Dendrohypopterygium arbusculum
Dendrocyathophorum decolyi
Canalohypopterygium tamariscinum
Catharomnion ciliatum

Calliergonaceae —
Calliergon cordifolium
Loeskhypnum badium
Leptodictyon riparium
Warnstorfia exannulata

Neckeraceae in part — *Baldwiniella kealeensis*

Leucodontaceae in part — *Felipponea montevidensis*

Phyllogoniaceae — *Phyllogonium fulgens*

Pterobryaceae —
Henicodium geniculatum
Pireella angustifolia
Orthostichopsis tetragona
Spiridentopsis longissima
Pterobryon densum
Neolindbergia cladomnioides
Muellerobryum whiteleggei

Cryphaeaceae in part — *Pilotrichopsis dentata*

Jaegerina scariosa
Symphysodon longicuspis
Symphysodontella cylindricea
Horikawaea redfearnii
Cryptogonium phyllogonoides
Calyptothecium praelongon
Pterobryopsis stolonacea
Hildebrandtiella endotrichelloides
Orthostichidium involutifolium

From Fig. 1A.

Right column

Dendroalsia abietina — Cryphaeaceae in part
Prionodon densus — Prionodontaceae
Campylophyllum halleri — Hypnaceae in part
Pteryginandrum filiforme — Pterigynandraceae in part
Rhytidiopsis robusta — Hylocomiaceae in part
Schoenobryum concavifolium — Cryphaeaceae in part
Cyptodon muelleri
Cyptodontopsis obtusifolia
Sphaerotheciella sphaerocarpa
Dendrocryphaea cuspidata
Cryphaea patens
Pseudoleskea andina — Leskeaceae in part
Dendropogonella rufescens

Rigodiadelphus robustus — Leskeaceae in part
Lescurea radicosa
Ptychodium plicatum
Symphyodon imbrocatifolius — Symphyodontaceae
Phyllodon truncatulus — Hypnaceae in part
Chaetomitriopsis glaucocarpa
Chaetomitrium borneense
Antitrichia curtipendula — Leucodontaceae in part
Bryonorrisia acutifolia — Anomodontaceae
Bissetia lingulata — Miyabeaceae
Homaliadelphus targionanus
Hylocomiastrum pyrenaicum — Hylocomiaceae in part
Trachypus bicolor — Meteoriaceae
Meteorium illecebrum
Cryptopapillaria penicillata
Diaphanodon blandum
Toloxis imponderosa
Duthiella wallichii
Neocladiella pendula
Trachypodopsis serrulata
Pseudospiridentopsis horrida
Chrysocladium flammeum
Meteoriopsis reclinata
Aerobryopsis longissima
Pseudotrachypus martinicensis
Floribundaria aurea
Barbellopsis trichophora
Barbella cubensis
Claopodium whippleanum — Leskeaceae in part
Fontinalis antipyretica — Fontinalaceae
Brachelyma subulatum
Dichelyma falcatum
Andoa berthelotiana — Hypnaceae in part
Myurium hochstetteri — Myuriaceae
Ctenidium molluscum — Hylocomiaceae in part
Hyocomium armoricum — Hypnaceae in part
Hylocomium splendens — Hylocomiaceae
Pleurozium schreberi
Ptilium cristacastrensis
Rhytidiadelphus triquetrus
Loeskeobryum brevirostre
Meteoriella soluta
Aerobryum speciosum — Brachytheciaceae
Pseudoscleropodium purum
Neodolichomitria yunnanensis
Scorpiurium deflexifolium
Palamocladium leskeoides
Entodontopsis leucostega
Stenocarpidiopsis saliciocola
Eurhynchiella acanthophylla
Platyhypnidium riparioides
Rhynchostegium serrulatum
Steerecleus serrulatus
Hygrohypnum smithii
Scleropodium obtusifolium
Cirriphyllum cirrosum
Brachythecium salebrosum
Myuroclada maximoviczii
Bryhnia novae-angliae
Homalothecium sericeum
Trachybryum megaptilum
Eurhynchium hians
Oxyrrhynchium rugosipes
Meteoridium remotifolium
Squamidium brasiliense
Zelometeorium patulum
Rhynchostegiella patula
Aerolindigia capillacea
Lindigia aciculata
Helicodontium capillare
Clasmatodon parvulus
Schwetschkea grateloupii
Homalotheciella subcapillata

(B)

To Fig. 1C.

