The Bryologist 102(4), pp. 634–650

The Transition to Pleurocarpy: A Phylogenetic Analysis of the Main Diplolepidous Lineages Based on *rbc*L Sequences and Morphology

EFRAIN DE LUNA and ANGELA E. NEWTON[1]
Departamento de Sistemática Vegetal, Instituto de Ecología, AC., Xalapa, Veracruz 91000, México

ALISON WITHEY
Department of Botany, Duke University, Durham, NC 27708, U.S.A.

DOLORES GONZALEZ
Departamento de Sistemática Vegetal, Instituto de Ecología, AC., Xalapa, Veracruz 91000, México

BRENT D. MISHLER
University Herbarium, Jepson Herbarium and Department of Integrative Biology, University of California, Berkeley, CA 94720-2465 U.S.A.

Abstract. *Hypotheses of character evolution require a phylogeny for polarization of transformations and optimization of state changes. Our goal is to provide a phylogenetic hypothesis for diplolepidous mosses to investigate the evolution of morphological features associated with the transition to pleurocarpy. We performed cladistic analyses of morphological and molecular data sets for exemplars of the main diplolepidous lineages. These analyses were designed to sample families commonly included in the Leucodontales, Hookeriales, and Hypnales with some exemplars also from the Bryales. Diplolepidous taxa (Bryaceae, Splachnaceae, Orthotrichaceae, Macromitriaceae, and Hedwigiaceae) were included as close outgroups, and* Dicranum scoparium, Grimmia apocarpa, *and* Funaria hygrometrica *were included as distant outgroups. We constructed a molecular data set derived from sequences of the chloroplast* rbc*L gene for 36 species, 22 of which were pleurocarp exemplars. In the molecular analysis, the bryalean pleurocarps were the sister group of acrocarp exemplars from the Bryales. However, in the analyses of combined morphological and* rbc*L data, the bryalean pleurocarps were the sister group of a clade that includes the 11 exemplars from the Leucodontales, Hypnales, and Hookeriales, thus pleurocarpy appeared monophyletic. Decay analyses suggested that the grouping of bryalean and hypnobryalean pleurocarps together was weak, whereas both the hypnobryalean and bryalean pleurocarp clades were individually robust. Present cladistic analyses provide an inferential basis for structural investigations of branching systems and the evolution of pleurocarpy in a phylogenetic context.*

Intriguing problems of character evolution in the diplolepidous mosses, such as the origin of pleurocarpy, have recently received renewed interest (Hedenäs 1994; La Farge-England 1996; Withey 1996*a*). The transition to pleurocarpy represents an event of morphological innovation in several features of the gametophyte. While relatively simple branching systems and naked branch primordia are common among acrocarps, pleurocarps are characterized by complex, iterative branching, and branch primordia surrounded by scale-like leaves and specialized leaf-like structures (pseudoparaphyllia). Available hypotheses explain the evolution of pleurocarpy as two events (Buck & Vitt 1986), or as a gradual process involving changes in several characters (Hedenäs 1994), some of which may have occurred independently in at least two lineages of mosses with diplolepidous alternate peristomes (Withey 1996*a*).

Empirical and methodological limitations have hindered the study of character evolution in pleurocarpous mosses. Empirically, it has been difficult to study the origin and early diversification of pleurocarp mosses due to different definitions of pleurocarpy (Buck & Vitt 1986; La Farge-England 1996; Meusel 1935; Schofield & Hébant 1984). An architectural analysis of modular construction and an ontogenetic approach are becoming crucial to the understanding of pleurocarpy as a combination of many independent features of the archegonial module, branching system, and structures derived from a single merophyte (Mishler & De Luna 1991; Newton & De Luna 1999). Morphological studies

[1] Present address: Department of Botany, Natural History Museum, London SW7 5BD, U.K.

0007-2745/99/634–650$1.85/0

by Hedenäs (1994, 1995) have revealed the conceptual and empirical difficulties of interpreting gametophyte diversity and peristome specializations in the pleurocarps. However, a molecular approach can provide independent data sets from gene sequences to facilitate the discovery of large scale patterns of relationships among phylogenetic groups in the diplolepidous mosses. Using this approach, a preliminary *rbc*L phylogeny for a limited number of pleurocarp taxa allowed Withey (1996*a*) to infer correlations among morphological characters associated with pleurocarpy.

