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CIRCUMSCRIPTION AND PHYLOGENY OF THE ORTHOTRICHALES (BRYOPSIDA) INFERRED FROM *RBCL* SEQUENCE ANALYSES[1]

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The affinities as well as the circumscription of the Orthotrichaceae (Bryopsida), one of the most diverse families of mosses, have been the focus of a controversy for much of the last century. We obtained *rbcL* sequences for 37 arthrodontous mosses, including 27 taxa of the Orthotrichales. The sequences were analyzed using maximum parsimony and neighbor joining in order to (1) test the monophyly of the Orthotrichales and the Orthotrichaceae; (2) determine their phylogenetic relationships; and (3) test the current subfamilial classification within the Orthotrichaceae. Both analyses suggest that the Orthotrichales are polyphyletic. The Erpodiaceae and the Rhachitheciaceae as well as *Amphidium* and *Drummondia,* two genera of the Orthotrichaceae, are shown to be of haplolepideous affinity. The Splachnales, the Bryales sensu lato, and the Orthotrichales form a monophyletic clade sister to the Haplolepideae. Both neighbor joining and maximum parsimony also suggest that the Orthotrichaceae are composed of two major lineages dominated either by acrocarpous or cladocarpous taxa. The monophyly of the family is, however, only well supported by Tamura's distances. The genera *Macrocoma, Macromitrium, Orthotrichum, Ulota,* and *Zygodon* all appear to be artificial assemblages. This study illustrates the contribution of *rbcL* sequence data to bryophyte systematics and, particularly, in determining the affinities of taxa lacking a peristome, whose characters are central to the classification of mosses.

Key words: bryophytes; evolution; mosses; Orthotrichales; Orthotrichaceae; phylogeny; *rbcL*; systematics.

The central concept in the Hennigian phylogenetic approach is the use of appropriate outgroups, which allows adequate polarization of character state transformation in the ingroup. A reconstruction of the evolutionary history of the Orthotrichaceae, one of the most diverse families of mosses, based on morphology has been hampered by the lack of a peristome in certain taxa, the absence of gametophytic characters that are informative at higher taxonomic levels, and consequently the absence of a consensus regarding the putative sister group and other outgroups to the order. Within the Bryophyta sensu lato, phylogenetic reconstructions using nucleotide sequence data have focused exclusively on the monophyly and the relationships of the division and its classes (e.g., Mishler et al., 1992, 1994; Waters et al., 1992; Capesius, 1995; Bopp and Capesius, 1996; Hedderson, Chapman, and Rootes, 1996) rather than on the evolutionary history of arthrodontous mosses.

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In arthrodontous mosses (Bryopsida; sensu Vitt, Goffinet, and Hedderson, 1997), the peristome teeth that line the mouth of the capsule and regulate spore dispersal are made of cell wall remnants. Three concentric layers, namely the outer (OPL), the primary (PPL), and the inner (IPL) peristomial layer, contribute to the teeth (Blomquist and Robertson, 1941). The number and the pattern of cell divisions occurring in these layers, particularly in the IPL, are central to the classification of arthrodontous mosses. Vitt (1984) recognized five types of articulate peristomes (i.e., four diplolepideous and one haplolepideous type), which characterize the subclasses of the Bryopsida (see also Edwards, 1979, 1984). Vitt, Goffinet, and Hedderson (1997) recently argued that morphological characters used to define these major peristome types are not phylogenetically informative and that higher level phylogenetic reconstruction of the Bryopsida will rely on independent sources of data such as ontogeny and gene sequences. The development of the funariaceous, bryaceous, and dicranaceous peristome types has been studied recently (Shaw, Anderson, and Mishler, 1989; Shaw, Mishler, and Anderson, 1989; Schwartz, 1994), but unless similar data are available for the orthotrichaceous peristome, polarizing the developmental pathways and thus defining homologies and monophyletic groups based on peristome architecture remain impossible.

The peristome of the Orthotrichales is diplolepideous and is characterized by the following features: ''an endostome with segments that alternate with exostome teeth; lack of basal membrane and with segments, which are not or are rarely keeled; and an exostome that has a thick, outer layer and a thin, inner layer'' (Vitt, 1982a). Crosby (1980) and Vitt (1981a) were the first to suggest that the ancestral arthrodontous peristome was diplolepideous. Crosby (1980) and later Shaw and Rohrer

(1984) considered that the *Bryum* type represented the most primitive among the diplolepideous types and consequently that the orthotrichaceous peristome was derived through reduction, a hypothesis also supported by Hedenäs (1994). Vitt (1984) argued that ''the evolution of the peristome has not been uni-directional'' and that the bryaceous peristome ''is a totally separate evolutionary line than either the orthotrichaceous or haplolepideous divergences'' from an ancestral funariaceous type (see also Vitt, 1981a). More recently, Lewinsky (1989) argued in favor of a distinct orthotrichaceous peristome type that is ''most likely to have evolved from a peristome with a formula of 4:2:4 with complete alignment of the cells in the IPL,'' thus the funariaceous type, but unlike Vitt (1984) she considers the orthotrichaceous peristome as possibly pivotal to the evolution of the dicranaceous and the bryaceous types.

The Orthotrichaceae sensu Vitt (1984; and not sensu Churchill and Linares, 1995) include over 500 species, distributed among 22 genera (see Goffinet, 1997a, b), with *Orthotrichum,* and *Macromitrium* accounting for more than two-thirds of the species richness (Vitt, 1982a). The family is cosmopolitan in distribution and is particularly prevalent in tropical montane forests. The Orthotrichaceae are traditionally characterized by a unique peristomial architecture (see above), as well as ''small, papillose upper leaf cells; no differentiated alar cells; large, usually mitriform calyptrae; terminal perichaetia with additional growth by lateral innovations'' (Vitt, 1982b). These characters are, however, by no means constant within the family, resulting in alternative hypotheses of familial circumscription. The controversy has focused particularly on the haplolepideous vs. the diplolepideous affinity of *Amphidium,* which lacks a peristome, and has atypical cell ornamentation (Brotherus, 1909 vs. 1925; Vitt, 1973 vs. Lewinsky, 1976), or of taxa whose peristome is reduced (*Drummondia*: Shaw, 1985 vs. Vitt, 1972). The current infrafamilial classification (Vitt, 1982a) reflects the four possible combinations of two characters, position of the female gametangia and ''shape'' of the calyptrae, and deviates little from Brotherus's concept early this century (Brotherus, 1925). In Vitt's (1982a) phylogenetic arrangement, the Zygodontoideae (Schimp.) Broth. (acrocarpous and cucullate calyptrae) are basal, followed by the Drummondioideae Vitt (cladocarpous and cucullate calyptrae), which are sister to a clade composed of the Orthotrichoideae Broth. and the Macromitrioideae Broth. (acrocarpous and cladocarpous, respectively, and both with mitrate calyptrae).

Addressing the phylogenetic relationships of the Orthotrichales and its delineation is essential for reconstructing the evolutionary history of orthotrichaceous genera. Goffinet (1997b) recently excluded the Microtheciellaceae from the Orthotrichales based on their pleurocarpy. Goffinet (1997a) also transfered two genera from the Orthotrichaceae to the Rhachitheciaceae. The peristomial architecture of the Rhachitheciaceae (Goffinet, 1997a) and of the Erpodiaceae Broth. (Crum, 1972; Edwards, 1979) suggests possible affinities to the Haplolepideae, and particularly the Seligeriales. The monophyly of the Orthotrichaceae and the Orthotrichales has recently also been questioned by De Luna (1995) while examining the systematic affinities of the Hedwigiaceae. Based on a phylogenetic analysis using morphological characters, De Luna (1995) concluded that the Orthotrichales merely represent an evolutionary grade, suggesting that a clade of predominantly cladocarpous Orthotrichaceae (Macromitrioideae, Drummondioideae) and the putatively related Erpodiaceae Broth., Microtheciellaceae Harrington and Miller, and Helicophyllaceae Broth.(see below) was sister to the pleurocarpous mosses, and separated from the acrocarpous Orthotrichaceae and their possible sister group, the Rhachitheciaceae Robins. by the Hedwigiaceae. Affinities between the Hedwigiaceae and the Orthotrichaceae have been proposed by other authors (Walther, 1983; Frey et al., 1995). Because of the gymnostomous nature of the Hedwigiaceae and a unique combination of gametophytic characters (acrocarpy, lack of costa, and presence of pseudoparaphyllia), the affinities of *Hedwigia* are unlikely to be resolved with traditional morphological characters.

Severe reduction trends in morphological characters, particularly with regard to the peristome, are thus the source of the controversies regarding the delineation of the Orthotrichales and its type family, the Orthotrichaceae. Ambiguous homologies between characters defining alternative peristome types hamper resolving the phylogenetic affinities of these taxa. Variation in the nucleotide sequence of the chloroplast gene *rbcL,* encoding for the ribulose 1,5 biphosphate carboxylase, has found a wide application in reconstructing the evolutionary histories within suprageneric taxa of vascular plants (Chase et al., 1993; Hasebe et al., 1995). The present study aims at setting the foundations for reconstructing the phylogeny of all orthotrichaceous genera based on morphology and for addressing the character evolution in this taxonomically and morphologically diverse family. Thus, the objectives were to use *rbcL* sequence data (1) to test the monophyly of the Orthotrichales and the Orthotrichaceae; (2) to determine the phylogenetic position of the Orthotrichales within the arthrodonteae, and thus identify its putative sister group; and (3) to test the monophyly and the phylogenetic relationships of the subfamilies proposed by Vitt (1982a).

