Annals of Botany **87**: 191–208, 2001
doi:10.1006/anbo.2000.1318, available online at http://www.idealibrary.com on IDEAL®

The Bryophyta (Mosses): Systematic and Evolutionary Inferences from an *rps4* Gene (cpDNA) Phylogeny

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Received: 31 May 2000 Returned for revision: 12 September 2000 Accepted: 3 October 2000

Phylogenetic analyses of nucleotide and amino acid sequences of the chloroplast protein coding gene *rps4* were performed for 225 species of mosses, representing 84 % of families recognized by Vitt (1984. In: Schuster RM, ed. *New manual of bryology*, *vol 2*. Nichinan: Hattori Botanical Laboratory), under the criterion of maximum parsimony with *Takakia* and *Sphagnum* as outgroups. Most parsimonious topologies converge to a scenario wherein the Andreaeidae are monophyletic and sister to the Bryidae (peristomate mosses), the Nematodonteae and the Buxbaumiaceae form a monophyletic lineage, the Diphysciaceae are sister to the Arthrodonteae and, within the latter, the Funarineae-Encalyptineae-Timmiaceae-Haplolepideae compose a monophyletic clade sister to remaining diplolepideous mosses. This hypothesis suggests that early in the evolution of the Arthrodonteae, two major lineages diverged, with opposite and alternate peristomes, respectively. Bootstrap support for the deep dichotomies is poor or lacking but increases when protein translations of *rps*4 sequences are included in the analysis. Several novel systematic hypotheses are raised, including (*a*) a diplolepideous rather than haplolepideous origin of the Pleurophascaceae; (*b*) an affinity of the Catascopiaceae with the Funariineae rather than the Bryineae; and (*c*) a close relationship of the Calomniaceae and Mitteniaceae to the Rhizgoniaceae. The advantages and disadvantages of a single gene phylogeny are discussed with respect to the identification of polyphyletic familial or suprafamilial taxa.

Key words: Bryophyta, mosses, phylogeny, *rps4*, evolution, peristome, parsimony, sequences.

INTRODUCTION

With an estimated 13 000 species (Crosby *et al*., 1999), the Bryophyta (i.e. mosses *sensu* Buck and Goffinet, 2000) compose a lineage of land plants whose diversity is second only to that of the angiosperms. Mosses, like liverworts and hornworts, are characterized by a life cycle that is dominated by the gametophytic or haploid phase. The sporophyte remains attached to the gametophyte during its short existence, which rarely exceeds 1 year. A single sporangium is produced at the apex of an unbranched axis, the seta. Spores are dispersed either through one or more vertical slits or, as is the case for most mosses, after dehiscence of a lid, called the operculum. In operculate mosses, the mouth of the capsule is lined by one or two rows of teeth, which compose the peristome. To a large extent, the mode of dehiscence and morphological features of the peristome provide the basis for the higher level classification of mosses (e.g. Vitt, 1984). However, these characters fail to offer substantial information to address the relationships among these lineages (Vitt *et al*., 1998). Although developmental studies of the peristome have yielded characters distinctive of lineages (Shaw *et al*., 1987, 1989*a*, *b*; Shaw and Anderson, 1988), the polarity of transformations between character states (in particular for the most crucial character, namely the pattern of cell division in the innermost layer contributing to the peristome) remains ambiguous in the absence of a robust phylogenetic hypothesis (Vitt *et al*., 1998; Goffinet *et al*., 1999). Many morphological characters of the gametophyte appear autapomorphic, extensively homoplasic or plagued by reversals (see Vitt, 1984) to the extent that they had, until recently (see Newton *et al*., 2000), been abandoned for formally addressing the higher level relationships among mosses.

The only attempt to formulate a phylogenetic hypothesis for mosses using morphological data was made by Mishler and Churchill (1984). Until recently their hypothesis has remained the only explicit phylogeny proposed for major lineages of mosses. According to their scenario, the Nematodonteae, that is, mosses whose peristome is constitute of whole cells *vs*. cell wall remnants (i.e. in the Arthrodonteae), constitute a basal grade, with the Buxbaumiineae sister to the Arthrodonteae. Vitt (1984) considered the peristome of the Buxbaumiineae to integrate nematodontous and arthrodontous elements, and suggested a rather basal position of this peristome type in the evolutionary history of the arthrodontous peristome.

DNA sequence data have become increasingly integrated in bryophyte systematics in the last 5 years. Most studies have focused either on the monophyly of bryophytes and

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0305-7364/01/020191+18 $35.00/00

their position in the evolution of land plants (e.g. Waters *et al*., 1992; Hedderson *et al*., 1996) or examined the relationships within major lineages (e.g. Capesius and Stech, 1997; Goffinet *et al*., 1998; Cox and Hedderson, 1999; De Luna *et al*., 1999, 2000; Buck *et al*., 2000; Cox *et al*., 2000; Goffinet and Cox, 2000; La Farge *et al*., 2000; Shaw, 2000). Thus far, only two studies have specifically addressed the relationships among major lineages of mosses, using exemplars of most major lineages. Newton *et al*. (2000) inferred phylogenetic relationships from analysing sequences of nuclear (*18SrRNA*) and chloroplast loci (*rbc*L, *rps4*, and trnL-trnF), whereas Beckert *et al*. (1999) tested phylogenetic hypotheses using the mitochondrial gene *nad5*. Differences in the phylogenetic conclusions reached by these authors pertain primarily to the early diversification of arthrodontous mosses. Newton *et al*. (2000) suggested that (1) *Diphyscium* was not related to *Buxbaumia*, but instead was sister to all Arthrodonteae; (2) that within the latter the Funariaceae represented the earliest derivation, immediately followed by the Timmiaceae; and (3) that the Haplolepideae together with the Encalyptaceae composed a sister group to the remaining diplolepideous mosses. The latter two conclusions were also reached by Goffinet *et al*. (1998), based on *rbc*L sequences, and by Cox and Hedderson (1999) using chloroplast (trnL-trnF and *rps4*) and nuclear (*18SrRNA*) data. In contrast, Beckert *et al*. (1999) proposed that the Haplolepideae share a common ancestor with the Funariaceae and the Timmiaceae, an hypothesis congruent with inferences from chloroplast data (Goffinet and Cox, 2000).

Phylogenetic inconsistencies among these studies may reflect past hybridization events, or represent artifacts due to constraints on the molecular evolution of the loci which affect patterns of homoplasy. The effect of the latter may be dependent on the taxon sampling and outgroup selection. Goffinet and Cox (2000) pointed out that lineages of reduced taxa, such as the Gigaspermaceae or Disceliaceae, which are routinely ignored in phylogenetic studies because they lack the morphological characters that would allow inferences about the evolution of these traits, may hold critical positions in the early evolutionary history of the arthrodontous clade or some of its major lineages. Inclusion of these taxa may therefore be essential for reconstructing basal divergences. Finally, the inference of polarization of character transformations within the mosses is dependent on the nature of the outgroup chosen. Hedderson *et al*. (1996) provided molecular evidence that *Takakia* belongs to the Bryophyta (i.e. mosses), an hypothesis supported by the subsequent discovery of the sporophyte generation (Smith and Davison, 1993; Renzaglia *et al*., 1997). The studies by Capesius and Stech (1997) and Beckert *et al*. (1999) did not include *Takakia*, and inferred relationships within mosses using liverwort taxa as outgroups. Evidence from 18S rDNA (Cox, 1998; Hedderson *et al*., 1998) suggest that *Takakia* and *Sphagnum* form the sister group to the arthrodonts, whereas combined 18S rDNA and chloroplast data (Newton *et al*., 2000) suggests that *Takakia* and the Sphagnideae compose the sister group to all other mosses.

The present study was therefore designed to sample extensively across the major lineages of mosses, while focusing on a single chloroplast locus (small ribosomal protein 4 or *rps4*) in an attempt to reconstruct the phylogeny of mosses using *Takakia* and *Sphagnum* as outgroups. This study specifically aims to address the following questions: (1) does peristome architecture as defined by Vitt (1984) define monophyletic groups; and (2) do the suprageneric systematic concepts adopted by Vitt (1984) concur with phylogenetic hypotheses inferred from a single chloroplast gene?

MATERIALS AND METHODS

Taxon sampling

Vitt (1984) accommodated mosses into 92 families. Exemplars for families for which *rps4* sequences were not currently available from GenBank were sampled from the following herbaria: BUF, DUKE, H, MO, and NY. Sixty-seven new taxa were targeted for this study.

DNA extraction and PCR

Exemplars of most families of mosses were sampled from dry herbarium material or recently collected material. Sequences were generated in two laboratories, Duke University, NC, USA and the University of Reading, UK. Detailed extraction protocols, complete PCR amplification and sequencing protocols followed by these laboratories can be found in Buck *et al*. (2000), and Cox and Hedderson (1999), respectively. Translation of nucleotide sequences into amino acid sequences was done using MacClade (Maddison and Maddison, 1999).

Sequence analysis

Amplified fragments comprised the *rps4* gene and the 3′ intergenic spacer. Phylogenetic relationships were inferred under the criterion of maximum parsimony using the coding sequences only. The sequences were trimmed at the 5′ end to exclude the primer annealing region. The Takakiaceae together with the Sphagnideae were used as outgroups, albeit without constraining their monophyly. The following search strategy was applied using PAUP* 4·0b3a (Swofford, 2000): heuristic searches were replicated 200 times, allowing for ten trees to be saved for each replicate, using the nearest-neighbour interchange (NNI) swapping option, and invoking the ‘steepest descent’ and the ‘collapse branches when maximum length is equal to zero’ options. All the resulting trees were swapped extensively using the TBR swapping option with the ‘steepest descent’ option turned off, and with a maximum limit of 20 000 trees to be retained. Strict and 90 % majority-rule consensus trees were generated for each analysis. Four data sets were analysed using this approach: complete nucleotide sequences, codon positions one and two only, and each of these two sets in combination with the amino acid translation of the sequences. The fast bootstrap procedure (i.e. a bootstrap without branch swapping) was preferred over a regular bootstrap analysis due to the computational (i.e. time) requirement for the latter to reach completion. The fast bootstrap analysis was conducted with 5000 replicates and the consensus of the

resulting trees used to assess the robustness of the branches. The presence of hierarchical structure in the four data sets was assessed by examination of the g_1 statistic inferred from the length distribution of 50 000 random trees (see Hillis and Huelsenbeck, 1992). The statistical significance of tree length differences between alternative topologies was assessed using the Templeton test (Templeton, 1983) as implemented in PAUP*. Phylograms were inferred under the assumptions of accelerated transformation between character-states (ACCTRAN option).

RESULTS

Sequence characterization and variability

Sequences were obtained for 67 taxa, and submitted to GenBank (Table 1). In addition to the sequences available from GenBank, we compiled a matrix of 225 taxa. These exemplars correspond to 190 genera distributed among 86 families of mosses, representing 84 % of the families accepted by Vitt (1984) in his classification of mosses.

