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Phylogenetic relationships among the ciliate arthrodontous mosses: evidence from chloroplast and nuclear DNA sequences

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Abstract: Parsimony and maximum likelihood analyses of combined *trnL* (UAA) 5′ exon – *trnF* (GAA) and *rps4* exon cpDNA, and 18S nrDNA sequences of 60 arthrodontous moss taxa indicate strong support for the monophyly of a clade containing the *Splachnineae*, *Orthotrichineae*, and diplolepideous alternate sub-orders. A clade including the *Splachnineae*, *Meesiaceae* and *Leptobryum* (*Bryaceae*) is similarly well supported and forms the sister group to a clade comprising the *Orthotrichineae* and the other diplolepideous alternate mosses. Within this latter clade a number of well supported lineages are identified, but relationships among these remain poorly resolved. These analyses indicate that the Splachnaceous and Orthotrichaceous peristomes have been independently derived from an ancestral 'perfect' bryoid peristome.

The understanding of phylogenetic relationships within the *Bryopsida* has particular problems rarely encountered in other land plant groups. Both the haploid (gametophyte) and diploid (sporophyte) generations are conspicuous and highly complex phases of the life cycle, and classifications based on a single generation can be constructed that would conflict with hypotheses based on the other (DIXON 1932). However, the notion of sporophytic conservatism (in contrast to gametophytic plasticity) has arisen due to the supposed reduction in exposure to environmental selection pressures experienced by the ephemeral and non-modular sporophyte (e.g. BUCK 1980). Consequently, modern classifications tend to be constructed using sporophytic characters to group taxa at the deeper phylogenetic levels.

The structure of the sporophytic peristome was first described in detail by PHILIBERT (1884a, b; TAYLOR 1962, for translation) and remains the basis of modern systems of moss classification (cf. reviews in EDWARDS 1979, 1984; VITT 1984). Electron microscopy has contributed immensely to the description of these complex structures (SHAW & ROHRER 1984; SHAW 1985a, b; SHAW 1986; LEWINSKY 1989). Futhermore, developmental studies of the three amphithecial layers that

give rise to the peristome [i.e. the outer peristomal layer (OPL), primary peristomal layer (PPL) and inner peristomal layer (IPL)] have provided further characters on which to base peristome homology assessments (Blomquist & Robertson 1941; Saito & Schimoze 1954; Saito 1956; Stone 1961; Edwards 1979; Shaw 1985a; Shaw & Anderson 1988; Shaw & al. 1989a, b; Schwartz 1994). Despite this increased awareness of the morphological complexity of the peristome, an understanding of evolutionary relationships among the major peristome types remains elusive (Vitt & al. 1998). Hence, the degree to which current classifications reflect phylogeny is largely unknown and has only recently begun to be addressed by modern phylogenetic techniques (Mishler & Churchill 1984, Mishler & al. 1992, Hedenäs 1994, De Luna 1995, Hedderson & al. 1998b). The most recent reassessment of higher-level relationships and classification is that of Vitt (1982, revised 1984; cf. Vitt & al. 1998).

Among the arthrodontous mosses, Vitt (1984; cf. Vitt & al. 1998) recognized five major peristome groupings: 1) Diplolepideous opposite (*Funariineae*, *Splachnineae*), 2) Diplolepideous Orthotrichaceous (*Orthotrichineae*), 3) Diplolepideous alternate (*Bryineae*, *Hypnineae*, *Leucodontineae* and *Hookeriineae*; most members of this group bear cilia on the endostome, and are thus frequently referred to as the ciliate mosses), 4) Diplolepideous flanged (*Encalyptineae*, *Buxbaumiineae*) and 5) Haplolepideous (*Pottiineae*, *Dicranineae*, *Fissidentineae*, *Seligeriineae*, *Grimmiineae*). It was suggested that the diplolepideous opposite (Funariaceous) peristome represents the plesiotypic state from which the Orthotrichaceaous, diplolepideous alternate and haplolepideous configurations arose. Transformation to the haplolepideous peristome was postulated to have occurred via an Encalyptaceous peristome, while the Orthotrichaceous peristome (having both modified diplolepideous opposite and diplolepideous alternate taxa) and diplolepideous alternate peristomes were thought to represent separate evolutionary divergences from the ancestral type.

Within the diplolepideous alternate mosses, an outstanding question concerns the monophyly and origin(s) of mosses with the pleurocarpous growth habit. The recent clarification and re-definition of gametophytic growth patterns by La Farge-England (1996) (whose definitions we adopt in this paper) has questioned again the non-monophyly of the pleurocarpous mosses. Many of the taxa previously considered pleurocarpous (e.g. Vitt 1984) have now been identified by La Farge-England as being cladocarps or acrocarps (i.e. the pseudo-pleurocarps). Thus, although previous treatments suggested that the pleurocarpous habit had arisen on multiple independent occasions, application of La Farge-England's definitions, shows pleurocarpy to be restricted to the *Hypnineae*, *Leucodontineae* and *Hookeriineae*, and to the families *Spiridentaceae* and *Hypnodendraceae* of the *Bryineae*. Buck & Vitt (1986) postulated that the *Hypnodendraceae* belong to the *Hypnales* (*Hypineae*, sensu Vitt 1984) based on the presence of pseudoparaphyllia protecting the branch initials. Cladistic evaluations of the phylogenetic position of the pleurocarpous mosses based on morphological data have been inconclusive (Hedenäs 1994).

The development of molecular techniques, and in particular methods of DNA sequencing, have provided a wealth of data from which to independently establish phylogenies and evaluate the evolution of morphological characters (e.g. Sibley &

AHLQUIST 1987; c.f. DONOGHUE & SANDERSON 1992). Recent phylogenetic analyses of 18S nuclear ribosomal DNA sequences have proven informative with regard to the deeper phylogenetic divergences within the mosses (HEDDERSON & al., unpubl.), and placed the *Funariineae* and the genus *Timmia* HEDW. (*Timmiaceae*, *Bryineae*) as the sister group to a largely unresolved clade containing the remaining arthrodontous mosses.

In this paper we present maximum parsimony and maximum likelihood analyses of three DNA regions to further examine relationships within the arthrodontous mosses. Two chloroplast DNA regions were sequenced, the *trnL* (UAA) 5′ exon – *trnF* (GAA) region (including the *trnL* 5′ exon, intron, the *trnL* 3′ exon and the *trnL* 3′ – *trnF* intergenic spacer; TABERLET & al. 1991) and the *rps4* gene (NADOT & al. 1995). In addition, published 18S rRNA gene sequences were used. Specific questions to be addressed are: a) What are the phylogenetic relationships among the *Splachnineae*, *Orthotrichineae* and the *Bryineae*? b) What are the phylogenetic origins of the pleurocarpous mosses? and c) What are the relationships among the major lineages of dipolepideous alternate mosses?

Materials and methods

Taxa included and sources of materials. The 60 taxa included in the analyses are listed in Table 1, with Genbank accession numbers. Voucher specimens for species from which DNA was extracted as part of this study are deposited at the herbaria indicated in Table 1. Members of the *Funariineae* and *Timmia* were used as outgroups, based on the analyses of HEDDERSON & al. (1998b). Ingroup taxa were chosen to represent all of the major families within the *Bryineae* and to include representatives of putatively related suborders. Taxa for which *rps4* and *trn* sequences were determined comprise 1 *Funariineae*, 1 *Timmia* sp., 2 *Encalyptineae*, 1 *Pottiineae*, 1 *Seligeriineae*, 2 *Grimmiineae*, 4 *Splachnineae*, 3 *Orthotrichineae*, 37 *Bryineae* (13 *Bryaceae*, 1 *Leptostomataceae*, 4 *Mniaceae*, 1 *Spiridentaceae*, 7 *Rhizogoniaceae*, 3 *Hypnodendraceae*, 3 *Bartramiaceae*, 2 *Aulacomniaceae* and 3 *Meesiaceae*), 2 *Hypnineae* (1 *Thuidiaceae* and 1 *Brachytheciaceae*) and 6 *Leucodontineae* (1 *Fontinalaceae*, 1 *Wardiaceae*, 1 *Hedwigiaceae*, 1 *Pterobryaceae* and 2 *Neckeraceae*). A second data set was constructed which included 40 of the above species for which 18S rDNA sequence data were also available.

