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Phylogenetic Relationships Among Basal-most Arthrodontous Mosses with Special Emphasis on the Evolutionary Significance of the Funariineae

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Abstract. *The classification of the Bryopsida (mosses) has been based primarily on the variation of sporophytic characters i.e., architectural features of the peristome teeth that line the capsule mouth. Five arthrodontous peristome types have been recognized. Whether peristome types define natural groups and how they are evolutionary related has, however, remained unclear. Nucleotide sequence data from one nuclear and two chloroplast loci are generated and compiled to test two contrasting hypotheses regarding the ancestral peristome type in the Arthrodonteae. The genomic data partitions are incongruent with regard to the phylogenetic signal they carry. All phylogenetic analyses converge toward the polyphyly of the Funariineae and the Funariaceae. The Funariaceae are defined by the loss of a codon in the* rps*4 gene.* Goniomitrium acuminatum, *the type of the genus, lacks this deletion, and is always resolved within the Haplolepideae. Consequently* Goniomitrium *is transferred to the Pottiaceae. The Ephemeraceae and Splachnobryaceae are tentatively retained as distinct, but with strong affinities to the Pottiineae. Neither the combined nor the independent data sets yield well supported topologies under the parsimony optimality criterion. Hence, the relationships among major lineages remain ambiguous. Inferences from chloroplast data alone yield a basal dichotomy between taxa with alternate peristomes (Orthotrichales and Bryales* sensu lato*) and those with opposite peristomes (Encalyptineae, Disceliaceae, Funariaceae, Timmiaceae, and the Haplolepideae). In contrast, analyses of the combined data resolve the Timmiaceae as sister to the split between the two peristomial lineages. It is hypothesized that the symmetric divisions of the IPL cells, is a synapomorphy for at least the Encalyptineae-Funariineae clade. The endostomial appendages of the timmiaceous peristome could, under either phylogenetic hypothesis, be regarded as homologous to the cilia in the bryoid peristome. Although the relationships among major lineages of arthrodontous mosses remain ambiguous, this study suggests that taxa with reduced or no peristomes, such as the Disceliaceae and the Gigaspermaceae, may be crucial in resolving the early evolutionary history of the Arthrodonteae when using DNA sequences.*

Arthrodontous mosses are defined by the architecture of their peristome teeth. In contrast to nematodontous mosses (the Polytrichales and Tetraphidales) whose teeth are solid, and built from whole cells, the teeth that line the mouth of arthrodontous mosses, are composed of cell wall remnants, and in particular of periclinal cell plates. The architecture of the peristome teeth varies among major lineages of arthrodontous mosses. Vitt (1981) recognized four main peristome types that differ in their basic architecture at maturity. The haplolepideous peristome or Dicranum-type peristome is typically reduced to an endostome of 16 teeth, whose inner surface is built from one and a half columns of cells. The remaining three types are diplolepideous, that is they are typically composed of two rows of teeth, with the outer teeth bearing remnants of two columns of cells (hence the name diplolepideous). In the Funaria-type peristome, the exostome teeth lie opposite the endostome segments. By contrast, in the Orthotrichum- and Bryum-types, the outer teeth alternate with the inner segments. The latter type is further unique by the presence of endostomial cilia between the segments, a character correlated to the presence of additional cells in the inner most peristomial layer (IPL). These architectural differences observed at maturity are complemented by ontogenetic variations in the type of cell divisions in the IPL. In the Funaria-type peristome, the divisions that lead to a doubling of the number of IPL cells from eight to 16 are all symmetric, and the new anticlinal walls are perfectly aligned, with the anticlinal walls of the adjacent primary peristomial layer (PPL – Shaw

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et al. 1989*a*), whereas in the remaining three types, these divisions are asymmetric, and hence the new wall, is not aligned with the adjacent PPL wall (Goffinet et al. 1999; Shaw et al. 1989*a*,*b*). Vitt (1984) later also considered the peristome of the Encalyptineae to be distinct enough to warrant its recognition as a distinct architectural type. In the Encalyptineae the teeth and segments appear opposite as in *Funaria* and in the Haplolepideae (see Vitt et al. 1998), but the exostome can also bear intermediate teeth alternating with the endostomial segments (Edwards 1984; Horton 1982).

The polarity of peristome-type evolution has remained controversial, due to the uncertainty regarding the sister-group to the Arthrodonteae, to the lack of a robust phylogeny for mosses, and to the difficulty of assessing homology among peristomial features (Goffinet et al. 1999; Vitt et al. 1998). Vitt (1981, 1984) proposed that the Funaria-type peristome represents the most basic peristome architecture from which other types are derived. By contrast, Shaw and Rohrer (1984) considered the peristome of *Funaria* to be the product of reduction from a ciliate bryalean type peristome. The opposite arrangement of peristomes would consequently appear as a derived feature. Recent reconstructions of the phylogeny of mosses from mitochondrial (Beckert et al. 1999), chloroplast (Goffinet et al., unpubl.), and nuclear (Newton et al. 2000) sequence variations converge toward the Diphysciaceae (i.e., *Diphyscium* Mohr, *Muscoflorschutzia* Crosby, and *Theriotia* Card.) forming the sister-group to the Arthrodonteae. Because the IPL division is asymmetric in *Diphyscium*, Goffinet et al. (1999) argued that this character should at present be considered plesiomorphic in the Arthrodontae, rather than derived from an ancestral symmetric division as suggested by Vitt (1981, 1984). This hypothesis does, however, not preclude an early origin of the Funariineae in the evolutionary history of the Arthrodonteae. Analyses of *rbc*L sequences resolved the Funariaceae sister to a clade comprising the Orthotrichineae, Dicranineae, Bryineae, and Splachnineae (Goffinet et al. 1998), with the Encalyptineae sister to either this large clade or only to the Dicranineae. Cox and Hedderson (1999), analyzing a more extensive taxon sample using chloroplast and nuclear data, examined the relationships within the Arthrodonteae rooted to *Funaria* based on evidence from a broader analysis of 18S rDNA sequences (Hedderson et al. 1996, 1998). Their analyses resolve the Orthotrichineae and the Splachnineae nested within the Bryineae, and the Encalyptineae and Haplolepideae composing a basal grade. Furthermore, they indicated that the Timmiaceae, that were resolved in a nested position within this basal grade, may be crucial for elucidating the relationships among major lineages of the Bryopsida (*sensu* Vitt 1984).