In all cases, amplifications yielded a single strong band that was accompanied by lighter accessory bands in some samples (Fig. 1). The latter did not, however, alter the high quality of the sequence obtained. Only a single taxon, namely *Ambuchanania leucobryoides* (Yamaguchi, Seppelt & Iwatsuki) Seppelt & Crum, yielded an amplification product whose sequence was considered unreliable and of low quality, and was thus not included. The sequence for *Pleurophascum occidentale* is partial but was retained for the analysis. The length of the product varied by about 200 bp between *Takakia*, the Sphagnales, Andreaeales, Andreaeobryales, Tetraphidales, *Oedipodium*, and the Polytrichales, *vs.* the Arthrodonteae, the latter yielding shorter fragments (Fig. 1). All arthrodontous mosses including the Buxbaumiaceae and the Diphysciaceae were characterized by a shorter fragment (±680 bp *vs.* 880 bp). The length differences were accounted for by a decrease in the size of the 3′ intergenic spacer in the Arthrodonteae. The coding sequences were easily aligned as indels were restricted to the loss of a codon (number 37) in the Funariaceae (Goffinet and Cox, 2000), and the loss of two codons (35 and 36) in *Theriotia lorifolia*, a member of the Diphysciaceae. Additional stop codons in the coding region were not detected suggesting the absence of pseudogenes within the data set. The variability and the distribution of parsimony informative characters among data partitions is presented in Table 2.

Tree statistics of the most-parsimonious trees

The limit of Most Parsimonious Trees (MPTs) to be saved was reached for each analysis. Two distinct islands were uncovered for the *rps4* + amino acid data set, and 15 000 trees were retained for each. Descriptions of the MPTs obtained for all four analyses are summarized in Table 3. Ninety percent majority-rule trees of all MPTs were constructed for each analysis and these are summarized in Figs 2 and 3. Only lineages at the family level or above that are relevant to the overall phylogeny of mosses are shown, except for novel phylogenetic hypotheses regarding specific genera or families. A phylogram of the first MPT (island 1) inferred from the full nucleotide data complemented by its protein translation is presented in Figs 4–6. Strict consensus trees were better resolved toward the base of the tree when these were inferred from nucleotide and amino acid data (Fig. 2B *vs.* A and Fig. 3B *vs.* A). The exclusion of 'hypervariable characters' (i.e. the third codon positions) resulted in a decrease in resolution in the summary trees (Figs 2A *vs.* 3A) except when protein sequences complemented the 1st and 2nd codon positions (Figs 2B *vs.* 3B), in which case, the consensus tree was better resolved but held at least one very unlikely relationship (see below). Trees generated by fast bootstrapping showed little consensus for most relationships, with most branches defining the ordinal relationships (*sensu* Vitt, 1984) being present in 73 % or less of these trees. However, bootstrap values increased slightly when amino acid sequences were incorporated into the analyses (Fig. 2B *vs.* A and Fig. 3B *vs.* A). The exclusion of the 3rd codon positions did not result in higher bootstrap values (Figs 3A *vs.* 2A and Figs 3B *vs.* 2B). Consistency indices (CI, based on informative characters only) improved upon inclusion of the amino acid data or upon the exclusion of the 3rd codon positions. The highest average CI was obtained for trees derived from analysing the variation contained in the 1st and 2nd codon positions in the presence of protein data (Table 3). Complementing the full *rps4* sequences with their translation product resulted in an increase of the average CI for characters of the 1st and 2nd codon positions (from 0·2504 to 0·2515 and from 0·2448 to 0·2560, respectively) and a decrease in average CI from 0·2276 to 0·2216 for those of the 3rd codon positions. Furthermore, optimization of amino acid character transformations on the tree inferred on *rps4* nucleotide sequences alone revealed a decrease in average CI compared to trees generated from *rps4* + protein data (i.e. a change from 0·2877 to 0·2753). The length of a tree obtained from analysing the 1st and 2nd codon positions upon which the amino acid data were superimposed was significantly worse under the Templeton test than that inferred from parsimony analysis of these three data partitions (1st and 2nd codon sites and amino acid sequences; $P < 0{\cdot}05$).

Phylogenetic relationships

Except for when the amino acid translations are analysed in combination with the full nucleotide data (Fig. 2B), the Andreaeobryaceae and the Andreaeaceae, sole families of the Andreaeidae, never compose a monophyletic lineage (Figs 2 and 3). Of the remaining three orders recognized by Vitt (1984), namely the Polytrichales, Tetraphidales and Bryales, the latter appears polyphyletic in all analyses as *Oedipodium* and *Buxbaumia*, sole members of their respective families, are consistently resolved outside the clade of arthrodontous mosses. The Oedipodiaceae appear either closely related to the Tetraphidaceae (Fig. 2A), intermediate between the Nematodonteae and the Arthrodonteae (Fig. 3B), sister to all the nematodontous mosses (Fig. 3A) or even in a position sister to all peristomate

TABLE 1. *Alphabetical list of taxa included in the present analysis. All vouchers of newly sequenced taxa (bold type-face) are deposited in the herbarium of Duke University (NC, USA) unless otherwise indicated*

Taxon	Voucher or reference	GenBank accession number
Acroporium pungens (Hedw.) Broth.	Buck *et al.* (2000)	AF143028
Adelothecium bogotense (Hampe) Mitt.	Buck *et al.* (2000)	AF143073
Amblyodon dealbatus (Hedw.) Bruch & W.P. Schimper	Goffinet & Cox (2000)	AF223062
Amphidium lapponicum (Hedw.) W.P. Schimper	***Schofield 98098***	AF222896
Anacamptodon splachnoides (Brid.) Brid.	Buck *et al.* (2000)	AF143031
Andreaea rothii Weber & D. Mohr	***Shaw s.n.***	AF306952
Andreaobryum macrosporum Steere & B.M. Murray	***Schofield 78094***	AF306953
Anomobryum julaceum (Gärtn., Meyer & Schreb.)Schimp.	Cox & Hedderson (1999)	AF023786
Anomodon rugelii (C. Müll.) Keissler	Buck *et al.* (2000)	AF143023
Aphanorrhegma serratum (W. J. Hooker & Wilson) Sull.	Goffinet & Cox (2000)	AF223047
Aplodon wormskioldii (Hornemann) R. Brown	***Belland 11024***	AF306964
Archidium alternifolium (Hedw.) Mitt.	***Mishler 3752***	AF306982
Archidium donnellii Aust.	***Risk 1536***	AF223054
Archidium tenerrimum Mitt.	***Risk & Kiser 5050***	AF306981
Atrichum angustatum (Brid.) Buch & Schimp.	***Hedderson 10393*** **(RNG)**	AF265356
Aulacomium androgynum (Hedw.) Schwägr.	Cox & Hedderson (1999)	AF023811
Aulacomium turgidum (Wahl.) Schwägr.	Cox & Hedderson (1999)	AF023809
Aulacopilum hodgkinsoniae (Hampe & C. Müll.) Broth.	***Vitt 28261*** **(ALTA)**	AF222897
Barbula unquiculata Hedw.	***Zander 1975*** **(BUF)**	AF306986
Bartramia halleriana Hedw.	***Akeryod et al. 4491*** **(RNG)**	AF265358
Bartramia stricta Brid.	Cox & Hedderson (1999)	AF023799
Bescherellia cryphaeoides (Müll. Hal.) M. Fleisch.	Buck *et al.* (2000)	AF143081
Blindia acuta (Hedw.) Br. Eur.	Cox & Hedderson (1999)	AF023781
Brachylema subulatum (P. de Beauv.) Card.	***Allen*** **Fontinalaceae Exc. 86**	AF306998
Brachymenium pulchrum Hook.	Cox & Hedderson (1999)	AF023788
Brachythecium austro-glareosum (Müll.) Paris	Buck *et al.* (2000)	AF143026
Brachythecium oxycladon (Brid.) Jäg.	Buck *et al.* (2000)	AF143025
Brachythecium plumosum (Hedw.) Schimp.	Buck *et al.* (2000)	AF143078
Brachythecium rutabulum (Hedw.) Br. Eur.	Cox & Hedderson (1999)	AF023818
Brachythecium salebrosum (Web. & Mohr.) Schimp.	Buck *et al.* (2000)	AF158176
Braithwaitea sulcata (Hook.) Jäg.	Cox & Hedderson (1999)	AF023820
Breutelia scoparia (Schwägr.) Jäg.	Buck *et al.* (2000)	AF143075
Bryhnia novae-angliae (Sull.) Grout	Buck *et al.* (2000)	AF143029
Bryobritonia longipes (Will.) Horton	Cox & Hedderson (1999)	AF023778
Bryoxiphium norvegicum (Brid.) Mitt.	Goffinet & Cox (2000)	AF223037
Bryum alpinum With.	Cox & Hedderson (1999)	AF023783
Bryum caespiticium Hedw.	Cox & Hedderson (1999)	AF023784
Bryum donianum Grev.	Cox & Hedderson (1999)	AF023785
Bryum stenotrichum C. M.	Cox & Hedderson (1999)	AF023787
Buxbaumia aphylla Hedw.	***Belland 16889***	AF306959
Calomnion complanatum (Hook. & Wils.) Lindb.	***Streimann 58086*** **(NY)**	AF307000
Campylium chrysophylum (Brid.) Lange	Buck *et al.* (2000)	AF143048
Catagonium nitens (Brid.) Card.	***Goffinet 5459***	AF307001
Catascopium nigritum (Hedw.) Brid.	***Longton 4592*** **(RNG)**	AF307003
Chamaebryum pottioides Thér. & Dixon	Goffinet & Cox (2000)	AF223051
Cinclidium stygium Swartz	Cox & Hedderson (1999)	AF023791
Clasmatodon parvulus (Hampe) Sull.	Buck *et al.* (2000)	AF143032
Climacium americanum Brid.	Buck *et al.* (2000)	AF143065
Conostomum tetragonum (Hedw.) Lindb.	***Goffinet 5755***	AF306990
Crossomitrium rotundifolium Herz.	Buck *et al.* (2000)	AF143070
Cryphaea glomerata Sull.	Buck *et al.* (2000)	AF143007
Ctenidium malacodes Mitt.	Buck *et al.* (2000)	AF143036
Curvirameum mexicanum (Thér.) Crum	Buck *et al.* (2000)	AF143062
Cyrtomnium hymenophyllum (Bruch & Schimp.) Holmen	Cox & Hedderson (1999)	AF023792
Dawsonia papuana Schliephacke & Geheeb	Hyvönen *et al.* (1998)	AF208419
Dendroligotrichum dendroides (Hedw.) Brother.	***Goffinet 5425***	AF306957
Dendroligotrichum squamosum (Hook. & Wils.) Card.	***Goffinet 5878***	AF306958
Diphyscium foliosum (Hedw.) Mohr	Goffinet & Cox (2000)	AF223034
Discelium nudum (Dicks.) Brid.	Goffinet & Cox (2000)	AF223063
Ditrichum pallidum (Hedw.) Hampe	***Nelson 13749***	AF306979
Drummondia obtusifolia C. Müll.	Goffinet & Cox (2000)	AF223038
Drummondia prorepens (Hedw.) Britton	***Vitt 26711*** **(ALTA)**	AF306977
Echinodium umbrosum (Mitt.) Jäg.	Buck *et al.* (2000)	AF143044
Encalypta armata Dusén	Goffinet & Cox (2000)	AF223039
Encalypta ciliata Hedw.	Goffinet & Cox (2000)	AF223040

