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Ordinal Phylogeny within the Hypnobryalean Pleurocarpous Mosses Inferred from Cladistic Analyses of Three Chloroplast DNA Sequence Data Sets: *trn*L-F, *rps*4, and *rbc*L

EFRAÍN DE LUNA
Departamento de Sistemática Vegetal, Instituto de Ecología AC., Xalapa, Ver. 91000, México

WILLIAM R. BUCK
New York Botanical Garden, Bronx, NY 10458-5126, U.S.A.

HIROYUKI AKIYAMA
Museum of Nature and Human Activities, Hyogo, 6-chome, Yayoigaoka, Sanda, Hyogo 669-1546, Japan

TOMOTSUGU ARIKAWA
Department of Biological Sciences, Graduate School of Science, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo, 113-0033, Japan

HIROMI TSUBOTA
Biological Science Course, Graduate School of Science, Hiroshima University, Kagamiyama 1-3-1, Higashi-hiroshima, Hiroshima 739-8526, Japan

DOLORES GONZÁLEZ
Departamento de Sistemática Vegetal, Instituto de Ecología AC., Xalapa, Ver. 91000, México

ANGELA E. NEWTON
Botany Department, Natural History Museum, London SW7 5BD, U.K.

A. JONATHAN SHAW
Department of Botany, Duke University, Durham NC 27708-0338, U.S.A.

Abstract. *Classification of families of hypnobryalean mosses into the Hypnales, Leucodontales, and Hookeriales has been taxonomically difficult. Several researchers have sequenced different genes for independent phylogenetic studies of these three pleurocarp groups. Our goal is to summarize available molecular data and compile the largest data set to infer phylogenetic relationships among families as basis for classification at ordinal level. Sequences of* rbc*L,* trn*L-F, and* rps*4 loci for 38 exemplars of most families of Hypnales, Leucodontales, and Hookeriales were analyzed to evaluate whether or not each of the three orders is monophyletic. Cladistic analyses of combined sequences, using five taxa in the Bryales as outgroups, reveal a robust clade (decay > 5) including all hypnobryalean pleurocarps. Within this group, one clade (decay = 2) includes only taxa of the Hookeriales, and is sister to a large monophyletic group (Hypnales* sensu lato*) containing all other taxa (decay = 2) previously in the Leucodontales and Hypnales. These relationships suggest that the ordinal level taxonomy needs to be reconsidered since major lineages detected do not correspond to the traditional Leucodontales or Hypnales. These two orders are not supported by any molecular evidence from* rbc*L,* trn*L-F, or* rps*4, either analyzed singly or in different combinations. Additionally, present results indicate the need for changes to the current system of three suborders of Hypnales and four of the Leucodontales. Phylogenetic reconstructions based on molecular data emphasize the need for a re-examination of the taxonomic relevance of morphological characters and corroborate previous interpretations of sporophytic morphological similarities as multiple transitions to similar solutions to epiphytism among the pleurocarps.*

Mosses in the subclass Bryideae (*sensu* Vitt 1984) have either haplolepidous or diplolepidous peristomes. Those with diplolepidous alternate peristomes have traditionally been classified in five orders: Bryales, Orthotrichales, Leucodontales, Hypnales, and Hookeriales (Buck & Vitt 1986; Crosby 1980; Robinson 1971). However, recent studies have put into question both the grouping of families

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constituting these orders and their inter-relationships, in particular the Orthotrichales and Bryales (De Luna 1995; Goffinet et al. 1998; Withey 1996). It also remains unclear whether or not all bryalean and hypnobryalean pleurocarps together constitute a monophyletic group (Cox & Hedderson 1999; De Luna et al. 1999; Hedenäs 1994). Certainly, the hypnobryalean mosses constitute a monophyletic group, as evaluated cladistically on the basis of morphological characters (Hedenäs 1994; Newton & De Luna 1999), sequences of the *rbc*L gene (De Luna et al. 1999), and the *trn*L-*trn*F and *rps*4 loci (Buck et al. 2000). But according to current phylogenetic studies of the relationships within this group of pleurocarps, the ordinal classification can be considered doubtful. There are preliminary indications that the Leucodontales (Buck et al. 2000; Hedenäs 1995; Newton 1993), Hypnales (Buck et al. 2000; Tsubota et al. 1999), and Hookeriales (Hedenäs 1996*a*,*b*) may not be monophyletic as classified by Buck and Vitt (1986).

The classification of approximately 52 families of hypnobryalean pleurocarpous mosses (about 6,500 species) into three groups (often treated at the ordinal rank) has been taxonomically difficult. This has been in part due to conflicting patterns of gametophyte and sporophyte character variation. For example, the morphological studies by Hedenäs (1994, 1995) and Newton and De Luna (1999) highlighted difficulties in the interpretation of homology in peristome transformations, particularly in the families of Leucodontales and Hypnales. Beside different features of peristomes, other kinds of characters have been emphasized in the classification of pleurocarps at the ordinal level. These include the appearance of the vegetative leaf costa, the shape of the median laminal cells, leaf cell papillae, and paraphyllia. A summary of the taxonomic history of the pleurocarpous mosses was given by Buck (1991) and Buck and Vitt (1986).

Previous discussions by Koponen (1979), Crosby (1980), Walther (1983), Vitt (1984), and Buck and Vitt (1986) have pointed out persistent taxonomic problems at the ordinal and subordinal level within the hypnobryalean mosses. Frey (1984), for example, anticipated that detailed studies of pleurocarpous mosses could be expected to result in a substantial revision of the ordinal classification. According to him, the Isobryales (*sensu* Crosby 1980; = Leucodontales, *sensu* Buck & Vitt 1986) and the Hypnales had long been regarded as ''very heterogeneous'' and of uncertain phylogenetic relationships (Frey 1984). Similarly, Crosby (1980) referred to the Isobryales as ''the dust bin order of the Bryidae.'' More explicitly, Koponen (1979) suggested that the Isobryales (= Leucodontales) are paraphyletic, and that some families could be included in the Hypnobryales (= Hypnales) and Eubryales (= Bryales). The last arrangement of orders, suborders, and superfamilies was suggested by Buck and Vitt (1986) ''to reflect the proposed phylogeny'' of pleurocarpous mosses. This hypothesis was based on many gametophytic and sporophytic features, but the evolutionary scenarios of character transformations were not formally analyzed. It was not until Hedenäs (1995) that ordinal grouping of families was evaluated. Recent progress in the large scale classification of pleurocarpous mosses has resulted from cladistic analyses of morphological (Hedenäs 1995; Newton & De Luna 1999) and molecular characters (Buck et al. 2000; De Luna et al. 1999). These phylogenetic studies revealed that the hypnobryalean pleurocarps form a monophyletic group, but suggested the Leucodontales and Hypnales may not be monophyletic as circumscribed by Buck and Vitt (1986).

Several researchers simultaneously have sequenced different genes for independent phylogenetic analyses of pleurocarp groups at various levels. Consequently, molecular data from three chloroplast DNA loci have become available for a wide range of species representing several families in the three hypnobryalean orders. Maeda et al. (2000) sequenced the *rbc*L gene for 35 species of Leucodontales. Arikawa and Higuchi (1999) and Tsubota et al. (1999) also sequenced *rbc*L for exemplars of Hypnales (32 and 16 species, respectively). In another study, Buck et al. (2000) sequenced the *rps*4 and *trn*L-*trn*F loci for a different set of 21 species of Leucodontales, 45 of Hypnales, and 8 species of Hookeriales. In the present study, new *rbc*L sequences were obtained for 19 taxa already sequenced for *trn*L-F and *rps*4 (Table 1). In total, *trn*L-F and *rps*4 sequences are currently available for 74 species and the *rbc*L gene has been sequenced in 102 species of hypnobryalean mosses.