Methods. Total genomic DNA was extracted using the method of EDWARDS & al. (1991), with subsequent cleaning using the Wizard DNA Clean-up Kit (Promega) and elution in 50 µl of nanopure water. Double-stranded DNA templates were prepared by polymerase chain reaction (PCR), employing 30 cycles of 1 min at 97 °C, 1 min at 52 °C and 3 min at 72 °C, preceded by an initial melting step at 97 °C, and followed by a final extension period of 7 min at 72 °C. Primers trnC and trnF (TABERLET & al. 1991) and rps5 and trnas (NADOT & al. 1995; CHASE, pers. comm.) were used to amplify the *trnL* (UAA) 5′ exon – *trnF* (GAA) region and the *rps4* gene respectively. Amplification was achieved using 2.5 units *Taq* polymerase (Perkin Elmer) in a 100 µl reaction volume (1 × thermostable buffer, 2.5 mM $MgCl_2$, 200 µm dNTPs, 300 µm primer). Fragments were cleaned on a QIAquick™ (Qiagen) PCR purification spin column and sequenced using each amplification primer in conjunction with the ABI Prism™ Dye Terminator Cycle Sequencing Ready Reaction Kit (P. E. Applied Biosystems). Sequencing products were resolved on an ABI (model 373, stretch) automated sequencing machine.

Sequence assembly and analyses. For each taxon and sequenced DNA region, forward (5′–3′) and reverse (3′–5′) sequences were assembled and checked for inaccurate base

Table 1. List of taxa included in this study with GENBANK accession numbers. Classification follows Vitt (1984). * indicates DNAs supplied by B. Goffinet

Taxon	Genbank numbers			Voucher specimen
	18S	rps4	trnL	
Funariineae				
Funaria hygrometrica Hedw.	X74114	AF023776	AF023716	Cox 148 (RNG)
Encalyptineae				
Encalypta rhapdocarpa Schwaegr.	AF023680	AF023777	AF023717	Hedderson 11814 (RNG)
Bryobrittonia longipes (Williams.) Horton.	AF023679	AF023778	AF023718	Vitt s.n. (ALTA)*
Splachnineae				
Tayloria lingulata (Dicks.) Lindb.	AF023690	AF023807	AF023732	Schofield 98443 (ALTA)*
Tayloria orthodonta (P. Beauv.) Demar.	----------	AF023808	AF023733	Pocs & al. 88110/BC (RNG)
Splachnum luteum Hedw.	AF023686	AF023805	AF023740	Hedderson 11837 (RNG)
Tetraplodon mnioides (Hedw.) Br. Eur.	AF023691	AF023804	AF023730	Söderström SN (RNG)
Orthotrichineae				
Macromitrium levatum Mitt.	----------	AF023813	AF023725	Pocs & al. 88102/N (RNG)
Orthotrichum lyelli Hook. & Tayl.	----------	AF023814	AF023727	Hedderson 5745 (RNG)
Ulota phyllantha Brid.	AF023692	AF023812	AF023726	Hedderson 11772 (RNG)
Bryineae				
Amblyodon dealbatus (Hedw.) B. S. G.	----------	AF023806	AF023731	Hedderson 3975a (RNG)
Aulacomnium androgynum (Hedw.) Schwaegr.	AF023688	AF023811	AF023728	Cox 149 (RNG)
Aulacomnium turgidum (Wahl.) Schwaegr.	AF023687	AF023809	AF023729	Hedderson 6385 (RNG)
Anomobryum julaceum (Gaertn., Meyer. & Schreb.) Schimp.	AF023701	AF023786	AF023739	Cox 112 (RNG)
Bartramia stricta Hedw.	AF023698	AF023799	AF023756	Longton 4871 (RNG)
Brachymenium pulchrum Hook.	AF023702	AF023788	AF023759	Swartz 2730 (RNG)
Braithwaitea sulcata (Hook.) Jaeg.	----------	AF023820	AF023745	Streimann 38403 (RNG)
Bryum alpinum Huds. ex With.	AF023700	AF023783	AF023738	Hedderson 11248 (RNG)
Bryum caespiticium Hedw.	AF023703	AF023784	AF023741	Hedderson 10985 (RNG)
Bryum donianum Grev.	AF023704	AF023785	AF023743	Hedderson 11190 (RNG)

Bryum stenotrichum C. M.	----------	AF023787	AF023742	HEDDERSON 10471 (RNG)
Cinclidium stygium SW.	AF023706	AF023791	AF023763	HEDDERSON 10477 (RNG)
Cyrtomnium hymenophyllum (B. S. G.) HOLMEN.	AF023707	AF023792	AF023764	HEDDERSON 4779 (RNG)
Goniobryum subbasilare (HOOK.) LINDB.	----------	AF023824	AF023753	STREIMANN 38105 (RNG)
Hypnodendron dendroides (BRID.) TOUW.	----------	AF023822	AF023746	BAKER 731 (RNG)
Hypnodendron camptotheca (PAR.) TOUW.	----------	AF023821	AF023747	CROSBY 14279 (H)
Rhodobryum giganteum (SCHWAEGR.) PAR.	AF023699	AF023789	AF023737	LONGTON 5073 (RNG)
Leptostomum macrocarpum (HEDW.) R. BR.	AF023696	AF023790	AF023744	FLETCHER SN (RNG)
Leptobryum pyriforme (HEDW.) WILS.	X80980	AF023802	AF023736	COX 121 (RNG)
Leptotheca boliviana HERZ.	----------	AF023816	AF023749	CHURCHILL 16400 (H)
Leptotheca gaudichaudii SCHWAEGR.	----------	AF023823	AF023750	STREIMANN 50993 (H)
Mielichhoferia elongata (HOOK.) HORNSCH.	AF023708	AF023793	AF023766	SHAW SN (RNG)
Mielichhoferia bryoides (HARV.) WIJK & MARG.	AF023709	AF023794	AF023765	HEDDERSON 11713 (RNG)
Meesia uliginosa HEDW.	AF023693	AF023803	AF023735	HEDDERSON 6481 (RNG)
Mnium hornum HEDW.	X80985	AF023796	AF023767	COX 115 (RNG)
Orthodontium lineare SCHWAEGR.	AF023697	AF023800	AF023768	HEDDERSON SN (RNG)
Paludella squarrosa (HEDW.) BRID.	----------	AF023810	AF023734	HEDDERSON 11810 (RNG)
Philonotis fontana (HEDW.) BRID.	AF023694	AF023801	AF023758	COX 117 (RNG)
Pohlia bolanderi (LESQ.) BROTH.	AF023710	AF023798	AF023761	SHAW SN (RNG)
Pohlia cruda (HEDW.) LINDB.	AF023712	AF023795	AF023760	HEDDERSON 10468 (RNG)
Plagiomnum affine (FUNCK) KOP.	AF023711	AF023797	AF023762	HEDDERSON 10461 (RNG)
Plagiopus oederi (BRID.) LIMPR.	----------	AF023833	AF023757	HEDDERSON 11784 (RNG)
Pyrrhobryum spiniforme (HEDW.) MITT.	----------	AF023832	AF023751	POCS & al. 88110/BJ (RNG)
Pyrrhobryum vallis-gratiae (HAMPE.) MANUEL.	AF023695	AF023825	AF023754	HEDDERSON 11755 (RNG)
Rhizogonium novae-hollandiae (BRID.) BRID.	----------	AF023827	AF023752	STREIMANN 36688 (RNG)
Rhizogonium lindigii (HAMP.) MITT.	----------	AF023826	AF023755	LINARES & CHURCHILL 3999 (H)
Spiridens reinwardtii NEES.	----------	AF023828	AF023748	EDDY 6232 (BM)
Timmia sibirica LIND. & ARNELL.	AF023678	AF023775	AF023715	HEDDERSON 6341 (RNG)
Hypnineae				
Brachythecium rutabulum (HEDW.) BR. EUR.	AF023713	AF023818	AF023772	HEDDERSON 11763 (RNG)
Thuidium tamariscinum (HEDW.) BR. EUR.	----------	AF023819	AF023770	HEDDERSON 11766 (RNG)