Another difficulty has been methodological because of the lack of cladistic analyses for the diplolepidous mosses to guide inferences on the evolution of pleurocarpy. Available classifications and postulated relationships among the Bryales, Leucodontales, Hypnales, and Hookeriales (Buck & Vitt 1986; Crosby 1980; Robinson 1971) are the current best estimates. However, such classifications do not provide an explicit and objective phylogenetic framework necessary for studies of character evolution. At the ordinal level, preliminary hypotheses of the phylogenetic relationships of pleurocarp taxa were developed by Hedenäs (1994, 1995, 1996*a,b*), but the results of these analyses should be treated with caution in view of his *a priori* dismissal of some morphological features. In his data matrices, similarities in the reduced peristomes in the pleurocarps have been coded as unknown because "characters cannot be homologized, or if it cannot be made sure that the reduced peristomes found have a common origin" (Hedenäs 1994, p. 226; but see Newton & De Luna 1999). However, such interpretations of homoplasy can only be made following the congruence test of homology i.e., after a cladogram has been produced (Mishler & De Luna 1991).

At the family level, some bryalean and hypnobryalean pleurocarp groups already have been analyzed cladistically, but this has had little impact on ordinal relationships (Anomodontaceae, Granzow-de la Cerda 1990; Neckeraceae, Hyvönen & Enroth 1994; Pterobryaceae, Newton 1993; Rigodiaceae, Zomlefer 1993; Spiridentaceae, Withey 1996*b*). Other cladistic studies (Cox & Hedderson 1999; De Luna 1995; Newton 1993; Withey 1996*b*) are also of limited application, but for a different reason: the sparse sampling across the pleurocarpous taxa. In summary, the large scale scheme of phylogenetic relationships among orders and families of pleurocarpous mosses remains inconclusive.

In this paper we provide a phylogenetic hypothesis for the large-scale relationships of diplolepidous pleurocarpous mosses. We address the question of whether the pleurocarpous mosses in the Bryales, Leucodontales, Hypnales, and Hookeriales together form a monophyletic group. This question is closely related to the problem of how pleurocarpy should be defined. We approach such morphological questions in a separate paper on the reconstruction of the evolution of features associated with pleurocarpy (Newton & De Luna 1999). Here, we present a molecular data set derived from *rbc*L gene sequences. The cladistic analyses of these data separately and in combination with the morphological data set from Newton and De Luna (1999) provide phylogenetic reconstructions that we use as an inferential basis for assessing whether pleurocarpy has evolved only once or several times among diplolepidous mosses.

Materials and Methods

Choice of representative families.—Traditionally, the classification of families with diplolepidous alternate peristomes into orders has been based on variation of two character systems: the features of the peristome, and the position of archegonia on branches. Thus, the mainly acrocarpous mosses in the Bryales and Orthotrichales have been regarded as basal to a "derived" group composed of pleurocarpous families in the Hypnales, Leucodontales, and Hookeriales (Buck & Vitt 1986; Crosby 1980; Robinson 1971). Although this is not a phylogenetic classification, we used it here as a basic scheme for sampling families. We also took into consideration a recent preliminary rearrangement within the Bryales following Withey (1996*a*). Thus, we designed the cladistic analyses to sample morphology and *rbc*L sequences from representative families of pleurocarpous mosses commonly included in the Bryales, Hypnales, Leucodontales, and Hookeriales.

We sampled the seven families from Clade "A" *sensu* Withey (1996*a*). This clade includes the acrocarpous Mniaceae and Bartramiaceae and the pleurocarpous Spiridentaceae, Hypnodendraceae (previously in the Hypnales, Buck & Vitt 1986), Cyrtopodaceae (previously in the Leucodontales), Racopilaceae (transferred from the Hypnales to the Bryales, Buck & Vitt 1986), and Rhizogoniaceae. The other seven families also in the Bryales *sensu* Vitt (1984), Leptostomataceae, Mitteniaceae, Schistostegaceae, Timmiaceae, Aulacomniaceae, Meesiaceae, and Catoscopiaceae, were not included in present analyses. The Bryaceae was included, but as one of several outgroups.