The Funariineae *sensu* Vitt (1984) comprise five families, namely the Funariaceae, Pseudoditrichaceae, Disceliaceae, Gigaspermaceae, and Ephemeraceae. Shaw (1984) excluded the monospecific Pseudoditrichaceae and transferred it near the Bryaceae (Bryineae) based on peristomial characters. The Gigaspermaceae and the Ephemeraceae are composed of gymnostomous taxa only. The peristome of the Disceliaceae is reduced; the endostome consists of a thin transparent membrane, adherent to the exostome, whereas the exostome is well developed and composed of sixteen teeth (Shaw & Allen 1985). The longitudinal anticlinal walls of the IPL appear aligned with those of the adjacent PPL, a feature present only in the Funariineae and the Encalyptineae (Shaw & Allen 1985). In the Funariaceae, the peristome is either well developed, reduced and simple, or lacking (Fife 1985). When well developed, the peristome of the Funariaceae is diplolepideous, with the endostome segments opposite or co-radially aligned to the exostome teeth. Cilia are always lacking and the peristome formula is typically 4:2:4, with the IPL lacking additional cellular divisions, which are characteristic of typical Bryales with cilia. The cells of the IPL are all similar, if not identical, in size as a consequence of the symmetric divisions (Shaw et al. 1989a). In the absence of sporophytic features for most taxa, the Funariineae have traditionally been defined by the similarities in vegetative characters, such as smooth cells, costate leaves, undifferentiated alar cells, and large calyptrae (Vitt 1982). Except for the Gigaspermaceae that are cladocarpous (LaFarge-England 1996), the Funariineae *sensu* Vitt are typical acrocarpous taxa (Fife 1980). Koponen (1981) erected the Splachnobryaceae, a small family restricted to the genus *Splachnobryum* C. Müll., and transferred it from the Pottiineae to the Funariineae, based on the diplolepideous rather than haplolepideous architecture of the peristome. Koponen (1981) further argued for *Splachnobryum* having a rather basal position among arthrodontous moss phylogeny, on the basis of the lateral solitary gametangia. The peristome is, however, reduced to a single row of 16 rather short teeth, whose architecture Allen and Pursell (2000) interpret as of the haplolepideous type. The circumscription of the Funariidae (*sensu* Vitt 1984), and the relationships among the families currently accepted within the order, have not been critically examined using a phylogenetic approach.

Assuming that *Diphyscium* and its close relatives compose the closest outgroup to the Arthrodonteae, this study will address the circumscription of the Funariineae, the relationships among its families, and examine the significance of the Funaria-type

peristome in the evolution of peristome types in the Arthrodonteae. Specifically, the following questions will be addressed 1) are the Funariineae *sensu* Vitt (1984) monophyletic? 2) what is the significance of the Funariineae in the early diversification of the Arthrodonteae? and 3) what are the trends in the evolution of peristomial characters in the Arthrodonteae?

Material and Methods

Taxon sampling.—Exemplars of arthrodontous taxa potentially representing early cladogenic events, with a main emphasis on the Funariineae, *sensu* Vitt (1984) were sampled (Table 1). Duplicate taxonomic sampling was performed for selected taxa as primarily analyses progressed, to confirm sequences; duplicate taxa were retained even when the additional sequences diverged by a single nucleotide. The matrix was completed by retrieving sequences from GenBank, primarily for diplolepideous mosses (Cox & Hedderson 1999). Vouchers are deposited in DUKE unless otherwise indicated in Table 1.

DNA extraction, PCR amplification, and sequencing.—Apical portions of stems or branches, or in the case of ephemeral taxa, operculate capsule(s), were removed from dried herbarium collections. DNA was extracted from plant tissues following the protocol outlined in Goffinet et al. (1998). Plant material was ground using a small glass test tube, in 250 μL of 2X CTAB (hexadecyltrimethylamonium bromide)-0.2% beta-mercaptoethanol, heated to 60°C, and incubated at this temperature for at least 30 min. An equal volume of chloroform-isoamyl (24:1) was added. The emulsified solution was centrifuged for one min. at 6,500 rpm, and the aqueous phase was transferred to a new tube to which an equal volume of ice cold isopropanol was added. DNA was precipitated at 4°C for 30–60 min. Tubes were centrifuged first for 10 min. at 13,000 rpm. The pellet was washed with 70% ethanol, and the tubes centrifuged for three min at 13,000 rpm. The pellet was dried in a vacuum centrifuge and suspended in 100 μL TE (Tris-EDTA, pH 8.0). Protocols (of) for the amplification(s) of the *trn*L-*trn*F and *rps*4 regions as well as the sequencing of the fragments followed those presented in Buck et al. (2000). Amplification and sequencing of the 18S rRNA gene followed the protocol described in Cox et al. (2000). Labeled fragments yielded by the sequencing reactions were separated on polyacrylamide gels (Long Range Singel; FMC Bioproducts), using an ABI Prism™ 373 or 377 automated DNA sequencer (Perkin Elmer). Nucleotide sequences were edited using Sequencher 3.0 (Gene Codes Corporation), entered in Paup version 4.0b2a (Swofford 1999) and manually aligned. The intron, exon, and spacer composing the *trn*L-*trn*F product were delimited by comparing the sequences with available GenBank accessions. Aligned *trn*L-*trn*F sequences were trimmed of the 5'exon of the *trn*L, and the *trn*F exon (leaving the *trn*L intron, *trn*L-3′ exon, and the *trn*L-*trn*F spacer), while the first 27 sites (including the annealing site of the primer) and the intergenic spacer following the stop codon were excluded from the *rps*4 sequences. Sequences of the 18S rRNA gene were trimmed at both ends at sites corresponding to the amplification primer annealing sites. All sequences obtained in this study were submitted to GenBank (Table 1).