Table 1 (continued)

Taxon	Genbank numbers			Voucher specimen
	18S	rps4	trnL	
Leucodontineae				
Fontinalis antipyretica Hedw.	AF023714	AF023817	AF023771	Hedderson 11849 (RNG)
Hildebrandtiella pachyclada Besch.	----------	AF023829	AF023774	Pocs & al. 88161/Q (RNG)
Porotrichum vancouveriensis (Kindb. ex Mac.) Crum.	----------	AF023830	AF023773	Hedderson 5774 (RNG)
Rhacocarpus purpurascens (Brid.) Par.	AF023685	AF023815	AF023724	Hedderson 11790 (RNG)
Thamnobryum alopecurum (Hedw.) Nieuwl.	----------	AF023834	AF023769	Cox 147 (RNG)
Wardia hygrometrica Hook.	AF023683	AF023782	AF023720	Hedderson 11709 (RNG)
Pottiineae				
Tortula ruralis (Hedw.) Gaertn., Meyer & Scherb.	AF023682	AF023831	AF023722	Cox 120 (RNG)
Seligeriineae				
Blinda acuta (Hedw.) Br. Eur.	AF023681	AF023781	AF023721	Hedderson 11828 (RNG)
Grimmiineae				
Ptychomitrium gardneri Lesq.	AF023689	AF023779	AF023719	Goffinet 580*
Scouleria aquatica Hook.	AF023684	AF023780	AF023723	Hedderson 5811 (RNG)

calling using SeqMan II (LaserGene System Software, DNAStar, Inc.). Consensus sequences were aligned manually using MegAlign (LaserGene System Software, DNAStar, Inc.) and regions of ambiguous alignment and incomplete data (e.g. at the beginning and end of sequences) were identified and excluded from subsequent analyses. Most insertion and/or deletion events occurred in regions that were subsequently excluded because of alignment difficulties. The remaining insertion and/or deletion events were coded as missing data. Sequences of the 18S rRNA gene were aligned with respect to secondary structure as detailed in HEDDERSON & al. (1998b).

Two combined data sets were constructed in McClade (ver. 3.06; MADDISON & MADDISON 1992); a *rps4/trn* data set comprising 60 taxa and an 18S/*rps4/trn* data set comprising 40 taxa (Nexus-formatted files of these alignments are available from the authors). Phylogenetic analyses were performed using PAUP* (test version d54–d55; SWOFFORD, pers. comm.) mounted on an Apple Macintosh PowerPC (8100/80 or 7600/132, 48MB RAM) or on a Silicon Graphics Indigo2 workstation. The g_1 statistic (HUELSENBECK 1991), based on the frequency distribution of 50000 random tree lengths (constructed using the RANDOM TREES procedure) was used to test for the presence of phylogenetic signal in the data sets. Maximum parsimony (MP) analyses were performed on each data set, implementing the heuristic search procedure with 100 random taxon addition replicates, and using the STEEPEST DESCENT and tree bisection and reconnection (TBR) branch-swapping options. All characters were equally weighted and character states were treated as unordered. Branches with a maximum length of zero were collapsed, and all most parsimonious trees were saved (MULPARS). Branch lengths of optimal trees were calculated using ACCTRAN character transformation. The probability of 'semi-strict' correct inference under the parsimony criterion was calculated according to GIVNISH & SYTSMA (1997). Support for individual nodes was evaluated using the decay index ('d.i'.) (BREMER 1988, MISHLER & al. 1991) aided by the programme AutoDecay 3.03 (ERIKKSON & WIKSTRÖM 1995), and by Parsimony Jackknife ('Jac'.) (version 4.22) percentages from 10000 Jackknife replicates (FARRIS & al. 1996).

Maximum likelihood (ML) analyses were performed using PAUP* under the Jukes-Cantor (JC69) (JUKES & CANTOR 1969) and general time-reversible (GTR) (YANG 1994a) models of nucleotide substitution, with rate variation among sites presumed to follow a gamma distribution ('dG') approximated by four rate categories (YANG 1994b). Tree scores for the most parsimonious trees (MPTs) were evaluated under the GTR+dG substitution model and the model parameter estimates of the tree yielding the highest likelihood were fixed in subsequent ML analyses. Heuristic searches employing TBR branch swapping were performed. Due to the large number of MPTs found in the analysis of the *rps4/trn* data set, 10 trees were chosen at random and GTR+dG ML analyses were performed as described above.

User-defined tree topologies were used to test alternative phylogenetic hypotheses for significant differences (evaluated at $p \leq 0.05$ unless stated otherwise) in tree length or likelihood using the Kishino-Hasegawa statistic (K. H.) (KISHINO & HASEGAWA 1989).

Results

rps4/trn data set. Alignment of the *rps4/trn* data set resulted in a total of 1492 positions, of which 698 were *rps4* sites and 796 *trn* sites; 519 sites were excluded due to ambiguous alignment in the non-coding region at the 5′ end of the *rps4* gene and in the *trn*L intron, and because of missing data at sequence extremities. Of the remaining 975 sites, 270 were parsimony informative, of which 182 (67.4%) were *rps4* (53 1st: 40 2nd: 89 3rd codon position) and 88 *trn* (32.6%). The frequency