Putative "true" pleurocarps *sensu* Buck and Vitt (1986) were sampled by including 13 exemplar species from the three currently recognized orders. Our goal was not to test or propose a classification of families. Rather, we wanted to represent putative major lineages by sampling a wide collection of hypnobryalean pleurocarpous mosses of diverse ordinal relationships. We selected four exemplar species from the Hypnales (four families), six species from the Leucodontales (six families), and three species from the Hookeriales (Table 1).

The selection of close outgroups for tree orientation was guided by previous cladistic studies by De Luna (1995) and Withey (1996*a,b*). These studies suggested a sister group relationship between the pleurocarpous mosses and a clade of cladocarpous families in either the Orthotrichales or the Bryales, respectively. To represent these orders we selected seven exemplar species of the following families: Bryaceae (2), Splachnaceae (1), Orthotrichaceae (1), Macromitriaceae (2), and Hedwigiaceae (1). Additionally, *Dicranum scoparium, Grimmia apocarpa,* and

TABLE 1. Exemplar species selected among diplolepidous mosses for *rbc*L gene sequencing. Pleurocarp groups represented are according to the classification of Buck and Vitt (1986). Sequence reference numbers following the species names indicate lab of origin (M = Berkeley, W = Duke, D = Xalapa, G = B. Goffinet). Genbank accession numbers are given where available. Voucher information is in Appendix 1.

Group represented	Exemplar species	Sequence
Grimmiales		
Grimmiaceae	*Grimmia* (=*Schistidium*) *apocarpa*	M–24
Dicranales		
Dicranaceae	*Dicranum scoparium*	M–11
Funariales		
Funariaceae	*Funaria hygrometrica*	M–49
Splachnales		
Splachnaceae	*Splachnum ampullaceum*	M–52
Orthotrichales		
Macromitriaceae	*Macromitrium longifolium*	G
	Schlotheimia brownii	G
Orthotrichaceae	*Orthotrichum pumilum*	M–83
Hedwigiaceae	*Hedwigia ciliata*	M–78
Bryales		
Bryaceae	*Bryum billardieri*	W
	Leptobryum pyriforme	M–39
Bartramiaceae	*Bartramia halleriana*	W
	Philonotis nitida	W
Cyrtopodaceae	*Cyrtopus setosus*	W
	Bescherellia elegantissima	W
Hypnodendraceae	*Hypnodendron vitiense*	W
	Hypnodendron menziesii	W
Mniaceae	*Plagiomnium cuspidatum*	M–82
Racopilaceae	*Racopilum convolutaceum*	W
Rhizogoniaceae	*Pyrrhobryum mnioides*	W
	Cryptopodium bartramioides	W
	Mesochaete undulata	W
Spiridentaceae	*Spiridens vieillardii*	W
	Franciella spiridentoides	W
Hypnales subord. Hypnineae		
Brachytheciaceae	*Brachythecium salebrosum*	M–32 (AF158176)
Fabroniaceae	*Anacamptodon splachnoides*	M–48
Thuidiaceae	*Thuidium delicatulum*	M–12 (AF158177)
Hypnales subord. Fontinalineae		
Fontinalaceae	*Fontinalis dalecarlica*	M–34
Hypnales subord. Hypnodendrineae		
Pleuroziopsidaceae	*Pleurozium schreberi*	M–59
Leucodontales subord. Pterobryineae		
Meteoriaceae	*Papillaria deppei*	D–6 (AF158172)
Prionodontaceae	*Prionodon densus*	D–21 (AF158174)
Pterobryaceae	*Pterobryon densum*	D–22 (AF158175)
Leucodontales subord. Leucodontineae		
Leucodontaceae	*Leucodon julaceous*	M–50
Leucodontales subord. Neckerineae		
Neckeraceae	*Neckera urnigera*	D–8 (AF158173)
Hookeriales		
Hookeriaceae	*Hookeria acutifolia*	M–22 (AF158170)
Hypopterigiaceae	*Hypopterigium tahitense*	W
	Hypopterigium tamariscinum	D–7 (AF158171)

Funaria hygrometrica were included as a set of more distant outgroups to properly locate an overall root for the trees that included the ingroup and the close outgroup taxa.