Anomodontaceae in part — *Herpetineuron toccae*
Myuriaceae in part — *Palisadula chrysophylla*
Hypnaceae in part — *Eurohypnum leptothallum*
Hypnum cupressiforme
Leskeaceae in part — *Leptopterigynandrum austro-alpinum*
Theliaceae — *Thelia lescurii*
Anomodontaceae in part — *Schwetschkeopsis fabronia*
Hypnaceae in part — *Taxiphyllum diplanatum*
Callicladium haldanianum
Meteoriaceae in part — *Lepyrodontopsis trichophylla*
Hypnaceae in part — *Caribaeohypnum polypterum*
Horridohypnum mexicanum
Anomodontaceae in part — *Curviramea mexicana*
Hypnaceae in part — *Chryso-hypnum diminutivum*
Hylocomiceae in part — *Puiggariopsis aurifolia*
Pterigynandraceae in part — *Iwatsukiella leucotricha*
Climaciaceae — *Climacium americanum*
Pleuroziopsis ruthenica
Leskeaceae in part — *Rozea pterogonioides*
Hypnaceae in part — *Platygyriella aurea*
Pterigynandraceae in part — *Trachyphyllum gastrodes*
Hylocomiceae in part — *Schofieldiella micans*
Pylaisiadelphaceae — *Platygyrium repens*
Aptychella proligera
Taxithelium planum
Brotherella recurvans
Pylaisiadelpha teniurostris
Fauriella tenuis
Isopterygium pulchellum
Pterogonidium pulchellum
Heterophyllium affine
Hypnaceae in part — *Rhacopilopsis trinitensis*
Isocladiella surcularis
Wijkia extenuata
Mastopoma subpiliferum
Pseudotrismegistia perundulata
Papillidiopsis complanata
Trichosteleum papillosum
Sematophyllaceae — *Schoeterella zygodonta*
Aptychopsis pyrophylla
Pterogoniopsis cylindrica
Sematophyllum demisssum
Donniella commutata
Chionostomum rostratum
Hydropogon fontinalioides
Colobodontium vulpinum
Meiothecium chlorocladum

From Fig. 1B

Conardia compacta — Amblystegiaceae in part
Dixonia thamnoides — Neckeraceae in part
Dolichomitra cymbifolia
Petrogonium gracile — Leucodontaceae in part
Renauldia mexicana — Amblystegiaceae in part
Isothecium myosuroides — Lembophyllaceae
Tripterocladium leucocladium
Dolichomitriopsis diversiformis
Bestia vancouveriensis
Rigodium toxarion
Camptochaete arbuscula
Lembophyllum divulsum
Weymouthia mollis
Orthostichella pentasticha — Neckeraceae in part
Pinnatella piniformis
Penzigiella cordata — Pterobryaceae
Neckera pennata — Neckeraceae in part
Metaneckera menziesii
Alsia californica — Leptodontaceae in part
Heterocladium dimorphum — Pterigynandraceae in part
Cryptoleptodon longisetus — Neckeraceae in part
Leptodon smithii — Leptodontaceae in part
Dozya japonica — Leucodontaceae in part
Forstroemia trichomitria — Leptodontaceae in part
Thamnobryum alopecurum — Neckeraceae in part
Porothamnium arbusculans
Echinodium umbrosum — Echinodiaceae
Porotrichodendron nitidum
Porotrichopsis flacca
Touwia ellipticum
Homalia trichomanoides
Curvicladium kurzii
Taiwanobryum speciosum — Leptodontaceae in part
Homaliodendron flabellatum
Noguchiodendron sphaerocarpum
Handeliobryum sikkimense
Himantocladium cyclophyllum
Neckeriopsis disticha
Limbella trichosta — Amblystegiaceae in part
Taxiphyllopsis iwatsukii — Hypnaceae in part
Tomenthypnum falcifolium — Brachytheciaceae in part
Callialaria curvicaulis — Amblystegiaceae in part
Cratoneuron filicinum
Anacamptodon splachnoides
Campyliadelphus stellatus
Campylium chrysophyllum
Hygroamblystegium varium
Orthotheciella varia
Eriodon conostomus — Brachytheciaceae in part
Cratoneuropsis relaxa
Hypnobartlettia fontana
Vittia pachyloma
Palustriella commutata
Pseudo-calliergon angustifolium
Drepanocladus aduncus
Gradsteinia andicola
Levierella fabroniaceae — Fabroniaceae in part
Erythrodontium longisetus — Entodontaceae
Mesonodon flavescens
Leskeella nervosa — Leskeaceae in part
Regmatodon declinatus — Regmatodontaceae
Pseudoleskeella tectorum
Lindbergia brachyptera
Dimerodontium balansae — Fabroniaceae in part
Leskeadelphus bolivianus
Pseudoleskeopsis imbricata
Homomallium adnatum — Hypnaceae in part
Pylaisiopsis speciosa
Elmeriobryum phillipense
Gollania cylindricarpa
Leptohymenium tenue — Hylocomiaceae in part
Macrothamnium macrocarpum
Bryostreimannia turgida — Amblystegiaceae in part
Calliergonella cuspidata — Hypnaceae in part
Foreauella orthotheca
Ectropothecium leptochaeton
Vesicularia vesicularis
Boulaya mitteni — Thuidiaceae
Helodium blandowii — Helodiaceae in part
Rauiella lagoensis
Haplocladium virgianum — Leskeaceae in part
Platylomella lescurii
Actinothuidium hookeri — Helodiaceae in part
Thuidium delicatulum
Cyrto-hypnum schistocalix
Thuidiopsis furfurosa