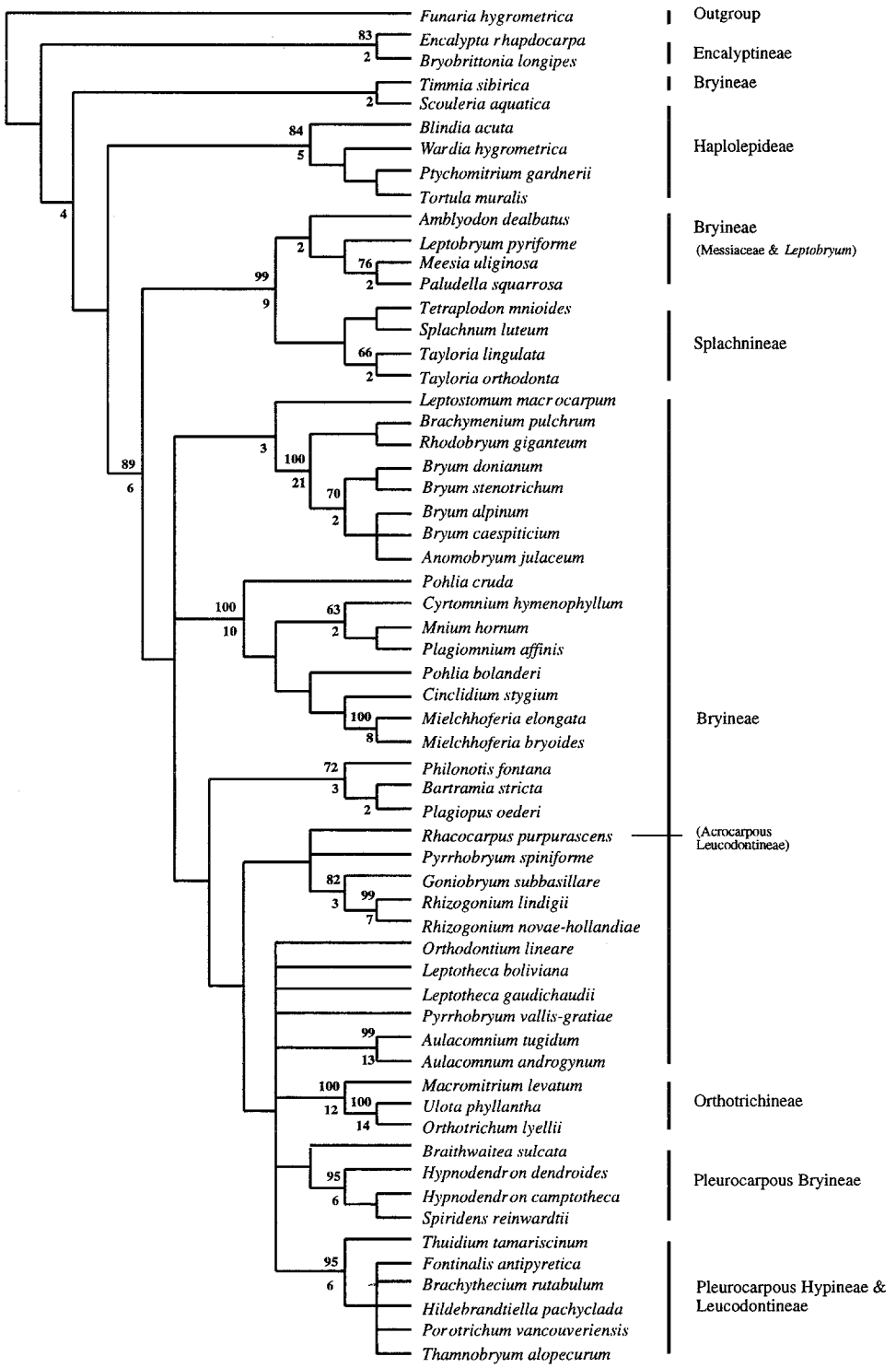


Fig. 1. The strict consensus of 396 MPT's (L=1161; C.I.=0.36, R.I.=0.60) found during equally weighted parsimony analysis of combined chloroplast *rps4* and *trn* sequences from 60 species representing all the suborders of ciliate *Bryales* and the main families of the *Bryineae*. Numbers above the branch are Jackknife percentages (>50%) based on 10000 replicates; numbers below are decay indices. Higher-level taxonomy follows Vitt (1984)

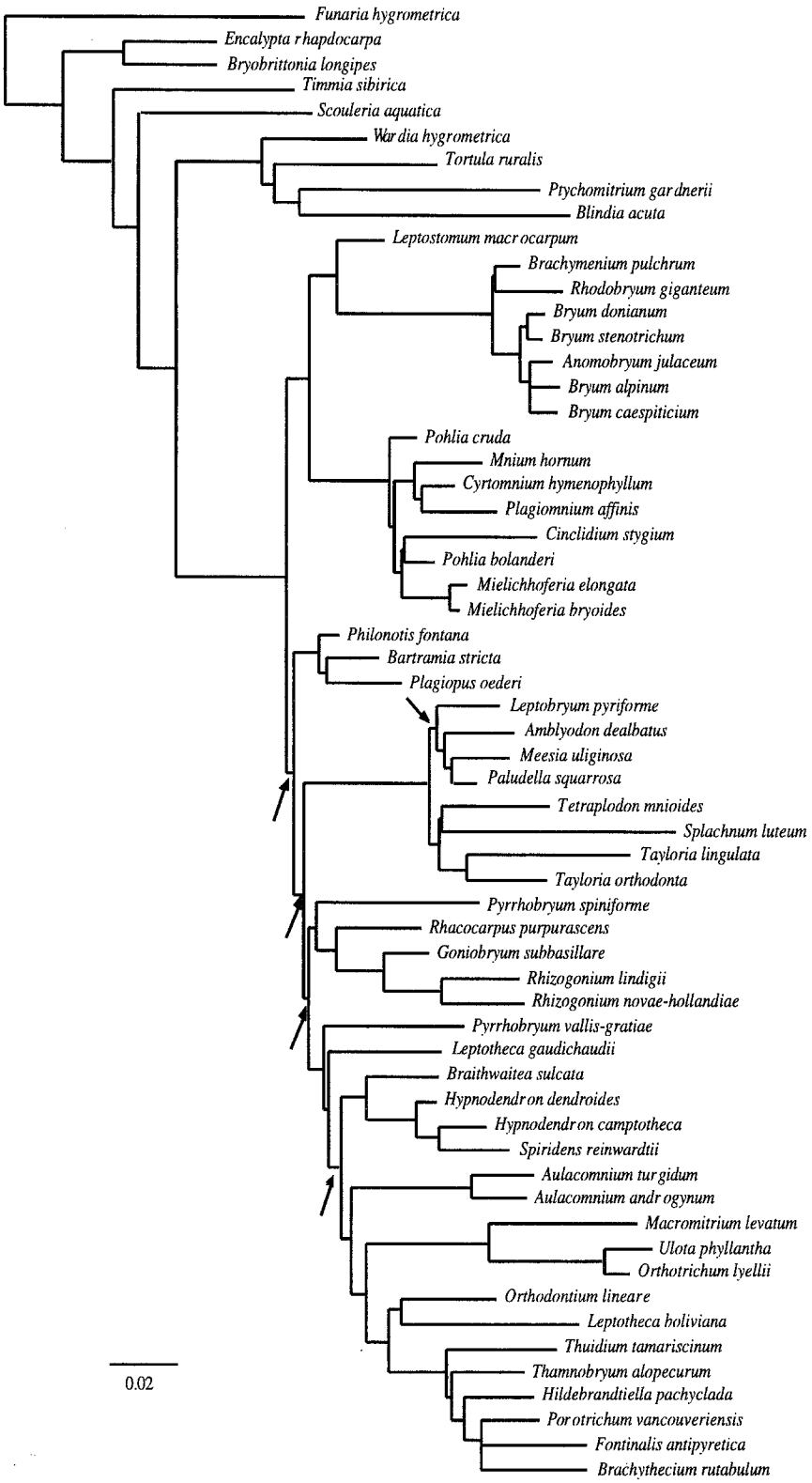
Table 2. Summary of parsimony analyses in ciliate arthrodontous mosses. *Incl.* Number of included characters, *Infor.* number of informative characters, *MPTs* most parsimonious trees, *Length* tree length, *CI* Consistency Index, *RI* Retention Index, *RC* Rescaled Consistency Index, *HI* Homoplasy Index, *gl* statistic based on 50000 randomly generated trees, CI_9 Standardized Consistency Index, *GS* Givnish & Sytsma statistic

	Incl.	Infor.	MPTs	Length	CI	RI	RC	HI	g_1	CI_9	GS Stat.
rps4/trn	975	270	396	1161	0.36	0.60	0.22	0.63	−0.634	0.842	0.628
18S/*rps4/trn*	2735	295	12	1041	0.42	0.62	0.26	0.58	−0.706	0.817	0.758

distribution of tree lengths for 50000 randomly generated trees was significantly left-skewed ($g_1 = -0.634$; $p \ll 0.01$), suggesting the presence of significant phylogenetic signal in the data set. MP analysis resulted in 396 MPTs (Length 1161, CI=0.36, RI=0.6), the strict consensus of which is shown in Fig. 1, with Jackknife support (>50%) indicated above branches and decay indices (>1) below. The estimated probability of recovering the correct phylogeny from these data is 0.63. Summary statistics for the parsimony analyses are presented in Table 2.