MATERIALS AND METHODS

Taxon sampling—Following the exclusion of five genera from the Orthotrichaceae (Goffinet, 1997a, b) the family now consists of 22 genera. While material has been seen for all taxa except for the recently described *Orthomitrium* (Lewinsky-Haapasaari and Crosby, 1996), extractions were not attempted for taxa known only from the type specimen (*Ceuthotheca* Lewinsky-Haapasaari, *Leiomitrium* Mitt., *Leratia* Broth.), or for which only very little material was available (*Stoneobryum* Norris and Robinson). DNA extractions were attempted for the remaining 17 genera, and for the most diverse genera, such as *Macromitrium* and *Orthotrichum,* several species belonging to morphologically distinct infrageneric taxa were tentatively included. DNA extractions were also attempted for representatives of all related families in the Orthotrichales. Furthermore, ten outgroup taxa were chosen, representing the Funariaceae, Splachnaceae, Encalyptaceae, Hedwigiaceae, the Haplolepideae (Ptychomitriaceae and Rhabdoweisiaceae), and the ciliate alternate peristomate mosses (Mniaceae and Thuidiaceae).

DNA extraction and PCR-DNA amplification—DNA was extracted, following a modification of Doyle and Doyle (1987), from apices of stems and branches, or single sporophytes sampled from dried herbar-

TABLE 1. Synthetic primers (5′–3′) used for sequencing the *rbcL* gene in mosses.

Forward primers	
427[a]	GCTTATTCAAAAACTTTCCAAGGCCCGCC
997	GGTAAACTTGAAGGAGAACG
Reverse primers	
295R	CTAATGGGTAAGCAACATAAGC
515R	CATCCTAATAATGGACGACC
678R[a]	GATTTCGCCTGTTTCGGCTTGTGCTTTATAAA
895R[a]	ACCATGATTCTTCTGCCTATCAATAACTGC
1081R	CCCAGTCTTGAGTGAAGTAAATACC

[a] Marks primers designed and provided by G. Zurawski; others were designed by authors.

ium or fresh collections. Gametophytic samples were cleaned of contaminating material in distilled water, then air-dried overnight and lyophilized prior to the extraction. One to 10 mg dry mass of each sample were placed in a spot plate, covered with liquid N_2, and ground to a fine powder once the liquid N_2 was completely evaporated. The powder was then suspended in 250 μL of 2X CTAB (hexadecyltrimethylammonium bromide)-1% beta-mercapto-ethanol extraction buffer heated at 60°C and the solution transferred to a microcentrifuge tube. The tubes were then placed in a 60°C water bath. After 45 min, 250 μL of CI (chloroform-isoamyl 24:1) were added and the tubes gently inverted for 10 min. Cell debris were then isolated by centrifuging the tubes for 10 min at maximum speed (e.g., 14 000 rpm). The aqueous phase was transferred to a fresh tube (the CI extraction can be repeated if the aqueous solution is not clear). Then 350 μL of 95% ethanol were added and the DNA allowed to precipitate at 4°C. After at least 2–3 h, the tubes were centrifuged for 5 min at maximum speed again to pellet the DNA. The ethanol was discarded and the pellet washed gently by adding 350 μL of 70% ethanol. The tubes were centrifuged again for 5 min at 7000 *g*. The ethanol was discarded and the pellet dried in a vacuum centrifuge. The DNA was then suspended in 100 μL of TBE (Tris-boric acid-EDTA) at 65°C for 10 min and kept at −20°C subsequently. The *rbcL* gene was amplified via the polymerase chain reaction (PCR) using Taq Polymerase (Promega Corporation, Madison, WI). The amplification reaction was performed in 50 μL volume including 50 mmol/L KCl, 10 mmol/L Tris-HCl, 0.1% Triton, 5% glycerol, 2.5 mmol/L $MgCl_2$, 20 mmol/L of each dNTP, 0.2 mmol/L of primers Z_1 and 1351R (Wolf, Soltis, and Soltis, 1994) and 0.2–30.0 ng of template DNA. The samples were exposed to the following temperature profiles using a Grant thermal cycler: one cycle of 94°C for 3 min and 85°C for 4 min, during which 1 unit of Taq DNA Polymerase would be added to each tube, and 30 cycles of 94°C for 1 min, 52°C for 1 min, 72°C for 2 min, and finally one segment of 72°C for 10 min. The double-stranded product subsequently served as the template for the amplification of single-stranded target DNA. This second PCR followed the same profiles as before, but the reaction solution included only one of the two PCR primers: Z_1 for amplification of the forward strand and 1351R for the reverse strand. The single-stranded product was precipitated with 20% PEG/2.5 mol/L NaCl, washed first with 70% EtOH and then with 95% EtOH, before being suspended in 7 mL of Tris buffer-EDTA (Morgan and Soltis, 1993).

Sequencing and alignment—Sequencing the single-stranded template followed the dideoxy chain termination method (Sanger, Nicklen, and Coulson, 1977) using the Sequenase® version 2.0 T7 DNA polymerase following the manufacturer's instructions (Amersham, Canada). The sequence of the primers used is given in Table 1. The sequencing products were electrophoresed on a 6% polyacrylamide gel (0.4 mm thickness; 1X TBE buffer), at 2400 V for 4 h. The gels were fixed in 10% acetic acid, washed with distilled water, and dried in an oven at 65°C for 30 min and autoradiographed for 36–48 h. The sequences were entered in MacClade (version 3.03) and aligned manually against available sequences of *Sphagnum palustre* and *Andreaea rupestris* (GenBank accession numbers L13485 and L13473, respectively; Manhart, 1994). The first and last 30 nucleotide sites, corresponding to the sequences of the PCR primers, were excluded from parsimony analyses.

Sequence and phylogenetic analysis—*RbcL* sequence variation was analyzed by neighbor-joining (NJ; Saitou and Nei, 1987), and maximum parsimony (MP; Fitch, 1971). Neighbor-joining analyses were performed using MEGA version 1.0 (Kumar, Tamura, and Nei, 1993) using Tamura's distance parameter, following the authors' ''Guidelines for choosing distance measures,'' and including both transitions and transversions. A ''bootstrap confidence level'' for the NJ tree was calculated over 100 replicates. Fitch parsimony analyses of nucleotide data were performed with PAUP version 3.1 (Swofford, 1993) on a MacIntosh PowerPC 7200/90 using the heuristic search with the following options in effect: keep all characters, multistate taxa interpreted as uncertainties, tree-bisection-reconnection branch swapping, steepest descent, collapse zero-length branches, and addition sequence ''as is.'' In an attempt to locate additional islands of shortest trees (Maddison, 1991), the search strategy further followed the steps recommended in Pryer, Smith, and Skog (1995) except that the search was only replicated 100 times. Bootstrap analysis (Felsenstein, 1985; Hillis and Bull, 1993) was performed with 100 bootstrap replicates of the heuristic search with the same set of options in effect. Relative support for branches was further determined by the decay analysis (Bremer, 1988; Donoghue et al., 1992) following the converse constraint method (Baum, Sytsma, and Hoch, 1994). The ACCTRAN (accelerated transformation optimization) option of PAUP was applied for calculations of branch lengths. The phylogenetic signal present in the *rbcL* sequence data was estimated by calculating the g_1 statistic of the distribution of tree length of 500 000 random trees produced with PAUP using the ''random tree'' option (Hillis and Huelsenbeck, 1992). Consistency (CI) and retention indexes (RI) and *f* values were calculated for all MPTs using PAUP. PAUP was also used to generate a matrix of absolute and mean distance between sequences. Average unit character consistencies (AUCC) were calculated for competing phylogenies in an attempt to select the tree with the strongest asymmetric distribution of homoplastic characters in the matrix (Sang, 1995). Costs in terms of number of additional steps required for alternative phylogenies were explored using the enforce constraint command during heuristic searches (Swofford, 1993). The same set of options as in the unconstrained searches was applied.

RESULTS

The *rbcL* gene was successfully sequenced for 22 taxa of the Orthotrichaceae distributed among ten genera, for representatives of two of the related families as well as for all selected outgroup taxa (Table 2). Amplification products were not obtained for *Florschuetziella* Vitt, *Leptodontiopsis* Broth., *Muelleriella* Dusén, *Pleurorthotrichum* Broth., and *Stenomitrium* (Mitt.) Broth., *Helicophyllum* Brid. The sequences obtained from PCR fragments generated using the PCR primers Z_1 and 1351R

TABLE 2. Taxa for which the *rbcL* gene sequence was obtained in this study (all vouchers deposited in ALTA unless otherwise noted).