Table 1 continued over page

TABLE 1. *Continued*

Taxon	Voucher or reference	GenBank accession number
Encalypta rhabdocarpa Schwägr.	Cox & Hedderson (1999)	AF023777
Enthostodon drummondii Sull.	***Shaw s.n.***	AF306961
Enthostodon laevis (Mitten) Fife	Goffinet & Cox (2000)	AF223043
Entodon brevisetus (Wils.) Lindb.	Buck *et al.* (2000)	AF143057
Entodontopsis leucostega (Brid.) Buck & Irel.	Buck *et al.* (2000)	AF143060
Ephemerum spinulosum Schimp.	Goffinet & Cox (2000)	AF223055
Fissidens bushii (Card. & Thér.) Card. & Thér.	***Goffinet 4526***	AF306988
Fissidens subbasilaris Hedw.	Goffinet & Cox (2000)	AF223056
Fontinalis antipyretica Hedw.	Cox & Hedderson (1999)	AF023817
Fontinalis dalicarlica Bruch & Schimp.	Buck *et al.* (2000)	AF143064
Forsstroemia trichomitria (Hedw.) Lindb.	Buck *et al.* (2000)	AF143006
Funaria hygrometrica Hedw.	Cox & Hedderson (1999)	AF023776
Garovaglia elegans (Dozy & Molk.) Bosch & Lac.	Buck *et al.* (2000)	AF143017
Gigaspermum repens (Hook.) Lindb.	Goffinet & Cox (2000)	AF223049
Glyphothecium sciuroides (Hook.) Hampe	Buck *et al.* (2000)	AF143016
Goniobryum subbasilare (Hook.) Lindb.	Cox & Hedderson (1999)	AF023824
Goniomitrium acuminatum Hook. & Wils.	Goffinet & Cox (2000)	AF223057
Grimmia pulvinata (Hedw.) Smith	***Christy 21771***	AF222900
Haplocladium virginianum (Brid.) Broth.	Buck *et al.* (2000)	AF143040
Haplohymenium triste (De Not.) Kindb.	Buck *et al.* (2000)	AF143022
Hedwigia ciliata (Hedw.) P. de Beauv.	***Belland 17819***	AF306997
Helicodontium capillare (Hedw.) Jaegr.	Buck *et al.* (2000)	AF143043
Helicophyllum torquatum (W.J. Hooker) Brid.	***Newton 4813*** **(herb. Newton)**	AF265357
Henicodium geniculatum (Mitt.) W.R. Buck	Buck *et al.* (2000)	AF143011
Hildebrandtiella pachyclada Besch.	Cox & Hedderson (1999)	AF023829
Homalotheciella subcapillata (Hedw.) Broth.	Buck *et al.* (2000)	AF143061
Hookeria acutifolia Hook. & Grev.	Buck *et al.* (2000)	AF143071
Hygroamblystegium tenax (Hedw.) C. Jensen	Buck *et al.* (2000)	AF143047
Hypnodendron camptotheca (Par.) Touw	Cox & Hedderson (1999)	AF023821
Hypnodendron dendroides (Brid.) Touw	Cox & Hedderson (1999)	AF023822
Hypnum imponens Hedw.	Buck *et al.* (2000)	AF143034
Hypnum lindbergii Mitt.	Buck *et al.* (2000)	AF143035
Hypopterygium tamarisci (Sw.) Müll. Hal.	Buck *et al.* (2000)	AF143077
Isopterygium tenerum (Hedw.) Sw.	Buck *et al.* (2000)	AF143037
Itatiella ulei (C. Müll.) G.L. Smith	Hyvönen *et al.* (1998)	AF208421
Lembophyllum divulsum (Hook. & f. Wils.) Lindb.	Buck *et al.* (2000)	AF143045
Lepidopilum scabrisetum (Schwägr.) Steere	Buck *et al.* (2000)	AF143066
Lepidopilum surinamense Müll. Hal.	Buck *et al.* (2000)	AF143067
Leptobryum pyriforme (Hedw.) Wils.	Cox & Hedderson (1999)	AF023802
Leptobryum stellatum (Herz.) Broth.	***Lewis 87-1222-d6***	AF306991
Leptobryum wilsonii (Mitt.) Broth.	***Goffinet 5608***	AF306992
Leptostomum macrocarpum (Hedw.) R. Br.	Cox & Hedderson (1999)	AF023790
Leptotheca boliviana Herz.	Cox & Hedderson (1999)	AF023816
Leptotheca gaudichaudii Schwägr.	Cox & Hedderson (1999)	AF023823
Lepyrodon pseudolagurus Allen	Buck *et al.* (2000)	AF143014
Leskea gracilescens Hedw.	Buck *et al.* (2000)	AF143042
Leskeodon cubensis (Mitt.) Thér.	Buck *et al.* (2000)	AF143072
Leucodon andrewsianum (Crum & Anders.) Reese	Buck *et al.* (2000)	AF143005
Leucodon brachypus Brid.	Buck *et al.* (2000)	AF143004
Leucoloma sp. nov.	***La Farge 5555*** **(ALTA)**	AF307005
Leucomium strumosum (Hornsch.) Mitt.	Buck *et al.* (2000)	AF143068
Loeskeobryum brevirostre (Brid.) Broth.	Buck *et al.* (2000)	AF143079
Lorentziella imbricata (Mitt.) Broth.	Goffinet & Cox (2000)	AF223052
Macromitrium richardii Schwägr.	***Goffinet 2648*** **(ALTA)**	AF306975
Macromitrium levatum Mitt.	Cox & Hedderson (1999)	AF023813
Meesia triquetra (Richter) Ångström	***Schofield 99251A***	AF306994
Meesia uliginosa Hedw.	***Schofield 93204***	AF306995
Meiotrichum lyallii (Mitt.) G.L.S. Merrill	Hyvönen *et al.* (1998)	AF208423
Mielichhoferia bryoides (Harv.) Wijk & Marg.	Cox & Hedderson (1999)	AF023794
Mielichhoferia elongata (Hook.) Hornsch.	Cox & Hedderson (1999)	AF023793
Mielichhoferia macrocarpa (Hook.) Bruch & Schimp.	*Hedderson 5487* (RNG)	AJ251311
Mittenia plumosa (Mitt.) Lindb.	***Streimann 5/12/96***	AF306999
Mitthyridium constrictum (Sull.) Robins.	***Withey 560***	AF306987
Mnium hornum Hedw.	Cox & Hedderson (1999)	AF023796
Muscoflorschuetzia pilmaiquen (Crosby) Crosby	**Isotype (H)**	AF306960
Myurium hochstetteri (Schimp.) Kindb.	Buck *et al.* (2000)	AF143018

Table 1 continued over page

TABLE 1. *Continued*

Taxon	Voucher or reference	GenBank accession number
Neckera pennata Hedw.	Buck *et al.* (2000)	AF143008
Neckeropsis disticha (Hewd.) Kindb.	Buck *et al.* (2000)	AF143010
Neomeesia palludella (Besch.) Deguchi	***Goffinet 5862***	AF306993
Neorutenbergia usagarae (Dix.) Bizot & Pócs	Buck *et al.* (2000)	AF143019
Oedipodium griffithianum (Dicks.) Schwägr.	***Schofield 98670***	AF306968
Orthodontium lineare Schwägr.	Cox & Hedderson (1999)	AF023800
Orthostichopsis tetragona (Hedw.) Broth.	Buck *et al.* (2000)	AF143012
Orthotrichum alpestre B.S.G.	**Vitt Orthotrichaceae Exc. 47**	AF306971
Orthotrichum anomalum Hedw.	**Vitt Orthotrichaceae Exc. 43**	AF306973
Orthotrichum lyellii Hook. & Tayl.	Cox & Hedderson (1999)	AF023814
Orthotrichum obtusifolium Brid.	***Schofield 98451***	AF306969
Paludella squarrosa (Hedw.) Brid	***Vitt 34205***	AF306996
Papillaria nigrescens (Hedw.) Jägr.	Buck *et al.* (2000)	AF143051
Pentastichella penstatichum (Mont.) Thér.	***Matteri 6527***	AF306973
Philonotis fontana (Hedw.) Brid.	Cox & Hedderson (1999)	AF023801
Phyllodrepanium falcifolium (Schwägr.) Crosby	Buck *et al.* (2000)	AF143074
Phyllogonium viride Brid.	Buck *et al.* (2000)	AF143020
Physcomitrium lorentzii Müll. Hall.	Goffinet & Cox (2000)	AF223046
Physcomitrium pyriforme (Hedw.) Hampe	Goffinet & Cox (2000)	AF223045
Pilosium chlorophyllum (Hornsch.) Müll. Hal.	Buck *et al.* (2000)	AF143059
Pilotrichella flexilis (Hedw.) Ångstr.	Buck *et al.* (2000)	AF143046
Pilotrichum fendleri Müll. Hal.	Buck *et al.* (2000)	AF143069
Plagiomnium affine (Funck) T. Kop.	Cox & Hedderson (1999)	AF023797
Plagiopus oederi (Brid.) Limpr.	Cox & Hedderson (1999)	AF023833
Plagiothecium cavifolium (Brid.) Z. Iwats.	Buck *et al.* (2000)	AF143080
Plagiothecium laetum Bruch & Schimp.	Buck *et al.* (2000)	AF143058
Platygyrium repens (Brid.) Schimp.	Buck *et al.* (2000)	AF143038
Pleuridium subulatum (Hedw.) Rabenh.	***Anderson 27634***	AF306980
Pleurophascum occidentale Wyatt & Stoneburner	***Wyatt 4385a***	AF306963
Pohlia bolanderii (Lesq.) Broth.	Cox & Hedderson (1999)	AF023798
Pohlia cruda (Hedw.) Lindb.	Cox & Hedderson (1999)	AF023795
Polytrichum pallidisetum Funck	***Goffinet 4581***	AF306956
Porotrichum vancouveriensis (Kindb. Ex Mac.) Crum	Cox & Hedderson (1999)	AF023830
Prionodon densus (Hedw.) Müll. Hall.	Buck *et al.* (2000)	AF143076
Pseudocryphaea dominguense (Spreng.) Buck	Buck *et al.* (2000)	AF143063
Pseudoscleropodium purum (Hedw.) Fleisch.	Buck *et al.* (2000)	AF143030
Pterobryella praenitens C. Müll.	***Streimann 56079*** **(NY)**	AF307002
Pterobryon densum Hornsch.	Buck *et al.* (2000)	AF143013
Ptychomitrium gardneri Lesq.	Cox & Hedderson (1999)	AF023779
Ptychomnion aciculare (Brid.) Mitt.	Buck *et al.* (2000)	AF143015
Pylaisiadelpha tenuirostre (Sull.) Buck.	Buck *et al.* (2000)	AF143053
Pyrrhobryum spiniforme (Hedw.) Mitt.	Cox & Hedderson (1999)	AF023832
Pyrrhobryum vallis-gratiae (Hampe) Manuel	Cox & Hedderson (1999)	AF023825
Rhabdoweisia crispata (Wither.) Lindb.	***Goffinet 4553***	AF222899
Rhachithecium papillosum (Williams) Wijk & Marg.	***Pócs & Lye 97123/A*** **(EGR)**	AF306978
Rhacocarpus purpurascens (Brid.) Par.	Cox & Hedderson (1999)	AF023815
Rhizogonium lindigii (Hampe) Mitt.	Cox & Hedderson (1999)	AF023826
Rhizogonium nova-hollandiae (Brid.) Brid.	Cox & Hedderson (1999)	AF023827
Rhodobryum giganteum (Schwägr.) Par.	Cox & Hedderson (1999)	AF023789
Rhytidiadelphus squarrosus (Hedw.) Warnst.	Buck *et al.* (2000)	AF143033
Sanionia georgico-uncinata (C. Müll.) Ochyra & Hedenäs	Buck *et al.* (2000)	AF143049
Schistostega pennata (Hedw.) Webr & D. Mohr	***Hedderson s.n.*** **(RNG)**	AF265359
Schlotheimia rugifolia (Hooker) Schwägr.	***Anderson 26352***	AF306976
Schwetschkeopsis fabronia (Schwägr.) Broth.	Buck *et al.* (2000)	AF143041
Scopelophila cataractae (Mitt.) Broth.	***Shaw & Ben*** **5/1988**	AF306983
Scouleria aquatica Hook.	***Schofield 102594***	AF306984
Sematophyllum demissum (Wils.) Mitt.	Buck *et al.* (2000)	AF143055
Sphagnum perichaetiale Hampe	***Shaw 9213***	AF306951
Sphagnum tenerum Sull.	***Shaw 9335***	AF307004
Spiridens reinwardtii Nees.	Cox & Hedderson (1999)	AF023828
Splachnobryum obtusum (Brid.) C. Müll.	***Churchill et al. 17135*** **(H)**	AF306962
Splachnum luteum Hedw.	Cox & Hedderson (1999)	AF023805
Splachnum sphaericum Hedw.	Goffinet & Cox (2000)	AF223059
Stegonia latifolia (Schwägr.) Broth.	***La Farge 10/08/90*** **(ALTA)**	AF222901
Takakia lepidozioides Hattori & Inoue	***Schofield 86563***	AF306950
Taxithelium planum (Brid.) Mitt.	Buck *et al.* (2000)	AF143054