In this paper we provide a summary of available data as a guide for further work and a phylogenetic hypothesis for the ordinal relationships within the pleurocarpous mosses. Our goal is to examine whether the Hypnales, Leucodontales, and Hookeriales are each monophyletic or not. We compiled *trn*L-F, *rps*4, and *rbc*L sequences for 38 species into the largest molecular data set yet to be brought to bear on this phylogenetic problem. Even though the individual data sets have been analyzed in separate publications, the current paper reports cladistic analyses based on the combined data matrix of the three chloroplast DNA sequences.

Materials and Methods

Exemplar species from Leucodontales, Hypnales, and Hookeriales.—In the classification contributed by Buck and Vitt (1986), 52 families of pleurocarpous mosses were

TABLE 1. List of 43 exemplar species included in present analyses to sample diversity in the Hypnales, Leucodontales, and Hookeriales and out-groups in the Bryales. The second column identifies the species whose sequences were used in our seven analyses. Lab indicates original source of DNA sequences as follows: A = Akiyama, Ar = Arikawa, B = Buck, D = De Luna, Ts = Tsubota, M = Mishler. Voucher data (V) for *rbc*L sequences are described in detail in the papers identified with a number as follows: 1) Maeda et al. 2000, 2) Buck et al. 2000, 3) De Luna et al. 1999, 4) Arikawa and Higuchi 1999, and 5) Tsubota et al. 1999. Voucher data for all *rsp*4, and *trn*L-F sequences are described in Buck et al. 2000.

		Lab source, vouchers and GenBank accession numbers					
		*rbc*L			*rps*4		*trn*L-F
Group represented	Exemplar species	Lab	V	Acc. #	Lab	Acc. #	Acc. #
BRYALES							
Bartramiaceae	*Bartramia halleriana* Hedw.	M	3	AF231090		—	—
	Breutelia scoparia (Schwaegr.) Jaeg.				B	AF143075	AF161168
Meesiaceae	*Leptobryum pyriforme* (Hedw.) Wils.	M	3	AF231072	B	AF023802	AF0237336
Cyrtopodaceae	*Bescherellia elegantissima* Duby	M	3	AF231097			
	Bescherellia cryphaeoides (C. Müll.) Fleisch.			—	B	AF143081	AF161174
Mniaceae	*Mnium hornum* Hedw.			—	B	AF023796	AF023767
	Mnium (= *Plagiomnium*) *cuspidatum* Hedw.	M	3	U87082		—	—
Rhizogoniaceae	*Pyrrhobryum vallis-gratiae* (Hampe) Manuel				B	AF023825	AF023754
	Pyrrhobryum mnioides (Hook.) Man.	M	3	AF231085		—	—
HYPNALES							
Amblystegiaceae	*Hygroamblystegium tenax* (Hedw.) C. Jensen	D	2	AF233565	B	AF143047	AF161140
Anomodontaceae	*Anomodon rugelii* (C. Müll.) Keissl.	A	1	AB019470	B	AF143023	AF161116
	Haplohymenium pseudotriste (C. Müll.) Broth.	A	1	AB019473		—	—
	Haplohymenium triste (De Not.) Kindb.				B	AF143022	AF161115
Brachytheciaceae	*Brachythecium plumosum* (Hedw.) Schimp.	D	2	AF233566	B	AF143078	AF161171
	Brachythecium salebrosum (Weber & Mohr) Schimp.	M	3	AF158176	B	AF143027	AF161120
	Pseudoscleropodium purum (Hedw.) Fleisch.	D	2	AF233567	B	AF143030	AF161123
Climaciaceae	*Climacium dendroides* (Hedw.) Weber & Mohr	A		AB019442		—	—
	Climacium americanum Brid.				B	AF143065	AF161158
Echinodiaceae	*Echinodium umbrosum* (Mitt.) Jaeger var. *glaucoviride* (Mitt.) Churchill	D	2	AF233568	B	AF143044	AF161137
Entodontaceae	*Entodon rubicundus* (Mitt.) Jaeger	Ts	5	AB029386		—	—
	Entodon brevisetus (Wils.) Lindb.				B	AF143057	AF161150
Fabroniaceae	*Anacamptodon splachnoides* (Brid.) Brid.	M	3	AF231077	B	AF143031	AF161124
Fontinalaceae	*Fontinalis dalecarlica* Bruch & Schimp.	M	3	AF231074	B	AF143064	AF161157
Hylocomiaceae	*Loeskeobryum brevirostre* (Brid.) Broth.			—	B	AF143079	AF161130
	Loeskeobryum cavifolium (Sande Lac.) Broth.	Ar	4	AB024658		—	—
	Rhytidiadelphus squarrosus (Hedw.) Warnst.	Ar	4	AB024667	B	AF143033	AF161126
Hypnaceae	*Ctenidium molluscum* (Hedw.) Mitt.	Ar	4	AB024657		—	—
	Ctenidium malacodes Mitt.			—	B	AF143036	AF161129
	Isopterygium tenerum (Sw.) Mitt.	D	2	AF233569	B	AF143037	AF161130
	Hypnum lindbergii Mitt.	Ts	5	AB029390	B	AF143035	AF161128

TABLE 1. Continued.

Group represented	Exemplar species	Lab source, vouchers and GenBank accession numbers					
		*rbc*L			*rps*4		*trn*L-F
		Lab	V	Acc. #	Lab	Acc. #	Acc. #
Lembophyllaceae	*Lembophyllum divulsum* (Hook.f. & Wils.) Lindb.	D	2	AF233570	B	AF143045	AF161138
Myriniaceae	*Helicodontium capillare* (Hedw.) Jaeger	D	2	AF233571	B	AF143043	AF161136
Plagiotheciaceae	*Entodontopsis leucostega* (Brid.) Buck & Ireland	Ar	4	AB024635	B	AF143060	AF161153
Sematophyllaceae	*Acroporium pungens* (Hedw.) Broth.	D	2	AF233572	B	AF143028	AF161121
	Pylaisiadelpha tenuirostris (Bruch & Schimp.) Buck	Ar	4	AB024641	B	AF143053	AF161146
	Taxithelium planum (Brid.) Mitt.	D	2	AF233573	B	AF143054	AF161147
	Trichosteleum papillosum (Hornsch.) Jaeger	D	2	AF233574	B	AF143056	AF161149
Thuidiaceae	*Thuidium delicatulum* (Hedw.) Schimp.	M	3	AF158177	B	AF143039	AF161132
LEUCODONTALES							
Cryphaeaceae	*Cryphaea sinensis* Bartr.	A	1	AB019457		—	—
	Cryphaea glomerata Sull.			—	B	AF143007	AF161100
Leptodontaceae	*Forsstroemia trichomitria* (Hedw.) Lindb.	A	1	AB019448	B	AF143006	AF161099
Leucodontaceae	*Leucodon secundus* (Harv.) Mitt.	A	1	AB019453		—	—
	Leucodon brachypus Brid.			—	B	AF143004	AF161097
Meteoriaceae	*Papillaria deppei* (C. Müll.) Jaeger	D	3	AF158172		—	—
	Papillaria nigrescens (Hedw.) Jaeger			—	B	AF143051	AF161144
Myuriaceae	*Myurium hochstetteri* (Schimp.) Kindb.	D	2	AF233575	B	AF143018	AF161111
Neckeraceae	*Neckera urnigera* C. Müll.	D	3	AF158173		—	—
	Neckera pennata Hedw.			—	B	AF143008	AF161101
Prionodontaceae	*Prionodon densus* (Hedw.) C. Müll.	D	3	AF158174	B	AF143076	AF161169
Pterobryaceae	*Pterobryon densum* Hornsch.	D	3	AF158175	B	AF143013	AF161106
	Trachyloma indicum Mitt.	A	1	AB019464		—	—
	Trachyloma diversinerve Hampe			—	B	AF143021	AF161114
Ptychomniaceae	*Ptychomnion aciculare* (Brid.) Mitt.	D	2	AF233576	B	AF143015	AF161108
Trachypodaceae	*Trachypus bicolor* Reinw. & Hornsch.	D	2	AF233577	B	AF143052	AF161145
HOOKERIALES							
Hypopterygiaceae	*Hypopterygium tamarisci* (Sw.) C. Müll.	D	3	AF158171	B	AF143077	AF161170
Hookeriaceae	*Hookeria acutifolia* Hook. & Grev.	M	3	AF158170	B	AF143071	AF161164
Pilotrichaceae	*Lepidopilum surinamense* C. Müll.	D	2	AF233578	B	AF143067	AF161160