Ⓒ

FIGURE 1. The optimal maximum likelihood tree of the combined *rps*4/*nad*5/nuc26S data set under the general time-reversible model with site rate variation (GTR+I+Γ : -ln likelihood = 54575.7; optimal parameters: A/C = 1.1166, A/G = 5.0600, A/T = 0.2165, C/G = 1.0611, C/T = 5.6457, G/T =1(fixed); f(A) = 0.3360, f(C) = 0.1836, f(G) = 0.1781, f(T) = 0.3027; α = 0.5730; pinvar = 0.3410). Nodes support by >95% posterior probability are highlighted. Taxonomic labels are applied to clades at the lowest rank applicable to the clade following the classification of Buck and Goffinet (2000): A) acrocarpous mosses - the two exemplars marked with an asterisk were originally labeled *Hymenostylium recurvirostre* and *Pottia truncata* and subsequently identified as *Ceratodon purpureus,* B) pleurocarpous mosses - part 1, C) pleurocarpous mosses - part 2.

taxon-dense and data-poor trees are not necessarily more accurate than taxon-sparse and data-rich trees *per se*, and *visa versa,* for a given set of taxa. The issue is perhaps most beneficially viewed with regard to specific nodes in the tree, which are most likely to require a balance of consideration for both these factors (Hillis *et al.* 2003). That is, while some nodes are relatively easy to resolve with either taxon-sparse or data-poor analyses, difficult to resolve nodes may require larger taxon samples, perhaps to break-up long-branches in the vicinity, or larger amounts of data to resolve short-branches in the tree, or, most probably, a balance of both of these factors. The analyses we present here are very taxon-dense in particular parts of the tree (e.g. the pleurocarpous mosses) while being relatively taxon-poor in others (e.g. within the nematodontous grade). Our aim was to provide an overview of molecular diversity among the mosses from each of the three genomic compartments, and as such we emphasized taxon sampling at the expense of data sampling (though clearly we consider the amount of data we collected to be enough to make sufficiently accurate trees), but we do not consider these trees to be more accurate with regard to any particular divergence merely due to the increased taxon sampling. It is, however, noteworthy that the analyses presented here are for the most part congruent with gene-rich studies regarding many of the deep-level relationships we identify.