Taxon	Voucher	GenBank accession number
ORTHOTRICHALES		
ORTHOTRICHACEAE		
Zygodontoideae		
Zygodon pungens[a] C. Müll.	*La Farge-England* 8097	AF005534
Zygodon obtusifolius Hook.	*Vitt* 38301	AF005535
Zygodon intermedius B.S.G.	*Vitt* 29262	AF005532
Zygodon reinwardtii (Hornsch.) Braun	*Goffinet* 636	AF005533
Amphidium lapponicum (Hedw.) Schimp.	*Vitt* 33854	AF005543
Orthotrichoideae		
Orthotrichum obtusifolium Brid.	*Vitt* 33870	AF005537
Orthotrichum anomalum Hedw.	*Goffinet* 4115	AF005538
Orthotrichum lyellii Hook. & Tayl.	*Goffinet* 3162	AF005536
Ulota lutea (Hook. f. & Wils.) Mitt.	*Fife* 8042	AF005540
Ulota obtusiuscula C. Müll. & Kindb.	*Goffinet* 3161	AF005539
Bryodixonia perichaetialis Sainsb.	*Fife* 8083	AF005541
Drummondioideae		
Drummondia prorepens (Hedw.) Britt.	*Vitt* 26711	AF005542
Macromitrioideae		
Schlotheimia brownii Schwaegr.	*Vitt* 27485	AF005522
Schlotheimia tecta Hook. f. & Wils.	*Schäfer-Verwimp* 9686	AF005520
Schlotheimia trichomitria Schwaegr.	*Schäfer-Verwimp* 6902	AF005521
Cardotiella quinquefaria (Hornsch.) Vitt	*Buck* 26230	AF005523
Groutiella apiculata (Hook.) Crum & Steere	*Goffinet* 2764	AF005527
Groutiella chimborazense (Mitt.) Florsch.	*Goffinet* 1173	AF005526
Macrocoma papillosa (Thér.) Vitt	*Matteri* 6521	AF005525
Macrocoma tenuis (Hook. & Grev.) Vitt subsp. *sullivantii* (C. Müll.) Vitt	*Breedlove* 69342	AF005524
Macromitrium incurvifolium (Hook. & Grev.) Schwägr.	*Streimann* 49345	AF005528
Macromitrium longifolium (Hook.) Brid.	*Goffinet* 656	AF005529
Macromitrium richardii Schwaegr.	*Goffinet* 2648	AF005530
Desmotheca apiculata (Dozy & Molk.) Lindb.	*Vinas* 96-4	AF005531
ERPODIACEAE		
Aulacopilum hodgkinsoniae (C. Müll.) Broth.	*Vitt* 28261	AF005545
Venturiella sinensis (Vent. in Rabenh.) Müll. Hal.	*Vitt* 34842	AF005546
RHACHITHECIACEAE		
Uleastrum palmicola (C. Müll.) Zander	*Vitt* 21162	AF005547
OUTGROUP TAXA		
FUNARIACEAE		
Funaria apophysata (Tayl.) Broth.	*Vitt* 27234	AF005514
Funaria hygrometrica Hedw.	*Priddle* 1408	AF005513
ENCALYPTACEAE		
Encalypta procera Bruch	*Vitt* 37966	AF005548
HEDWIGIACEAE		
Hedwigia ciliata (Hedw.) P. Beauv.	*Goffinet* 3324	AF005517
MNIACEAE		
Mnium thomsonii Schimp.	*Vitt* 35884	AF005518
PTYCHOMITRIACEAE		
Ptychomitrium gardneri Lesq.	*Ireland* 7038 (PMAE)	AF005549
RHABDOWEISIACEAE		
Rhabdoweisia crenulata (Mitt.) Jameson	*Vitt* 36707	AF005544
SPLACHNACEAE		
Tayloria lingulata (Dicks.) Lindb.	*Schofield* 98443	AF005515
Splachnum sphaericum Hedw.	*Goward* 95-1470	AF005516
THUIDIACEAE		
Abietinella abietina (Hedw.) Fleisch.	*Goffinet* 4106	AF005519

[a] This specimen is tentatively identified as *Zygodon pungens* C. Müll., a species hitherto not known from Africa (Malta 1926), but may represent a new species.

TABLE 3. Distribution and frequency (%) of constant and phylogenetically informative sites over all taxa and over the Orthotrichaceae only; characters with ambiguous or missing data are included.

	All taxa				
	Codon position			Total	Orthotrichaceae (total)
	First	Second	Third		
Constant	368 (83.6)	407 (92.5)	148 (33.6)	923 (69.9)	1158 (87.8)
Phylogenetically informative	30 (6.1)	18 (4.1)	109 (24.7)	157 (11.9)	108 (8.2)

were, as expected, 1320 bases long (±90% of the total length of the gene). Alignment of the sequences with known sequences of *Sphagnum pallustre* and *Andreaea rupestris* did not require the inclusion of gaps. Phylogenetically informative characters are preponderant at the third codon position followed by the first and the second codon site (Table 3). *RbcL* sequence variation yields 108 sites that are potentially phylogenetically informative within the Orthotrichaceae. Informative characters are distributed fairly evenly across the sequence (Fig. 1). The distribution of 500 000 random trees using all characters had a skewness index g_1 of −0.55; excluding the first and second codon position yielded a similar index (g_1 = −0.54), while trees generated solely from characters of these two first codon sites had a higher skewness index (g_1 = −0.39).

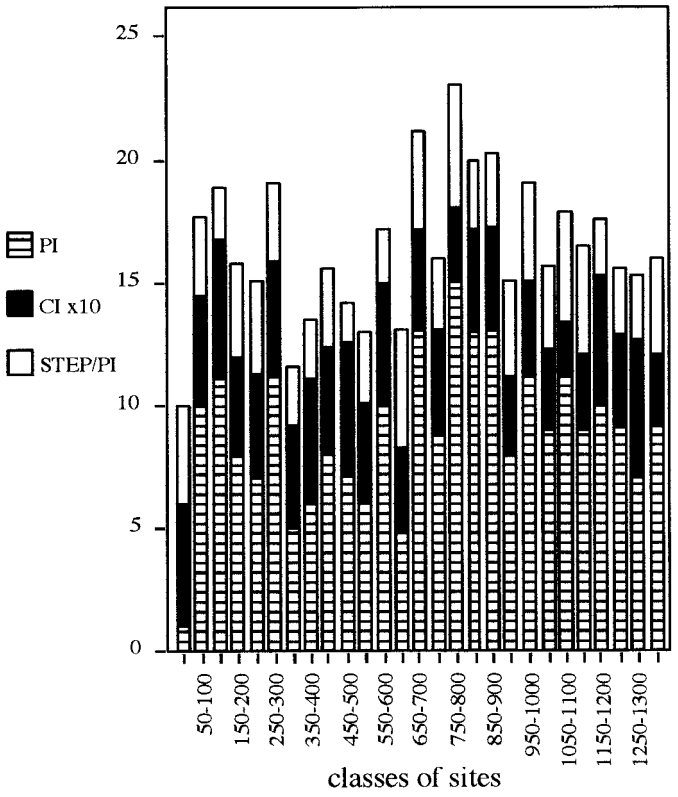

Fig. 1. Distribution of phylogenetically informative (PI) characters, the average consistency index (CI), and the average number of steps per phylogenetically informative character along the *rbcL* sequence based on tree shown in Fig. 3. Each class contains 50 nucleotide sites, except for the first class that includes only sites 31–50. Cross-lined bars = total number of phylogenetic informative characters; solid bars = average CI multiplied by 10; hollow bar = total number of steps per total number of phylogenetically informative characters.

Using the maximum parsimony criterion with *Sphagnum* and *Andreaea* as the outgroup yields 39 most parsimonious trees (MPT) distributed among three islands of size 21, 6, and 12 (Fig. 2). The MPTs are 964 steps long and have a CI of 0.390 and a RI of 0.624. The *f* value of the trees varies between 13 306 and 19 198. Both methods of analysis agree on the polyphyly of the Orthotrichales and place the Erpodiaceae and the Rhachitheciaceae as well as the orthotrichaceous genera *Amphidium* and *Drummondia* in a monophyletic clade with *Ptychomitrium* and *Rhabdoweisia* (Figs. 3–4). Constraining the search for the inclusion of the Erpodiaceae and Rhachitheciaceae in the Orthotrichales results in trees that are 17 and 15 steps longer than the shortest unconstrained trees, respectively. Including *Amphidium* and *Drummondia* in the Orthotrichaceae also increases the length of the most parsimonious topology (+25 steps), and furthermore these two genera remain more closely related to each other than they do to any other genus of the Orthotrichaceae. The NJ tree agrees with the strict consensus tree over all MPTs in the following relationships (Figs. 3–4): (1) *Funaria* occupies a basal position among arthrodontous mosses; (2) the Orthotrichales and the Splachnales form a monophyletic clade sister to the ciliate mosses; and (3) the Haplolepideae, including *Amphidium*, *Drummondia*, *Venturiella*, and *Uleastrum*, form a monophyletic group sister to derived Diplolepideae. *Hedwigia* is sister to the two ciliate mosses in 36 MPTs (Fig. 2) as well as in the NJ tree (Fig. 4) and basal in the diplolepideous clade (excluding the Funariaceae) in the remaining three trees (Fig. 2). Compared to MP, NJ provides similar or slightly higher bootstrap values for major lineages (Haplolepideae, combined Splachnales and Orthotrichales, Orthotrichaceae, and ciliate mosses), but like MP, NJ fails to yield strong support for their relationships (Figs. 3, 4).