Morphological data.—The morphological studies by Hedenäs (1994, 1995) have revealed the difficulty of interpreting variation in the gametophyte and specializations in the peristomes in pleurocarpous mosses. We re-examined many of the same morphological characters, but added others derived from the previous studies by De Luna (1992), Newton (1993), and Withey (1996*b*). Several specimens were studied from the exemplar species included in the present cladistic analyses. These observations were complemented by additional specimens representing other species from the same genera. Relevant descriptions in the literature were also consulted for each taxon. The morphological character analyses are presented in detail separately (Newton & De Luna 1999).

DNA sequence data.—Molecular data are increasingly being used to infer phylogenetic relationships among major clades of mosses, liverworts, and hornworts (Bopp & Capesius 1995; Mishler et al. 1992, 1994; Waters et al.

TABLE 2. Primers for amplification and sequencing the *rbc*L gene in pleurocarp mosses for present studies.

PCR amplification	
Forward primers	
M34	5′-GGATTTAAAGCTGGTGT-3′
RH1	5′-ATGTCACCACAAACAGAAACTAAAGC-3′
Reverse primers	
M1390R	3′-GACGACGAACACTTTAAACCTTTC-5′
Primers for sequencing	
Forward primers	
M34	5′-GGATTTAAAGCTGGTGT-3′
RH1	5′-ATGTCACCACAAACAGAAACTAAAGC-3′
M636	5′-GCGTTGGAGAGATCGTTTCT-3′
Reverse primers	
M1390R	3′-GACGACGAACACTTTAAACCTTTC-5′
M740R	3′-CGATGACGTCCATGTAC-5′

1992). Within the mosses, variation in the nuclear 18s rRNA gene has been explored for a general outline of relationships (Hedderson pers. comm.). Also, sequences of the chloroplast *rbc*L gene have been used for an overview of embryophyte phylogeny (Mishler pers. comm.). Among diplolepidous mosses, this is the first attempt to document *rbc*L sequences from pleurocarps. Gene sequences of the *rbc*L were obtained in three laboratories: University of California (Berkeley, California, coded as "M"), Duke University (Durham, North Carolina, coded as "W"), and Instituto de Ecología (Xalapa, México, coded as "D"); the origin of sequences is identified in Table 1. Two sequences included to represent the Orthotrichales were kindly send by Bernard Goffinet (Duke University). Procedures for extraction, amplification, and sequencing of taxa marked as "M" and "W" (Table 1) can be found through Mishler (pers. comm.) and Withey (1996*b*), respectively. Details of procedures for DNA extraction, amplification, and sequencing for taxa marked "D" are presented here since these were modified substantially from the regular CTAB method of Doyle and Doyle (1987).

DNA extraction.—Total DNA for PCR amplification was prepared using a modification from the Doyle and Doyle (1987) protocol as follows. Clean moss gametophytes were first finely sliced with a razor blade to yield an homogeneous pulp (about 125 to 200 mg, fresh weight). This pulp was deposited into an Eppendorf tube with 400 μl of CTAB 1× added for extraction during 30 minutes. If this extraction time was not sufficient, the pulp was kept and reused for subsequent extractions (15 minutes) as needed in some taxa. The extract solution was recovered into another Eppendorf tube and mixed with 200 μl of Chloroform-Isoamidic alcohol for 5–10 minutes at room temperature. This mix was centrifuged (13,000 rpm, 10 min) and about 400 μl of supernatant were recovered. Absolute alcohol (600 μl) was added and the solution was stored at −20°C for two hours. Precipitated DNA was recovered by centrifuging (13,000 rpm, 10 min), washing the pellet with ethanol (70%, 200 μl), and vacuum drying for five mins. This DNA extract was suspended in 20 μl of TE and purified using a low-melting point agarose gel (0.6% Sigma). The high molecular-weight DNA was cut out from the gels and diluted to a concentration of 0.1 ng per μl. Each sample was incubated at 65°C to dissolve the agarose before preparation of the PCR reactions.