Phylogenetic analysis.—All analyses and tests were performed using PAUP version 4.0b2a (Swofford 1999) on a Macintosh G3 400 MHz. Maximum parsimony (MP, Fitch 1971) was chosen as the optimality criterion for the phylogenetic analyses. Parsimony analyses were performed with equal weighting of characters and transformations. For each analysis searches were replicated 100 times, with random sequence addition. The steepest descent option was selected. A partition homogeneity test was performed using the same set of options to examine whether phylogenetic signal carried by the nuclear and chloroplast sequences are congruent or whether they recover distinct histories. Consistency indices (CI) and rescaled consistency indices (RC) were calculated with PAUP. Fifty thousand random trees were generated and the g_1 (Huelsenbeck 1991) statistic describing the tree length frequency distribution was compute with Paup. Relative strength of support for particular branches was estimated using bootstrap analysis (Felsenstein 1985; Hillis & Bull 1993) based on 100 bootstrap replicates of the heuristic search with the same set of options in effect as above (except for 10 search replicates). Support for the branches was evaluated by Bremer support analysis, using the program Autodecay (Eriksson 1998).

Finally, constraint trees were employed to assess the effect of alternative phylogenetic hypotheses on the tree length. Tree scores of the constrained trees were compared to that of unconstrained trees by non-parametric (Templeton 1983) and parametric (Kishino & Hasegawa 1989) testing.

Results

Sequence variation.—Sequences for the 18S rRNA and *rps*4 genes and for the *trn*L-*trn*F region were assembled for 39 taxa. 18S rDNA sequences were not obtained for *Discelium nudum* (Disceliaceae) and the taxon is therefore not included in the combined analysis. Eighty-two sequences were generated in the course of this study (Table 1). Alignment of sequences resulted in a matrix of 3,430 characters of which 807 were excluded, either because these sites corresponded to the amplification primer sequences, or the homology assumptions required for these sites were considered ambiguous (the matrix is available from the senior author). The 2,623 characters included in the analyses consisted of 1,740 nt from the18S rRNA gene, 570 nt of the *rps*4 gene and 313 nt of the *trn*L-*trn*F region. Approximately 13% (i.e., 331) of the sites used, were parsimony informative, with the 18S rDNA accounting for 34% (i.e., 111 sites) of these sites, whereas the *rps*4 gene and the *trn*L-*trn*F region carried 149 (i.e., 45% of all informative sites) and 71 (i.e., 21%) informative sites, respectively. Although indels per se were not examined for their phylogenetic signal, all members of the Funariaceae except *Goniomitrium acuminatum* shared the absence of a codon in the *rps*4 gene. The distribution of the tree length of 50,000 randomly generated trees from the data used here, was significantly left-skewed ($p < 0.01$), suggesting that the data set appears more structured than what would be expected from a random set of data, and thus that the data may carry the signal necessary for resolving the

phylogenetic history of the taxa (Hillis & Huelsenbeck 1992). The partition homogeneity test indicates that the chloroplast and nuclear data partitions are incongruent ($p < 0.01$). That is, the chloroplast and nuclear sequences appear to possess conflicting historical signals based on the assumptions implicit in the use of the parsimony criterion and the options invoked during the tree search procedure.

Phylogenetic analyses.—All analyses consistently resolve the Funariaceae and Funariineae as polyphyletic lineages. *Goniomitrium acuminatum,* which lacks the codon loss in the *rps*4 shared by all other Funariaceae, appears nested within the Haplolepideae. The Ephemeraceae and Splachnobryaceae are also resolved within the Haplolepideae. The phylogenetic position of the Splachnobryaceae is determined primarily by chloroplast data, as analyses of 18S rDNA sequence data result in it being nested within the Funariaceae. However, *Splachnobryum* does possess the codon that is absent from the other members of the Funariaceae. Enforcing the monophyly of either the Funariaceae or the Funariineae *sensu* Vitt (1984) using the 18S sequences results in topologies whose length is significantly worse ($p < 0.001$).

Phylogenetic inferences from a combined data set resulted in two most parsimonious hypotheses (tree length = 1,363, CI with autapomorphic characters excluded = 0.441, RC = 0.384; Fig. 1). In both trees the Timmiaceae are resolved as the sister-group to the remaining ingroup taxa. The Archidiaceae are consistently nested within the Haplolepideae. The Orthotrichales, Splachnales (including Meesiaceae), and Bartramiales form a monophyletic group characterized by a bootstrap percentage (BP) of 86 and a decay index (DI) of three. In the sister clade to this diplolepideous alternate clade, the Haplolepideae share a common ancestor with a lineage comprising the Gigaspermaceae, Encalyptineae, and the Funariineae. Within the latter clade the Funariaceae appear more closely related to the Encalyptaceae than to the Gigaspermaceae. None of the branches defining the relationships among these major lineages is defined by BP higher than 50, and the DI vary between one and two. Considering the possible heterogeneity of the signal carried by both partitions (nuclear and chloroplast data), separate analyses were performed. When using the 18S rDNA sequences alone, at least 5,000 equally most parsimonious trees (tree length = 414, CI without autapomorphies = 0.496, and RC = 0.502) were obtained (result not shown). The Funariaceae (excl. *Goniomitrium acuminatum*) were resolved sister to clade composed of all other Arthrodonteae, wherein the Timmiaceae were resolved as the basal-most lineage (see also Cox et al. 2000; Newton et al. 2000). Within the large clade, neither the Encalyptaceae, the Gigaspermaceae, nor the Haplolepideae was defined as a monophyletic taxon, and the Orthotrichaceae were nested within the Haplolepideae. Similarly, the genera *Aphanorrhegma* (Funariaceae) and *Lorentziella* (Gigaspermaceae) were resolved as para- or polyphyletic.