The remaining genera of the Orthotrichaceae (thus excluding *Amphidium* and *Drummondia*) form a strongly supported monophyletic family in the phylogenetic reconstruction using Tamura's distance parameter (Fig. 4). Among the 39 MPTs found in the cladistic analysis (Fig. 2), 15 trees (38%), distributed between island 1 and 3 (each with nine and six trees, respectively), support the monophyly of the family. The trees of these two islands differ mainly by the position of *Encalypta* (Fig. 2). In island 3 (mean *f* values: 13 943), *Encalypta* is sister to the Haplolepideae (Fig. 2B), while in islands 1 and 2 it occupies a position basal to the dichotomy between Haplolepideae and the derived Diplolepideae (Fig. 2C; mean *f* value overall: 16 634). Comparison of the distribution of homoplasy among characters from two selected trees contrasted mainly by the position of *Encalypta* (island 1 vs. island 3), yields an AUCC value of 0.71 for both,

suggesting no difference in the distribution of homoplastic characters (Sang, 1995). All other indexes being equal, the tree with the lowest *f* value (see Farris, 1972) is shown (Fig. 3). The Orthotrichaceae are composed of two major lineages: one including all Macromitrioideae (*Cardotiella, Desmotheca, Groutiella, Macrocoma, Macromitrium,* and *Schlotheimia*) as well as *Zygodon obtusifolius,* and one uniting the remaining species of *Zygodon* with the Orthotrichoideae (*Bryodixonia, Orthotrichum,* and *Ulota*). Large genera such as *Macromitrium, Orthotrichum,* and *Zygodon* appear paraphyletic, independent of the method of analysis. In the NJ analysis, as well as in all MPT of island 2, *Desmotheca* is basal in a clade centered around *Macromitrium,* while the other MPT suggest a derived position, sister to *Macromitrium richardii.*

DISCUSSION

RbcL sequence data—*RbcL* sequence data are routinely used to reconstruct evolutionary histories among (Chase et al., 1993; Hasebe et al., 1995) or more rarely within (e.g., Haufler and Ranker, 1995) genera of vascular plants. With 30% variable sites, including 12% of sites with changes that may be phylogenetically informative, the variation in the nucleotide sequences of the *rbcL* gene among moss taxa may yield sufficient characters for reconstructing the evolutionary history of the taxa included in this study. The distribution of informative characters appears rather uniform (Fig. 1) and is similar to that observed in vascular plants, i.e., with no obvious hot spot detectable (Olmstead and Sweere, 1994). The distribution of randomly generated trees has a left-hand skewness with a g_1 value (-0.55) significantly smaller than the critical value (-0.10 or -0.08 for 100 or 250 characters, respectively; $P = 0.01$) furnished by Hillis and Huelsenbeck (1992), suggesting that our *rbcL* data set is more structured than a random data set of equal size and that it may contain significant phylogenetic signal (Hillis and Huelsenbeck, 1992). Unlike Conti, Fischbach, and Sytsma's (1993) observation that the third codon position introduces ''noise,'' a lower g_1 value is observed when the matrix is restricted to the third codon position (-0.54 vs. -0.39), suggesting that the changes at the third codon position are more structured. Analyses of a data set restricted to the third codon positions only yield topologies congruent with those obtained with the complete data set, whereas a search excluding changes at the third codon position results in a consensus tree incongruent with monophyletic concepts of either the ciliate mosses, or the Haplolepideae (results not shown). This observation would suggest that the first two codon sites carry more homoplasies than the third position, which may thus be more informative. Alternatively, the taxon sample may be too disparate for variability at the more conserved positions 1 and 2 to be mostly phylogenetically informative. The significance of the difference between the g_1 value based on our data set and that of a random matrix may, as a result, be due to differences in the frequency of character states rather than in the congruence among characters in both data sets (Källersjö et al., 1992). This second hypothesis most likely applies to our data since our restricted taxon sample represents major lineages of arthrodontous mosses with a long evolutionary history (Frey, 1977, 1990).

An indication of the accuracy of our sequences as well as for the presence of pseudogenes may be obtained from the comparison of amino acid sequences with the distribution of active sites (Kellog and Juliano, 1997). All amino acid residues of the active site found in spinach by crystallography (Andersson et al., 1989) are conserved among studied bryophyte taxa, except for position 404 (amino acid numbering), which is scored as polymorphic (Arg in addition to the conserved Gly) for six taxa, and thus excluded from phylogenetic analyses in MP. This observation may provide some preliminary support for the accuracy of the sequences obtained.

Circumscriptions of the Orthotrichales—The Orthotrichales are currently composed of four families: the Erpodiaceae, Helicophyllaceae, Orthotrichaceae, and Rhachitheciaceae (Goffinet, 1997b). *RbcL* sequence data analyzed using either the neighbor joining or the maximum parsimony criterion indicate that the Orthotrichales form a polyphyletic taxon. Both the Erpodiaceae (*Aulacopilum hodgkinsoniae, Venturiella sinensis*) and the Rhachitheciaceae (*Uleastrum palmicola*) are indeed placed in a clade with haplolepideous taxa (Figs. 3, 4). This clade also includes the ''orthotrichaceous'' genera *Amphidium* and *Drummondia.* The monophyly of this haplolepideous clade is strongly supported (MP: decay index [DI] of 4, bootstrap value [BV] = 77%; NJ: BV = 94%). Constraining these taxa to a relationship within a monophyletic Orthotrichales is furthermore very costly in terms of parsimony, requiring up to 25 additional steps. Variation in the nucleotide sequence of the *rbcL* gene is thus congruent with earlier hypotheses based on morphology and cytology, suggesting haplolepideous affinities of these taxa (for *Amphidium* see Anderson and Crum, 1958; Vitt, 1970, 1973; *Drummondia*: Shaw 1985; Erpodiaceae: Edwards, 1979; Rhachitheciaceae: Goffinet, 1997a). More extensive sampling of haplolepideous taxa would be needed before ordinal affinities of *Amphidium* and *Drummondia* are elucidated (see also below, affinities of excluded taxa).

De Luna (1995) recently argued that the Orthotrichales represented an evolutionary grade, reached by two lineages. The cladocarpous lineage (including, e.g., Erpodiaceae, Macromitrioideae) was sister to the Leucodontales and separated from the acrocarpous line (e.g., Rhachitheciaceae, Orthotrichoideae) by the Hedwigiaceae. Affinities between the Hedwigiaceae and the Orthotrichaceae have also been proposed by other authors (Walther, 1983; Frey et al., 1995). Constraining the heuristic search to include *Hedwigia ciliata* in the Orthotrichaceae (as defined in Figs. 3 and 4, and with no further relationships specified within this clade) yielded 12 trees that not only are 16 steps longer than the MPTs in the unconstrained search, but also share a monophyletic Orthotrichaceae. The incongruence between De Luna's morphological study (De Luna, 1995) and the present molecular analysis may be due to inconsistent and inadequate taxon sampling with the absence of representatives of the Leucodontineae from the molecular analysis and of bryalean taxa from the morphological analysis. The latter analysis further includes taxa that are here shown

to be unrelated to the Diplolepideae (i.e., Erpodiaceae, Rhachitheciaceae) and might be affected by the subsequent misinterpretations of the nature of the peristome of these families (scored as an exostome instead of an endostome). A closer relationship between the Hedwigiaceae and the Orthotrichaceae cannot be excluded, but if the Hedwigiaceae were closely related to the Orthotrichaceae, *rbcL* data suggest that the Orthotrichaceae would most likely remain a natural group. This phylogenetic hypothesis seems to be supported by 18S sequence data analyses (C. Cox and T. Hedderson, personal communication, University of Reading, U.K.). The branch that supports the Hedwigiaceae–cladocarpous Orthotrichales–Leucodontales lineage is supported in De Luna's analysis (1995) by three synapomorphies, namely plagiotropic growth, presence of pseudoparaphyllia, and differentiated perichaetial leaves. Two of these characters are reversed in the cladocarpous Orthotrichales, leaving a single character that actually supports a relationship of these taxa with the Hedwigiaceae and Leucodontales, namely plagiotropic growth. This character is, however, homoplastic among many families and genera of mosses (Meusel, 1935) and may thus not be truly informative at the ordinal level.

Analysis using the distance method yields a single tree (Fig. 4) that shows strong support for the monophyly of the Orthotrichaceae, a phylogeny congruent with that of 15 of the 39 MPTs found in the cladistic analysis. Käss and Wink (1995) and Barker, Linder, and Harley (1995) also used both methods (MP and NJ) for phylogenetic reconstructions based on *rbcL* sequence data, and the results of both analyses were fairly congruent. Kim, Rohlf, and Sokal (1993) critically examined the accuracy of the NJ method under different constraints on a random data set and found that this method ''has the highest accuracy overall.'' Russo, Takezaki, and Nei (1996) recently compared the efficiency of various tree-building methods in recovering a known phylogeny and concluded that NJ ''gives as good a result as the more time consuming. . .methods.'' The congruence between the NJ tree and 15 MPTs is therefore here interpreted as supporting the monophyly of the Orthotrichaceae.