The many incongruencies between genes in our analyses and, more generally, between our study and previous studies, highlight the necessity of identifying the sources of conflicting signals in phylogenetic analyses. The incongruencies may be 'real' in the sense that they reflect different evolutionary histories between the nuclear and two cytoplasmic genomes, via mechanisms such as lateral gene transfer (Bergthorsson *et al.* 2004) or differential retention in lineages of copies of duplicated genes (where paralogs can wrongly be assumed to be orthologs), but such conclusions are often the explanation of last resort (because they have such profound biological implications) when all other possibilities have been eliminated. Alternatively, as is most likely here, they are the result of analytical artifacts introduced by differing taxon samples and applied methodologies, although human error (data collection and manipulation errors) is often difficult to discount. Of particular concern here is the failure of the applied models to adequately reflect the underlying processes of molecular evolution that gave rise to the taxon gene sequences. Substitution models are typically chosen as best-fitting from a selected few possible models (e.g. using Modeltest), but rarely are they tested for adequacy — a model may be the best-fitting of those available but at the same time fail to reflect the underlying substitution processes adequately (Bollback 2001). Furthermore, in a standard model-based methodology substitution models are applied such that the substitution process described by the instantaneous rate matrix (Q; Swofford *et al.* 1996) remains constant across the entire tree within a particular data partition (be it gene, codon-site, or how ever the data are divided). Nevertheless, as we go deeper in the tree below species-level diversity it is increasingly unlikely that constancy of this substitution process holds, regardless of changes in overall rate of change (i.e. branch length variation). The older a divergence the longer have its descendant lineages had time to diverge in substitution dynamics — often, we need only look at taxon base compositions to confirm that indeed the substitution process itself has changed through time. Failing to model these changes can have detrimental effects on phylogenetic accuracy (Foster 2004), but the extent to which particular analyses are affected is usually unknown. In our analyses, we constructed a data set with the largest possible taxon sample containing all three genes, where those genes exhibited no evidence of severe incongruence (as evidenced by < 95% p.p. of conflicting resolution of lineages). By doing so we attempted to at least minimise the effects associated with intra-taxon conflict, whatever its origin, between data partitions with the expectation that inter-taxon resolution and statistical support would be maximised in the tree.

Early-branching peristomate lineages

The relationships among the early-diverging peristomate moss lineages have proven especially difficult to resolve with confidence (Newton *et al.* 2000, Hyvönen *et al.* 2004, Cox *et al.* 2004, Wahrmund *et al.* 2010), and our analyses here provide only a little further insight. The two nematodontous lineages, Tetraphidales and Polytrichales, compose a monophyletic group only when inferences were made based on the mitochondrial genes *nad*2 and *nad*5 (78% maximum likelihood bootstrap; Beckert *et al.* 2001). In the current analysis of the *nad*5 gene (S.I. Figure S.2.2) we found no evidence of a sister-group relationship between the two groups (the

*nad*2 gene in the Beckert *et al.* study did not provide any statistical support for the monophyly of the nematodontous mosses). The *rps*4 gene alone strongly supported the Tetraphidales as the earliest diverging lineage of peristomate mosses, with the gymnostomous taxon *Oedipodium* and the (at least partially) arthrodontous taxon *Buxbaumia* forming a clade with the Polytrichales (S.I. Figure S.2.1). By contrast, previous analyses of the *rps*4 gene have placed *Oedipodium* as the earliest diverging lineage, with the Tetraphidales and *Buxbaumia* forming a clade sister to the Polytrichales, although these relationships received no statistical support (Goffinet *et al.* 2001). When combined with the *nad*5 and nuc26S data, the statistical support from our *rps*4 analyses for the resolution of the Tetraphidales as the earliest-diverging lineage was eroded (i.e. <95% pp. Figure 1A), as was the support for the placement of *Oedipodium* and *Buxbaumia* within a clade with the Polytrichales. Inferences from additional loci combined with morphological data also failed to unambiguously resolve the affinities of the Tetraphidales (Hyvönen *et al.* 2004). Given the ambiguity, the origin of the arthrodontous peristome, its derivation from a nematodontous one, and even the fundamental homology between the peristome of the Tetraphidales and Polytrichales, remains obscure.

Early-branching arthrodontous lineages

The Diphysciales are well established as the first diverging lineage of arthrodontous mosses (Newton *et al.* 2000, Beckert *et al.* 2001, Cox *et al.* 2004, Magombo 2003, Wahrmund *et al.* 2010) and our analyses corroborate this result (Figure 1A). The Funariidae (*sensu* Goffinet *et al.* 2007), uniting the Gigaspermales, Funariales, and Encalyptales, are resolved as a monophyletic group but without significant support (see also Goffinet & Cox 2000, Hedderson *et al.* 2004). The monophyly of the clade consisting of the Funariales and Encalyptales is supported by the presence of a 71kb inversion in the single-copy region of the chloroplast genome (Goffinet *et al.* 2007), a seemingly strong synapomorphy for the group that was recovered by our and some other phylogenetic analyses (Goffinet & Cox 2000, Cox *et al.* 2004).