The analysis of the chloroplast data yielded two most parsimonious trees (Fig. 2), of length 889, characterized by a consistency index (CI) of 0.56 (0.46 when autapomorphic characters are removed) and a rescaled consistency index (RC) of 0.39. Here the Haplolepideae, Funariaceae (excl. *Goniomitrium acuminatum*), and the Gigaspermaceae are monophyletic. Furthermore, these taxa compose a natural group that is sister to the Timmiaceae. This inclusive clade shares a common ancestor with the remaining diplolepideous mosses. This topology is retained upon the exclusion of the third codon position of the *rps*4 gene, or the inclusion of *Discelium* in the analysis. In the resulting single MPT in the latter analysis (tree length = 909, CI = 0.56, RC = 0.38) *Discelium* is nested between the Gigaspermaceae and the Funariaceae-Encalyptaceae clade (Fig. 2). Most basal internodes remain defined by low BT and DI values.

Discussion

The Funariineae are primarily defined by vegetative characters, because many taxa (genera or even families) are gymnostomous or characterized by otherwise reduced peristomes. Such a systematic concept of the Funariineae does not withstand phylogenetic testing. Neither nuclear nor chloroplast data, whether analyzed in combination or separately, support a monophyletic Funariineae as traditionally defined (e.g., Vitt 1984). Moreover, the monophyly of these taxa is even strongly rejected by the data presented here. The Ephemeraceae, a small, but widespread family of minute ephemeral mosses, are consistently resolved within the Haplolepideae. The lack of a peristome and the lack of strong gametophytic differentiation of the Ephemeraceae preclude reciprocal testing of this hypothesis using morphological data at present. A placement within the Pottiineae, and maybe even within the Pottiaceae, is certainly a viable hypothesis, considering that the Pottiaceae include many taxa having undergone severe morphological reduction (see Zander 1993). The affinities of the Ephemeraceae within the Haplolepideae are currently being further examined and preliminary results, based on *rps*4 data for species of *Micromitrium* corroborate a relationship of the Ephemeraceae with the Pottiaceae (Goffinet et al., unpubl.). *Goniomitrium acuminatum,* traditionally included in the Funariaceae (Fife

TABLE 1. Taxa for which the 18S rRNA and *rps*4 genes and the *trn*L-*trn*F region have been included in the analyses (all vouchers for which sequences were generated during the course of this study are deposited in DUKE unless otherwise noted; * refers to collections represented by axenic cultures maintained by Michael Christianson, University of Kansas).

Taxon	Voucher or reference	GenBank accession number (18S/*rps*4/*trn*L-*trn*F)
BUXBAUMIINEAE		
DIPHYSCIACEAE		
Diphyscium foliosum (Hedw.) Mohr	*Goffinet 4492*	AF230415/AF223034/AF229891
Theriotia lorifolia Card.	*Mitzutani 13257*	AF223007/AF223036/AF229892
FUNARIINEAE		
DISCELIACEAE		
Discelium nudum (Dicks.) Brid.	*Smith 47503* (NYSM)	—/AF223063/AF229920
FUNARIACEAE		
Aphanorrhegma serratum (Hook. f. & Wils.) Sull. 1	*Goffinet s.n.*	AF223018/AF223047/AF229904
Aphanorrhegma serratum (Hook. f. & Wils.) Sull. 2		AF223019/AF223048/AF229905
Entosthodon bonplandii (Hook.) Mitt.	*Goffinet 6326*	AF223013/AF223042/AF229899
Entosthodon laevus (Mitt.) Fife	*Goffinet 5601*	AF223014/AF223043/AF229900
Funaria apophysata (Taylor) Broth.	*Vitt 27234*	AF223012/AF223041/AF229898
Funaria hygrometrica Hedw.	Cox & Hedderson (1999)	X74114/AF023776/AF03716
Goniomitrium acuminatum Hook. & Wils.	*Streimann 48855* (H)	AF223028/AF223057/AF229914
Physcomitrella patens (Hedw.) Bruch & Schimp.	*Whitehouse s.n.*	AF223015/AF223044/AF229901
Physcomitrium lorentzii C. Müll.	*Goffinet 5348*	AF223017/AF223046/AF229903
Physcomitrium pyriforme (Hedw.) Hampe	*Goffinet s.n.*	AF223016/AF223045/AF229902
EPHEMERACEAE		
Ephemerum spinulosum Schimp.	*Goffinet 4524*	AF223026/AF223055/AF229912
GIGASPERMACEAE		
Chamaebryum pottioides Thér. & Dix.	*Oliver, Tölken & Venter 575*	AF223022/AF223051/AF229908
Gigaspermum repens (Hook.) Lindb. 1	*Schofield 90527*	AF223020/AF223049/AF229906
Gigaspermum repens (Hook.) Lindb. 2	*McGill**	AF223021/AF223050/AF229907
Lorentziella imbricata (Mitt.) Broth. 1	*Schinini 24785* (NY)	AF223023/AF223052/AF229909
Lorentziella imbricata (Mitt.) Broth. 2	*Rushing* (voucher 822)*	AF223024/AF223053/AF229910
ENCALYPTINEAE		
ENCALYPTACEAE		
Bryobrittonia longipes (Williams) Horton	Cox & Hedderson (1999)	AF023679/AF023778/AF023718
Encalypta armata Dusén	*Goffinet 5613*	AF223010/AF223039/AF229896
Encalypta ciliata Hedw.	*Schofield 98872*	AF223011/AF223040/AF229897
Encalypta rhaocarpa Schwaegr.	Cox & Hedderson (1999)	AF023680/AF023777/AF023717
ORTHOTRICHINEAE		
ORTHOTRICHACEAE		
Drummondia obtusifolia Mitt.	*Goffinet 5586*	AF223009/AF223038/AF229895
Orthotrichum lyellii Hook. & Tayl.	Cox & Hedderson (1999)	AF025291/AF023814/AF023727
Zygodon intermedius Bruch & Schimp.	*Goffinet 4580*	AF223032/AF223061/AF229918

TABLE 1. Continued.