Table 1 continued over page

TABLE 1. *Continued*

Taxon	Voucher or reference	GenBank accession number
Tayloria lingulata (Dicks.) Lindb.	Cox & Hedderson (1999)	AF023807
Tayloria orthodontia (P. Beauv.) Demar.	Cox & Hedderson (1999)	AF023808
Tayloria splachnoides (Schwägr.) Hook.	***De Luna 209***	AF306965
Tetraphis geniculata Milde	***Schofield 103022***	AF306955
Tetraphis pellucida Hedw.	***Goffinet 4542***	AF306954
Tetraplodon lamii Reimers	***De Sloover 43076***	AF306966
Tetraplodon mnioides (Hedw.) Br. Eur.	Cox & Hedderson (1999)	AF023804
Thamnobryum alleghaniense (Müll. Hal.) Nieuwl.	Buck *et al.* (2000)	AF143009
Thamnobryum alopecurum (Hedw.) Nieuwl.	Cox & Hedderson (1999)	AF023834
Thelia asprella (Schimp.) Sull.	***Goffinet 4520***	AF306989
Thelia lescurii Sull.	Buck *et al.* (2000)	AF143039
Theriotia lorifolia Card.	Goffinet & Cox (2000)	AF223036
Thuidium delicatulum (Hedw.) Bruch & Schimp.	Buck *et al.* (2000)	AF143039
Thuidium tamariscinum (Hedw.) Br. Eur.	Cox & Hedderson (1999)	AF023819
Timmia austriaca Hedw.	Goffinet & Cox (2000)	AF223035
Timmia megapolitana Hedw.	***Schofield 97957***	AF222902
Timmia sibirica Lind. & Arnell	Cox & Hedderson (1999)	AF023775
Tortula ruralis (Hedw.) Gaertn., Meyer & Schreb.	Cox & Hedderson (1999)	AF023831
Trachyloma diversinerve Hampe	Buck *et al.* (2000)	AF143021
Trachypus bicolor Reinw. & Hornsch.	Buck *et al.* (2000)	AF143052
Uleastrum paraguense (Besch.) Buck	***Zardini & Aquino 32310***	AF222898
Ulota crispa (Hedw.) Brid.	***Goffinet 4535***	AF306972
Ulota phyllantha Brid.	Cox & Hedderson (1999)	AF023812
Voitia hyperborea Greville & Arnott	***Scotter* 7/27/1990** (ALTA)	AF306967
Wardia hygrometrica Hook.	Cox & Hedderson (1999)	AF023782
Weissia ludiviciana (Sull.) Reese & Lemmon -	***Reese 11412* (BUF)**	AF306985
Zelometeorium patulum (Hedw.) Manuel	Buck *et al.* (2000)	AF143050
Zygodon viridissimus (Dicks.) Brid.	***Goffinet 4580***	AF306974

TABLE 2. *Variation, parsimony informativeness, and base composition of the* rps4 *gene, its codon-defined partitions and its protein translation*

	Rps4 total	1st codon position	2nd codon position	3rd codon position	Amino acids
Number of:					
constant characters	152	59	86	7	52
autapomorphic	90	33	34	23	41
parsimony informative	328	98	70	160	97
(%)	58	52	37	84	51
Composition					
A	0·40	0·35	0·38	0·48	
C	0·14	0·21	0·17	0·06	
G	0·14	0·21	0·15	0·06	
T	0·32	0·23	0·30	0·43	

TABLE 3. *Parameters relating to the four combinations of data partitions of the* rps4 *gene sequences and their amino acid translations, statistics of optimal trees obtained from their analysis under maximum parsimony*

	Rps4	*Rps4* + AA	*Rps4* (3rd codon omitted)	*Rps4* + AA (3rd codon omitted)
Number of characters	570	760	380	570
g_1 statistic	−0·2266	−0·2348	−0·1790	−0·1669
Length of MPT	2584	3451	1154	1993
Number of MPTs	20·000	30·000	20·000	20·000
CI	0·2682	0·2822	0·3076	0·3191
CI (autapomorphies excluded)	0·2369	0·2483	0·2595	0·2736
RI	0·6803	0·6046	0·7223	0·7325
RE	0·1824	0·1960	0·2222	0·2338

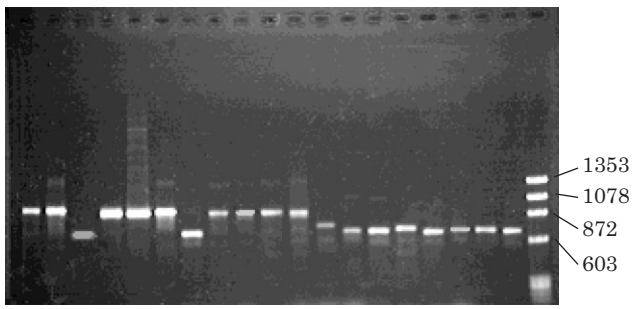

FIG. 1. Size variation in the amplicon comprising the *rps4* gene and the 3′ intergenic spacer. Lane 1. *Takakia lepidozioides*; 2. *Sphagnum perichaetiale*; 3. *Ambuchanania leucobryoides*; 4. *Andreaea rupestris*; 5. *Andreaeobryum macrocarpon*; 6. *Oedipodium griffithianum*; 7. *Buxbaumia aphylla*; 8. *Tetraphis pellucida*; 9. *T. geniculata*; 10. *Polytrichum pallidisetum*; 11. *Dendroligotrichum dendroides*; 12. *Theriotia lorifolia*; 13. *Diphyscium foliosum*; 14. *Timmia austriaca*; 15. *Enthostodon laevis*; 16. *Encalypta armata*; 17. *Fissidens subbasilaris*; 18. *Neomeesia paludella*; 19. *Catagonium nitens*; 20. phi-X DNA digested by *Hae*III (numbers on right indicate approximate size of fragments in nucleotides).

mosses (Fig. 2B). The Buxbaumiineae are also consistently resolved as a polyphyletic assemblage, but the affinities of its two families remain somewhat uncertain: the Diphysciaceae form a sister clade to the arthrodontous mosses (i.e. the Bryales) in all analyses except when the reduced set of nucleotide characters is analysed (Fig. 3A), whereas the Buxbaumiaceae are affiliated with the nematodontous mosses, being either sister to the Tetraphidaceae (Fig. 2) or the Polytrichaceae (Fig. 3). The Nematodonteae (i.e. the Tetraphidales, Buxbaumiaceae and Polytrichales) are resolved as a monophyletic group in the strict consensus upon inclusion of amino acid data (Figs 2B and 3B), and in 90 % of the MPTs derived from nucleotide data alone (Figs 2A and 3A).

The remaining Bryales (i.e. *sensu* Vitt but excluding the Oedipodiaceae and Buxbaumiaceae) compose a monophyletic group in all analyses. Although the relationships are poorly supported by bootstrap percentages, several trends emerge. The Funariineae *sensu* Vitt (1984) are always resolved as a polyphyletic. Except for when the first and second codon positions are analysed independently (Fig. 3A), the Funariaceae, Gigaspermaceae and Disceliaceae form a polyphyletic group, with the Encalyptaceae nested between them. Together these four families are resolved as a natural lineage when amino acid sequences complement the nucleotide data in the analysis, and even in the absence of the protein data this lineage occurs in 99 % of all most parsimonious trees recovered (Figs 2 and 3). The Timmiaceae are resolved in 90 % (Figs 2A and 3A) or all (Figs 2B and 3B) of the MPTs with this lineage of Funariineae-Encalyptineae. The genera *Drummondia* and *Scouleria*, which compose a monophyletic group in all most parsimonious trees in each of the four analyses, represent in most or all cases a sister group to the large clade comprising the Timmiaceae, Encalytineae and Funariineae (TEF-clade; Figs 2B, 3A and B). Only when all data partitions of the *rps4* sequences are analysed together does the *Drummondia*/*Scouleria* clade show the expected affinities to the Haplolepideae (Fig. 2A). By contrast *Bryoxiphium*, the sole member of the Bryoxiphiaceae, exhibits affinities to other haplolepideous mosses, except when only the first and second codon positions are included in the analyses, in which case *Bryoxiphium* appears sister to *Drummondia* and *Scouleria*.

The inclusion of amino acid sequences in the analyses leads to the Haplolepideae and *Bryoxiphium* composing the sister group to the above described TEF-clade in all optimal topologies (Figs 2B and 3B). This relationship is also present in 99 % of all most parsimonious trees recovered when the full nucleotide data set is analysed. By contrast, the exclusion of the third codon position in the absence of protein data results in the Haplolepideae being resolved as sister to the clade comprising diplolepideous mosses with alternate peristomes. Noteworthy here is the consistent inclusion of the Archidiineae in a nested position within the Haplolepideae, mostly close to the Grimmiineae and the Seligeriineae (Fig. 4). Similarly, the Schistostegaceae occur in all optimal topologies in the Haplolepideae, rather than within the Bryineae (Fig. 4). By contrast, the Pleurophascaceae, a family containing two species of cleistocarpous and aperistomate mosses, is consistently (i.e. in all most parsimonious trees) resolved in a sister position to the Bryaceae (see below), rather than within the Haplolepideae (Fig. 5). Furthermore, three analyses (e.g. Fig. 4) concur with Goffinet *et al.* (1998) and Stech (1999) in resolving the genus *Amphidium* in the Haplolepideae. One exception occurred when the third codon positions were excluded in the presence of protein data, in which case *Amphidium* was resolved as the most basal Arthrodontous moss (Fig. 3B). The monospecific Catascopiaceae were consistently resolved within this large clade (TEF-clade + Haplolepideae), rather than the Bryineae. In three of the four analyses, the affinities of the Catascopiaceae lay with the Funariineae/Encalyptineae ± Timmiaceae (Figs 2B and 3A,B), but the family was more closely related to the Haplolepideae and *Bryoxiphium* when the full nucleotide data set was analysed alone (Fig. 2A).

Diplolepideous mosses with alternate peristomes compose a monophyletic lineage in all MPTs inferred from the four analyses. The Orthotrichaceae are consistently resolved as monophyletic. Species of *Pohlia* (Bryaceae) always associate with *Phyllodrepanium* and the Mniaceae, as do the Splachnaceae with the Meesiaceae and *Leptobryum* (Bryaceae). The remaining Bryaceae, with the exception of *Orthodontium*, consistently compose a monophyletic lineage with *Pleurophascum* and the Leptostomataceae. The position of *Pleurophascum* in the Bryaceae is tentative since the sequence is not complete, and should thus be tested further. *Orthodontium lineare* never occurred close to other Bryaceae, but instead consistently showed affinities to *Leptotheca* (Rhizogoniaceae), and in particular to *L. boliviana*. Species of both *Leptotheca* and *Orthodontium* formed a monophyletic group when the third codon positions were excluded from the analyses (Fig. 3A and B).