PCR amplification.—Primers used for amplification are those listed in Table 2. The amplification of the *rbc*L gene was set up with Amplitaq DNA polymerase using the conditions recommended by the manufacturer (Perkin-Elmer) and carried out in an automated thermocycler. Purified DNA (10 μl) was subjected to 30 PCR cycles in 50 μl of reaction volume. Temperature profiles for each cycle consisted of 94°C (one minute) for denaturation, 48°C (one minute) for annealing, and 72°C (two minutes) for extension. After the last cycle, samples were incubated at 72°C (seven minutes). Upon completion of reactions, the excess of deoxy terminators and Taq polymerase was removed with a phenol/chloroform extraction.

Gene sequences.—Amplified PCR products were sequenced with the AmpliTaq cycle sequencing kit (Perkin-Elmer), performing successive rounds of denaturation, annealing, and extension. Each sequencing reaction contained a PCR-amplified template, a primer, and fluorescent dideoxynucleotides. Sequence products were then purified with a phenol/chloroform extraction. Samples were loaded on a 4.25% acrylamide-bis-acrylamide gel and electrophoresed at 47 W. These reactions were analyzed with a sequencer ABI model 373A (Perkin-Elmer).

Phylogenetic analyses.—The molecular data matrix included 1,320 nucleotides for 36 taxa. Alignment of sequences was performed visually. The morphological data set for 39 exemplars consisted of 91 characters, with from two to eight states. The independent treatment of this data and cladistic results are reported elsewhere (Newton & De Luna 1999). A combined data matrix was also produced, including only those 28 taxa for which both *rbc*L sequences and morphological data were available. The two Nexus files are available from the authors on request.

Cladistic analyses using the molecular data alone and the molecular data combined with the morphological data are reported here—see Newton and De Luna (1999) for analysis of the morphological data. All searches for most parsimonious cladograms were performed with the program PAUP 3.1.1 (Swofford 1993). Given the number of taxa included (36 *rbc*L; 28 combined), the only available option was to execute multiple heuristic searches. Each heuristic exploration evaluates an island of trees derived from a single random starting tree. A large number of different starting trees are necessary to avoid limiting the search to one island (Maddison 1991). We performed replicated heuristic searches (with PAUP steepest descent option in effect), in which a starting tree is built by random stepwise addition of taxa and then swapped to completion (PAUP branch swapping option = TBR) saving all most parsimonious trees (PAUP option MULPARS = ON). We replicated this tree search procedure 300 times for each analysis.

The data were analyzed first under equal weighting, then the molecular characters were analyzed using the character-state weighting method of Albert et al. (1993). This approach simultaneously accounts for differential

probabilities of change in transition/tranversion ratios and codon position biases, through the use of three symmetric step matrices (one for each codon position). Values for parameters in the model were estimated from previous studies of *rbc*L evolution in land plants as discussed by Albert et al. (1992, 1993). For the three codon positions respectively, the character state weights for transitions/tranversions applied were: 0.552/0.662; 0.637/0.747; 0.404/0.513.

One estimation of historical pattern in a data set is the shape of the tree-length frequency distribution of all possible trees (skewness–Huelsenbeck 1991). The g1 statistic is <0 for a left-skewed distribution, indicating phylogenetically informative data. Using the "random trees" option in PAUP, we estimated the statistics of the frequency distribution of lengths from a sample of 10,000 trees for both the *rbc*L data and the combined data sets.

The stability of branches or cladograms was examined with additional analyses excluding particular taxa from the tree search. Topological stability was explored by the use of different combinations of exemplars to represent large clades. The relative support for different branches in the most parsimonious trees was evaluated by relaxing parsimony one step at a time and seeing which branches remained supported the longest (the decay or branch support index, Bremer 1994; Mishler et al. 1991). Levels of clade support for both the *rbc*L and the combined data sets were conducted with PAUP by doing heuristic searches using TBR and saving all trees up to four steps longer than the most parsimonious. Decay indices (di) were taken from examining the strict consensus of each increasingly suboptimal class of trees.