TABLE 2. Comparison of results from seven analyses of three sequence data sets for pleurocarp mosses. Analyses 1–7 correspond to those described in the text. In all analyses, 386 gapped sites were excluded. In analyses 1–6, the total of excluded sites (excl) is the sum of gaps plus the particular size of the data set excluded in each analysis. The number of characters for an analysis is specified in terms of the number of sites included (incl), variable (var), and informative (inf). The percentage of informative sites is respect to the total of included sites (inf/incl). Other columns are: number of trees found in heuristic analyses (# MPT), length of most parsimonious trees (Length), consistency index (CI), rescaled consistency index (RC), g_1 statistic, and the data decisiveness score (DD).

Analyses	Excl	Incl	Var	Inf	%	# Trees	Length	CI	RI	RC	g_1	DD
1. *trn*L-F	2,312	484	191	98	20.2	> 38,000	477	0.545	0.424	0.231	−0.309	0.371
2. *rps*4	1,174	622	243	141	22.6	> 18,000	633	0.517	0.423	0.218	−0.612	0.384
3. *rbc*L	1,492	1,304	359	200	15.3	1	946	0.455	0.399	0.182	−0.452	0.335
4. *trn*L-F + *rps*4	1,690	1,106	434	239	21.6	2,518	1,154	0.509	0.376	0.191	−0.558	0.327
5. *trn*L-F + *rbc*L	1,008	1,788	550	298	16.6	18	1,478	0.467	0.362	0.169	−0.428	0.297
6. *rps*4 + *rbc*L	870	1,926	602	341	17.7	663	1,625	0.466	0.376	0.175	−0.598	0.318
7. three sets	386	2,410	793	439	18.2	18	2,154	0.472	0.357	0.168	−0.611	0.298

grouped in the Hypnales (25 families in three suborders), Leucodontales (24 families, four suborders), and Hookeriales (three families, one suborder). This classification was regarded by Arikawa and Higuchi (1999), Buck et al. (2000), Maeda et al. (2000), Tsubota et al. (1999) and our current study as the basic scheme for the even sampling of families in each order. The different sampling of representative families of pleurocarpous mosses resulted in 102 species sequenced for *rbc*L and 74 species sequenced for both *trn*L-F and *rps*4. A combined molecular data matrix was prepared including only those 38 exemplars for which the three data sets were available. In some cases, it was necessary to combine data from different species of the same genus in order to include a sample of a family. For example, the Entodontaceae are represented by an entry in the data matrix with the combined sequences of *Entodon brevis* (*rps*4, *trn*L-F) and *E. rubicundus* (*rbc*L). Ten pleurocarp exemplars were constructed with this type of combination of data. In total, the sequence data matrix includes 25 exemplars from the Hypnales, 10 of the Leucodontales, and three of the Hookeriales (Table 1).

Outgroups.—The choice of outgroups for our analyses was guided by previous cladistic studies (Cox & Hedderson 1999; De Luna et al. 1999; Hedenäs 1994; Newton & De Luna 1999). These analyses revealed hypnobryalean exemplars of pleurocarpous mosses forming a robust monophyletic group. The same studies also suggested families in the Bryales, especially the Rhizogoniaceae, as the potential sister groups of the hypnobryalean clade. In the present study, we included as outgroups five genera to represent five families of the Bryales as follows: *Bescherellia* (Cyrtopodaceae), *Breutelia* (Bartramiaceae), *Leptobryum* (Bryaceae), *Mnium* (Mniaceae), and *Pyrrhobryum* (Rhizogoniaceae). The particular selection of exemplar taxa from a family was also restricted by the availability of the three sequence data sets for the same species. Only the Cyrtopodaceae were represented by an entry with the combined sequences from two species: *Bescherellia cryphaeoides* (*rps*4, *trn*L-F) and *B. elegantissima* (*rbc*L). We used these five families of the Bryales as outgroups to root the tree, but without addressing the issue of their position among the diplolepidous mosses relative to the hypnobryalean clade. In the current paper, we wished to explore general patterns of relationships within the hypnobryalean mosses at the ordinal level. The questions of the monophyly of the Bryales and the phylogenetic relationships of the Rhizogoniaceae are still too complex and resolving these must await particular cladistic analyses with extensive sampling of exemplars in those groups (see Cox et al. 2000).

Gathering of molecular data.— We compiled previously available sequences of the *trn*L-*trn*F region and the *rps*4 and *rbc*L genes from chloroplast DNA for 43 moss taxa (Table 1). Sequences were obtained in five laboratories: Duke University (Durham, NC), Hiroshima University (Japan), Instituto de Ecología (Xalapa, México), Kobe University (Japan), and National Science Museum (Japan). Total DNA was extracted following protocols as described in Arikawa and Higuchi (1999), Buck et al. (2000), De Luna et al. (1999), Maeda et al. (1999), and Tsubota et al. (1999). The same papers should be consulted for lists of amplification primers, polymerase chain reaction protocols, details of the sequencing reactions, and voucher information. In general, DNA was extracted from herbarium or fresh samples of gametophytes using a CTAB protocol, and PCR amplified with Amplitaq DNA polymerase. Sequence reactions were processed with Dye terminator Cycle sequencing kits. Labeled sequence products were electrophoresed with automated DNA sequencer models ABI Prism 310, ABI 373A, and ABI Prism 373.

Phylogenetic analyses.—Each of the three sequence data sets were aligned first independently and then a combined data set was constructed using MacClade (ver. 3.06, Maddison & Maddison 1992). The Nexus file is available from the first author. The *rbc*L gene had no gaps or alignment problems, but gapped sites and sections with ambiguous alignment in the *trn*L-F region and the *rps*4 gene were excluded from all analyses. Excluding all gaps, the combined molecular data matrix contains 2,410 sites, of which 484 were from the *trn*L-F region, 622 from *rps*4, and 1,304 were *rbc*L sites. Among included sites, there were 439 informative positions, of which 98 were of the *trn*L-F region, 141 *rps*4, and 200 of the *rbc*L gene (Table 2). Since the three sequences derive from the same genome and it is void of recombination, each character set should recover the same historical pattern. However, evolutionary rates differ among loci (Clegg & Zurawski 1991) and even among partitions within the *rps*4 gene and the *trn*L-F region, as Buck et al. (2000) showed. Thus, independent analyses were done to reveal the contribution of each character suite to the combined analysis.