Of the putative ingroup taxa, *Wardia hygrometrica* Hook. (*Wardiaceae*) is resolved among the haplolepideous groups (Fig. 1). The remaining ingroup taxa form a well supported clade (Jac. 89; d.i.=6) sister to the haplolepideae, within which several major groupings are discernible. A clade consisting of the *Meesiaceae*, *Leptobryum pyriforme* (Hedw.) Wils. (*Bryaceae*) and the *Splachnineae* is very strongly supported (Jac. 99%; d.i.=9), and forms the sister group to the remaining taxa. The *Mniaceae*, *Pohlioideae* and *Mielichhoferioideae* form a very strongly supported monophyletic group (Jac. 100%; d.i.=10), as do the *Bryoideae* (*Bryum*, *Brachymenium*, *Rhodobryum*, *Anomobryum*); *Leptostomataceae* is resolved, with somewhat weaker support, (d.i.=3) as sister to the latter. These two clades form a trichotomy with the clade containing the remaining taxa. The monophyly of the *Bartramiaceae* is moderately well supported (Jac. 72%; d.i.=3), and this family is resolved as sister to a clade containing a weakly supported group including some *Rhizogoniaceae* and *Rhacocarpus pupurascens* (Brid.) Par. (*Hedwigiaceae*). This grouping is placed sister to a largely unresolved clade containing Rhizogoniaceous taxa, *Orthodontium lineare* Schwaegr. (*Bryaceae*) and the following apparently monophyletic groups; *Aulacomniaceae*, *Orthotrichineae*, pleurocarpous *Bryineae*, *Hypnineae* and *Leucodontineae*. The clade containing the *Hypnodendraceae* and *Spiridentaceae* is well supported (Jac. 95%; d.i.=6), as is a clade containing *Hypnineae* and *Leucodontineae* (excluding *Rhacocarpus purpurascens*) (Jac. 95%; d.i.=6).

Of the 10 MPTs chosen, the tree with the highest likelihood provided the following estimates of the GTR+dG model parameters: rmatrix=(1.47, 5.37, 0.48, 1.10, 5.85, 1, corresponding respectively to A-C, A-G, A-T, C-G, C-T, G-T substitution types with G-T arbitrarily set to 1), proportion of characters invariant (Pinvar)=0.33, shape parameter of gamma distribution (Shape) = 0.67 (PAUP* notation). The GTR+dG substitution model provided a significantly better fit to the data than the JC69+dG model ($-2\log\Lambda = 1201.7, \chi^2 : p \ll 0.01$). Figure 2 shows one of the three most likely trees (Ln-likelihood=8259.19) based on the GTR+dG model (branches marked with an arrow are too short to be visible at the

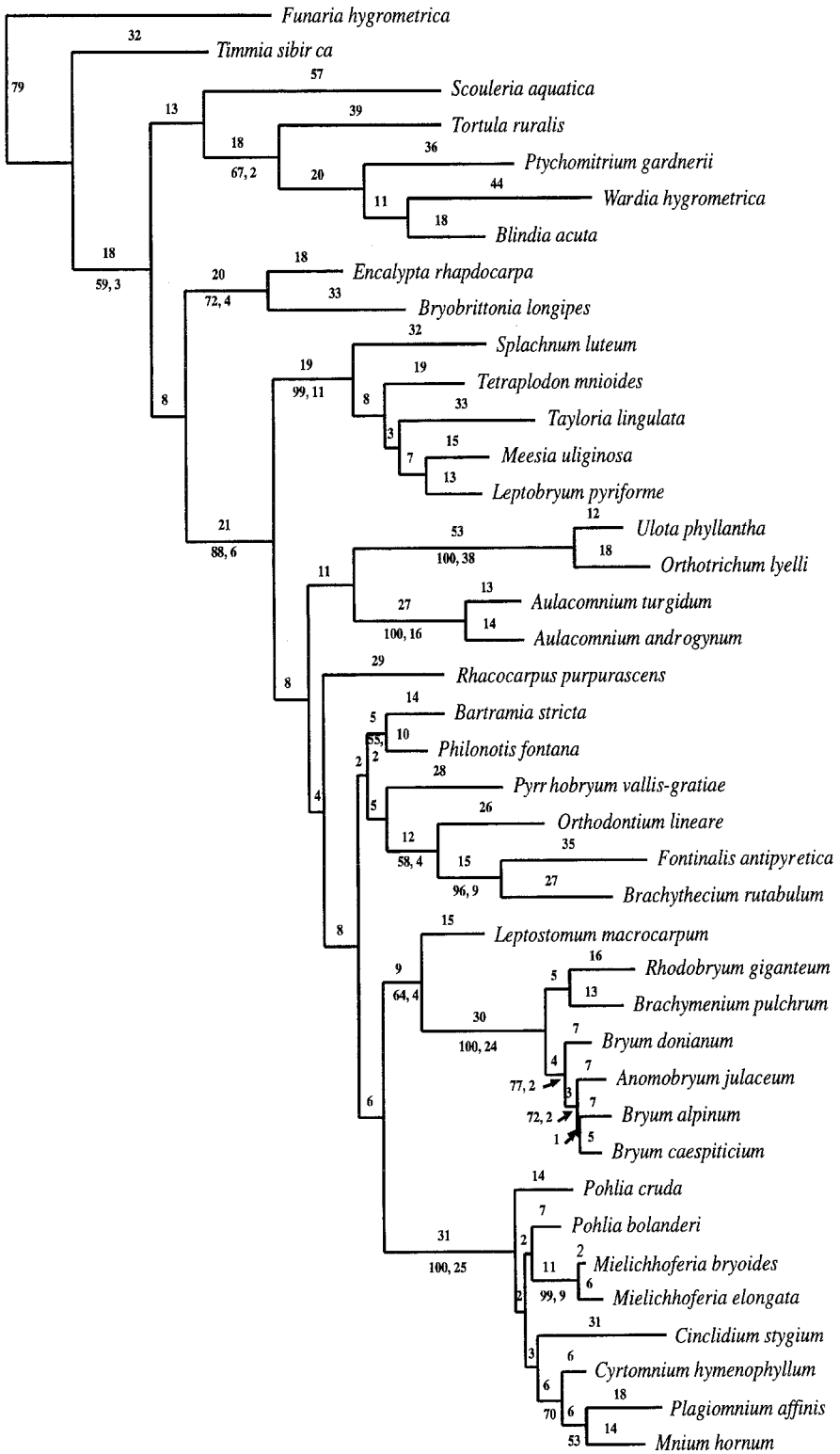

Funaria hygrometrica
Encalypta rhapdocarpa
Bryobrittonia longipes
Timmia sibirica
Scouleria aquatica
Wardia hygrometrica
Tortula ruralis
Ptychomitrium gardnerii
Blindia acuta
Leptostomum macrocarpum
Brachymenium pulchrum
Rhodobryum giganteum
Bryum donianum
Bryum stenotrichum
Anomobryum julaceum
Bryum alpinum
Bryum caespiticium
Pohlia cruda
Mnium hornum
Cyrtomnium hymenophyllum
Plagiomnium affinis
Cinclidium stygium
Pohlia bolanderi
Mielichhoferia elongata
Mielichhoferia bryoides
Philonotis fontana
Bartramia stricta
Plagiopus oederi
Leptobryum pyriforme
Amblyodon dealbatus
Meesia uliginosa
Paludella squarrosa
Tetraplodon mnioides
Splachnum luteum
Tayloria lingulata
Tayloria orthodonta
Pyrrhobryum spiniforme
Rhacocarpus purpurascens
Goniobryum subbasillare
Rhizogonium lindigii
Rhizogonium novae-hollandiae
Pyrrhobryum vallis-gratiae
Leptotheca gaudichaudii
Braithwaitea sulcata
Hypnodendron dendroides
Hypnodendron camptotheca
Spiridens reinwardtii
Aulacomnium turgidum
Aulacomnium androgynum
Macromitrium levatum
Ulota phyllantha
Orthotrichum lyellii
Orthodontium lineare
Leptotheca boliviana
Thuidium tamariscinum
Thamnobryum alopecurum
Hildebrandtiella pachyclada
Porotrichum vancouveriensis
Fontinalis antipyretica
Brachythecium rutabulum
0.02