With the exclusion of the Erpodiaceae, Rhachitheciaceae, and Hedwigiaceae from the Orthotrichales, the order is now reduced to the Orthotrichaceae and the Helicophyllaceae. In the absence of *rbcL* data, the phylogenetic relationship between these two families remains dubious due to unique gametophytic characters, such as reduced ventral and dorsal leaves, and lateral leaves that are inrolled when dry, and the absence of a peristome in *Helicophyllum torquatum*, the sole species of the Helicophyllaceae (Vitt, 1982c). Buck and Vitt (1986) argued in favor of a close relationship with the Racopilaceae (ciliate diplolepideae), as suggested by earlier authors (Brotherus, 1909, 1925; Fleischer, 1920; Dixon, 1932; Reimers, 1954). The perichaetia are, however, produced terminally on the main axis (De Luna, 1995), a condition that is a priori incompatible with a placement in a pleurocarpous lineage (La Farge-England, 1996). Other loci are currently being tested for addressing the affinities of the Helicophyllaceae. A putative phylogenetic relationship between the Orthotrichaceae and the Cryphaeaceae and the monotypic Wardiaceae (Walther, 1983) was not examined in the present study. *Wardia hygrometrica* should be excluded from the Orthotrichales based on well differentiated alar cells and prosenchymateous cells. Combined with the acrocarpous condition (Welch, 1943), these characters may indicate a bryalean origin, a hypothesis recently examined using 18S gene sequences (Hedderson, Cox, and Gibbings, 1997). The Cryphaeaceae have traditionally been placed among the Leucodontineae (Vitt, 1984; Buck and Vitt, 1986). A relationship of this family to the Hedwigiaceae, and thus the Orthotrichales, has been considered and rejected (De Luna, 1995), but may need to be reexamined in the light of the discovery of cladocarpous species of *Cryphaea* sensu lato (La Farge-England, 1996).

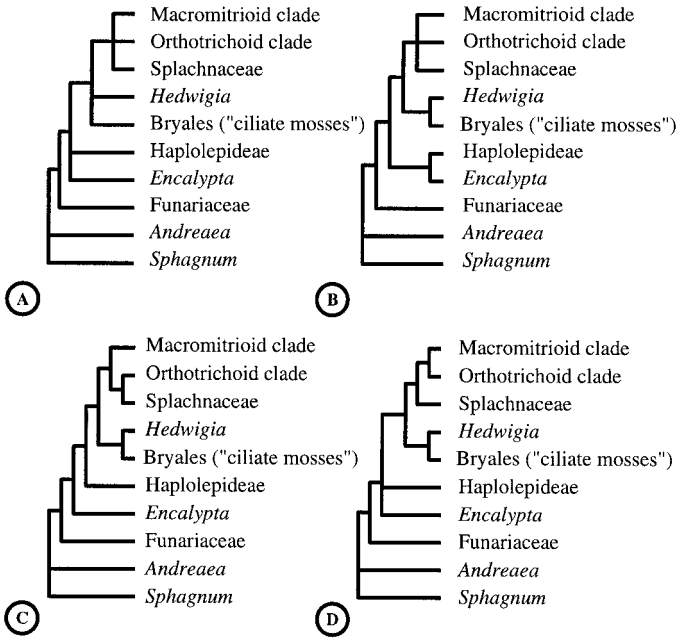

Fig. 2. Summary consensus trees of most parsimonious Fitch trees found in a heuristic search using *rbcL* sequence data with *Sphagnum palustre* and *Andreaea rupestris* as outgroups. (A): strict consensus of all three islands (39 MPTs; tree 964 steps; CI: 0.390; RI: 0.624); (B): 50% majority rule tree of island 3 (12 MPTs; mean *f* value: 13 943 ± 656); (C): 50% majority rule tree of islands 1 and 2 (27 MPTs; mean *f* value: 16 633 ± 1 308); (D): 50% majority rule tree of all 15 MPTs showing the Orthotrichaceae monophyletic (mean *f* value: 16722 ± 2015).

Ordinal relationship of the Orthotrichales—The affinities of the Orthotrichales, here restricted to the Orthotrichaceae, as indicated by comparisons of the *rbcL* sequences, are within a diplolepideous lineage including the Splachnales and the ciliate mosses. This clade shares a common ancestor with the Haplolepideae, and together they form a sister group to the Funariales (Figs. 3–4). This topology is congruent with Vitt's hypothesis (Vitt, 1981a) that the opposite diplolepideous peristome is primitive among arthrodontous mosses and that the haplolepideous peristome is derived from such an ancestral type (Vitt, 1984). Alternative hypotheses proposed by Lewinsky (1989; Orthotrichales basal to dichotomy between haplolepideae and ciliate diplolepideae) or by Shaw and Rohrer (1984) and Crosby (1980; ciliate diplo-

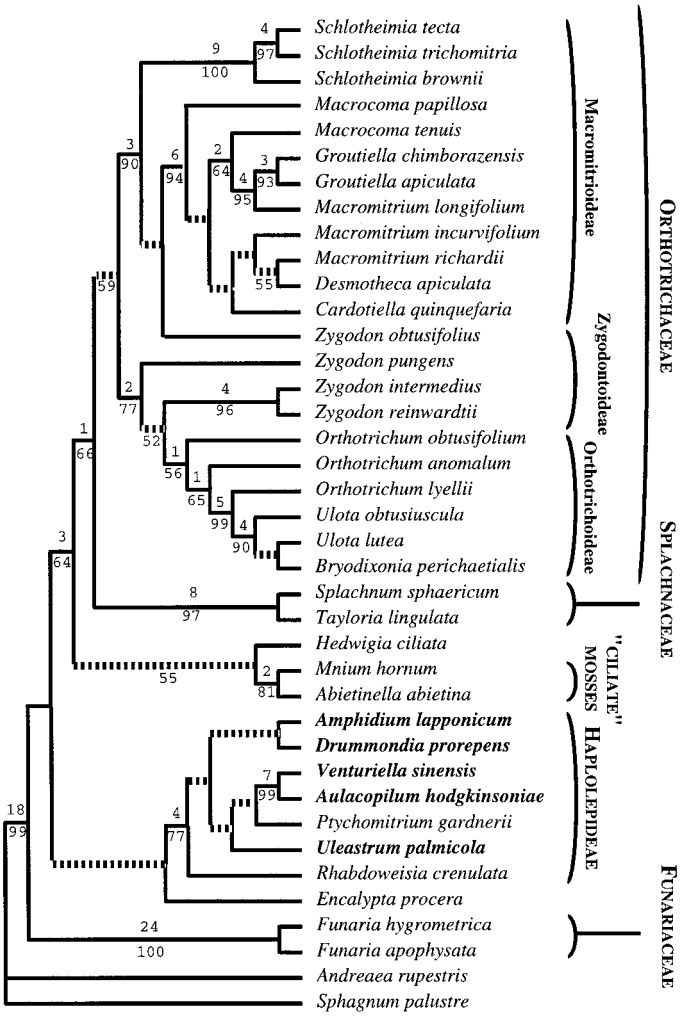

Fig. 3. One of 39 most parsimonious Fitch trees found based on *rbc*L sequence data and using *Sphagnum palustre* and *Andreaea rupestris* as outgroups. The dotted lines identify branches not present in the strict consensus tree. Bootstrap values (% of 100 replicates) 50% or higher are given below the branch, and decay indexes are presented above the branch. Taxa excluded from the Orthotrichales are in boldface.

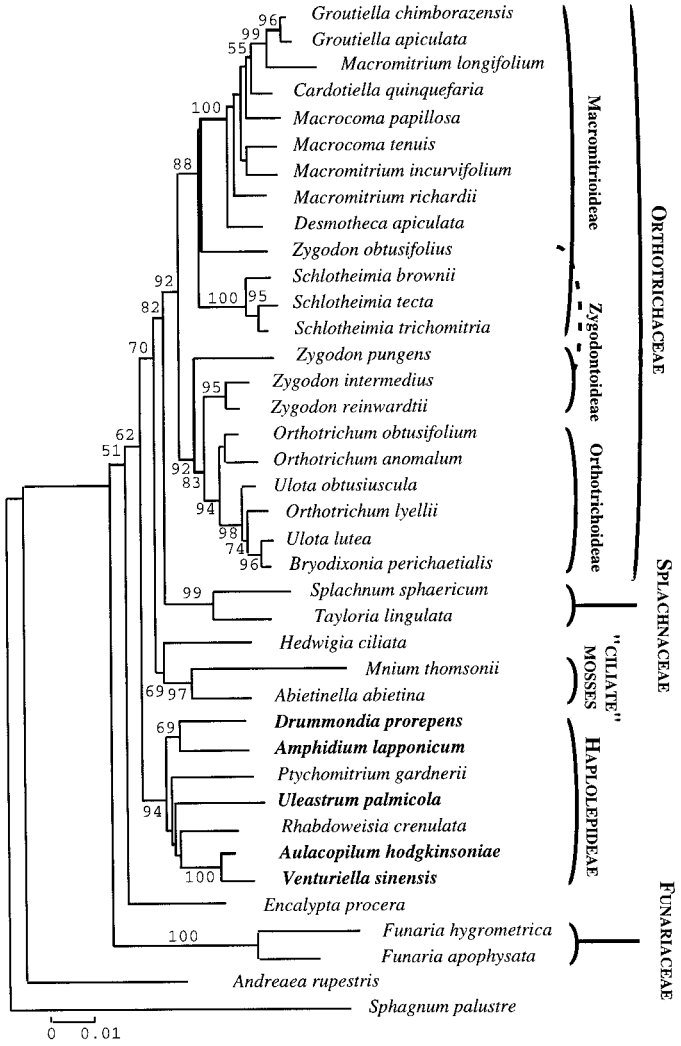

Fig. 4. Phylogenetic tree reconstructed using the neighbor-joining method, with Tamura's distance parameter including both transitions and transversions. Bootstrap values (% of 100 replicates) 50% or higher are plotted on the tree. Taxa excluded from the Orthotrichales are in boldface. Abbreviations for generic names in the Orthotrichaceae follow those of Table 4.

lepideae are the most primitive mosses) both require five additional steps.