The search for cladograms was performed with PAUP 4.0b2a (Swofford 1999). Seven analyses with the Fitch parsimony model were conducted: each of the three sets of molecular data alone, three combinations of pairs of data sets, and the three data sets together (Table 2). Given the 43 taxa included, the only available option was to execute multiple heuristic explorations of the tree universe. We attempted 600 replicated heuristic searches for each of the seven analyses. In every replicate, we used

different starting trees built by random stepwise addition of taxa; branch swapping (TBR) was allowed to complete, saving all most parsimonious trees (MULPARS=on). In the first replicate of analyses ONE (*trn*L-F only) and TWO (*rps*4 only), the number of MPTs found was over 38,000 and over 16,000, respectively. In both cases, the 600 replicates were accomplished by holding only 100 trees at each step (steepest descent option in effect) for the TBR swapping algorithm, and saving only 100 trees of same score or larger than that of the MPTs found in the initial replicate (477 steps for analysis ONE, and 633 steps for analysis TWO). Such a strategy allowed us to go beyond one heuristic replicate, but found no shorter trees in either analysis. Other five analyses were allowed to swap to completion without such restrictions in each replicate. Strict consensus trees were constructed in cases when multiple MPTs were found, and one tree for each analysis was selected for reconstruction of branch lengths optimized according to the ACCTRAN algorithm in PAUP.

We calculated the Data Decisiveness (DD) score as a measure of information content in the data to allow the selection among different trees. A low score describes an ''undecisive'' or phylogenetically uninformative data set that produces topologies slightly different in length, and thus does not allow choice among alternative cladograms. A ''decisive'' or phylogenetically informative matrix yields topologies of very different length. The DD score measures the degree of difference in length and it is calculated according to the procedure in Kitching et al. (1998).

As an estimation of phylogenetic structure in each data set, we explored the g_1 statistic (skewness, Huelsenbeck 1991) that describes the frequency curve of tree length values for all possible trees derived from a data matrix. Using the ''RANDOM TREES'' option in PAUP 4, we estimated g_1 from random samples of 100,000 trees for each of the seven sets of data. Relative branch support within a most parsimonious tree (decay index, Bremer 1994) was estimated according to the reverse constraint PAUP method implemented in AutoDecay ver. 3.0 (Eriksson & Wikström 1995). Bootstrap percentages were calculated with 10,000 replications using the FAST option in PAUP 4. Constraint trees were used to calculate lengths of alternative hypothesis of monophyly of each of the three orders.

RESULTS

A comparison of general features of the most parsimonious trees (MPTs) found under seven different analyses is summarized in Table 2. Sequences of the *rps*4 data set contain proportionally more informative sites (22.6%) than the other two data sets taken individually or in any combination. In contrast, *rbc*L has the lowest proportion of phylogenetically informative sites (15.3%) relative to the number of sites included. On the other hand, the consistency index (CI) is higher in MPTs found with the *trn*L-F data set, and the lowest CI is in the tree inferred with the *rbc*L alone. However, in terms of the number of MPTs, the *trn*L-F data set is the most prolific, whereas the *rbc*L data set produces only one MPT.

Analyses of individual data sets.—Different resolution levels of phylogenetic relationships are reconstructed in our analyses with the three data sets taken individually. The two strict consensus trees derived from thousands of MPTs produced with the *trn*L-F sequences or *rps*4 have poor resolution (Figs. 1–2). In each consensus tree, none of the few clades resolved with these two data sets corresponds with any of the three orders of pleurocarps (*sensu* Buck & Vitt 1986). In contrast, analyses of the *rbc*L sequences alone resulted in a single most parsimonious tree (Fig. 3), which is completely resolved and shows a basal split of the 38 pleurocarp exemplars into two sister groups. One clade incorporates two of our three exemplar species of the Hookeriales; the second clade contains all other pleurocarp taxa we sampled. Internally, this large clade shows several subgroups, but none corresponds to the Leucodontales or Hypnales (*sensu* Brotherus 1925; Buck & Vitt 1986). Most subgroups include exemplars from both orders. Interestingly, two of these small clades are consistent with taxonomic concepts of families, for example three exemplars of the Brachytheciaceae are recovered as a monophyletic group.

Analyses of pairs of data sets.—The strict consensus tree of 2,518 MPTs inferred from the combination of *trn*L-F + *rps*4 sequences (analysis FOUR) is poorly resolved (results not shown), with similar clades to those found in analyses ONE and TWO. None of the few clades recovered is congruent with the three orders of pleurocarps. The other two strict consensus trees from analyses of combined *trn*L-F region + *rbc*L (analysis FIVE) and *rbc*L + *rps*4 sequences (analysis SIX) also have poor resolution (results not shown).

Analyses of three data sets combined.—Only 18 MPTs resulted from multiple heuristic searches using the combined *rbc*L + *trn*L-F region + *rps*4 data (analysis SEVEN). The strict consensus of these trees is almost completely resolved (Fig. 4). One clade includes our three representative taxa of the Hookeriales plus *Ptychomnion,* and it is sister to a large monophyletic group containing all other hypnobryalean taxa. None of the internal groups within this large clade corresponds to the Leucodontales or Hypnales (*sensu* Buck & Vitt 1986). Exemplars from both the Leucodontales and Hypnales are mixed and dispersed across several small clades. Some of these groups are the same as those recovered with the analyses of *rbc*L alone (the group including *Forsstroemia, Neckera, Echinodium,* and *Lembophyllum,* for example, Fig. 4). However, the combined tree has several novel clades that were not found in separate analyses (the group that includes *Thuidium, Hygroamblystegium,* four exemplars of Sematophyllaceae, and *Isopterygium,* for example, Fig. 4). Levels of clade support (decay index) vary from one to five or greater (Fig. 4).

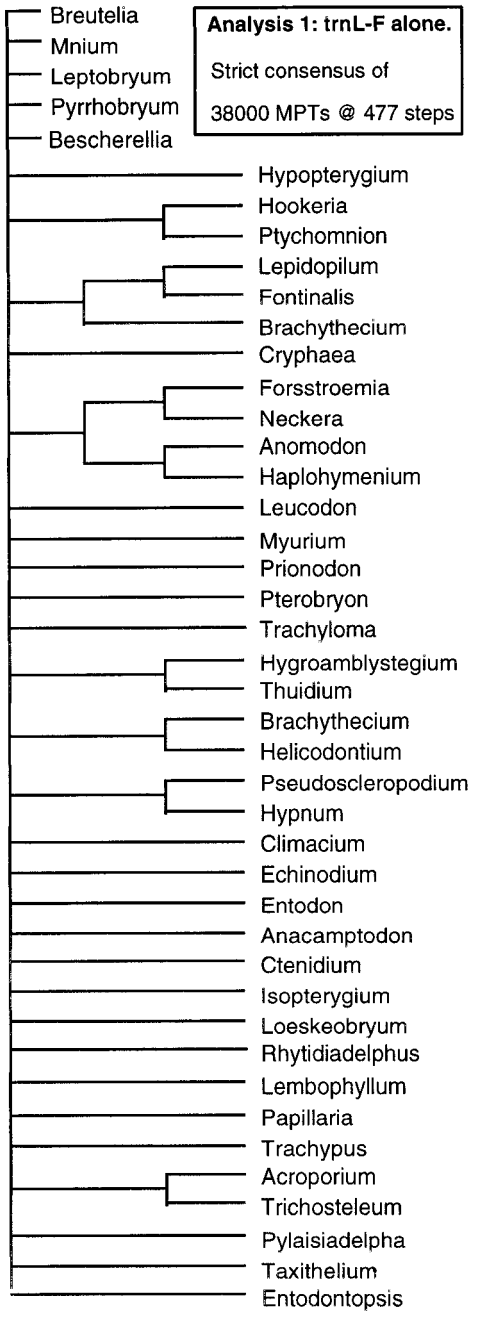

FIGURE 1. Strict consensus of 38,000 equally most parsimonious trees (477 steps, CI=0.545, RI=0.424) found in the replicated heuristic search (analysis 1) based on sequences of the *trn*L-F region for 38 exemplar species selected from the Hoookeriales, Leucodontales, and Hypnales. The tree was oriented with members of the Bryales. Complete species names are given in Table 1.