scale of the Figure and have been arbitrarily lengthened). The topology of the strict consensus of the three trees differs from Fig. 2 only in the lack of resolution among *Bryum caespiticium* HEDW., *B. alpinum* WITH. and *Anomobryum julaceum* (GAERTN., MEYER & SCHREB.) SCHIMP. The GTR+dG ML tree differs from the MPT's with respect to the groupings of the major clades identified in the parsimony analysis. The *Leptostomataceae/Bryoideae* clade is resolved as the sister group to the *Mniaceae/Pohlioideae* clade, and together these form a clade co-ordinate with the remaining ingroup taxa. The next three nodes resolve the *Bartramiaceae*, the *Splachnineae/Meesiaceae* clade and the *Rhacocarpus purpurascens/Rhizogoniaceous* clade, respectively, as sister to the remaining taxa. The clade *Orthodontium lineare* to *Thamnobryum alopecurum* (HEDW.) NIEUWL. (*Neckeraceae*) of the MP analyses is fully resolved in the ML tree with *Orthodontium lineare* and *Leptotheca boliviana* HERZ. (*Rhizogoniaceae*) forming the sister clade to the *Hypnineae/Leucodontineae* clade. The GTR+dG ML tree is not significantly more likely than the MP tree used to derive the model parameters (K.H test: $p=0.393$ under the GTR+dG model).

18S/rps4/trn data set. The combined 18S/*rps4/trn* data set yielded 3494 aligned positions. A total of 759 sites were excluded due to ambiguity in sequence alignment and incomplete data at the sequence extremities. Of the remaining 2735 positions, 295 were parsimony informative, of which 67, 150 and 78 were from the 18S, *rps4* and *trn* sequences, respectively. The frequency distribution of lengths for 50000 randomly generated trees from this data set is significantly left-skewed ($gl=-0.706$; $p \ll 0.01$), indicating the presence of phylogenetic signal. The probability of recovering the correct topology with this data set is 0.76 (Table 2). MP analysis recovered 12 equally optimal trees (Length 1041, CI=0.42, RI=0.62), one of which is shown in Fig. 3, with branch lengths indicated above branches, and Jackknife percentages (>50%) and decay indices (>1) below. The remaining 11 trees differ from the topology shown in Fig. 3 with regard to the relationships between *Bryum caespiticium*, *B. alpinum* and *Anomobryum julaceum* and to the placement of *Pohlia bolanderi* (LESQ.) BROTH. and *Cinclidium stygium* SW. (*Mniaceae*) within the *Mniaceae/Pohlioideae* clade.

The *Splachnineae/Orthotrichineae*/diplolepideous alternate clade is again very well supported (Jac. 88%; d.i.=6), with *Wardia hygrometrica* placed among the haplolepideous taxa (Fig. 3). The well supported clades recovered in the MP analyses of the previous data set are again resolved (e.g. *Splachnineae/Meesiaceae*; *Mniaceae/Pohlioideae*). The *Meesiaceae/Splachnineae* clade is resolved as sister to the remaining taxa. At the next two nodes, the *Orthotrichineae* + *Aulacomniaceae*, and secondly, *Rhacocarpus purpurascens* are split from the remaining taxa. The *Leptostomataceae* + *Bryoideae* is sister to the *Mniaceae* + *Pohlioideae*, and

Fig. 2. One of three maximally likely (Ln-likelihood=−8259.19) trees obtained from analyses of combined chloroplast *rps4* and *trn* sequences. Arrows indicate those branches which were too short to be depicted at the scale of the Figure presented, and which have been arbitrarily lengthened. The two trees not presented differed only with respect to relationships in the clade containing *Bryum caespiticium*, *B. alpinum* and *Anomobryum julaceum*. Scale bar indicates % substitutions per site


Funaria hygrometrica
Timmia sibir ca
Scouleria aquatica
Tortula ruralis
Ptychomitrium gardnerii
Wardia hygrometrica
Blindia acuta
Encalypta rhapdocarpa
Bryobrittonia longipes
Splachnum luteum
Tetraplodon mnioides
Tayloria lingulata
Meesia uliginosa
Leptobryum pyriforme
Ulota phyllantha
Orthotrichum lyelli
Aulacomnium turgidum
Aulacomnium androgynum
Rhacocarpus purpurascens
Bartramia stricta
Philonotis fontana
Pyrrhobryum vallis-gratiae
Orthodontium lineare
Fontinalis antipyretica
Brachythecium rutabulum
Leptostomum macrocarpum
Rhodobryum giganteum
Brachymenium pulchrum
Bryum donianum
Anomobryum julaceum
Bryum alpinum
Bryum caespiticium
Pohlia cruda
Pohlia bolanderi
Mielichhoferia bryoides
Mielichhoferia elongata
Cinclidium stygium
Cyrtomnium hymenophyllum
Plagiomnium affinis
Mnium hornum

this entire group is in turn sister to a clade consisting of the *Bartramiaceae*, *Rhizogoniaceae*, *Orthodontium lineare*, *Hypnineae* and *Leucodontineae*.

Of the 12 MPTs, the topology with the highest likelihood provided the following GTR+dG model parameter estimates: rmatrix = (1.33 4.93 0.88 1.15 5.73) pinvar=0.666 shape=0.62. The GTR+dG substitution model again provided a significantly better fit to the data than did the JC69+dG model ($-2 \log \Lambda = 834.6$, $\chi^2 : p \ll 0.01$). Figure 4 depicts one of two most likely topologies (Ln-likelihood = 11042.38) recovered under the GTR + dG model (branches indicated by an arrow are too short to be visible at the scale of the Figure and have been arbitrarily lengthened). The two trees differ only in the placement of *Bryum caespiticium*. These topologies are not significantly more likely than the tree used to estimate the GTR+dG ML model parameters (K.H. test: p=0.1415). A GTR+dG ML analysis with the starting tree computed by stepwise addition found two most likely trees (Ln-likelihood = 11042.54; not shown). These place the *Orthotrichaceae* and then the *Aulacomniaceae*, respectively, as sister to the remaining ingroup taxa. Although suboptimal, these trees are not significantly less likely than the optimal trees recovered and shown in Fig. 4.

Discussion

The phylogenetic relationships resolved in these analyses conflict substantially with those implied by previous classifications based largely on peristomes (e.g. VITT 1984), especially if taxa at the same rank are interpreted as co-ordinate monophyletic groups. However, peristome 'types' per se are not formal cladistic characters, but rather character complexes that may be further broken down into putatively homologous characters. Thus the extent to which the molecular phylogeny conflicts with peristome characters remains unclear.

If the haplolepideae are sister to the Ciliate/*Splachnineae*/*Orthotrichineae* clade, as our analyses suggest, the asymmetric division in the IPL at the eight cell stage constitutes a likely synapomorphy for the two (cf. VITT & al. 1998). The small number of taxa sampled for the haplolepideae precludes any extensive evaluation of relationships within the group, due partly to the probable presence of 'long branches' for these disparate taxa (FELSENSTEIN 1978). However, it is clear that the gymnostomous *Wardia hygrometrica* belongs with the haplolepideous taxa as discussed more fully elsewhere (HEDDERSON & al. 1998a).