The Rhizogoniaceae are always resolved as a polyphyletic assemblage, with species of *Leptotheca* either allied to *Orthodontium* or to such families as the Hedwigiaceae or Orthotrichaceae (Figs 2 and 3). *Pyrrobryum vallis-gratiae*

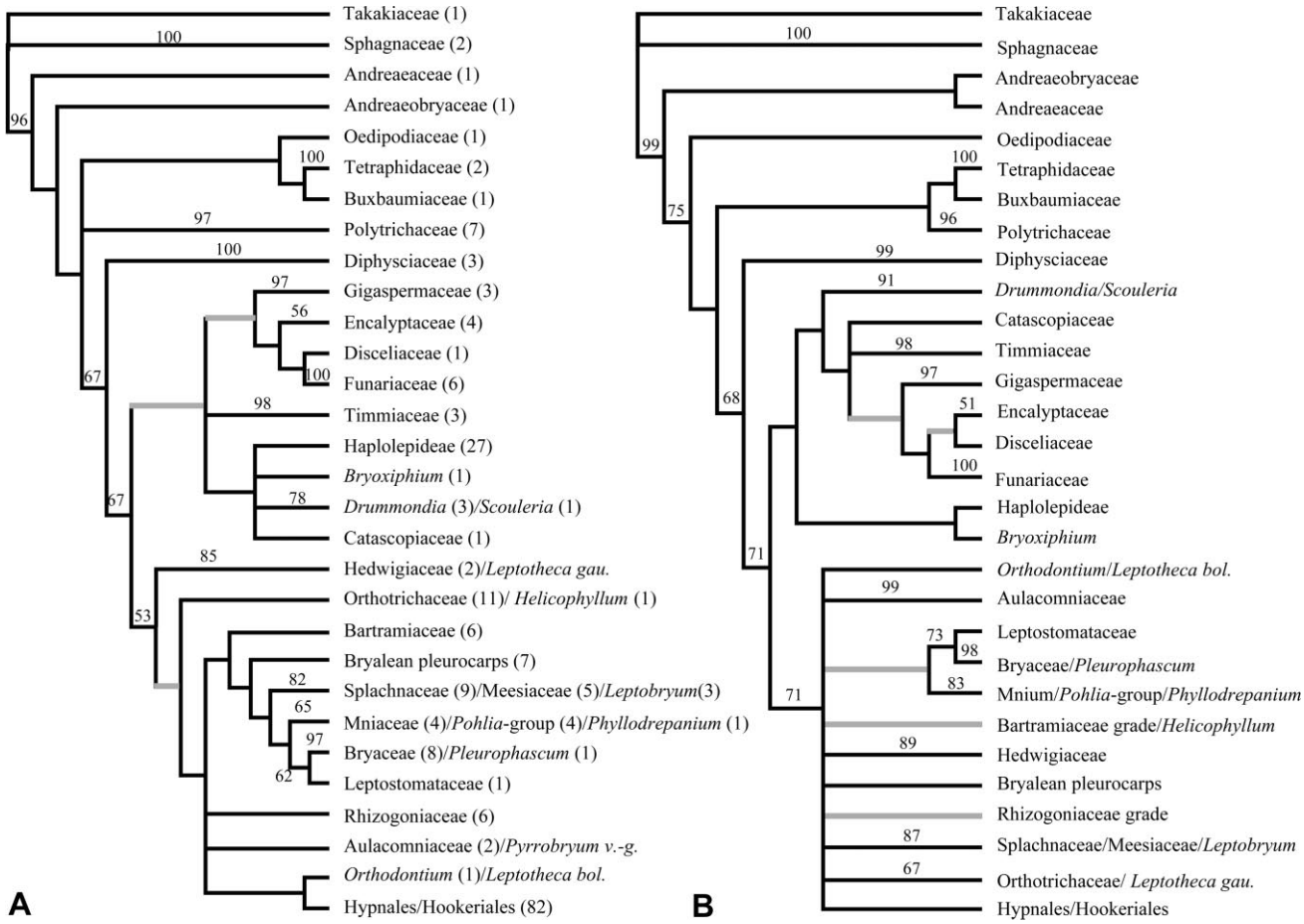

FIG. 2. A, Summarized 90 % majority-rule consensus tree of 20 000 most parsimonious trees inferred from analysing the *rps4* gene sequences (i.e. all codon positions included). Values following taxa refer to number of exemplars composing the lineage. B, Summarized 90 % majority-rule consensus tree of 30 000 most parsimonious trees inferred from analysing the *rps4* gene sequences (i.e. all codon positions included) in combination with their amino acid translation. Grey branches correspond to branches collapsing in the strict consensus. Values above branches correspond to frequencies of occurrence of branches among the trees recovered from 5000 fast bootstrap replicates.

also never associated with its congener *P. spiniforme*. By contrast, the monogeneric families Calomniaceae and Mitteniaceae consistently occurred as sister to *P. spiniforme* and the *Rhizogonium-Goniobryum* clade, respectively. Members of these five genera (except for *P. vallis-gratiae*) formed a monophyletic group only when nucleotide sequences alone were analysed. The Bartramiaceae formed a monophyletic group only when all the nucleotide sites were included in the analysis with or without the protein sequences. Members of the Cyrtopodaceae, Hypnodendraceae, Spiridentaceae and *Pterobryella*, here termed the Bryalean pleurocarps, composed a monophyletic lineage only when the 3rd codon positions were included in the analysis (Figs 2A and 3B). Finally, all exemplars of the Hypnineae *s. l.* and Hookeriineae, a group traditionally named the pleurocarps (Hypnidae *sensu* Buck *et al.*, 2000), formed a monophyletic lineage in all MPTs.

DISCUSSION

Phylogenetic analyses of nucleotide sequences of the *rps4* gene and its amino acid translations using *Takakia* and the Sphagnidae as an outgroup converge towards an overall evolutionary history of mosses wherein (1) the mono- or paraphyletic Andreaeidae are ancestral to a dichotomy between nematodontous and arthrodontous mosses; (2) the Buxbaumiineae are polyphyletic, with the Diphysciaceae composing a sister lineage to the remaining Bryales *sensu* Vitt (1984); and (3) the Funariineae, Encalyptineae, Timmiaceae and the Haplolepideae compose a monophyletic lineage sister to mosses with alternating peristomes (e.g. Bryineae, Orthotrichineae, Hypnineae). Furthermore, the extensive taxon sampling suggests placements for several groups that are at odds with traditional classifications (e.g. Pleurophascaceae, Catascopiaceae).

Suitability of the data

The g_1 statistic (Table 3) of the distribution of 50 000 random trees indicates that all four combinations of data partitions used here are significantly more structured than random data sets of equal size (see reference values provided by Hillis and Huelsenbeck, 1992), suggesting that the DNA sequence variation is not random with respect to phylogeny.

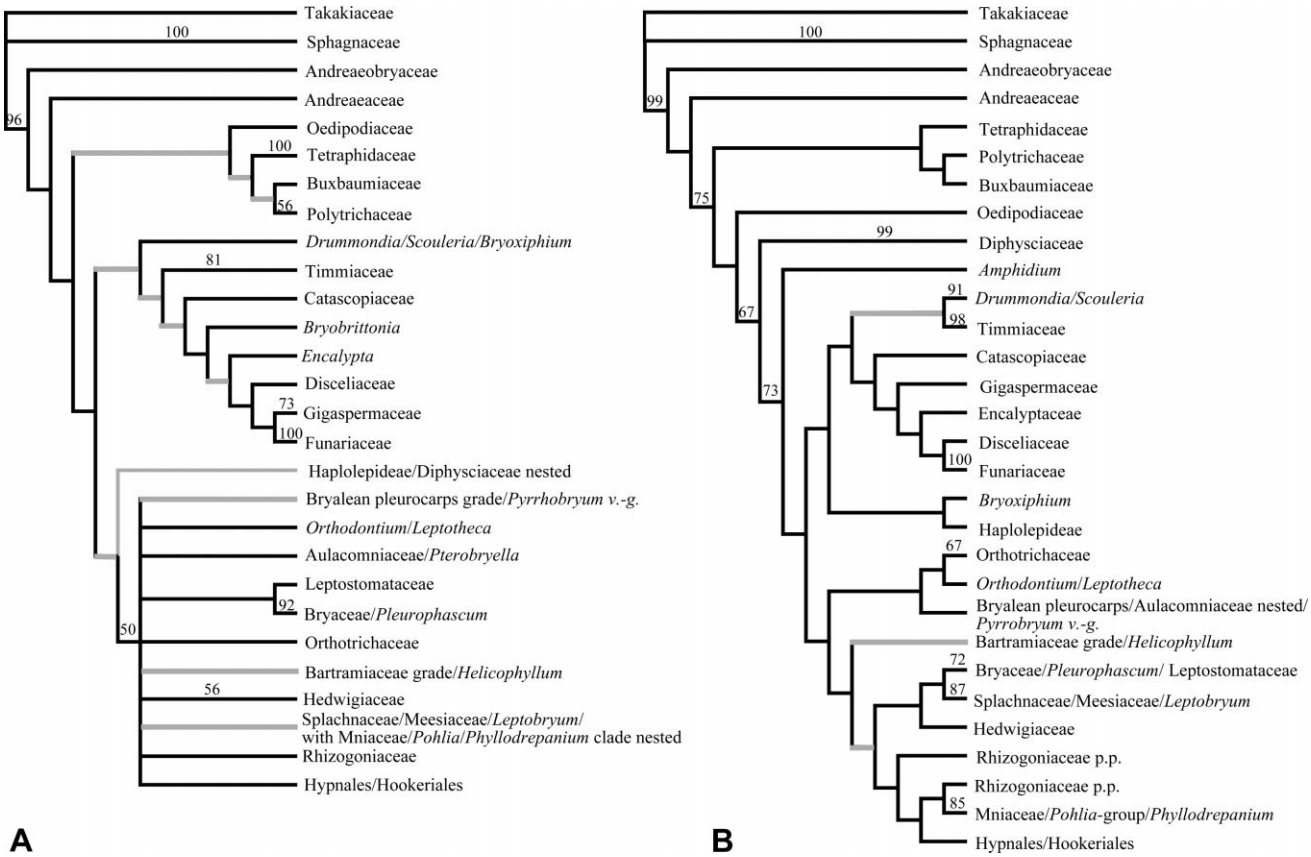

FIG. 3. A, Summarized 90 % majority-rule consensus tree of 20 000 most parsimonious trees inferred from analysing the first and second codon positions of *rps4* gene sequences (i.e. excluding the third codon positions). B, Summarized 90 % majority rule consensus tree of 20 000 most parsimonious trees inferred from analysing first and second codon positions of *rps4* gene sequences in combination with the amino acid translation of the complete sequences. Grey branches correspond to branches collapsing in the strict consensus. Values above branches correspond to frequencies of occurrence of branches among the trees recovered from 5000 fast bootstrap replicates.

Although the congruence among most parsimonious topologies inferred from four different combinations of nucleotide and protein partitions is high with regard to the above relationships, the deeper dichotomies (i.e. at the suprafamilial level) are in general poorly supported by the bootstrap (< 73 %).