Relevant clades, such as the entire hypnobryalean group, are well supported (decay > 5). Optimization of changes on this branch show 27 states shared for this large clade (Fig. 5). The Hookeriales group decays in trees only two steps longer than the MPT, although there are 24 changes reconstructed for that branch (Fig. 5).

DISCUSSION

Systematic implications at the ordinal level.— Our combined molecular data matrix of 38 pleurocarp exemplars suggests some phylogenetic patterns and relationships at the ordinal level (Fig. 5). A consistent pattern is found in analyses, of either single or combined data sets, that are sufficiently resolved (analyses 3, 6, & 7). The Hookeriales are recovered as a monophyletic group, but the Leucodontales and Hypnales collapse and exemplars of these two orders form a single large clade. The alternative hypothesis of monophyly of these two orders was evaluated using a constraint tree with the combined data matrix. Shortest trees were 2,190 steps (CI = 0.464, RI = 0.336). These trees are 36 steps longer that the MPTs found without constraints (2,154 steps, Table 2). The consensus of many possible unconstrained trees of that length is completely unresolved due to competing topologies. The hypothesis of the Leucodontales and the Hypnales as monophyletic groups cannot be supported by our analyses of three sequence data sets. Only two orders can be recognized: Hookeriales and Hypnales *sensu lato.* The second order includes all families previously in the Leucodontales and Hypnales *sensu stricto.*

Our analyses support previous interpretations of the Hookeriales as a group (Buck 1988; Buck et al. 2000; Crosby 1974; Vitt 1984), although the circumscription and relationships of the individual families will have to await detailed analyses. Among exemplars included in our analyses, the Hypopterygiaceae, Hookeriaceae, Pilotrichaceae, and Ptychomniaceae form a monophyletic group and certainly belong in this order. The first three familes have commonly been classified in the Hookeriales, but the last family (Ptychomniaceae) has usually been included in the Leucodontales (Buck & Vitt 1986), following Fleischer (1922) and Brotherus (1925). The Ptychomniaceae has been associated with the Trachypodaceae and Meteoriaceae (Vitt 1984), but Buck and Vitt (1986) placed the family in the Garovagliacanae, with the Garovagliaceae, Myuriaceae, and the Lepyrodontaceae. However, the position of Ptychomniaceae in the Hookeriales is not novel, since Robinson (1975) intuitively classified this family close to the Hookeriaceae, and Buck et al. (2000) found it related to the Hookeriales. The alternative relationship of the Ptychomniaceae with other Hypnales *sensu lato* was examined using a constraint tree. Shorter trees under this constraint (2,373 steps, CI = 0.0, RI = 0.0, RC

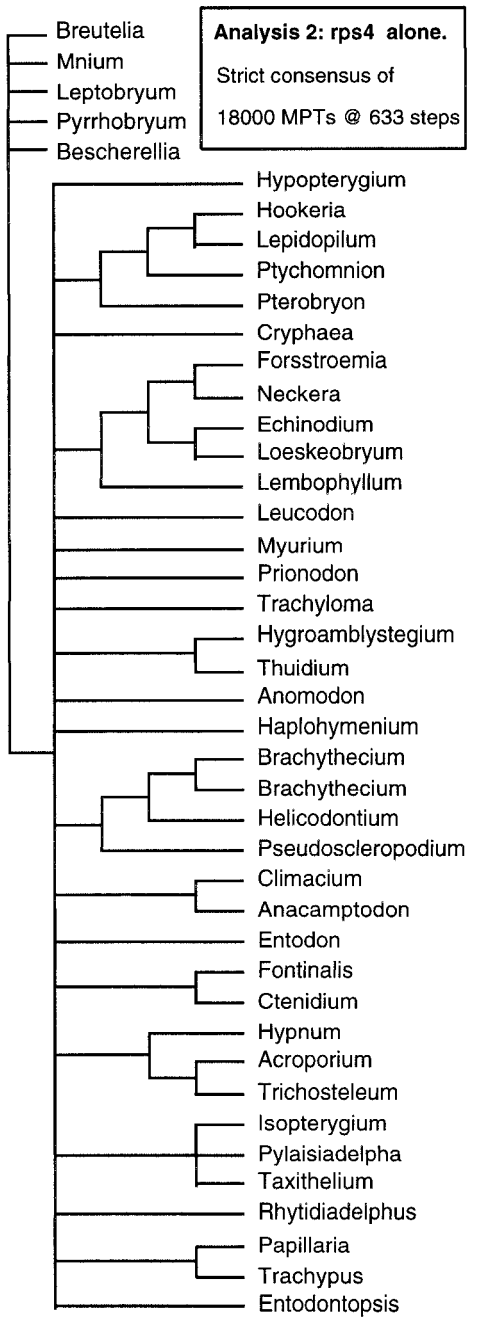

FIGURE 2. Strict consensus of 18,000 equally most parsimonious trees (633 steps, CI=0.517, RI=0.423) found in the replicated heuristic search (analysis 2) based on sequences of the *rps*4 loci for the same 38 species of pleurocarp mosses as in analysis 1. Table 1 lists complete species names.

= 0.0) are 219 steps longer than MPTs in unconstrained analyses. Our combined molecular analyses reject the traditional placement of the Ptychomniaceae in the Leucodontales. Results from constrained analyses also suggest that classification of the Ptychomniaceae in the Hookeriales would be more acceptable than its classification in the Leucodontales.

The second order supported by our molecular analyses, Hypnales *sensu lato,* includes families previously classified in the Leucodontales and Hypnales *sensu stricto* (Buck & Vitt 1986). Separate cladistic analyses of the Leucodontales and Hypnales based on *rbc*L data also found that neither order was monophyletic (Maeda et al. 2000; Tsubota et al. 1999). Similarly, recent analyses of 91 morphological characters for 39 representative species (Newton & De Luna 1999) and *trn*L-F and *rps*4 sequences for 78 pleurocarp exemplars (Buck et al. 2000) proposed that the Leucodontales and Hypnales were not monophyletic groups. Our study reaches the same conclusion using a broader character sampling (*rbc*L gene added) for 38 exemplars. The alternative hypotheses of monophyly of the Hypnales and Leucodontales were evaluated searching for most parsimonious trees using two constraint trees, each one defining just one of the orders as monophyletic. Under the topological constraint that the Hypnales are monophyletic, the most parsimonious trees are 2,175 steps long (CI = 0.468, RI = 0.345). It therefore requires at least 21 additional character state changes in comparison to the MPTs found in this study. In comparison, if the Leucodontales are monophyletic, the MPTs are 2,188 steps (CI = 0.465, RI = 0.345, RC = 0.161), 34 steps longer than the hypothesis presented here (Fig. 4). Therefore, it now seems clear from the concurrence of results with previous analyses and indications from constrained topologies that neither of these two orders (Leucodontales and Hypnales – *sensu* Buck & Vitt 1986) can be regarded as monophyletic.

Systematic implications at the subordinal level within the Hypnales sensu lato.—Present results indicate the need for changes not only at the ordinal level, but also to the current system of seven suborders of Hypnales and Leucodontales proposed by Buck and Vitt (1986). In their system, 25 families in the Hypnales were classified in three suborders: Hypnineae, Fontinalineae, and Hypnodendrineae. In the Leucodontales, they classified 24 families in four suborders: Climaciineae, Neckerineae, Leucodontineae, and Pterobryineae. Our phylogenetic analyses revealed several small clades of families in the large Hypnales + Leucodontales group, but none of these subgroups corresponds to any of the seven suborders proposed by Buck and Vitt (1986).