It is clear from our analyses that the *Bryineae* is non-monophyletic, and some lineages within this group as currently defined share more recent common ancestry with the *Orthotrichineae*, *Splachnineae* or pleurocarpous mosses than with other members of the suborder. The 4:2:4 (OPL, PPL, IPL) aligned cell patterns and

Fig. 3. One of 12 MPT's (L=1041; C.I.=0.42; R.I.=0.62) from equally weighted parsimony analyses of combined nuclear 18S rRNA gene, and chloroplast *rps4* and *trn* sequences of 40 species representing the sub-orders of the ciliate *Bryales* and the main families of the *Bryineae*. Numbers above branches are branch lengths. Numbers below the branch are, respectively, Jackknife support percentages (>50%) based on 10000 replicates, and decay indices

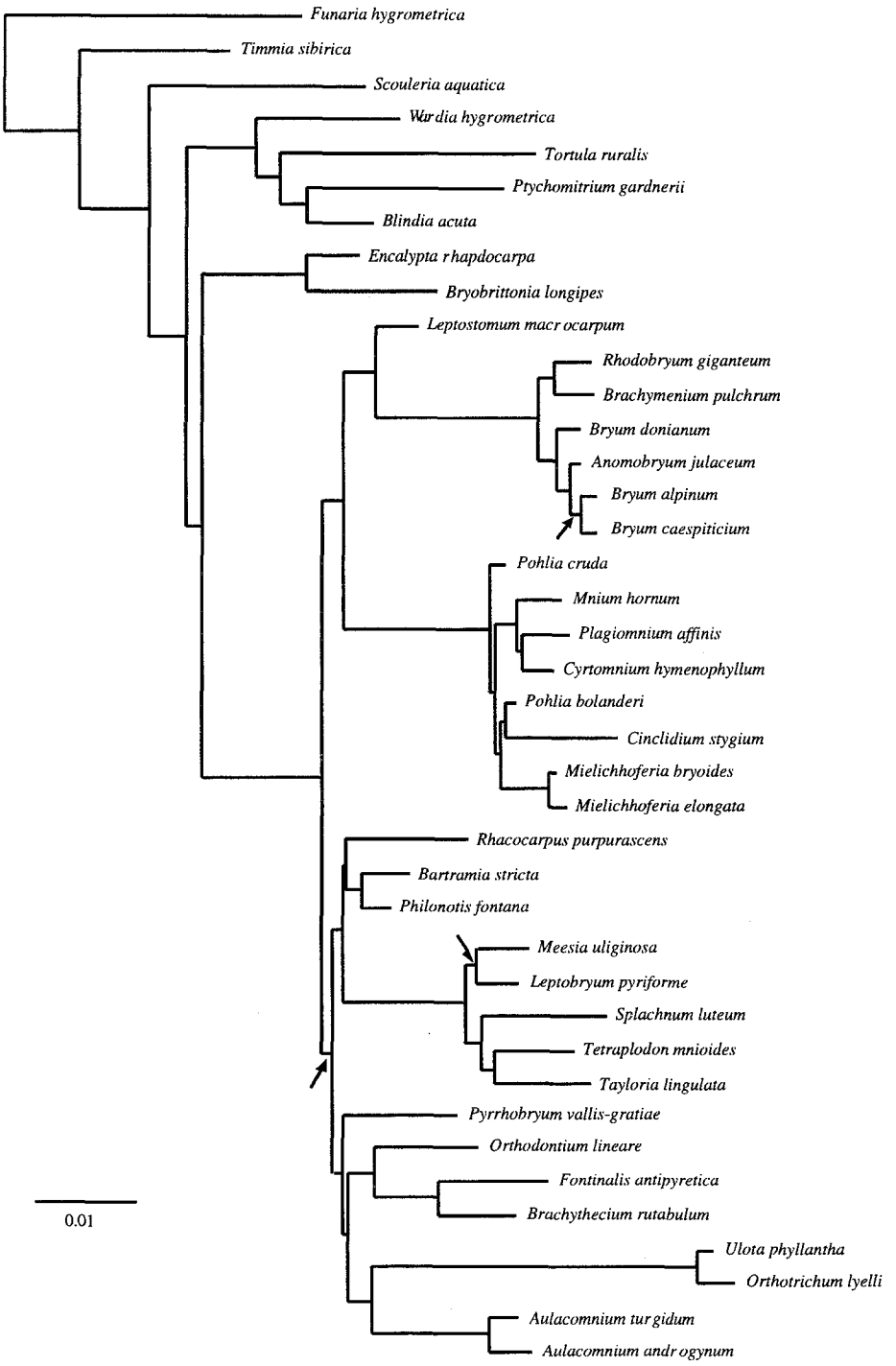


Fig. 4. One of two maximally likely trees (Ln-likelihood=11042.36) from analyses of combined 18S rDNA, and chloroplast *rps4* and *trn* sequences. Arrows indicate those branches which were too short to be depicted at the scale of the Figure presented, and which have been arbitrarily lengthened. The second optimal tree differs only in the placement of *Bryum caespiticium* as the sister taxon to *Anomobryum julaceum*. Scale bar indicates % substitutions per site

opposite positioning of the peristome teeth exhibited by the *Funariineae*, *Splachnineae* and some *Orthotrichineae* are thus probably non-homologous since, on balance, the topologies of Figs. 1–4 imply that the Splachnaceous and Orthotrichaceous cell patterns are independently derived by reduction from the 4:2:6/8 pattern of the *Bryineae*. There are no published detailed ontogenetic data for the Splachnaceous or Orthotrichaceous peristomes (but see SCHWARTZ 1994), and it is possible that the alignment of cell walls at maturity may represent a secondary displacement after an asymmetric division.

The monophyly of the group comprising the *Splachnineae*, *Orthotrichineae* and the diplolepideous alternate mosses is strongly supported in the analysis of the chloroplast *rps4/trn* data set. While a number of major lineages are well resolved within the ingroup, their relationships to each other vary between analyses. It is evident that the *rps4/trn* data do not provide sufficient characters for confident resolution of relationships among these lineages and, even with the addition of the more conserved 18S rDNA sequences, support for many internal branches is lacking. We discuss some of the better supported lineages below.

The *Splachnineae-Meesiaceae* lineage. The *Splachnineae* is very well supported as the sister group to the *Meesiaceae*. This relationship indicates that the Splachnaceous peristome has been derived from the diplolepideous alternate configuration, as the alternative, and less parsimonious reconstruction, would postulate that the diplolepideous alternate configuration had arisen twice. The placement of *Leptobryum pyriforme* with the *Meesiaceae* agrees well with overall gametophytic similarity (CRUM & ANDERSON 1981). In the MP analyses of the *rps4/trn* data, *Leptobryum* is embedded within the *Meesiaceae*, while ML analyses of both data sets place *Leptobryum* as the sister taxon to the *Meesiaceae*. This latter topology is more congruent with peristome morphology, as it would suggest that the Meesiaceous peristome had evolved once by reduction from a ciliate diplolepideous alternate peristome, such as that of *Leptobryum*. At present there are few apparent morphological synapomorphies for the *Splachnineae/Meesiaceae* clade, but the presence in all these taxa of axillary hairs with single clavate hyaline apical cells and several coloured basal cells may provide one such character. These hairs are often cited as a defining character of the *Splachnaceae* (e.g. KOPONEN 1994), but those of the *Meesiaceae* and *Leptobryum* are very similar, and our own observations suggest they fall well within the variation exhibited by the *Splachnaceae*.

The *Orthotrichineae-Aulacomniaceae* lineage. The placement of the *Orthotrichineae* is ambiguous, being either resolved near the pleurocarpous taxa (Figs. 1, 2, 4) or in a more basal position (Fig. 3). It is noteworthy that the cladistic analyses of morphological characters by HEDENÄS (1994) also suggested a relatively close relationship between the *Orthotrichaceae* (represented by *Schlotheimia*) and the pleurocarps. Analyses of 18S rRNA gene sequences (HEDDERSON & al. 1998b) indicated a sister relationship between *Orthotrichineae* and *Aulacomniaceae*. This relationship is not contradicted by the MP analyses of the *rps4/trn* chloroplast data (Fig. 1) and occurs in 55% of the 396 MPTs. Although ML analyses are not able to confidently reject an alternative placement of *Orthotrichineae* as sister to the remaining ingroup taxa (i.e. such topologies are less likely, but not significantly so under the GTR model), the weight of evidence from molecular analyses (Figs. 3, 4)

and morphological similarities (Hedderson & al. 1998b) suggests a close (perhaps sister) relationship with the *Aulacomniaceae*. If this is the case, then the Orthotrichaceous peristome has arisen by reduction from a diplolepideous alternate ancestor, as suggested by Shaw & Rohrer (1984) and Shaw (1986). If the alternate arrangement of peristome segments is homologous in the *Bryineae* and *Orthotrichineae*, this arrangement would appear to be the plesiotypic state within the *Orthotrichineae* (cf. Vitt & al. 1998).