The phylogeny obtained by either NJ or MP analysis (Figs. 3, 4) also agrees with Vitt (1984) with regard to the monophyly of a lineage composed of the Splachnales, Orthotrichales and the ciliate mosses. The relationships among these lineages remain however, ambiguous (Fig. 2). The most parsimonious scenario points toward the Splachnales and the Orthotrichales being sister groups. Vitt (1984), by contrast, proposed that the Orthotrichales are sister to the ciliate mosses, a topology that would only require one additional step based on our *rbcL* data set. Koponen (1977, 1983) considered the genus *Brachymitrion* to be the most primitive extant member of the Splachnaceae. Unlike in related genera, the thickening on the PPL is heavier than on the OPL, and the PPL also has strong trabeculae (Koponen, 1977, 1982). Both these features are shared with the *Funaria* and the *Bryum* peristome type (Shaw and Rohrer, 1984). If the Orthotrichales and the Splachnales formed a monophyletic group, both orders would most likely remain natural orders, with the Orthotrichales defined by thick-walled laminal cells. The heavier thickening on the OPL could have arisen independently in both lineages or be a synapomorphy for both, with subsequent reversal in *Brachymitrion.* In terms of peristome evolution, these lineages would form a plesiomorphic sister group to the ciliate mosses, the latter one being defined by the asymmetric division of the IPL leading to the development of cilia. If we consider the alternative scenario where the Splachnales are sister to a clade composed of the Orthotrichales and the ciliate mosses, the most parsimonious topology based on our data suggests that within the latter, the ciliate mosses remain monophyletic. An extensive taxon sampling in the Bryales is, however, needed before their evolutionary relationship can be addressed more critically. The Ortho-

trichales and the Bryales sensu lato each share one plesiomorphic state with *Brachymitrion* (Splachnales): either completely aligned cell divisions (Orthotrichales; Lewinsky, 1989) or heavier thickening of the PPL and ventral exostomial trabeculae (typical ciliate mosses). The ciliate mosses have traditionally been defined by a strongly asymmetric division in IPL leading to the development of cilia. While some ''ciliate'' mosses actually lack cilia (Buck and Vitt, 1986), have a thickened OPL (*Eucamptodontopsis,* A. Newton, personal communication, Smithsonian), and others even have opposite peristomes (*Garovaglia* div. sp.; During, 1977; Nishimura and Watanabe, 1992), none has yet been found to have a first-late symmetric division in the IPL. An asymmetric division is elsewhere found only among haplolepideous taxa. Considering that the Rhachitheciaceae are here shown to be of haplolepideous affinity, their peristome with a 2:1 formula most likely results from reduction, through the loss of the asymmetric first-late division. The asymmetric division of the ciliate mosses may not be homologous to that of the haplolepideae (Shaw, Mishler, and Anderson, 1989). Assuming that the genetic complexity behind the asymmetric division in both groups is similar, we cannot exclude the possibility that a loss of it or a reversal to a symmetric division may be possible among ciliate mosses and may even occur in such genera as *Mielichhoferia* with a peristome formula of 4:2:4 (Shaw and Crum, 1984; Shaw and Rohrer, 1984). Since the possibility of a reversal to a symmetric division cannot be excluded, a bryalean origin of the Orthotrichaceae will need to be further examined in comparison with a broad sample of ciliate taxa.

Hedenäs (1994) considered *Schlotheimia* to be pleurocarpous and, based on this interpretation, suggested that the Orthotrichaceae ''are rather close to the clade where most pleurocarpous mosses belong.'' He further hypothesized that ''the transition to pleurocarpy must have been gradual,'' and in this scenario the Orthotrichaceae would occupy an intermediate position. The Macromitrioideae differ, however, from typical pleurocarps by several characters and should rather be considered cladocarpous (La Farge-England, 1996). In genera where both cladocarpy and acrocarpy occur, the former seems to be restricted to terminal taxa, suggesting that the trend is from acrocarpy to cladocarpy with no obvious cases of reversals (La Farge-England, 1996). Such a general trend may suggest that in the Orthotrichaceae too, the primitive condition is acrocarpy. Thus if the Orthotrichales are indeed reduced ciliate mosses, their putative sister group would most likely belong to a group of acrocarpous Eubryales.

Subfamilial phylogenetic relationships—The ten orthotrichaceous genera remaining in the present analysis are distributed among two clades that are moderately to strongly supported by either method of analysis, with bootstrap values (BV) ranging from 77 to 94% and decay indexes (DI) of 2 and 3. The orthotrichoid clade combines the Zygodontoideae (*Zygodon* sections *Zygodon* and *Bryoides*) and the Orthotrichoideae (*Bryodixonia, Orthotrichum,* and *Ulota*), while the macromitrioid clade includes all the Macromitrioideae (*Cardotiella, Desmotheca, Groutiella, Macrocoma, Macromitrium,* and *Schlotheimia*) as well as *Zygodon obtusifolius* (*Zygodon* sect. *Obtusifolii*). *Zygodon obtusifolius* has retained several characters considered here plesiomorphic, such as acrocarpy, smooth, cucullate calyptrae, but exhibits also some derived features reminiscent of some Macromitrioideae (strongly bulging cells, coarse papillae, undifferentiated basal cells, etc.; see Goffinet, 1997c). If *Z. obtusifolius* is to be retained within the Zygodontoideae, the unexpected relationship with the Macromitrioideae as proposed by *rbcL* sequence data may be an artifact due to a long branch attraction (Hendy and Penny, 1989) or an indication of hybridization involving chloroplast capture from a macromitrioid taxon (Soltis and Kuzoff, 1995). In the latter case, the topology obtained here may represent the ''correct'' gene tree, but deviates from the true phylogeny of the taxa (Doyle, 1992). This hypothesis is currently being tested by comparing sequence data of the nuclear gene 18S.

With the exclusion of the Drummondioideae, Vitt's (1982a) phylogenetic arrangement of subfamilies would have the Zygodontoideae (cucullate calyptrae) basal to a dichotomy between the Orthotrichoideae and the Macromitrioideae (both typically or exclusively have large mitrate calyptrae). The sister group relationship between the orthotrichoid and the macromitrioid clades proposed here deviates from Vitt's (1982a) phylogenetic concept of the family by the inclusion of the Zygodontoideae in the former clade, and parsimony would need to be relaxed by six steps for his concept to be satisfied (without constraining the affinities of *Z. obtusifolius*). The monophyly of *Zygodon* and thus Zygodontoideae is not supported by *rbcL* sequence data either, and the relationship of sections *Zygodon* and *Bryoides* to the Orthotrichoideae sensu stricto remain unresolved. If *Zygodon* is indeed paraphyletic, section *Bryoides* would most likely be the most primitive taxon, given that it shares the plesiomorphic state of ''smooth laminal cells'' with either a splachnaceous or a typical bryaceous ancestor. The two sections of *Zygodon* differ on average by 40 mutations, while only 28 changes separate section *Zygodon* from the Orthotrichoideae, compared to 43 changes between section *Bryoides* and the Orthotrichoideae. Such divergences may be indicative of the monophyly of a clade composed of section *Zygodon* and the Orthotrichoideae and support a single origin of papillae that would define this clade.

With regards to the Macromitrioideae, both methods of analysis yield two strongly supported monophyletic clades (Figs. 2, 4): one composed of all three species of *Schlotheimia,* the other including all remaining Macromitrioideae (i.e., *Cardotiella, Desmotheca, Groutiella, Macrocoma,* and *Macromitrium*). Vitt, Koponen, and Norris (1993) suggested that *Schlotheimia* and *Cardotiella* should be placed in a separate subfamily based on the smooth and distinctly lobate calyptra. *RbcL* data do not support such relationship as ten more steps are needed to unite *Schlotheimia* and *Cardotiella* into a monophyletic clade. The species of *Schlotheimia* differ, on average, from other Macromitrioideae (including *Cardotiella*) by 47 bases. By contrast, the average distance between these other macromitrioid genera is only 23 bases. If rates of molecular evolution are assumed to be similar among these taxa (a reasonable assumption considering both clades are predominantly phyllodioicous, and epiphytic in tropical montane forests; see Britten, 1986), dif-

ferences in nucleotide sequences may indicate a relatively ancient divergence between *Schlotheimia* and the other Macromitrioideae. Among the morphological characters that separate *Schlotheimia* from other Macromitrioideae, those related to the calyptrae (shape, anatomy, outline) are shared only with *Cardotiella* (Vitt, 1981b), whereas the remaining ones (cell areolation, color of leaves) are plesiomorphic or autapomorphic (Goffinet, 1997c) and thus phylogenetically uninformative. The pleisiomorphic state of ''smooth laminal cells'' may, as was argued in the case of *Zygodon* sect. *Bryoides,* be indicative of the primitive position of *Schlotheimia* in the evolution of the Macromitrioideae. Considering both the morphological and molecular divergences between *Schlotheimia* and the remaining Macromitrioideae, and the strong support for the monophyly of both clades on molecular grounds, accommodating *Schlotheimia* in its own subfamily may better reflect the evolutionary relationship among these clades.