The suitability of a data set is often assessed by the frequency of dichotomies well supported by bootstrapping. The overall lack of support in the *rps4* phylogeny is consistent with extensive taxon sampling combined with the comparatively small number of characters sampled. Sanderson and Donogue (1989) demonstrated that an increase in numbers of exemplars is accompanied by a decrease in the consistency index, and thus an increase in homoplasy. It is the latter that accounts for the incongruence among trees obtained by bootstrapping, and the lack of swapping as implemented in the fast bootstrap analysis probably accentuates this effect as the search for most parsimonious topologies is relaxed. Poor overall support for a topology does not, of course, preclude the phylogeny from being accurate; for example, the traditional pleurocarps, i.e. Hypnineae, Leucodontiineae and Hookeriineae, which are represented here by 82 exemplars, compose a monophyletic group in all analyses presented here (see also Buck *et al*., 2000; De Luna *et al*., 2000), yet fail to obtain a bootstrap value of 50 % or more. However, since the true phylogeny is rarely known, and such is the case for mosses, the accuracy *per se* of the data cannot, in those cases, be established. What remains is circumstantial evidence. Does the information from the data significantly differ from a random data set of identical dimensions? Does the most parsimonious gene tree differ significantly from alternative phylogenetic hypotheses? How does the resulting phylogeny agree with traditional morphology-based concepts?

As is typical for protein coding genes, rates of evolution differ among codon positions, with the third codon positions accumulating mutations at a higher rate than the first or the second positions (Olmstead *et al*., 1998). Consequently the former partition is often characterized by a higher level of homoplasy. Nevertheless, the third codon positions may carry a phylogenetic signal that contributes to the overall resolution of the relationships (Lewis *et al*., 1997). The exclusion of the third codon positions from the analyses resulted in a dramatic decrease in resolution in the strict consensus and a general decrease in bootstrap values, suggesting greater character conflict or less phylogenetic signal in these remaining sites, or simply the lack of enough characters. An alternative to excluding the partition is to

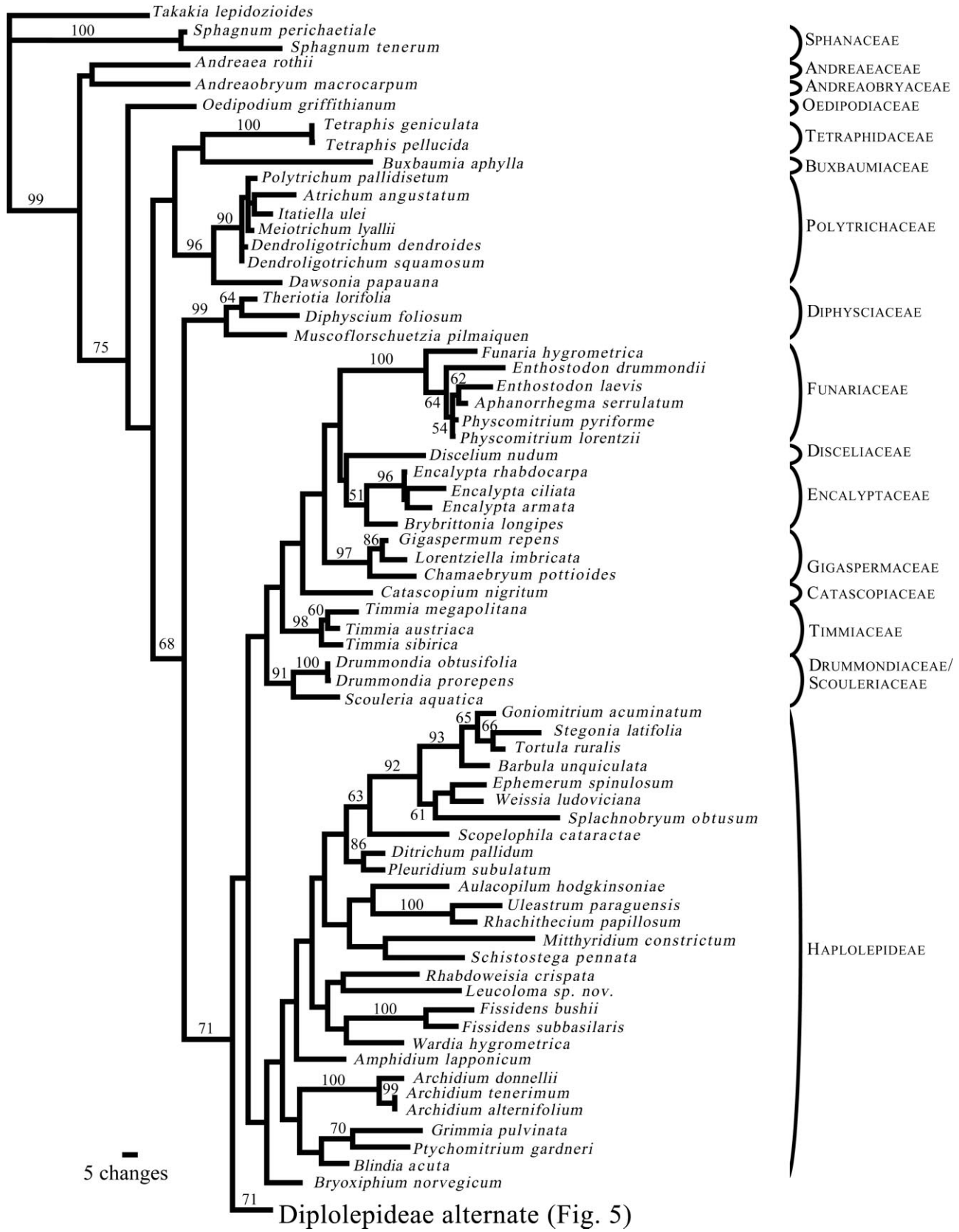

FIG. 4. Portion of the phylogram of the first most parsimonious tree (island one) inferred from the analysis of *rsp4* gene complemented by its amino acid translation. Only relationships among and within basal lineages are shown here. Branch lengths are obtained following optimization by accelerated transformation of character-states.

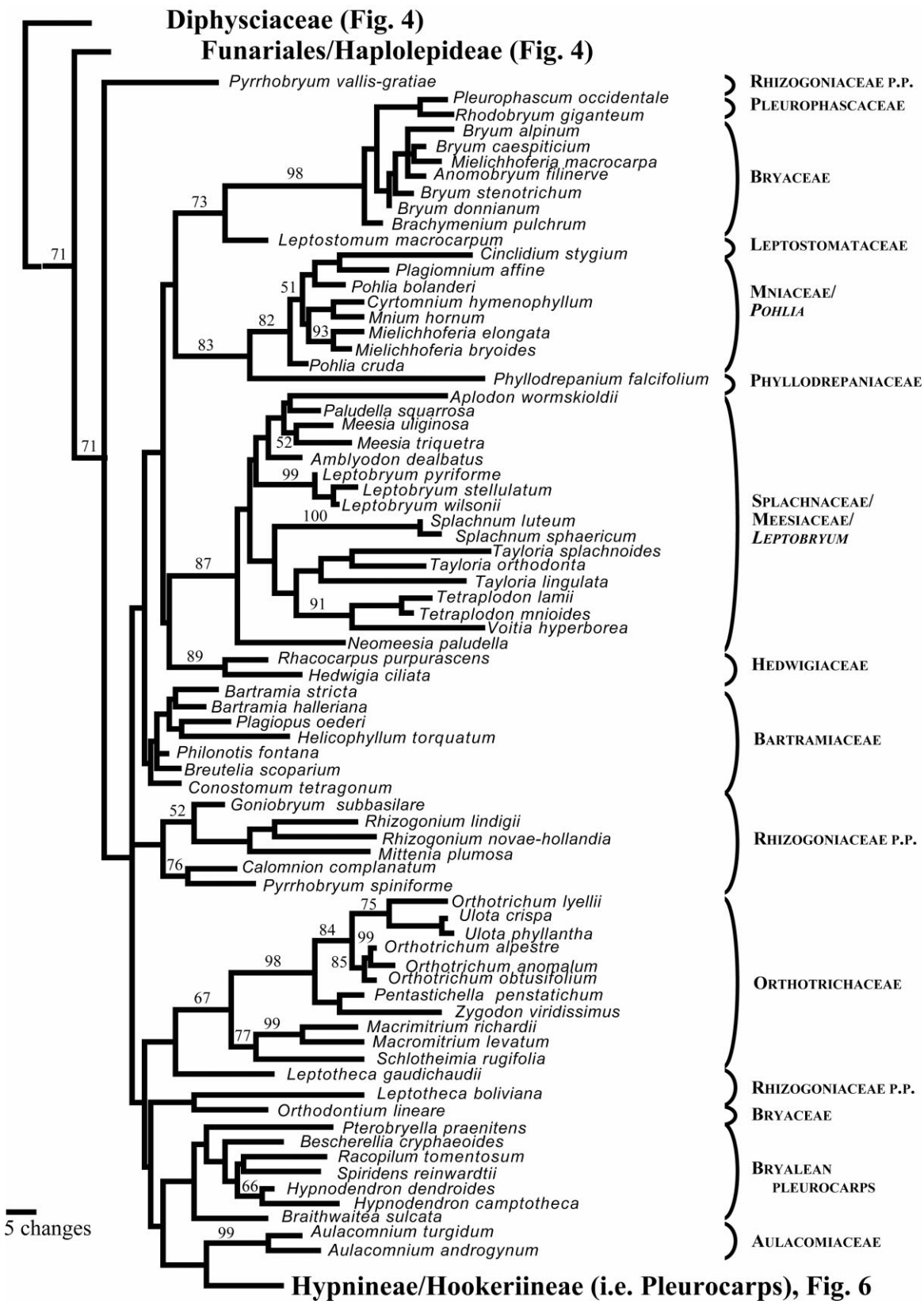

FIG. 5. Portion of the phylogram of the first most parsimonious tree (island one) inferred from the analysis of *rsp4* gene complemented by its amino acid translation. Only relationships within the clade comprising mosses with opposite peristomes are shown here. Branch lengths are obtained following optimization by accelerated transformation of character-states.

downweigh the synonymous changes relative to the non-synonymous changes. Complementing the sequences by their amino acid translation as proposed by Agosti *et al.* (1996) allows for the non-synonymous changes to be given more weight by occurring twice in the data set, once as a nucleotide and once as an amino acid change. Clearly non-synonymous mutations, too, may occur in parallel and the inclusion of amino acid sequences in the data set would thus accentuate the effect of such homoplasious character-states on reconstructing the phylogeny. Examination of the consistency indices for different codon partitions for a tree based on the nucleotide data alone and one inferred from variation in both nucleotide and amino acid sequences reveals that the inclusion of amino acid sequences in the analysis resulted, as would be expected *a priori*, in a decrease of the average consistency index for characters in the third codon positions, and an increase in this index for the first and second character partitions. Consequently, the phylogenetic hypothesis inferred from the expanded data set draws more on the information carried by the characters expected *a priori* to be less variable, namely positions one and two, and may therefore be considered as a more accurate representation of the relationships within mosses given the data at hand.