The relationships among the three sub-classes, Funariidae, Dicranidae, and Bryidae, remain ambiguous. The Funariidae are resolved as the sister-group to the clade consisting of the Dicranidae (haplolepideae) and Bryidae (diplolepideous-alternate peristomate mosses) but lacking strong support (Figure 1A), by contrast to the inferences from multiple genes (Cox *et al.* 2004). Other analyses suggest that the Funariidae are most closely related to the haplolepideae: based on multiple genes (Goffinet & Cox 2000), *nad*5 (Beckert *et al.* 2001), *rps*4 (Goffinet *et al.* 2001, Hedderson *et al.* 2004), or *nad*5 plus *rps*4 (S.I. Figure S.2.7). However, the latter result is not confirmed by our analyses here (S.I. Figure S.2.1) which support the clade uniting the haplolepideae and Bryidae (94% p.p.). The correct resolution of these lineages remains elusive, and may well rest upon resolution of the members of the so-called 'proto-haplolepideae' (Hedderson *et al.* 2004), some of which were resolved as most closely related to the Bryidae in our *rps*4 tree (S.I. Figure S.2.1) and as most closely related to the Funariidae in previous analyses of the same gene (Goffinet *et al.* 2001).

The ambiguity among the three sub-classes is exacerbated when *Timmia* is considered. In previous analyses, *Timmia* has been resolved either as the sister-taxon to the Funariidae (Cox *et al.* 2004) or the sister-taxon to the clade uniting Dicranidae and Bryidae (Cox *et al.* 2004), as either the sister-taxon to (Goffinet & Cox 2000), or within the clade uniting Dicranidae and Funariidae (Goffinet *et al.* 2001, Beckert *et al.* 2001), or as the sister-taxon to the Dicranidae alone (Hedderson *et al.* 2004). In the *rps*4 analyses presented here, *Timmia* is strongly supported within the Encalyptaceae (S.I. Figure S.2.1), while the nuc26S analysis places it strongly in a clade with two haplolepideous taxa, *Distichium* (Ditrichaceae) and *Timmiella* (Pottiaceae) (S.I. Figure S.2.3). *Timmia*'s placement within the Encalyptaceae seems most likely an artifact as it does not share the inversion in the chloroplast genome common to members of the Encalyptaceae (Goffinet *et al.* 2007).

The three main lineages of arthrodontous mosses, Funariidae, Dicranidae, and Bryidae are defined by distinct combinations of architectural and ontogenic features of their peristome. The architecture of *Timmia*'s peristome is unique but it shares critical developmental stages with the peristome of *Funaria* (i..e, the symmetric division in cells contributing to the inner surface of the endostome; Budke *et al.* 2007). With the ambiguity in the sequence of divergence of these four taxa, the fundamental question of which peristome-type

composes the ancestral architecture (Bryum-type *fide* Crosby 1980, or Funaria-type *fide* Vitt 1984) remains unanswered.

The haplolepideae (Dicranidae)

The monophyly of mosses with a haplolepideous peristome (Vitt 1984) has been consistently supported by phylogenetic inferences (Goffinet & Cox 2000, LaFarge *et al*. 2000, Cox *et al*. 2004, Tsubota *et al*. 2004). The circumscription of the Dicranidae as proposed by Vitt (1984) has only changed slightly, and only as the affinities of taxa with atypical, reduced, or no peristome are reassessed, suggesting that the haplolepideous architecture of the peristome (Shaw *et al*. 1989) evolved only once. The circumscription of the Dicranidae has been expanded to include taxa such as the Drummondiaceae (Goffinet *et al*. 1998), *Wardia* (Hedderson *et al*. 1999), Splachnobryaceae (Goffinet & Cox 2000, Werner *et al*. 2004) and the Ephemeraceae (Goffinet & Cox 2000, Werner *et al*. 2007), and is here further broadened to accommodate *Bryowijkia*, a genus previously included in the Hedwigiaceae (Vitt 1984) or Trachypodaceae (Vitt & Buck 1984). Should the placement of *Bryowijkia* be confirmed, then the interpretation of its peristome as being diplolepideous (Vitt & Buck 1984) must be questioned. The circumscription of the Dicranidae has also been contracted as *Bryobartramia,* long considered a member of the Pottiales (Vitt 1984), is shown to share a common ancestor with *Encalypta* (Hedderson *et al*. 2004, Goffinet *et al*. 2007).