Brotherus (1925, as Pseudo-Macromitrioideae), Walther (1983), and Crum (1987) isolated *Desmotheca* from other Macromitrioideae on the basis of its dimorphic sterile and fertile branches. Vitt (1990), however, argued that excluding *Desmotheca* from the Macromitrioideae would most certainly result in the paraphyly of the later subfamily. Based on variation in the *rbcL* sequences, the phylogenetic affinities of *Desmotheca* clearly lay with *Macromitrium* sensu lato, but remain ambiguous with regard to its sister group within this clade. Whether *Desmotheca* should be retained in its own subfamily, and placed sister to the Macromitrioideae (excluding *Schlotheimia*), or be inserted within the latter, is not clear. In 33 MPTs (including the 15 that share a monophyletic Orthotrichaceae) *Desmotheca* occupies a derived position (Fig. 3), nested between two *Macromitrium* species. In the remaining six MPTs as well as in the NJ tree (Figs. 3, 4), *Desmotheca* is sister to the Macromitrioideae (*Schlotheimia* excluded). The phenetic distance between *Desmotheca* and other macromitrioid genera (Table 4; excluding *Schlotheimia*) are, on average, similar to those between *Orthotrichum* and *Ulota* (20 bases), in the Orthotrichoideae, suggesting that placing *Desmotheca* in a distinct subfamily may not be appropriate.

Based on our phylogenetic analyses of the *rbcL* sequence variation, the Orthotrichaceae could be regarded as composed of two subfamilies, the Orthotrichoideae and the Macromitrioideae, with the latter further divided into two tribes ''Macromitriae'' and ''Schlotheimiae.'' Alternatively, the two subfamilies may deserve recognition at the family level as suggested by Churchill and Linares (1995) and the Macromitriaceae would then be composed of two subfamilies, the Macromitrioideae and the ''Schlotheimioideae.'' The status of the Zygodontoideae, and thus the relationship of the two main subgenera to the Orthotrichoideae, needs further study.

Phylogenetic relationships of Orthotrichaceous genera—Analysis of *rbcL* sequence using either the MP or the NJ method suggests that four of the five larger genera, namely *Macromitrium, Orthotrichum, Ulota,* and *Zygodon,* are not monophyletic. The paraphyly of *Zygodon* has been addressed earlier. *Bryodixonia* is a monotypic genus endemic to New Zealand. Sainsbury (1945) argued

TABLE 4. Pairwise comparison of *rbcL* nucleotide sequences within the Orthotrichaceae. Below diagonal is absolute distance; above diagonal is average distance. Distance values used for intrageneric comparisons (see text) are in boldface.[a]

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1 *S. tecta*	—	0.006	0.012	0.035	0.036	0.035	0.040	0.040	0.036	0.049	0.033	0.031	0.032	0.035	0.045	0.033	0.045	0.039	0.042	0.041	0.047	0.047
2 *S. trichomitria*	8	—	0.012	0.036	0.036	0.033	0.040	0.040	0.038	0.047	0.036	0.033	0.032	0.033	0.041	0.030	0.041	0.034	0.038	0.036	0.042	0.042
3 *S. brownii*	16	16	—	0.030	0.031	0.030	0.033	0.033	0.033	0.041	0.031	0.029	0.035	0.035	0.041	0.031	0.041	0.034	0.038	0.038	0.043	0.043
4 *C. quinquefaria*	**46**	**48**	**39**	—	0.014	0.015	0.017	0.017	0.017	0.025	0.016	0.018	0.038	0.036	0.045	0.035	0.048	0.039	0.043	0.045	0.049	0.049
5 *M. tenue*	**48**	**47**	**41**	**19**	—	0.012	0.011	0.013	0.015	0.020	0.015	0.017	0.036	0.037	0.042	0.031	0.044	0.033	0.039	0.039	0.044	0.044
6 *M. papillosa*	**46**	**44**	**39**	**20**	16	—	0.016	0.017	0.018	0.024	0.020	0.020	0.036	0.036	0.042	0.030	0.044	0.036	0.041	0.039	0.044	0.044
7 *G. chimborazense*	**53**	**53**	**44**	**22**	**15**	21	—	0.003	0.014	0.016	0.019	0.020	0.042	0.042	0.045	0.034	0.049	0.040	0.042	0.045	0.049	0.049
8 *G. apiculata*	**53**	**53**	**44**	**22**	**17**	23	4	—	0.017	0.016	0.022	0.020	0.045	0.044	0.045	0.037	0.051	0.042	0.044	0.046	0.049	0.051
9 *Mt. incurvifolium*	**48**	**50**	**43**	**23**	**20**	24	**19**	23	—	0.023	0.014	0.017	0.036	0.034	0.044	0.032	0.047	0.038	0.044	0.042	0.047	0.047
10 *Mt. longifolium*	**64**	**62**	**54**	**33**	**26**	31	**21**	21	30	—	0.030	0.029	0.048	0.049	0.050	0.042	0.055	0.045	0.048	0.050	0.053	0.055
11 *Mt. richardii*	**44**	**47**	**41**	**21**	**20**	26	**25**	29	18	40	—	0.013	0.033	0.035	0.045	0.032	0.048	0.037	0.045	0.045	0.050	0.050
12 *D. apiculata*	**41**	**43**	**38**	**24**	**23**	27	**26**	26	**23**	**38**	**17**	—	0.037	0.035	0.045	0.030	0.045	0.036	0.042	0.041	0.046	0.046
13 *Z. intermedius*	**42**	**42**	**46**	**50**	**48**	47	**55**	59	**48**	**64**	**43**	**49**	—	0.009	0.031	0.034	0.024	0.018	0.023	0.023	0.026	0.026
14 *Z. reinwardii*	**46**	**44**	**46**	**47**	**49**	48	**56**	58	**45**	**65**	**46**	**46**	12	—	0.030	0.036	0.021	0.014	0.019	0.020	0.023	0.021
15 *Z. pungens*	**60**	**54**	**54**	**60**	**56**	56	**59**	59	**58**	**66**	**59**	**59**	41	39	—	0.037	0.036	0.027	0.032	0.033	0.036	0.035
16 *Z. obtusifolius*	44	40	41	46	41	40	45	49	42	55	42	39	45	47	49	—	0.042	0.035	0.037	0.037	0.042	0.042
17 *O. lyellii*	**60**	**54**	**54**	**63**	**58**	58	**65**	67	**62**	**72**	**64**	**60**	**32**	**28**	**47**	55	—	0.015	0.017	0.009	0.012	0.009
18 *O. obtusifolium*	**51**	**45**	**45**	**52**	**44**	47	**53**	55	**50**	**60**	**49**	**47**	**24**	**18**	**35**	46	20	—	0.011	0.017	0.020	0.017
19 *O. anomalum*	**56**	**50**	**50**	**57**	**52**	54	**56**	58	**58**	**63**	**60**	**56**	**31**	**25**	**42**	49	22	15	—	0.017	0.018	0.017
20 *U. obtusiuscula*	**54**	**48**	**50**	**59**	**52**	52	**59**	61	**56**	**66**	**60**	**54**	**30**	**26**	**43**	49	**12**	**22**	**22**	—	0.011	0.009
21 *U. lutea*	**62**	**56**	**56**	**65**	**58**	58	**65**	65	**62**	**70**	**66**	**60**	**34**	**30**	**47**	55	**16**	**26**	**24**	14	—	0.005
22 *B. perichaetialis*	**62**	**56**	**56**	**65**	**58**	58	**65**	67	**62**	**72**	**66**	**60**	**34**	**28**	**46**	55	**12**	**22**	**22**	12	6	—

[a] *B. = Bryodixonia; C. = Cardotiella; D. = Desmotheca; G. = Groutiella; M. = Macrocoma; Mt. = Macromitrium; S. = Schlotheimia; O. = Orthotrichum; U. = Ulota; Z. = Zygodon.*

for a generic distinction from *Ulota* on the basis of ''the highly differentiated and conspicuous perichaetial bracts and the diminutive calyptra'' and the immersed capsule. In addition, the perichaetial leaves have prorate basal laminal cells (Goffinet, 1997c), a feature otherwise unknown from the Orthotrichoideae. *Bryodixonia* does, however, share many of the characters found in the genus *Ulota,* such as very thick-walled cauline cells, the differentiated marginal cells of the lamina, and the flexuose to crisped leaves. *Bryodixonia* may thus be patristically very derived; however, in cladistic terms it may not deserve taxonomic recognition at the generic level. *RbcL* sequences of *Ulota lutea* and *Bryodixonia perichaetialis* differ only by six mutations, a degree of divergence that is similar to that found between species of *Groutiella* (4) or *Schlotheimia* (8–16), but, moreover, it is less than the divergence recorded between the two species of *Ulota* (14; Table 4). Immersed capsules are characteristic of many mosses that are taxonomically unrelated but share a xerophytic habitat (Vitt, 1981a). Within the Orthotrichaceae completely immersed capsules are characteristic for *Schlotheimia* sect. *Stegotheca,* and a shortening of the setae leading ultimately to an immersed capsule occurs in *Orthotrichum* subg. *Gymnoporus* sect. *Leiocarpa,* subg. *Pulchella* sect. *Rivularia,* and sect. *Diaphana,* as well as in subg. *Orthotrichum* (Lewinsky, 1993). Considering the low degree of morphological and molecular divergence of *Bryodixonia,* segregation at the generic level does not seem appropriate.