Although our results do not support current subordinal classifications, one clade in the molecular MPT (Fig. 5) is consistent with an existing suborder in an older classification. The group formed by the Lembophyllaceae (*Lembophyllum*), Echinodiaceae

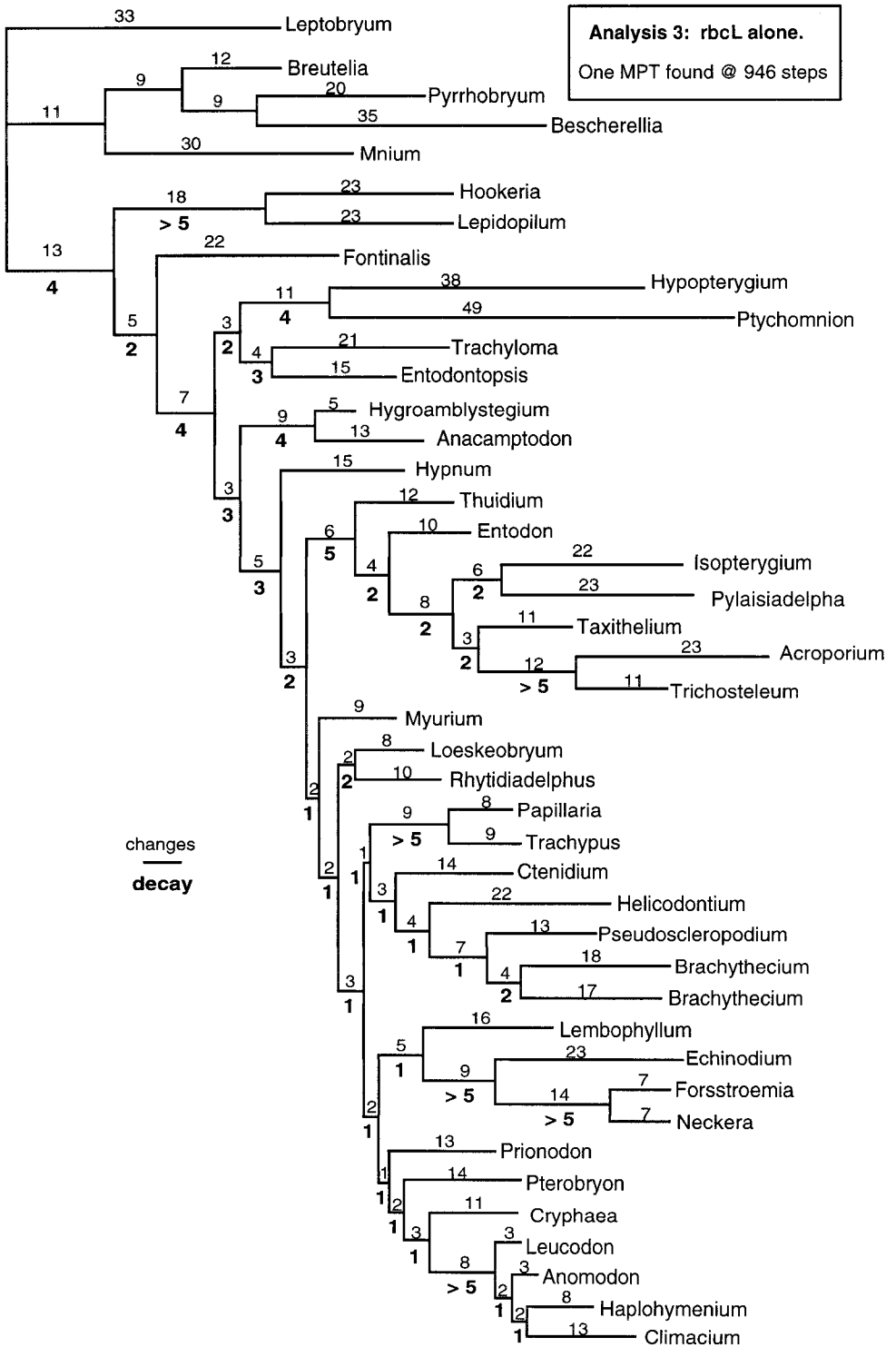

FIGURE 3. Single most parsimonious tree (946 steps, CI = 0.455, RI = 0.399) found in the replicated heuristic search using sequences of the *rbc*L gene alone (analysis 3) for the same pleurocarp mosses as in analyses 1 and 2. Numbers above branches are branch lengths (ACCTRAN), indicative of the distribution of character state changes. Numbers below branches are estimated values of the decay or branch support index (in bold).

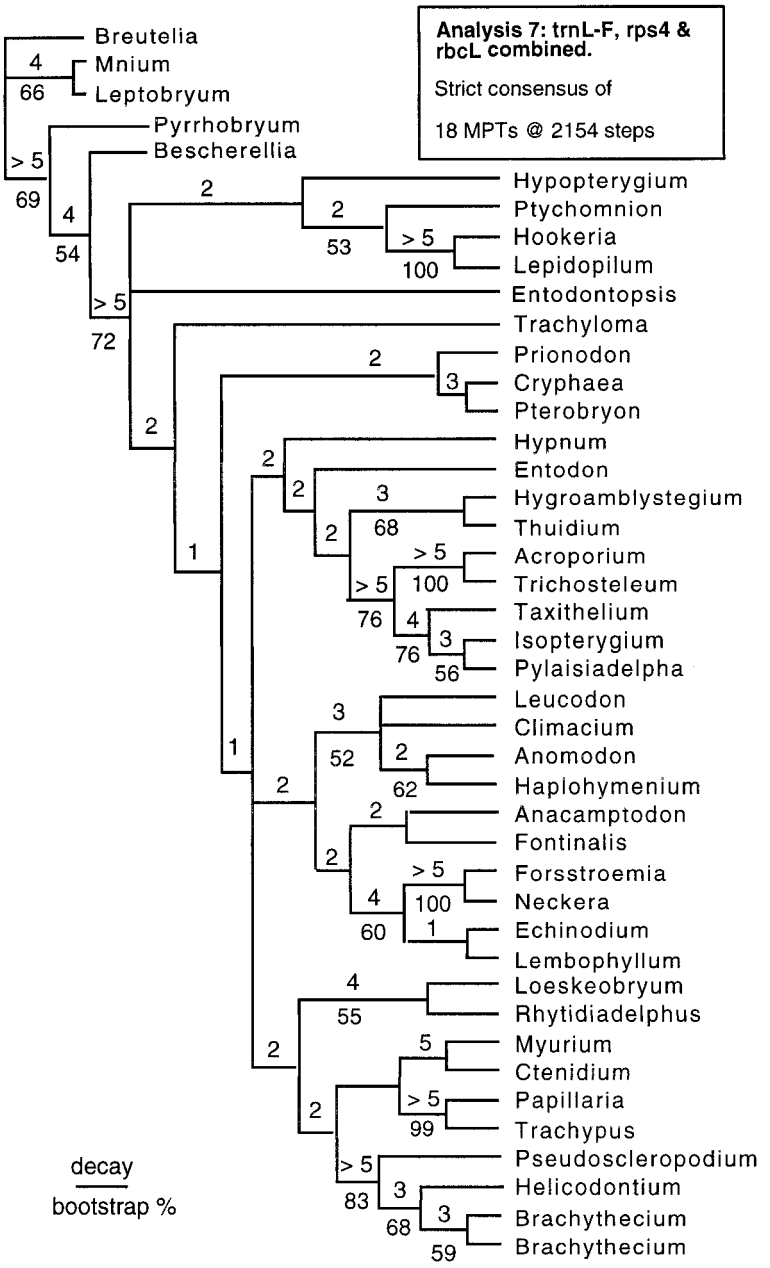

FIGURE 4. Strict consensus of 18 equally most parsimonious trees (2,154 steps, CI = 0.472, RI = 0.357) found in the replicated heuristic search (analysis 7) based on combined sequences of the *trn*L-F, *rps*4, and *rbc*L loci for 38 exemplar species selected from three orders of pleurocarpous mosses (Table 1). The same five out-group species of Bryales were used for tree orientation as in previous six analyses. Numbers above branches are estimated values of the decay or branch support index. Numbers below branches are bootstrap percentages (only values over 50% are indicated).