Phylogenetic relationships

The phylogenetic hypothesis for deep divergences among major lineages of mosses (e.g. ordinal level *sensu* Vitt, 1984) as inferred here from analysing *rps4* sequences under maximum parsimony (Figs 2 and 3), agrees overall with the scenario proposed by Mishler and Churchill (1984) or Vitt (1984), except for the following relationships: (1) the Andreaeaceae and Andreaeaobryaceae may not be sister taxa; (2) the Nematodonteae (i.e. Tetraphidaceae and Polytrichaceae) compose a monophyletic lineage; and (3) the Buxbaumiineae *sensu* Vitt (1984) are polyphyletic, with the Diphysciaceae sister to the Arthrodonteae and the Buxbaumiaceae with stronger affinities to either the Tetraphidaceae or the Polytrichaceae. Furthermore, the gymnostomous (or cleistocarpous) family Oedipodiaceae, traditionally considered allied to the Splachnaceae, is here shown to be of more ancestral origin—in some cases (Fig. 2B) even predating the dichotomy between nematodontous and arthrodontous mosses, a hypothesis that is congruent with that presented by Newton *et al.* (2000) based on molecular and morphological data.

The Andreaeidae *sensu* Vitt (1984) can be defined by features of spore development including a multiseriate protonema, parenchymatous protonemal appendages, the dehiscence of the sporangium along four or more longitudinal lines, calyptra formation, and the mode of pigmentation of the exothecial cells (Murray, 1988; Newton *et al.*, 2000). The phylogenetic signal emerging from the parsimony analysis of the nucleotide and amino acid data corroborates that of other molecular data sets (Newton *et al.*, 2000) in supporting the monophyly of the Andreaeidae. This subclass comprises two lineages: the Andreaeaceae and the Andreaeobryaceae, which differ by a suite of characters described in great detail by Murray (1988). Phylogenetic analyses of morphological characters alone under maximum parsimony resulted in the polyphyly of the Andreaeidae (Newton *et al.*, 2000), with the Andreaeobryaceae and the Andreaeaceae sister to *Takakia*, and the peristomate mosses, respectively. For the four characters for which *Takakia* and *Andreaeobryum* share the same state and differ from *Andreaea*, one is synplesiomorphic among land plants (absence of axillary hairs), one is a potential synapomorphy with the Bryidae (long tapering shape of the foot), and two are potentially autapomorphic for the *Takakia-Andreaeobryum* clade (presence of mucilage hairs, lack of regular lines of dehiscence along sutures of thin-walled cells). Schuster (1997) raised some doubts on the homology of mucilage papillae between these taxa, and the irregular dehiscence in *Andreaeobryum* could be seen as a reversal, or a synplesiomorphy. The characterization of the foot of *Andreaea* as conical *vs.* long tapered has little bearing on the phylogenetic reconstruction since it is autapomorphic. Weighted against the autapomorphies of the Andreaeidae, there seems little morphological evidence left in support of polyphyly of the Andreaeidae.

The Bryidae *sensu* Vitt (1984), which comprise all peristomate mosses, is resolved as a natural group (Figs 2 and 3). This lineage is further characterized by imperforate water conducting cells, the presence of filamentous protonemata, terminal antheridia, and cylindrical columella in the sporangium (Newton *et al.*, 2000). Two basic peristomial architectures occur in these mosses. The nematodontous peristome, which is composed of a single row of teeth made of several layers of whole cells—rather than one or two rows of teeth built from cell wall remnants as in the arthrodontous type—characterizes the Polytrichales and the Tetraphidales. Mishler and Churchill (1984) considered the Nematodonteae a paraphyletic assemblage on the basis of air spaces occurring in the Polytrichales and Bryales, but lacking in the Tetraphidales, Sphagnales and Andreaeales, as well as in *Takakia* (Renzaglia, pers. comm.). Analysis of multiple gene sequence data and morphological features corroborated the hypothesis of paraphyly, but suggested that the Tetraphidales and not the Polytrichales were sister to the Bryales (Newton *et al.*, 2000). Successively weighting transformations led Hyvönen *et al.* (1998) to a similar hypothesis. Bootstrap support for this topology did not, however, exceed 54 % in either study. Characters defining a relationship of the Tetraphidales to the Bryales are the 'induction of brood bodies by abscisic acid', and the 'gametophyte transfer cells in placenta with labyrinthine walls on inner tangential walls' (Newton *et al.*, 2000). Analyses of *rps4* data, particularly in combination with the amino acid sequences, yield the Nematodonteae as a monophyletic lineage. Similarly, parsimony analyses of *18SrRNA* sequences resolved nematodonts as a natural lineage (Hedderson *et al.*, 1998). Further studies, focusing on the Nematodonteae and modelling the rate of molecular evolution of the different loci, are needed to critically test these alternative phylogenetic hypotheses.

Based on *rps4*, the Tetraphidales may include *Oedipodium* and *Buxbaumia*. The association between *Tetraphis* and *Oedipodium* is resolved based on variation in nucleotide sequences only (Figs 2A and 3A). Inclusion of the amino

acid sequences in the analysis confines the Oedipodiaceae to a basal position within the Bryidae (Fig. 2B), a hypothesis congruent with that inferred from multiple gene sequences (Newton *et al*., 2000). Vitt (1982, 1984) considered the Oedipodiaceae to be allied to the Splachnaceae. The only species of *Oedipodium* is gymnostomous (i.e. lacking a peristome), a feature widely homoplasious in mosses (Vitt, 1981) and thus not necessarily indicative of shared ancestry. Although the gametophytic and sporophytic characters may be reminiscent of the Splachnaceae (see Vitt, 1982), they are not incompatible with other families such as the Funariaceae and even the Tetraphidaceae; or they may even indicate an early divergence within the Bryidae. Analysis of morphological data concurs with the general molecule-based hypothesis that *Oedipodium* is not a member of the Arthrodonteae, but is instead related to the Nematodonteae or its ancestor (Hyvönen *et al*., 1998; Newton *et al*., 2000; this study).

In all most parsimonious topologies (Figs 2 and 3), the Buxbaumiaceae, a family of species that have an extremely reduced gametophyte but with a massive sporophyte, are resolved with close affinities to the Nematodonteae, in a position sister to either the Tetraphidaceae (Figs 2A,B) or the Polytrichaceae (Fig. 3A,B). The Diphysciaceae, traditionally considered allied to the Buxbaumiaceae (Mishler and Churchill, 1984; Vitt, 1984) are instead consistently resolved sister to the Arthrodonteae. Vitt (1984) had argued that the affinities of *Buxbaumia* were with the Arthrodonteae, as the peristome comprises multiple rows of teeth of which the innermost are composed of cell plates. However, one could similarly attribute more weight to the presence of whole cells in the outer peristome teeth, a feature diagnostic of nematodontous peristomes. The position of *Buxbaumia* as inferred from molecular characters tends to suggest that the nematodontous elements in the peristome of *Buxbaumia* are homologous, if only by their architecture, to those found in the Tetraphidales and Polytrichales.

The polyphyly of the Buxbaumiineae was also found by Hyvönen *et al*. (1998) and Newton *et al*. (2000) based on total evidence from multiple gene sequences. Noteworthy in this regard is the incongruence between most parsimonious topologies inferred from nuclear or chloroplast gene sequences. Analysis of 18S rDNA sequence data reveals the Buxbaumiineae as monophyletic, whereas *rbc*L data resolves them as a paraphyletic assemblage, with *Diphyscium* sister to *Funaria* and *Buxbaumia* with ambiguous affinities (Hyvönen *et al*., 1998). Although the Buxbaumiineae are consistently resolved as a paraphyletic assemblage in our analyses, *Buxbaumia* shares with the Diphysciaceae and all Arthrodonts, a similar size amplimer, approx. 200 bp shorter than in the Polytrichales, Tetraphidales, Andreaeidae and Sphagnideae. The difference in size is accounted for by an indel in the 3′ intergenic spacer. The intergenic spacer in *Marchantia polymorpha* (GenBank accession X04465) is 228 bp and thus similar in size to that found in the Sphagnidae, Andreaeidae, Polytrichales and Tetraphidales. The spacer is highly variable, making it impossible to assess homology among sequences and thus to assert the positional homology of the indel. The comparison with *Marchantia* does, however, suggest that the spacer suffered a deletion prior to the evolution of the Arthrodonteae. Based on the phylogeny recovered here and elsewhere (Newton *et al*., 2000) the deletion observed in the Buxbaumiaceae and the arthrodonts, including the Diphysciaceae is thus probably homoplasious.

A sister relationship of the Diphysciaceae to the 'true' arthrodonts was also hypothesized by Beckert *et al*. (1999) and Newton *et al*. (2000) based on sequence variation in the mitochondrial locus *nad5* and combined molecular characters from the chloroplast and the nuclear genome respectively. Such phylogenetic association is, however, not supported by independent analysis of nuclear data. As discussed above, analysis of 18S rDNA data by Hyvönen *et al*. (1998) and Cox (1998) suggest that the Buxbaumiineae are monophyletic, whereas the inclusion of chloroplast data (Newton *et al*., 2000) leads to a phylogenetic hypothesis congruent with the one obtained by *rps4* data. Parsimony analysis of 18S rDNA data resulted in the Buxbaumiineae nested within a monophyletic nematodontous clade (Cox 1998; see also Hedderson *et al*., 1998 who only included *Diphyscium* as an exemplar). The present study is the only one wherein the family Diphysciaceae is represented by its three genera, namely *Diphyscium*, *Muscoflorschuetzia* and *Theriotia*. The latter two genera lack a peristome, whereas species of *Diphyscium* bear a well-developed peristome of an architectural type similar to that of the peristome in *Buxbaumia*. Edwards (1984) and Vitt (1984) considered the peristome of *Diphyscium* to be arthrodontous in origin. It differs from that of *Buxbaumia* by its rather reduced exostome, whose teeth are arranged into a single row and are composed of cell remnants only. Furthermore, Shaw *et al*. (1987) showed that development of the *Diphyscium* peristome conforms to the haplolepideous pattern. A position of *Diphyscium* sister to the Bryales, as suggested by chloroplast and mitochondrial data, is thus fully congruent with characters of the peristome. The incongruence between plastid and nuclear data could be an artifact of different sampling strategies, incorrect modelling of the data, or distinct evolutionary histories, and perhaps of hybridization events, and therefore warrants further study.

Within the Bryidae, mosses are primarily subdivided according to the architecture of their peristome. Given the exception previously addressed by Goffinet and Cox (2000), the families of the Funariineae are monophyletic and, together with the Encalyptineae, they compose a natural lineage in most if not all optimal topologies (Figs 2 and 3). They are further typically associated with the Timmiaceae and the Catascopiaceae. The sole species of the Catascopiaceae lacks an endostome. It has traditionally been aligned within the Bryineae (Vitt, 1984), on the basis of vegetative characters (Vitt, 1982). Following Vitt's (1984) concept of peristome types, the TEF-clade comprises three distinct architectures, namely the Funaria-, Encalypta-, and Bryum-type. As argued by Cox and Hedderson (1999), Goffinet and Cox (2000), and Cox *et al*. (2000), the peristome of *Timmia* is best regarded as opposite rather than alternate as in the Bryum-type. Consequently, the clade comprising the funariaceous taxa, the Encalyptaceae, and the Timmiaceae

can be diagnosed by their diplolepideous peristome with opposite arrangement of endostome and exostome.