The backbone phylogeny of the Dicranidae is poorly supported except for a) *Timmiella anomala*, *Distichium capillaceum*, and *Clastoblastella chilense* composing a clade sister to, or a grade leading to, the remainder of taxa; b) *Bryoxiphium norvegicum*, *Drummondia obtusifolia* and *Scouleria aquatica* forming the next clade or grade, and c) the monophyly of a broad assemblage including most Dicranaceae, most Ditrichaceae, Pottiaceae, Rhabdoweisiaceae, Fissidentaceae, Schistostegaceae, etc, but excluding the Grimmiaceae, Ptychomitriaceae and Leucobryaceae (Figure. 1A). Whether the Dicranales as defined by Goffinet *et al*. (2009) or Vitt (1984 as Dicranineae) compose a monophyletic lineage is not clear: except for the above-mentionned taxa the Dicranales compose in most optimal reconstructions at best a paraphyletic group leading to the Pottiales but support to reject the monophyly is lacking given the overall weakness of the backbone phylogeny derived from the three loci. However, when inferences are drawn only from *rps*4 and *nad*5 data alone, the successive nodes of the grade are well-supported (S.I. Figure S.2.7). The polyphyly of the Dicranales is further accentuated in the latter phylogeny by the shared ancestry of the Leucobryaceae and Eustichiaceae with the Archidiales. Within the Dicranales, the circumscription of the Dicranaceae, Ditrichaceae and Rhabdoweisiaceae is in critical need of reassessment, as suggested by Stech (1999a,b) and La Farge et al. (2000, 2002). By contrast the circumscription of the Grimmiales as proposed by Tsubota *et al*. (2004) withstands additional sampling, although the inclusion of the Seligeriaceae is supported by inferences from cytoplasmic loci only (S.I. Figure S.2.7). The affinities of the Grimmiales remain ambiguous in all reconstructions. Similarly the sister-group to the Pottiales is still unknown.

Overall, the lack of resolution of the successive cladogenic events marking the macroevolutionary history of the Dicranidae suggests that diversification was rapid following the evolution of the haplolepideous peristome. Indeed, an overall slow substitution rate in mosses provides an inadequate explanation for the short branches defining putative lineages considering that more recent cladogenic events are seemingly defined by rather unambiguous synapomorphies (e.g., the well supported Pottiales, Leucobryaceae, or Grimmiaceae). If a rapid radiation occurred, resolving the branching order within the Dicranidae may required intensive sampling (i.e., 10 or more loci), as was needed for the resolution of the relationships within the Saxifragales for example (Jian *et al*. 2008). Resolution of the relationships and the circumscription of major lineages within the haplolepideous mosses is essential to critically assess the phylogenetic and ecological significance of transformations of gametophytic and sporophytic characters in particular those linked to transitions to extreme habitats.

The acrocarpous diplolepideous-alternate peristome mosses

Within the unambiguously monophyletic Bryidae (i.e., mosses with a diplolepideous-alternate peristome mosses), the superorder Bryanae (*sensu* Goffinet *et al.* 2009 — Bryanae and Rhizogonianae but excluding Hypnodendrales *sensu* Buck & Goffinet 2000) composed of essentially acrocarpous taxa is a grade from which the pleurocarpous mosses (Hypnanae) are derived. Although the paraphyly of the Bryidae has long been suspected (e.g. Cox & Hedderson 1999, Newton *et al.* 2000), the sister-group to the pleurocarpous mosses remains unknown. The combined analyses presented here place the Orthotrichales as the well-supported sister-group to the 'rhizogoniaceous grade' of (with successive branching) Orthodontiales, Rhizogoniales, Aulacomniales, Hypnodendrales, and the pleurocarpous mosses (Bell *et al.* 2007). This order of branching among the groups is mainly due to support from the mitochondrial *nad*5 gene (S.I. Figure S.2.2) - the chloroplast *rps*4 data (S.I. Figure S.2.1) provide little supporting or contradictory evidence, as noted by Bell *et al.* (2007). Our analyses of the nuc26S gene data indicate highly unusual groupings, for instance, the Ptychomniales and Aulacomniales being more closely related to the Bryales (S.I. Figure S.2.3). These latter relationships most likely reflect analytical artifacts, perhaps a failure to adequately model compensatory changes in stem regions of the 26S rRNA molecule (Dixon & Hillis 1993). Nevertheless, these results need to be confirmed with additional data from the nuclear genome.