The morphological analyses of De Luna (1995), concerned primarily with the relationships of the *Hedwigiaceae*, found the cladocarpous *Orthotrichineae* to be more closely related to the pleurocarps than the acrocarpous *Orthotrichineae*. The *Hedwigeaceae*, (except *Rhacocarpus*), were placed in the grade between these two groups. Although only three taxa of the *Orthotrichineae* were sampled in our analyses, the strong support for the grouping of the cladocarpous *Macromitrium* with the acrocarpous *Orthotrichum* and *Ulota* conflicts strongly with the findings from morphology. Similarly, the analyses of 18S rRNA gene sequences by Hedderson & al. (1988b) clearly unite *Rhacocarpus* and *Hedwigia* and resolve them sister to the *Aulacomnium* and *Orthotrichineae* lineage. The analyses presented here provide no strong support for the placement of *Rhacocarpus* (hence *Hedwigiaceae*), but it seems clearly to fall outside the pleurocarpous taxa. The presence of pseudoparaphylia in the *Hedwigiaceae* thus implies the separate evolution of pseudoparaphyllia in this group and in the pleurocarps (cf. Ireland 1971).

The *Mniaceae-Bryaceae* lineage. The *Bryaceae* as presently circumscribed is a gametophytically diverse assemblage, characterized sporophytically by the possession of elongate capsules with a conspicuous neck that tapers to the seta (Crum & Anderson 1981). These characters are not unique to the *Bryaceae* and are found in other families of the *Bryineae* such as the *Meesiaceae* and *Aulacomniaceae*. Similarly, no unique characters group *Pohlia* and *Bryum*, although traditionally a close relationship between these genera has been assumed. Given the lack of unique defining characters for the *Bryaceae*, it is perhaps not surprising that the phylogenetic analyses presented here show that the family is highly polyphyletic.

The well supported relationship of *Pohlia* and *Mielichhoferia* to the *Mniaceae* in the MP and ML analyses of the chloroplast *rps4/trn* data set is congruent with the findings of the nuclear 18S rRNA analyses of Hedderson & al. (1998b). The nodose cilia and presence of sunken stomata in the *Mniaceae* and *Pohlia* may represent sporophytic synapomorphies for this clade, while the formation of more than one gametophore bud from a single protonemal cell (Allsop & Mitra 1958) may also be unique to this clade.

While high support indices (Jac. 100; d.i.=25) and inter-genomic congruence can leave little doubt concerning the validity of the Mniaceous/Pohliaceous clade, it appears to be highly incongruous gametophytically. However, similarity between the juvenile leaves of certain *Mniaceae* (e.g. *Mnium hornum* Hedw.) and the mature leaves of *Pohlia/Mielichhoferia* suggests the possibility of a heterochronic shift in developmental evolution (Gould 1977, Mishler 1986, Mishler & De Luna 1991). Such a hypothesis could only be validated after careful ontogenetic observation and is well beyond the scope of this paper. Nevertheless, we suggest that this clade may

represent an ideal study group for the investigation of the rôle of heterochrony in moss evolution.

The sister relationship between the *Leptostomataceae* and *Bryaceae* (which here consists of only the *Bryoideae*) is in agreement with the placement by VITT (1984: fig. 29; cf. BROTHERUS 1924). The placement of this entire clade as sister to the Mniaceous clade is similarly in accordance with more traditional classificatory concepts, which recognize similarity between *Rhodobryum* and members of the *Mniaceae* such as *Plagiomnium* (e.g. NYHOLM 1954).

The genus *Orthodontium*, usually placed within the *Bryaceae* due to perceived sporophytic similarities, is not related to either the Mniaceous or Bryaceous lineages, but appears to be more closely related to the pleurocarpous taxa. As noted above for *Leptobryum* (traditionally also placed in the Bryaceae), *Orthodontium* shows very little gametophytic similarity to the other Bryaceae, and also exhibits a number of substantial differences in features such as spore ornamentation.

The *Bartramiaceae*, *Rhizogoniaceae* and pleurocarpous taxa. The *Bartramiaceae* is supported as a monophyletic taxon in both MP analyses and placed sister to the *Rhizogoniaceae*/pleurocarps, but without support for this position. The *Rhizogoniaceae* appear to be polyphyletic, with *Leptotheca boliviana* HERZ., *L. gaudichaudii* SCHWAEGR. and *Pyrrhobryum vallis-gratiae* (HAMPE.) MANUEL more closely related to the pleurocarps than to the clade containing *Rhizogonium lindigii* (HAMP.) MITT, *R. novae-hollandiae* (BRID.) BRID. and *Goniobryum subbasillare* (HOOK.) LINDB. (Figs. 1, 2). Although their exact placement remains somewhat ambiguous, our data provide no support for the relationship of *Rhizogoniaceae* to the *Mniaceae* as suggested by the analyses of KOPONEN (1988).

The pleurocarpous taxa of the *Hypnineae* and *Leucodontineae* emerge as a very well supported clade separate from the pleurocarpous *Bryineae* in the MP analyses of the *rps4/trn* data sets. Consequently, these analyses provide no evidence for the placement of the *Hypnodendraceae* in the *Hypnales* (*-ineae* sensu VITT 1984) as suggested by BUCK & VITT (1986). Similarly, the sister relationship of *Braithwaitea* to the *Hypnodendraceae/Spiridentaceae* in these analyses does not support the removal of *Braithwaitea* from a position close to the *Hypnodendraceae*, as suggested by TOUW (1971) and BUCK & VITT (1986). The absence of pseudoparaphyllia in the *Spiridentaceae* suggests their secondary loss in this taxon, as they are present in both *Braithwaitea* and *Hypnodendron*. As noted by NORRIS & KOPONEN (1996), the presence of macronemata (normally of feature of the *Bryineae*) in the *Hypnodendraceae* would be anomalous within the *Hypnineae*. With the removal of the *Hypnodendraceae* and *Hedwigiaceae* from *Hypnineae* and *Leucodontineae*, respectively, none of the morphological characters cited by BUCK & VITT (1986) as synapomorphous for the clade containing Hypnalean, Leucodontalean and Hookerialean mosses remain as uncontradicted synapomorphies BUCK & VITT 1986: fig. 65, tab. 1).

While the two pleurocarpous clades (sensu LA FARGE-ENGLAND 1996) do not form a monophyletic group in any of the MPTs of the *rps4/trn* analysis, the clade is present in those trees one step longer (i.e. 1162). These suboptimal trees are not significantly longer than the MPTs under the Kishino-Hasegawa test (p=0.81), and furthermore a topology constraining the monophyly of the pleurocarps is not significantly less likely than the tree presented in Fig. 2 under the GTR+dG ML

model (K.H. test, $p > 0.05$). Hence, the monophyly of the pleurocarps cannot be rejected with the current data set.

The analyses presented here resolve some novel and well-supported groups within the ciliate mosses. At present, many of these have no obvious non-molecular synapomorphies, but continued analysis of morphological characters in a formal cladistic context will undoubtedly provide these for at least some of the clades. An intriguing corollary to the results presented here is that gametophytic characters may have considerably more phylogenetic and taxonomic utility than they have hitherto been allocated. At very least, the molecular data suggest new avenues for the continued exploration of morphology and anatomy of mosses. Within this morphological framework, recognition that the *Splachnineae*, *Orthotrichineae* and pleurocarpous mosses have arisen from within the *Bryineae* (as circumscribed traditionally) is crucial to understanding evolutionary trends within the arthrodonts.

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