Taxon	Voucher or reference	GenBank accession number (18S/*rps*4/*trn*L-*trn*F)
SPLACHNINEAE		
SPLACHNACEAE		
Splachnum sphaericum Hedw.	*Goward 95–1470* (UBC)	AF223030/AF223059/AF229916
Tayloria scabriseta (Hook.) Mitt.	*Churchill 15606*	AF223031/AF223060/AF229917
SPLACHNOBRYACEAE		
Splachnobryum obtusum (Brid.) C. Müll.	*Buck 29822* (NY)	AF223029/AF223058/AF229915
BRYINEAE		
BARTRAMIACEAE		
Bartramia stricta Hedw.	Cox & Hedderson (1999)	AF023698/AF023799/AF023756
BRYACEAE		
Leptobryum pyriforme (Hedw.) Wils.	Cox & Hedderson (1999)	X80980/AF023702/AF023736
MEESIACEAE		
Amblyodon dealbatus (Hedw.) Bruch & Schimp.	*Schofield 89289*	AF223033/AF223062/AF229919
TIMMIACEAE		
Timmia austriaca Hedw.	*Schofield 98363*	AF223006/AF223035/AF229892
Timmia siberica Lind. & Arnell	Cox & Hedderson (1999)	AF023678/AF023775/AF023715
FISSIDENTINEAE		
FISSIDENTACEAE		
Fissidens subbasilaris Hedw.	*Goffinet 5263*	AF223027/AF223056/AF229913
DICRANINEAE		
BRYOXIPHIACEAE		
Bryoxiphium norvegicum (Brid.) Mitt.	*Schofield 102331*	AF223008/AF223037/AF229894
ARCHIDIINEAE		
ARCHIDIACEAE		
Archidium donnellii Aust.	*Risk 1536*	AF223025/AF223054/AF229911
POTTIINEAE		
POTTIACEAE		
Tortula ruralis (Hedw.) G.M.S.	Cox & Hedderson (1999)	AF023682/AF023831/AF023722
GRIMMIINEAE		
GRIMMIACEAE		
Ptychomitrium gardneri Lesq.	Cox & Hedderson (1999)	AF023689/AF023831/AF023722
Scouleria aquatica Hook.	Cox & Hedderson (1999)	AF023684/AF023780/AF023723