(*Echinodium*), Neckeraceae (*Neckera*), and Leptodontaceae (*Forsstroemia)* corresponds closely to the suborder Neckerineae *sensu* Brotherus (1925), who classified the first three families and the Phyllogoniaceae together. Our exemplars do not include a member of the Phyllogoniaceae. A study in progress using *rbc*L data to investigate in detail the relationships of the Neckeraceae (Sastre de Jesús, pers. comm.) will allow the evaluation of the relationship of Phyllogoniaceae to the Neckerineae *sensu* Brotherus (1925). This group of four families is different from the current concept of the Neckerineae *sensu* Buck and Vitt (1986). They placed together the Neckeraceae, Symphyodontaceae, and

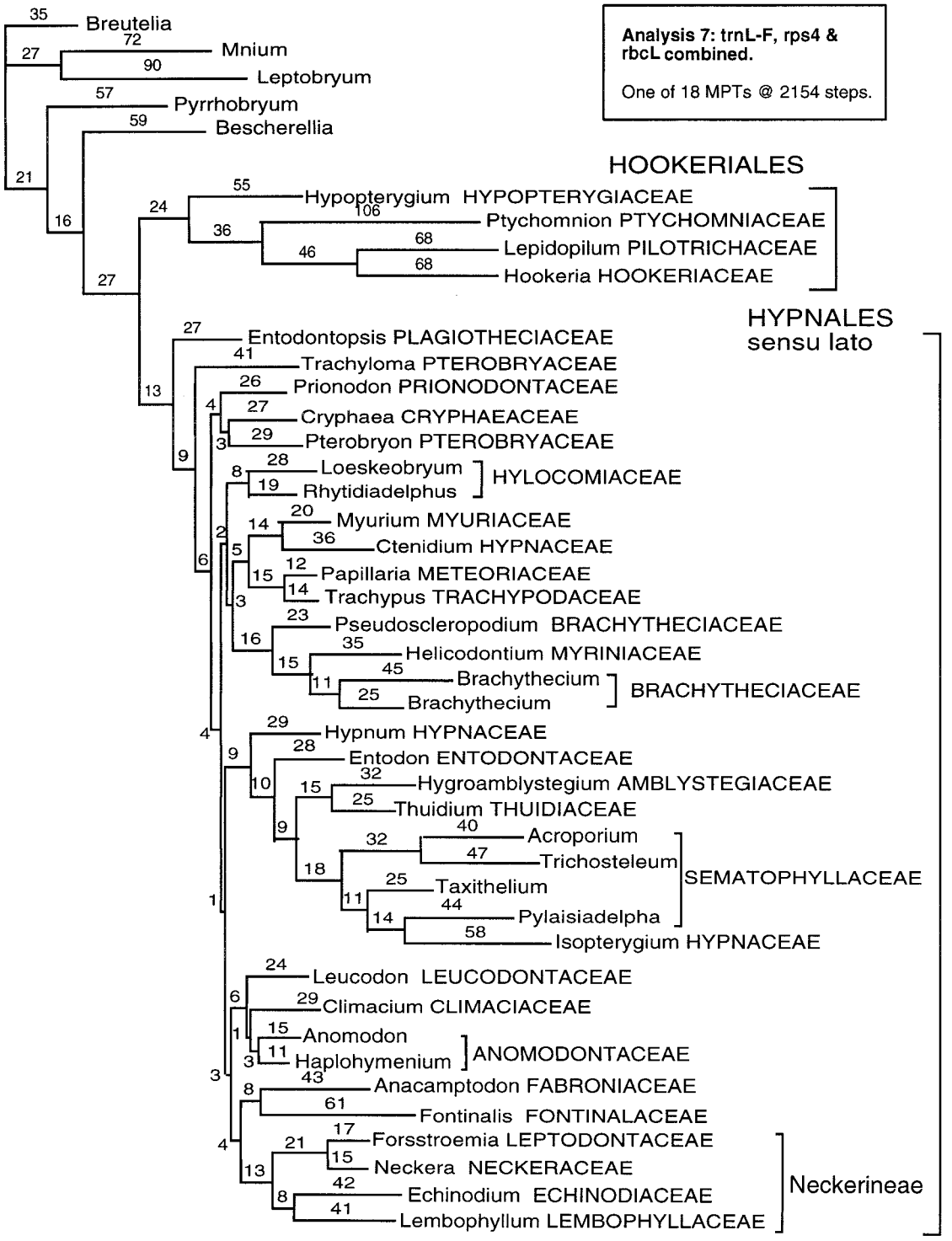

FIGURE 5. One tree selected from the set of 18 most parsimonious trees found in analysis 7 (three data sets combined). Numbers are branch lengths (ACCTRAN). This tree summarizes taxonomic implications of the current paper. Two clades are recognized at ordinal level: Hookeriales and Hypnales *sensu lato*. One clade is considered at subordinal level: Neckerineae *sensu* Brotherus (1925).

Sorapillaceae. Exemplars of the latter two families are critical in resolving whether the Neckerineae *sensu* Buck and Vitt (1986) can be rejected or whether the Neckerineae *sensu* Brotherus (1925) should be recognized, as our cladistic analyses of *rbc*L alone and three molecular data sets combined (Fig. 5) seem to suggest. The close relationship of the Leptodontaceae and Neckeraceae revealed by our molecular analyses, was recognized earlier while interpreting morphological similarities among *Forsstroemia* and *Neckera* (Ireland 1974). This close relationship was also perceived by Brotherus (1925), as implied by his placement of *Leptodon* in the Neckeraceae. Recently, a cladistic analysis of *rbc*L data from a larger sample of species of *Forsstroemia* and Cryphaeaceae, Leucodontaceae, and Neckeraceae led Maeda et al. (2000) to conclude that *Forsstroemia* should be included in the Leptodontaceae as the sister group of the Neckeraceae. Therefore the Leptodontaceae, as represented by *Forsstroemia* in our analyses, also should be included in the suborder Neckerineae (*sensu* Brotherus 1925).

Phylogenetic information of individual data sets.—This is the first study integrating three molecular data sets in order to investigate phylogenetic patterns within the pleurocarpous mosses. The use of *rbc*L, *trn*L-F, and *rps*4 sequence data in studies of relationships of mosses is still very recent (Cox & Hedderson 1999; Goffinet et al. 1998; Withey 1996, for example), and no generalizations seem possible now as to the relative value of each of the three molecular data sets for particular taxonomic levels in mosses, except those derived from many studies in vascular plants. These studies show that sequences of the *rbc*L gene change relatively slowly, at a rate that is appropriate to recover phylogenetic signal at high taxonomic levels (Pryer et al. 1995, for example). In mosses, Goffinet et al. (1998) found that 12% of sites in *rbc*L contained phylogenetic information to resolve relationships within the Orthotrichales. In contrast, other studies indicate a relatively fast rate of change in sequences of the *trn*L-F region and *rps*4 gene (Clegg & Zurawski 1991; Gielly & Taberlet 1994). In mosses, Buck et al. (2000) found that rates differed among data partitions of the *trn*L-F and *rps*4 loci and also among codon sites.