The monophyly of *Ulota* (including *B. perichaetialis*) is compromised by *O. lyellii* in the NJ tree and in 13 MPT (in 13 other MPTs their relationship is not resolved). Strong affinities of *O. lyellii* for *Ulota* (MP: 99% BV and DI of 5) and *U. obtusiuscula* in particular (13 MPTs) may indicate that among the different lineages of *Orthotrichum,* subg. *Gymnoporus* (Braithw.) Limpr. is the most closely related to *Ulota.* Among the characters that subg. *Gymnoporus* sect. *Leiocarpa* (see description in Lewinsky, 1993) shares with *Ulota,* only the long flexuose vegetative leaves may be derived within the Orthotrichoideae and thus be indicative of common ancestry. The genus *Ulota* (even if including *Bryodixonia*) is morphologically well defined from *Orthotrichum,* suggesting that the paraphyly of *Orthotrichum,* if confirmed, would need to be resolved by dividing the genus into discrete entities rather than broadening the concept of *Orthotrichum* by including *Ulota.* The relationships of the subg. *Orthophyllum* Delogn. (*O. obtusifolium*) and subg. *Orthotrichum* (*O. anomalum*) are not unambiguously resolved either: they form sister taxa in 13 MPTs as well as the NJ tree, while in 13 other MPTs, *O. obtusifolium* is sister to a clade composed of the remaining Orthotrichoideae. *Orthotrichum obtusifolium* had been placed together with the related *O. gymnostomum* Brid. in the genus *Nyholmiella* (see Lewinsky, 1993, for history), based on ''the obtuse leaves with plane or incurved leaf margin and incrassate leaf-cells with a stout central papillae on each side'' (Lewinsky, 1993). Patterns in peristome ornamentation are similar to those observed elsewhere in the genus (Lewinsky, 1993), and it may therefore be more parsimonious to retain subg. *Orthophyllum* in *Orthotrichum.*

Within the macromitrioid clade (Fig. 4) the relationships remain ambiguous too (except for *Schlotheimia* see above), either because *Macromitrium* and *Macrocoma* truly are paraphyletic, or because of an insufficient taxon sample. The genus *Macromitrium* is, with over 250 species, by far the most speciose genus of the Orthotrichaceae (Vitt, 1982a). Mitten (1869) divided the genus in four sections, to which Buck (1991) recently added sect. *Reverberatum.* Two of these have recently been raised to the genus level, namely *Micromitrium* (now *Groutiella* Steere) and *Macrocoma* Grout. The genus *Macromitrium* remains, however, morphologically extremely diverse in terms of size of the plant, degree of differentiation of the basal cells, shape of the urn, and laminal cell shape, orientation, and ornamentation. The relatively high cost in terms of parsimony, for a monophyletic *Macromitrium* (nine steps) may be seen as just one other indication that the genus as currently defined is still a heterogeneous assemblage. *Groutiella* differs from *Macromitrium* by the marginal limbidium of hyaline elongate cells and a short calyptra covering only the upper portion of the urn. Except for *G. tomentosa, Groutiella* is restricted to the Neotropics, where ten species occur. The sister species in all shortest trees is *Macromitrium longifolium,* a neotropical endemic, rather than any of two paleotropical taxa (*M. richardii* is known from Africa and the Americas [van Rooy and van Wyk, 1992; Vitt 1993] and belongs to *M. ligulare* group of the Old World; *M. incurvifolium* occurs throughout the Pacific Ocean [Vitt and Ramsay, 1985]). Whether these putative affinities of *Groutiella* for neotropical *Macromitria* indicate a common ancestry with a distinct neotropical lineage of *Macromitrium* needs to be further investigated.

The genus *Macrocoma* is composed of two subgenera, subg. *Trachyphyllum* (*M. papillosa*) and subg. *Macrocoma* (*M. tenuis*), that differ by a series of characters, but particularly by the well-developed peristome of the former (Vitt, 1980). Our molecular data suggest that these two taxa, too, form an artificial group; three additional steps are needed to restore the monophyly of *Macrocoma. Macrocoma papillosa* is found in a basal position among macromitrioid taxa (excluding *Schlotheimia*) in 33 MPTs (Fig. 4), while in the remaining six it is found in a more derived position with both species of *Groutiella, Macromitrium longifolium,* and *Macrocoma tenuis.* While the Macromitrioideae are typically cladocarpous (i.e., with their perichaetia terminal on lateral branches) and have dimorphic leaves between stem and branches, *M. papillosa* is clado- and acrocarpous (i.e, with perichaetia terminal on lateral branches and on the stem) and the leaves are not differentiated into stem and branch leaves (Goffinet, 1997c). True acrocarpy also occurs in subg. *Macrocoma* (e.g., *M. braziliensis* [Mitt] Vitt), while other taxa of this subgenus are strictly cladocarpous (e.g., *M. tenuis* [Hook. & Grev.] Vitt). Acrocarpy and cladocarpy have not been reported before from the same taxon, not to mention from the same individual (see La Farge-England, 1996). The combination of plesiomorphies such as undifferentiated stem and branch leaves, terminal cauline gametangia, and the complete double peristome may be a strong indication that subg. *Trachyphyllum* is a primitive clade not only when compared to subg. *Macrocoma* (Vitt, 1982c), but maybe even with regard to the evolution of the Macromitrioideae.

Affinities of excluded taxa—Critically addressing the relationships of the taxa here excluded from the Orthotrichaceae is beyond the scope of the present study and would need a broader sampling of haplolepideous taxa. A suite of unique characters combined with the lack of characters that are phylogenetically crucial may always hamper determining sister group relationships based on morphology only. Crum (1987) already suggested that gametophytic characters may not suffice to resolve the phylogenetic relationship of *Drummondia.* The same opinion prevails with regards to *Amphidium.* Brotherus (1925), Anderson and Crum (1958), and Vitt (1973, 1982b, 1984) placed *Amphidium* near *Rhabdoweisia.* Our results, though preliminary, do not suggest that these genera are closely related, and future study may need to consider alternative relationships, as for example with *Glyphomitrium,* due to the overall similarity of *Amphidium lapponicum-Glyphomitrium daviesii.*

Presence of a peristome allows for a more explicit hypothesis to be made regarding the systematic position of the Erpodiaceae and the Rhachitheciaceae. Edwards (1979), as part of a review of the haplolepideous peristome, examined the peristome architecture of *Venturiella* and concluded that the teeth are ''strongly dorsally trabeculate, and also have a rudimentary unthickened basal exostome,'' and that ''these characters are of a haplolepideous peristome although not of the dicranaceous type'' but of a distinct type, the *Seligeria* type. This peristome type is characterized by little ventral thickening, strong dorsal trabeculae, and an exostome reduced to a thin, smooth membrane adhering to the trabeculae. This combination of characters has also been observed in the Rhachitheciaceae and has been interpreted as a possible indication of haplolepideous affinities of the family (Goffinet, 1997a). Molecular data thus tend to confirm these hypotheses, and consequently the peristome of the Rhachitheciaceae and the Erpodiaceae should be regarded as derived, through reduction, from a typical haplolepideous peristome. Though the monophyly of a group of taxa sharing the *Seligeria*-type peristome has not been critically examined, the nearly identical peristomes of *Rhachithecium, Glyphomitrium* (Ptychomitriaceae), and *Blindia* (Seligeriaceae) may be seen as an indication of close phylogenetic relationships, despite gametophytic differences. Alternatively, the Rhachitheciaceae may be more closely related to *Rhabdoweisia* in the Dicranales, considering the similarities in the overall habit, leaf shape, and cell shape and differentiation.

Phylogenetic conclusions—Sequence data of the chloroplast gene *rbcL* are useful in circumscribing the Orthotrichales, particularly with regard to the systematic position of taxa lacking peristome features that are central to the classification of mosses. Analyses of the variation in the nucleotide sequence using either the parsimony or the distance method strongly suggest that the Orthotrichales are polyphyletic and that the Erpodiaceae and the Rhachitheciaceae are of haplolepideous affinities as suggested by their *Seligeria*-type peristome. The Orthotrichaceae too, are shown to be an artificial assemblage due to the current inclusion of *Amphidium* and *Drummondia,* two genera better placed among the Haplolepideae. The Orthotrichaceae are only distantly related to the latter clade and should rather be considered a member of a derived diplolepideous clade. The relationship to the Splachnales and the ciliate mosses remains unsettled, but at present all three lineages are best considered monophyletic. Molecular data do not support Vitt's (1982a) subfamilial phylogeny and instead suggest that the Orthotrichaceae are composed of two lineages. The first consists of the Zygodontoideae and the Orthotrichoideae, while the second includes all Macromitrioideae. Putative basal taxa within these clades may be represented by *Zygodon* sect. *Bryoides* and *Schlotheimia,* respectively, which are characterized by smooth laminal cells.

The Orthotrichaceae are now composed of 20 genera: *Bryodixonia, Cardotiella, Ceuthotheca, Desmotheca, Florschuetziella, Groutiella, Leiomitrium, Leptodontiopsis, Leratia, Macrocoma, Macromitrium, Muelleriella, Orthomitrium, Orthotrichum, Pleurorthotrichum, Schlotheimia, Stenomitrium, Stoneobryum, Ulota,* and *Zygodon.* Examination of cladistic relationships and associated phenetic distances suggests that the monotypic genus *Bryodixonia* may be better regarded as a patristically derived species of *Ulota.* Our molecular data furthermore reveal that larger genera such as *Macromitrium* and *Zygodon* may merely represent evolutionary grades. Gene data obviously have made a significant contribution in resolving the circumscription of the Orthotrichaceae and the relationship of the main lineages. Above all, however, this molecular study has laid the foundation for critically reexamining the morphological characters that are central to the generic concept used in the Orthotrichaceae.

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