Results

Analysis of rbcL sequence data.—The sequences of the *rbc*L gene were 1320 bases long. These were aligned with previously known sequences from *Sphagnum, Andreaea,* and several haplolepidous mosses (Mishler pers. comm.). Alignment among sequences resulted in 206 informative sites and did not require the inclusion of gaps. The cladistic analysis of sequence data alone yielded three most parsimonious trees (742 steps, CI = 0.352, RI = 0.528). The strict consensus tree (Fig. 1) is almost completely resolved and reveals the existence of three main clades (A, B, C) relevant to the question of the evolution of pleurocarpy. One group (clade A, Fig. 1) incorporates families of pleurocarpous and some acrocarpous mosses traditionally placed in the Bryales. This is consistent with clade "A" found in previous studies by Withey (1996*a*). A second clade (Fig. 1, B), sister to the first, includes taxa traditionally classified in the Hookeriales, Leucodontales, and Hypnales. However, these orders are not recovered as monophyletic groups, except for the few exemplars of the Hookeriales (*Hookeria* and *Hypopterygium*). The Hedwigiaceae (represented by *Hedwigia*) is placed as sister group to clades A and B. The third clade (C, Fig. 1) consists of representatives of the acrocarpous Bryales and is sister to A + B + Hedwigiaceae. Consequently, the *rbc*L data places the pleurocarpous taxa in two separate clades.

Evaluations of tree stability in relation to exemplar sampling were obtained from simple experiments involving the exclusion of particular taxa from the analyses. The three main clades in the *rbc*L tree were stable under different combinations of particular exemplar species (results not shown), or when single ingroup taxa were excluded. Similarly, the removal of distant (Dicranales, Grimmiales) or close outgroups (Orthotrichales) had no effect on tree topology.

The character-state weighting analysis resulted in only a single optimal tree. This had the same topology as one of the three MP trees (shown as Fig. 2).

The level of clade support as measured by decay indices varied from one to five or greater. In terms of the *rbc*L data alone, clade A is the most robust group since it persisted until parsimony was relaxed three steps. Optimization of changes on this branch show at least eight states supporting this clade (Fig. 2). Interestingly, the other main groups (B and C) decay in trees only one step longer than the MP trees (Fig. 1), although there are six and twelve character changes reconstructed for each branch respectively (Fig. 2), demonstrating why branch length *per se* can be so misleading as a measure of support. None of the three main clades nor the large clade containing *Hedwigia* + A + B was found in the consensus of trees four steps longer than the MP trees.

Analysis of rbcL sequences and morphology combined.—Multiple heuristic searches using a data set with the 91 morphological characters (Newton & De Luna 1999) and *rbc*L sequences combined yielded only two most parsimonious trees (1162 steps, CI = 0.373, RI = 0.444). The strict consensus reveals three main clades (D, E, F) relevant to the question of the evolution of pleurocarpy (Fig. 3). The first group (D) incorporates representatives of pleurocarpous mosses traditionally classified in the Hookeriales, Leucodontales, and Hypnales. The question of monophyly of each of these orders is outside the scope of this current study, requiring more extensive taxon sampling, and the resolution of the taxa included in this clade is consequently not discussed further. The second clade (E) is sister to D and includes exemplars of pleurocarpous families in the Bryales, such as the Racopilaceae, Hypnodendraceae, Spiridentaceae, and Cyrtopodaceae. Thus, clade [D + E] includes all and only the pleurocarpous mosses sampled in this study. It includes representatives of Clade B plus the pleurocarp exemplars from Clade A in the molecular tree (Fig. 1). The Rhizogoniaceae (as represented by *Pyrrhobryum*) is sister to the clade