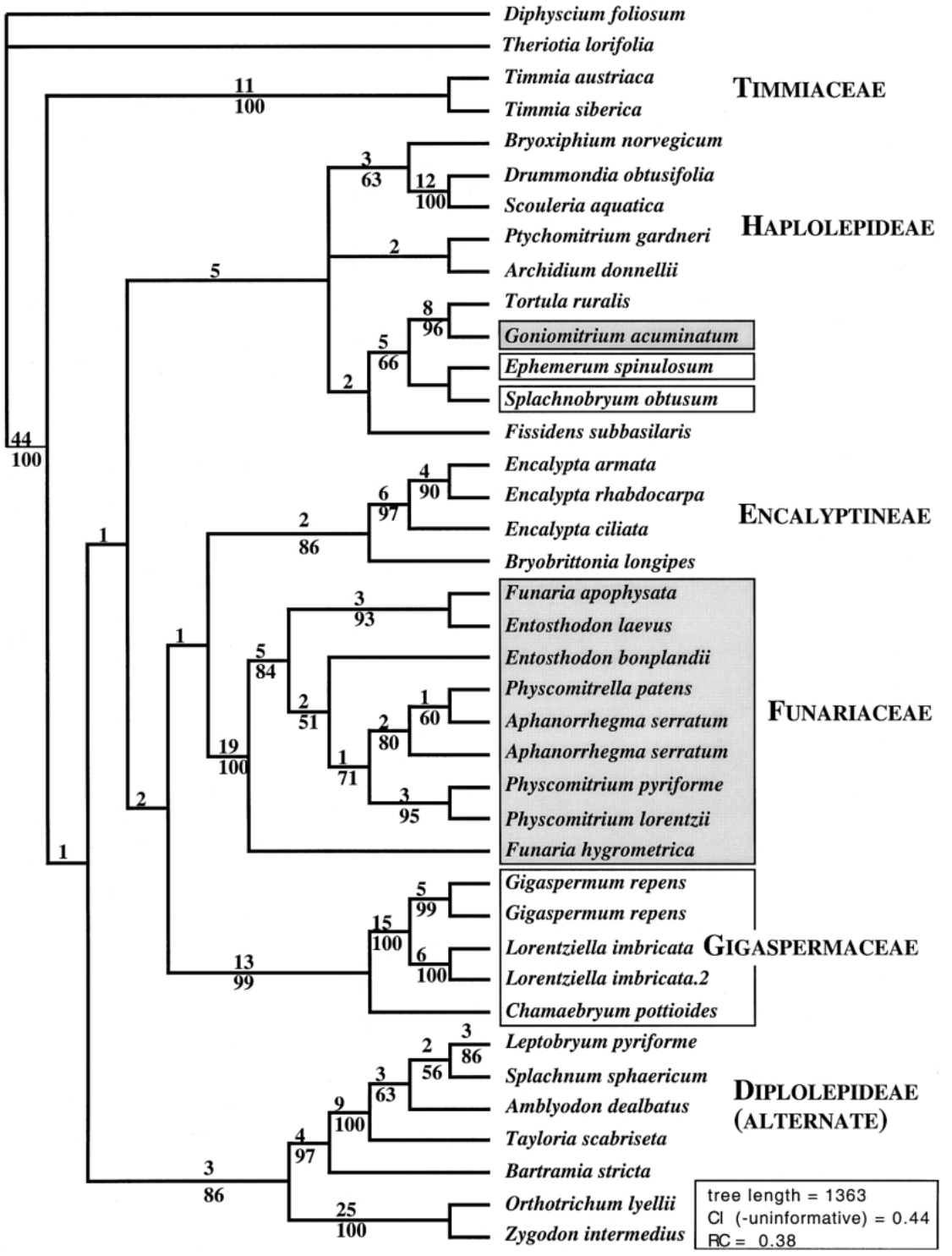

FIGURE 1. Strict consensus of two equally most parsimonious trees obtained from analyzing the nuclear and chloroplast data combined. Bootstrap percentages (> 50%) are presented below the branches and decay indices above. Boxed taxa belonged to the Funariineae *sensu* Vitt, and the shaded boxes further more composed the Funariaceae *sensu* Vitt (1984).

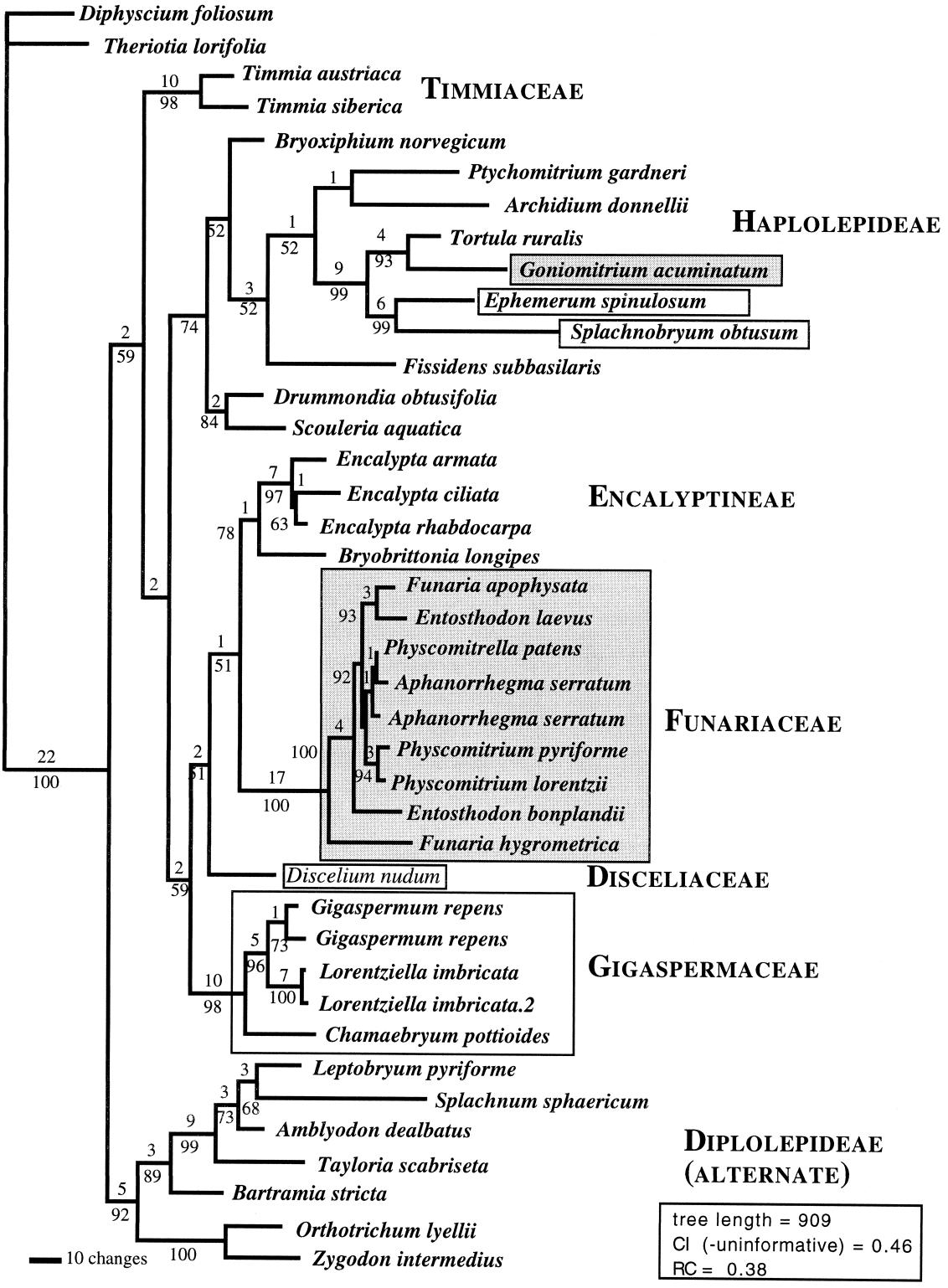

FIGURE 2. Phylogram of single most parsimonious trees obtained from analyzing chloroplast data (*rps*4 and *trn*L-*trn*F region) independently for a taxon sample that includes *Discelium*. Bootstrap percentages (> 50%) are presented below the branches and decay indices above. Boxed taxa belonged to the Funariineae *sensu* Vitt, and the shaded boxes further more composed the Funariaceae *sensu* Vitt (1984).

1985) and considered with strong affinities to *Funaria* (Stone 1981), is also shown to be more closely related to the Haplolepideae and to *Tortula* (Pottiaceae) in particular (BT = 96 and DI = 8). The genus *Goniomitrium* comprises five species, and is defined by an overall reduce morphology (Fife 1985). The phylogenetic affinities of *Goniomitrium* within the Pottiaceae is beyond the scope of this study and will be examined critically elsewhere.