Although the haplolepideous peristome is typically composed of an endostome only, the position of these inner segments is the same as that of the segments in the Funaria-type peristome, and the arrangement of the haplolepideous peristomes is thus opposite (Vitt *et al.*, 1998). The Haplolepideae, excluding the Pleurophascaceae, compose a monophyletic lineage in most optimal topologies (Figs 2 and 3). The Drummondiaceae-Scouleriaceae clade and the Bryoxiphiaceae are consistently resolved near the base of this large lineage of opposite peristomate mosses, but their affinities are ambiguous. *Bryoxiphium* is gymnostomous, whereas *Drummondia* and *Scouleria* have a peristome that, although reduced, fits the haplolepideous architectural type (Churchill, 1985; Shaw, 1986). The support for the monophyly of the Timmiaceae-Funariaceae-Encalyptaceae-Haplolepideae clade is low (< 50 % bootstrap value), but it is present in most or all of the most parsimonious topologies inferred from *rps4* data and their amino acid translations (Figs 2 and 3). Maximum parsimony analysis of mitochondrial data yielded relationships within the Bryales that overlap perfectly with the present topology, although, again, support from bootstrap replicates was lacking (Beckert *et al.*, 1999). This hypothesis is incongruent with the relationships inferred from data sets that are constructed from, or include, nuclear 18S rDNA gene sequences (e.g. Cox, 1998; Hedderson *et al.*, 1998; Goffinet and Cox, 2000). Optimal topologies inferred from these data suggest a closer relationship between the Encalyptales, haplolepideae, and remaining diplolepidous taxa, to the exclusion of *Diphyscium*, the Funariales and Timmiaceae, although bootstrap support is lacking for the relevant node. However, analyses of combined data from the 18S rDNA and chloroplast genome (including the *rps4* gene) by Newton *et al.* (2000) and Cox *et al.* (2000) suggest the same relationships and provide moderate bootstrap support at the relevant node (75 % and 67 %, respectively).

The remaining diplolepideae, i.e. those with an alternate arrangement of their peristomes, are consistently resolved as a monophyletic group, with low to moderate bootstrap values (Figs 2 and 3). This lineage includes the Orthotrichineae, the Bryineae, and the combined Hypniineae-Leucodontineae clade. Included here are also the Splachnineae, whose peristome development is consistent with this phylogenetic placement even though *Splachnum*, the only member with a double peristome, has endostome segments that are opposite and fused to the exostome teeth (see Goffinet *et al.*, 1999 for further discussion). The monophyly of the Meesiaceae/Splachnaceae/*Leptobryum* clade, and the *Pohlia s. l.*/Mniaceae clade, is well supported here, and is corroborated by previous analyses of combined nuclear 18S rDNA and chloroplast data, plus separate analyses of the two genomic partitions (Cox, 1998; Cox and Hedderson, 1999; Cox *et al.*, 2000). Similarly, the polyphyly of the Rhizogoniaceae is congruent with results from previous studies (Cox and Hedderson, 1999). The traditional pleurocarpous mosses form a monophyletic clade in all most parsimonious reconstructions. Although the strict consensus of all most parsimonious trees suffers from low resolution within this clade, the majority-rule consensus tree resolves the Hookeriineae as monophyletic and sister to the clade composed of the Hypnineae and the Leucodontineae. The latter two suborders are each polyphyletic (Fig. 6) as previously suggested by Buck *et al.* (2000) and De Luna *et al.* (2000).

The major clades resolved by analysing *rps4* sequence data and their protein translation typically agree with taxa as defined by Vitt (1984) on the basis of peristome characters. However, the phylogenetic significance of these characters remains ambiguous, as already suggested by Vitt *et al.* (1998). The classification of mosses is based on features of the peristome and, within the Arthrodonteae, peristome types are defined based on combinations of states pertaining to four characters: peristome opposite or alternate, the presence of cilia between adjacent endostomial segments, and finally, the pattern of cell division with the inner peristomial layer (IPL). Ancestral character-state reconstruction relies heavily on polarization using outgroup comparison; however, all basal lineages of mosses (i.e. Takakiaceae, Sphagnaceae, Andreaeaceae, Andreaeobryaceae) lack a peristome. Furthermore, the problem is exacerbated by the fact that alternative states of some characters define sister lineages, making reconstruction of ancestral states ambiguous or impossible. Considering, for example, the dichotomy between the Nematodonteae and the Arthrodonteae (Fig. 2B), how do we decide which architecture is plesiomorphic? Finally, the lack of ontogenetic data for critical taxa such as the Timmiaceae, and ambiguous phylogenetic relationships of others (such as the Orthotrichaceae), preclude any objective reconstruction of the evolution of peristome-types [see Goffinet and Cox (2000) and Cox *et al.* (2000) for further discussion].

Single gene phylogeny and bryophyte systematics

The reconstruction of the phylogeny of mosses is, despite the tremendous progress achieved within the last 5 years, still in its infancy. Although little consensus has been reached regarding the relationships among major lineages of mosses, molecular data are contributing greatly by offering new suites of characters to test systematic concepts, and for assessing the affinities of taxa with reduced morphologies (e.g. Goffinet *et al.*, 1998; Hedderson *et al.*, 1999; Buck *et al.*, 2000; Goffinet and Cox, 2000). These studies highlight the difficulty of circumscribing suprageneric taxa based on morphology, revealing in particular the polyphyletic (and not just paraphyletic) nature of several families or suborders, such as the Funariaceae and Funariineae (Goffinet and Cox, 2000), the Bryaceae and Bryineae (Cox and Hedderson, 1999), the Orthotrichaceae and Orthotrichineae (Goffinet *et al.*, 1998), and the Hypnineae (Buck *et al.*, 2000). These cases of polyphyly all involve taxa that appear, from molecular evidence, to be only distantly related. In the present study, additional cases are hypothesized where taxa circumscribed *sensu* Vitt (1984) are polyphyletic: the Bryineae due to a nested position of the Schistostegaceae within the Dicraniineae; the Dicraniineae based on the Pleurophascaceae, which are consistently resolved sister to a clade of bryaceous taxa; and the

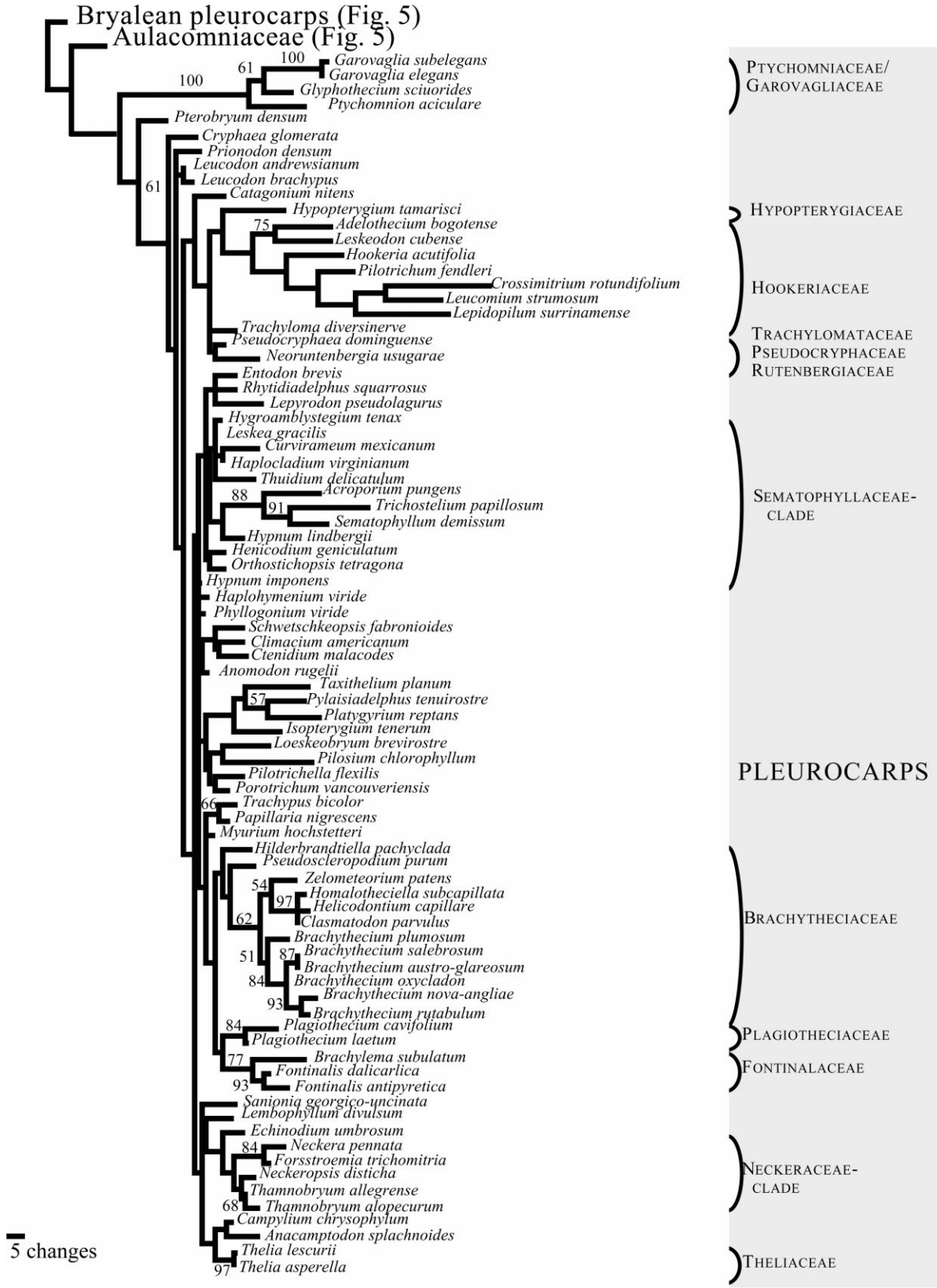

FIG. 6. Portion of the phylogram of the first most parsimonious tree (island one) inferred from the analysis of *rsp4* gene complemented by its amino acid translation. Only relationships within the clade comprising mosses with alternate peristomes are shown here. Branch lengths are obtained following optimization by accelerated transformation of character-states.

Tetraphidales, due to the position of the Calomniaceae within the Rhizogoniaceae. The latter family is also shown to include the Mitteniaceae, which had been recognized at the ordinal level based on unique peristomial architecture by Shaw (1985). Furthermore, molecular data suggest that the affinities of the Catascopiaceae lie with taxa defined by opposite peristomes rather than within the Bryineae (see Vitt, 1984).

Phylogenetic hypotheses inferred from a single gene should be viewed with caution, as the gene phylogeny may not overlap with the organismal phylogeny (Doyle, 1992). Some of the relationships obtained by analysing the *rps4* gene are, however, corroborated by nuclear data (e.g. Ephemeraceae: Goffinet and Cox, 2000). An assumption of monophyly of an ingroup is essential for any phylogenetic study aimed at reconstructing the evolutionary histories of taxa and the transformations of the characters that define them. Although multiple outgroups are typically used in such studies, if the polyphyly of the ingroup involves a distantly related taxon then polyphyly may go unnoticed. With approx. 300 sequences currently available and with another 100 or more soon to be published, *rps4* is the most extensively sequenced gene in mosses, spanning the widest systematic range. Although the *rps4*-based phylogeny shows little support in many of its branches, the core of most families is resolved as monophyletic and hence *rps4* sequence data offer a suitable framework for a preliminary assessment of the monophyly of supraspecific moss taxa.

ACKNOWLEDGEMENTS

This study was made possible by financial support from Duke University to A.J. Shaw and B. Goffinet, by NSF-grant DEB-9806955 to A.J. Shaw and B. Goffinet, financial support to C.J. Cox from The University of Reading, and a NERC (U.K.) Advanced Research Fellowship to T.A.J. Hedderson. We thank Catherine La Farge and William R. Buck for the sequences of *Leucoloma sp. nov.* and *Pterobryella praenitens*, respectively, Heinar Streimann (CBG) for sending fresh material of *Mittenia plumosa*, Angela Newton (BM) for material of *Helicophyllum torquatum* and the curators of BUF, EGR, H, MO, and NY for permission to sample collections for DNA extraction. Finally, we are thankful for the comments made by an anymous reviewer.

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