Bartramiales and Hedwigiales are resolved as the first diverging lineage within the Bryanae (Figure 1A). However, the Splachnales were excluded from the tree due to incongruence among partitions — *rps*4 placed the Splachnales in this basal-most position, but the nuc26S resolved them nested within the Hypnales (again most likely an analytical artifact). Support for the basal-most position of Splachnales within the Bryanae is weak: 83% p.p. (*rps*4: S.I. Figure S.2.1; 71–77 p.p. Bell *et al.* 2007) and contradicted by the *nad*5 data (80% p.p; see also Cox & Hedderson 1999, Cox *et al.* 2004). Similarly, support for the sister-relationship between Bartramiales and Hedwigiales is lacking, though not statistically contradicted, in our analyses (see also Cox *et al.* 2000, Goffinet *et al.* 2001, Bell *et al.* 2007). Yet, remarkably, nuc26S provides very strong support for the grouping of all other Bryanae to the exclusion of the Bartramiales and Hedwigiales (S.I. Figure S.2.3), possibly artifactually, but congruent with the multigene analysis of Cox *et al.* (2004).

The pleurocarpous mosses

Through evolutionary time, the transfer of the female sex organ to the apex of short specialized branches marks the origin of the Hypnanae, the most diverse lineage of mosses, comprising approximately 6,000 species. For much of the 20th century these species were accommodated into three main lineages, recognized at differents ranks, but equivalent to the Hookeriales, Hypnales, and Leucodontales (Vitt 1982, 1984). The pioneering study by Hedenäs (1994) noted that these concepts were unlikely to withstand critical cladistic analysis of morphological characters, and the first inferences from DNA sequence data (DeLuna *et al.* 1999) lent credence to that hypothesis. The polyphyly of the Leucodontales was demonstrated by DeLuna *et al.* (1999) and Buck *et al.* (2000) and the order has not been recovered as a monophyletic entity since (Tsubota *et al.* 2004, Ignatov *et al.* 2007). Its families have been transferred to the Hypnales, except for the Ptychomniaceae, which are now considered the sister lineage to all other pleurocarpous mosses (Buck *et al.* 2004). The circumscription of the Hookeriales, the sister-group to the Hypnales, has remained unchanged since Vitt (1984) although the number of recognized families has increased from three to seven (Buck *et al.* 2004). In the analyses presented here the Hypopterygiaceae are resolved within the Hypnales rather than the Hookeriales, as previously suggested by Pedersen & Newton (2007), but none of the nodes separating the Hypopterygiaceae from the Hookeriales are well-supported, and hence a shared ancestry with other Hookeriales as proposed by Buck *et al.* (2004) cannot be rejected. In contrast to the well-supported monophyly of the Hookeriales s. str., a unique shared ancestry for all Hypnalean taxa remains doubtful (Figure 1B; Tsubota *et al.* 2004, Olsson *et al.* 2009a). The lack of synapomorphies for the Hypnales may be a consequence of their rapid diversification after divergence from the Hookeriales (Shaw *et al.* 2003b) that may have followed the diversification of angiosperms and the development of the angiosperm forest ecosystems (Pedersen & Newton 2007). A rapid diversification coupled with a low rate of mutations (Stenøien 2008) may

also account for the conspicuous lack of robustness throughout the backbone of the optimal phylogeny of pleurocarpous mosses inferred from all three loci, a weakness highlighted earlier by Goffinet *et al.* (2001) and Tsubota *et al.* (2004) and observed again most recently by Olsson *et al.* (2009b). Regardless of the character source and the taxon sampling, few well-supported lineages of hypnalean taxa are recovered and these lineages rarely match the families defined by Vitt (1984) or contemporary workers. The phylogenetic inference presented here includes several large robust monophyletic lineages in the Hypnales, that can be roughly compared to the Pterobryaceae, the Meteoriaceae, the Brachytheciaceae (Figure 1B), Sematophyllaceae *s. lat.* (including the Pylaisiadelphaceae), and the Amblystegiaceae (Figure 1C). Except maybe for the extensively studied Meteoriaceae (Quandt *et al.* 2004, Huttunen & Quandt 2007) and Lembophyllaceae (Quandt *et al.* 2009), these families in the sense of Goffinet *et al.* (2009) are polyphyletic, due to the scattered distribution of some discrete outliers. One other clade includes most Neckeraceae, but not the type genus, a hypothesis contrary to the recent study by Olsson *et al.* (2009a) wherein inferences were drawn from more variable loci (but see also Olsson *et al.* 2009b). Noteworthy here may be the resolution of the monogeneric Echinodiaceae, a taxon not sampled by Olsson *et al.* (2009) within the Neckeraceae (Figure 1C). Unlike most families of pleurocarpous mosses, the Thuidiaceae share a common ancestor, although it is unique only if the Helodiaceae and *Haplocladium* (Leskeaceae) are accepted within a broader concept of the family. A core group of Cryphaeaceae composes a robust lineage to the exclusion of *Dendroalsia* and *Pilotrichopsis* (Figure 1B).