The phylogenetic relationships of *Splachnobryum,* a pantropical genus also composed of minute mosses, have remained controversial. Robinson (1971) and Crum and Anderson (1981) among others, placed the genus within the Pottiaceae, whereas Vitt (1982, 1984) retained the genus within the Splachnaceae as proposed earlier by Brotherus (1924). Koponen (1981) accommodated the genus in its own family, and argued against affinities with the Pottiaceae, on the basis of diplolepideous features of the peristome. Affinities to the Splachnaceae were rejected due to the lack of differentiated hypophysis. Instead, Koponen proposed to transfer the Splachnobryaceae to the Funariineae. As mentioned above, the Funariineae have been defined mainly by vegetative characters. Many of these characters are, however, considered plesiomorphic in mosses (Crosby 1980; Vitt 1982), and could be reacquired following reduction (e.g., loss of papillae results in smooth cells). The most parsimonious inferences from nuclear sequences are congruent with Koponen's hypothesis, even though the genus is resolved in a nested position within the Funariaceae. Such paraphyly of the Funariaceae seems, however, incompatible with *Splachnobryum* lacking the loss of a codon within its *rps*4 sequence, a character otherwise shared by all other Funariaceae sampled (except *Goniomitrium acuminatum,* see above). In contrast, phylogenetic inferences from chloroplast data suggest that *Splachnobryum* is allied to the Haplolepideae. Allen and Pursell (2000) recently re-examined the peristome of *Splachnobryum* and argued for a haplolepideous rather than diplolepideous architecture, an interpretation congruent with the chloroplast-based phylogenetic hypothesis presented here.

Phylogenetic inferences regarding the early diversification of Arthrodontous mosses based on nuclear (18S rDNA) and chloroplast (*rps*4 and *trn*L-*trn*F) sequences separately yielded incongruent results. However, neither the nuclear nor the chloroplast data yielded topologies with internal branches well supported by bootstrap values or decay indices. The nuclear data resolved many well established taxa as polyphyletic (e.g., the Haplolepideae were polyphyletic and included the Orthotrichaceae; the Encalyptaceae were paraphyletic; and so were Aphanorrhegma and Lorentziella based on two conspecific samples) whereas the chloroplast data consistently resolved at least the peristomate taxa in major groups in accordance with traditional peristome-based systematic concepts (e.g., Funarineae, Dicranineae). We will therefore discuss primarily the topologies inferred from chloroplast data.

In the analyses of the combined data or the chloroplast data alone the Encalyptaceae form a sister group to the Funariaceae. The peristome of *Funaria hygrometrica* is composed of two rows, with the endostome segments positioned opposite the exostome teeth. The segments are raised on a high basal membrane formed from an IPL that is composed of 32 identical cells as a result of perfectly symmetric divisions (Shaw et al. 1989*a*). The peristome of the Encalyptaceae varies in architecture (Edwards 1984; Horton 1982, 1983), with regard to the number and structure of the teeth. Edwards (1984) found no evidence of sesquilepideae (i.e., a 2:3 cell pattern between the PPL and IPL characteristic of the Dicranum-type peristome). The endostome segments never bear a vertical line on their outer surface, and are always facing the exostome teeth, as in the Funaria-type peristome. Some of the species have narrow intermediate teeth that alternate with the segments. This situation is not homologous to that found in the Bryum-type peristome, since, in the latter, the segments bear remnants of the longitudinal anticlinal wall of the PPL on the outer surface (Edwards 1984). This arrangement is therefore only analogous to that of the Bryales *sensu lato.* Developmental studies further indicate that the divisions in the IPL are also aligned with the plane of the adjacent PPL wall, although the timing may be different from that observed in *Funaria* (Shaw, pers. comm.). The sister relationship between the Encalyptineae and the Funariineae, as suggested by chloroplast data, is consequently not incongruent with the sporophytic features. This phylogenetic hypothesis is, although the most parsimonious, poorly supported and in conflict with analyses based on broader character sampling (see Cox et al. 2000; Newton et al. 2000). The ancestor to this group may be mostclosely related to the Disceliaceae (Fig. 2). The peristome of *Discelium,* albeit reduced (Shaw & Allen 1985), is consistent with defining this broad clade by an opposite peristome. Furthermore, anatomical studies of immature capsules reveal that the anticlinal walls of the IPL are all perfectly aligned with those of the PPL, suggesting that the divisions are symmetric (Shaw & Allen 1985).

The Gigaspermaceae are resolved as the sistergroup to this broad diplolepideous-opposite clade. Its members are, however, consistently gymnostomous, and therefore offer no information for recon-

structing ancestral peristome types. The development of the sporophyte in the Gigaspermaceae has been studied by Rushing and Snider (1980). The ontogenetic patterns of cell divisions within the amphithecium of *Lorentziella imbricata*, a species included here, does not follow that of the fundamental square as in other mosses, and the divisions within the layers are not synchronized. Rushing and Snider (1980) do not specifically address each set of divisions, but from their figure 7, within which three of the eight IPL have undergone an anticlinal division, it appears that the new walls are laid down in the same plane as the adjacent PPL walls. The divisions can thus be characterized as symmetric. This mode of IPL cell division may serve as a synapomorphy for the clade comprising the Gigaspermaceae, Encalyptineae, and Funariineae.

Inferences from chloroplast data suggest that the sister-group to this Funarialean-Encalyptalean clade is the Haplolepideae; a hypothesis retained upon analysis of the combined data set, but in conflict with the analyses by Cox et al. (2000) and Newton et al. (2000). The peristome of the Haplolepideae is typically single, and composed of the endostome only. When the exostome is present, the teeth are always poorly developed and adherent to the endostomial segments, and clearly positioned opposite to the latter. That this observation is not an artifact can be established by the position of the teeth with regard to the anticlinal walls of the PPL. The teeth are indeed always formed between two consecutive anticlinal walls of the PPL (Shaw et al. 1989*b*), as in the Funaria-type peristome and can thus only lie opposite the exostomial teeth (Vitt et al. 1998). The clade composed of the Funariineae, Encalyptineae, and the Haplolepideae can therefore be characterized by its opposite peristomes. Unlike in the Funariineae, the development of the haplolepideous peristome proceeds through a stage characterized by an asymmetric division. Whether the asymmetric division in the ILP should be considered plesiotypic compared to the symmetric one of the Funariineae and Encalyptineae is not clear.