In our current analyses, the general pattern in terms of topological resolution of strict consensus trees (Figs. 1–2) suggests that there is more historical pattern in the *rbc*L alone (Fig. 3) than in the other two sequences analyzed (*trn*L-F region and *rps*4 gene) either alone or combined. Nevertheless, the CI, RI, RC, g_1 and DD values (Table 2) reveal that the *trn*L-F and *rps*4 sequences are better than the *rbc*L gene in terms of phylogenetically informative data. On one hand, the *trn*L-F sequences resolved sister groups of genera, such as *Anomodon* and *Haplohymenium*, or *Acroporium* and *Trichosteleum* (Fig. 1). These pairs of genera are placed in the Anomodontaceae and Sematophyllaceae, respectively, on the basis of morphological similarities. On the other hand, the *rps*4 sequences recovered groups, such as *Hookeria* and *Lepidopilum*, that seem reasonably consistent with their traditional placement in closely related families.

In terms of the clades recovered, the combined analysis of three loci has novel groups that were not found in each separate analyses. All our exemplars of the Hookeriales, for example, only form a clade in the combined tree (Fig. 4). This synergistic effect of data combination also increases the branch support values for those branches that are found in separate analyses. The clade that includes *Neckera*, *Forsstroemia*, *Echinodium*, and *Lembophyllum* has a decay index of one with *rbc*L data (Fig. 3), for example, but it goes up to four in the combined analysis (Fig. 4). However, these features of combined analyses do not seem to derive from the *trn*L-F and *rps*4 sequences, since the consensus tree did not have enough resolution. The strict consensus tree from an analysis with a larger taxon sampling than ours was not resolved either (Buck et al. 2000). Only when excluding hypervariable regions (i.e., the spacer and third codon positions in the *rps*4 gene), did they reveal the Hookeriales and Hypnales, and other lineages within the Hypnales. Thus, *trn*L and *rps*4 may not be the best sequences to use for phylogenetic reconstruction within the pleurocarps, especially when taxon sampling is limited. The addition of *rbc*L characters had a beneficial effect on clade resolution and branch support and we were able to discover the same major clades (Hookeriales and Hypnales) even though our taxon sampling was smaller than that of Buck et al. (2000).

Based on current results we can recommend that sequencing of the *rbc*L gene should be pursued further at the subordinal and family levels within the hypnobryalean mosses. Besides, it should be noted that the mean length of terminal branches is about 15 changes in the *rbc*L gene (Fig. 3). This might indicate a potential for further resolving power even at generic levels, especially in the Hookeriales where the terminal branch length varies from 23 to 49 changes in four exemplars. In turn, it seems that the faster *trn*L-F and *rps*4 sequences would potentially be more useful than the *rbc*L at generic levels and below. Additional genes or spacers, such as *atp*B (Hoot et al. 1995), *mat*K (Steele & Vilgalys 1994), *ndh*F (Olmstead & Sweere 1994), 18S (Soltis et al. 1999), and ITS (Baldwin et al. 1995) re-

main to be explored for their utility for phylogenetic reconstructions within the pleurocarps.

Morphological evolution.—The current phylogenetic reconstruction of the pleurocarp relationships has implications for the interpretation of the taxonomic relevance of morphological characters from the gametophyte and sporophyte. The collapse of the orders Hypnales and Leucodontales in this and other studies based on molecular data (Buck et al. 2000; Maeda et al. 2000; Tsubota et al. 1999) suggests that many of the gametophytic and sporophytic characters traditionally used to circumscribe these groups are convergent. Morphological studies (Hedenäs 1994, 1995; Newton & De Luna 1999) using cladistic methodology also show breakdown of the traditional ordinal classifications, indicating that the perceived general patterns do not withstand more precise examination. It seems, indeed, that many of the morphological characters may reflect multiple transitions and adaptations to similar habitats in the pleurocarps.

In a ''Generalized Conspectus'' of the higher level taxa of the mosses, Vitt (1984, pp. 738–740) summarized the ''defining apotypic states and the characteristic plesiotypic states'' for these three groups (as the suborders Hypnineae, Leucodontineae, and Hookerineae). When considered in the light of the current results, the possibility that many of these features represent convergence rather than shared evolutionary history becomes apparent. The ''perfect'' hypnoid peristome, similar to that seen in the sister group Bryales (Cox et al. 2000), would seem to represent the plesiomorphic condition in the traditional pleurocarps, with multiple reductions to produce the different variations seen in the ''Leucodontales''. The alternative, that the taxa with hypnoid peristomes share a common ancestor, and form the sister group to the taxa with reduced peristomes, is in conflict with the molecular sequence data. The Hookeriales, as an order, are supported here and in other studies, but as yet no published morphological character(s) seem to be strong synapomorphies for the entire group. Detailed cladistic analyses of this order, as a hypothesized monophyletic group, may disclose such characters, and will facilitate studies of evolutionary patterns in the other pleurocarpous mosses.

The traditional pleurocarpous mosses are supported by molecular analyses (Buck et al. 2000; Cox et al. 2000; De Luna et al. 1999), and also share some morphological characters. The costae, even in taxa where they are well developed, reaching to the leaf apex and multistratose, are formed of cells uniform in transverse section (homogenous costa of Hedenäs 1994) with no differentiation of deuters, stereids, or sub-stereids. Rhizoids formed at the abaxial base of the leaves showing the ''distal-contact'' branching pattern (Newton & De Luna 1999) might also be a synapomorphy. These rhizoids remain unbranched until they contact a firm substrate, but then form a densely branched rhizoidal plate. However, the positioning of perichaetia on reduced lateral branches may not be a synapomorphy for the traditional hypnobryalean pleurocarps. A morphologically similar condition is found in the taxa placed in the Eubryalean pleurocarps, but the two groups of pleurocarps are separated by undoubted acrocarps (*Orthodontium, Aulacomnium;* Cox et al. 2000), indicating that this arrangement of the perichaetia has evolved in parallel in the two groups. When viewed on a smaller scale, at the family and genus level, many morphological features may indeed represent localized homologies, with individual groups adapting to similar habitats and similar environmental problems by adopting similar morphologies. At the broader scale, however, these similar morphologies are not homologous, and will adversely affect understanding of the relationships of the taxa involved.

Conclusions

Our molecular analyses have corroborated that the hypnobryalean pleurocarps are monophyletic and that this group consists of two taxonomically important lineages at the ordinal level: the Hookeriales and the Hypnales *sensu lato.* Both clades are weakly supported, as suggested by decay indices of two in the combined analysis. The Hypnales *sensu lato* includes taxa previously in the traditional Leucodontales and Hypnales. Available taxon sampling and molecular characters from three loci studied here also suggest that the current system of three suborders of Hypnales and four of Leucodontales need to be re-evaluated. Furthermore, our phylogenetic hypothesis suggests the re-establishment of the Neckerineae (*sensu* Brotherus 1925).

The combination of sequences evolving at different rates seem to be complementary. Combined analyses of three molecular data sets yield novel clades that were not found in separate analyses. Also, support values for same branches are higher in the combined analyses than in those found in separate analyses. Further research on the phylogenetic relationships within particular groups of pleurocarps need to evaluate the contribution of DNA sequence data. More phylogenetic studies could corroborate our interpretation that *rbc*L data seems better than the *trn*L-F and *rps*4 sequences at resolving phylogenetic relationships at the ordinal and subordinal levels within the pleurocarps, but that the latter two loci would also be useful at familial and generic levels.

Two developments are driving current research

in moss systematics: cladistic methods and the use of diverse DNA sequences. These analytical and empirical factors combined have made it possible to address the phylogeny and classification of the main lineages of mosses (e.g., papers in this Symposium issue). In that framework, our goal beyond this paper is to continue promoting an international collaborative effort to further study the phylogenetic relationships within the pleurocarpous mosses.

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