Perhaps the most notable result of the our phylogeny is the widely polyphyletic nature of the Hypnaceae, a family traditionally defined by the ecostate leaves, elongate-linear cells, and a well developed double peristome with a high endostomial membrane. Vitt (1982) had doubted that the circumscription of the family would withstand critical examination: a conjecture that has been borne out by the phylogenetic analysis presented here (Figure 1B&C) and earlier (Tsubota *et al.* 2004).

Phylogenetic diversity

Biodiversity can have multiple meanings in different contexts and can consequently be estimated using different metrics depending on the purpose. Phylogenetic diversity (PD) quantifies the amount of evolutionary change (branch lengths on a phylogenetic tree) that separates taxa, and can provide information that complements diversity estimates based on numbers of species or some other taxonomic unit (Faith 1992, 1994). We observed that PD and taxic diversity give highly correlated estimates of how biodiversity is partitioned among some of the major clades of mosses; indeed correlation coefficients were greater than 0.9 between estimates based on loci from the three different genomes (plastid, mitochondrial, nuclear), and between these molecular estimates and numbers of genera. One implication of this consistency is that the generic level in the Linneaean taxonomic hierarchy for mosses is not as arbitrary as one might perhaps suggest. That is, a genus of Funariales, for example, encompasses about the same amount of phylogenetic diversity as does a genus of Bartramiaceae.

PD estimates can in some cases provide insight into the tempo and mode of evolutionary diversification. In the peatmosses (*Sphagnum*), the Neotropics contain an extremely high level of species richness, but much less PD than predicted from those species numbers (Shaw *et al.* 2003a). One possible explanation from that observation is that species richness of Neotropical peatmosses reflects a recent radiation. Similarly, the lower than predicted (from taxic diversity) levels of PD within hypnalian pleurocarpous genera may be related to a relatively recent and sudden radiation of this clade (Shaw *et al.* 2003b). Higher than predicted levels of PD in the Dicranidae could, conversely, reflect extinction, leading to more distinct; i.e., relictual, genera. Other explanations such as differences in substitution rates among clades cannot be ruled out, but it is noteworthy that the patterns are highly correlated across the three genomes, supporting a more general interpretation. Phylogenetic estimates of biodiversity do not substitute for more traditional taxon-based metrics, but can generate hypotheses about evolutionary processes.

Concluding remarks

Our analyses highlight the difficulty of obtaining robustly supported groupings of the main lineages of mosses. Despite the antiquity of the main lineages (Newton *et al*. 2007), the overall rate of moss molecular evolution appears to be rather slow (Stenøien 2008) suggesting that many loci will most likely be required to confidently establish some major divergences. At a shallower taxonomic level, the many instances of polyphyly among the families of haplolepidous and pleurocarpous mosses re-inforce the need to address phylogenetic hypotheses within a broader context than those defined by traditional families.

Obtaining statistically robust resolutions of the relationships, firstly, among the nematodontous taxa, *Oedipodium*, *Buxbaumia*, and the arthrodontous mosses, and, secondly, among the Funariidae, Dicranidae, and Bryidae, are the two most difficult problems currently facing moss phylogenetics, with respect to deep evolutionary divergences. The conflicting resolutions of the Funariidae plus Dicranidae versus the Dicranidae plus Bryidae hypotheses may be indicative of an evolutionary event resulting in authentic phylogenetic conflict (such as an early hybridization event between two of these lineages); the cytoplasmic phylogenetic signal for the former relationship is strong, but always overruled (although without support) when the nuclear rRNA loci are included. Yet, the alternative explanation of this conflict being an artifact resulting from model mis-specification during the phylogenetic analyses needs to be disproved before the implications of a hybridization event deep in moss evolution can be fully assimilated.

Although some crucial nodes in the moss phylogenetic tree remain poorly resolved and therefore of minimal value for the reconstruction of ancestral character states, the completion of the first entire moss genome (*Physcomitrella*: Rensing *et al*. 2008), and the ability to characterize transcriptomes at particular ontogenetic stages, provides an opportunity for a complementary experimental approach to moss systematics. The resolution of gene networks underlying the development of specific traits should permit an assessment of the homology among distinct peristome types and among the transitions of gametangial production from the stem to the branches that occurred during the evolution of pleurcarpy. Solving these puzzles will be essential to understanding the innovations that mark the major cladogenic events in the evolution of the mosses.

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