The Timmiaceae and a clade composed of the Orthotrichineae and Bryineae *sensu lato* form a grade at the base of the Arthrodonteae (Figs. 1–2). The development of the timmiaceous peristome has not been studied, except for the latest stages (Murphy 1988). Prior to the deposition of the wall material, anticlinal walls of IPL adjacent to those of the PPL, appear in some cases well aligned, whereas in others, they are not (Murphy 1988). The pattern deviates from the perfect alignment of all walls in well-developed peristomes of the Funariineae (Schwartz 1994, see also Goffinet et al. 1999), but is similar to that observed in the Splachnineae and the Orthotrichineae. Partial asymmetry may represent an ancestral type of division from which more severe asymmetries have evolved (i.e., those typical of the Haplolepideae and the Bryales *sensu lato*; Shaw et al. 1989*a*,*b*). Weak asymmetries occur also in *Tetraphis* (Goffinet et al. 1999; Shaw & Anderson 1988), whereas in *Diphyscium*, they are more pronounced (Shaw et al. 1987). If indeed the divisions in *Timmia* are asymmetric, then the symmetric division represents a synapomorphy for the Encalyptineae and Funariineae.

The peristome of the Timmiaceae is unique among diplolepideous mosses in the architecture of the endostome. This inner row is composed of a high basal membrane supporting 64 filamentous appendages, arranged into groups of four that are opposite the exostome teeth at maturity. As in other opposite peristomes, these appendages lack any anticlinal wall remnants of the PPL on their outer surface (Murphy 1988; Shaw & Rohrer 1984). Whether the opposite arrangement of the peristomes is an apomorphy or a plesiomorphy for the clade comprising the Funariineae, Encalyptineae, Haplolepideae, and Timmiaceae (as presented in Fig. 1) remains unclear since the endostome of *Diphyscium* (the *Theriotia* peristome was not examined) consists of a pleated membrane that lacks individual segments. The exostome of *Diphyscium* is composed of 16 teeth separated by 16 intermediate teeth. The latter are characterized by a median vertical wall on their inner surface, and are fused to the outer pleats of the endostome (Edwards 1984). The true teeth thus alternate with the pleats. A basal membrane of the endostome is particularly pleated in taxa with alternate peristomes (see Shaw et al. 1989*a*), but also albeit less so, in lineages with opposite peristomes (Edwards 1984; Schwartz 1994). The pleats themselves are thus not an indication of the arrangement of the peristomes. Consequently, the lack of differentiated segments in *Diphyscium* precludes from determining its ''putative'' arrangement. Inclusion of the nuclear data in the analysis (or analyzing the nuclear data alone) resolves *Timmia* sister to a dichotomy between lineages with either opposite or alternate peristome, suggesting that the latter peristome configuration is derived.

The typical Bryum-type peristome bears cilia between the segments. The occurrence of cilia is seemingly correlated to additional divisions in the IPL, leading to at least 48 cells composing the IPL. In the Orthotrichaceae, cilia are always lacking and the IPL is composed of 32 cells at most. The relationships of the Orthotrichaceae remain ambiguous (Cox & Hedderson 1999; Cox et al. 2000; Goffinet et al. 1998), but a sister-group relationship to the Bryales *sensu lato* as resolved here, rather than a nested position within the latter, cannot be ruled out. Depending on the homology assumption made

for the appendages of the Timmiaceae, cilia could, based on the chloroplast based phylogeny have arisen in the ancestor to either the Arthrodonteae (excluding the ''Diphysciaceae'') or to the Bryales only. The appendages present in the endostome of the Timmiaceae are monomorphic, whereas in the Bryales *sensu lato*, these are dimorphic; cilia and segments have distinct architectures (Shaw & Rohrer 1984). The appendages present in the Timmiaceae are similar to cilia in that they lack remnants of the anticlinal PPL walls on the outer surface, and in their position opposite the teeth. The endostome of the Timmiaceae is also characterized by additional divisions leading to more than 32 cells in the IPL (Murphy 1988; Shaw & Rohrer 1984), as is also typical of ciliate bryoid peristomes. It is thus possible that at least some appendages of the timmiaceous endostome are homologous to the cilia of the typical Bryum-type peristome, as suggested by Shaw and Rohrer (1984). Support for this hypothesis will depend on the phylogenetic affinities of the diplolepideous alternate mosses lacking cilia (e.g., Orthotrichales, and Splachnales; see Goffinet et al. 1999) and the interpretation of their peristome (see Cox et al. 2000).

The phylogenetic relationships among basalmost arthrodontous mosses hypothesized here based on chloroplast data are congruent with inferences made from mitochondrial sequences (Beckert et al. 1999), but are incongruent with those derived from analyses of the nuclear 18S rRNA gene (this study, and see also Cox et al. 2000; Hedderson et al. 1996, 1998; Newton et al. 2000). Whether this conflict results from ancestral hybridization or reflects the inadequacy of either set of characters for deep-level reconstructions needs to be addressed further. However, both the nuclear and the chloroplast do concur with regard to the polyphyly of the Funariineae and the Funariaceae. These results highlight again the usefulness of the molecular characters in addressing the circumscription of taxa characterized by morphological reduction (i.e., loss or reversal of characters). Our analyses reveal further that taxa lacking critical morphological features should not be omitted from phylogenetic analyses because of their limited contribution in resolving trends of morphological evolution, as these taxa, such as the Disceliaceae or the Gigaspermaceae, may be highly relevant for resolving the relationships among lineages of arthrodontous mosses.

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