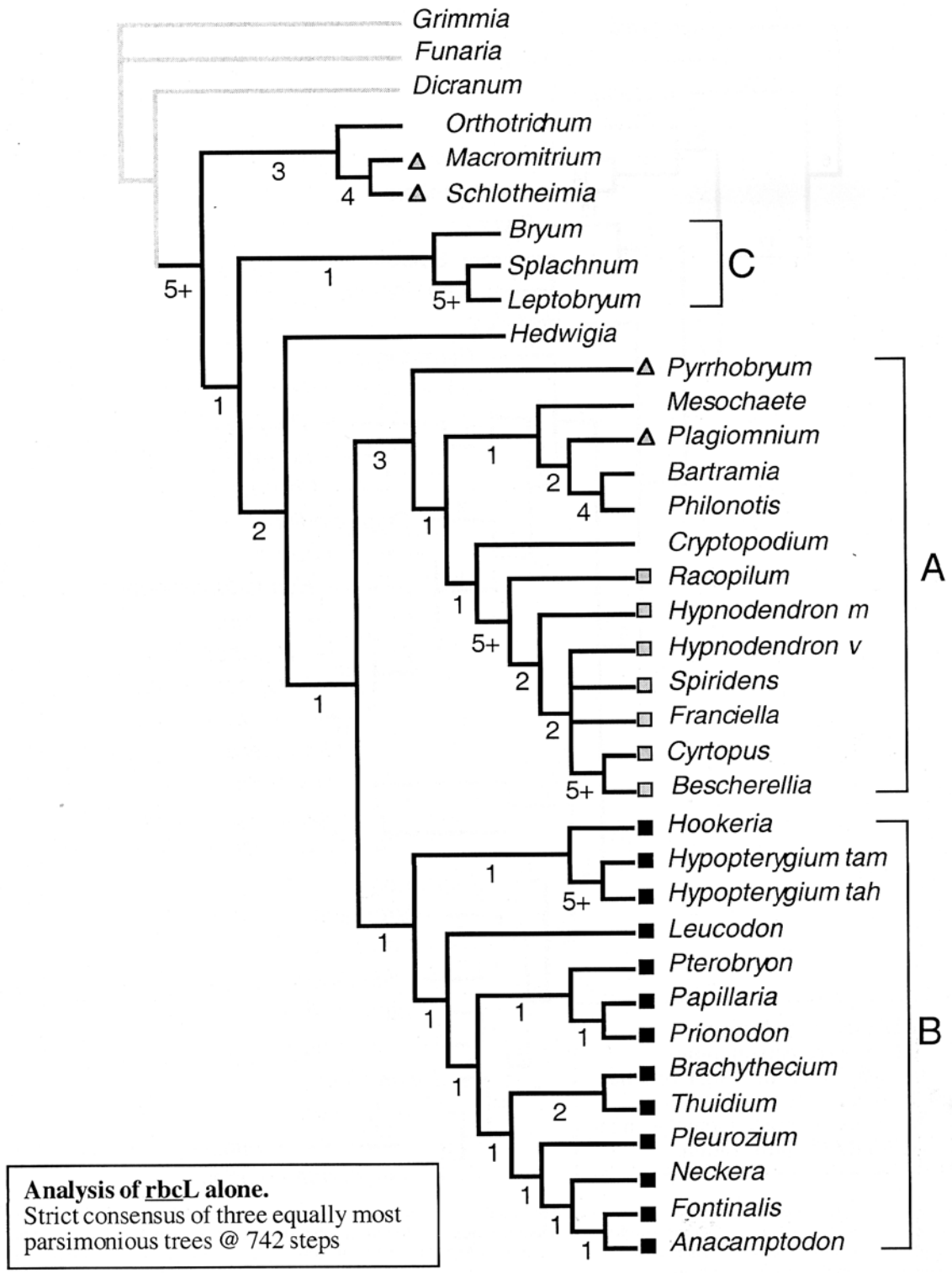

FIGURE 1. Strict consensus of three equally most parsimonious trees (742 steps, CI = 0.352, RI = 0.528) found in a heuristic search based on sequences of the *rbc*L gene for 36 exemplar species selected from the Bryales (A, C), Leucodontales, Hypnales, and Hookeriales (B). The tree was rooted with *Dicranum, Funaria* and *Grimmia*. Numbers below the branches are estimated values of the decay index (number of steps in suboptimal trees to collapse a particular clade). Taxa with black squares are exemplars of the hypnobryalean pleurocarps, gray squares are representative taxa of the bryalean pleurocarps, and triangles indicate cladocarp taxa.

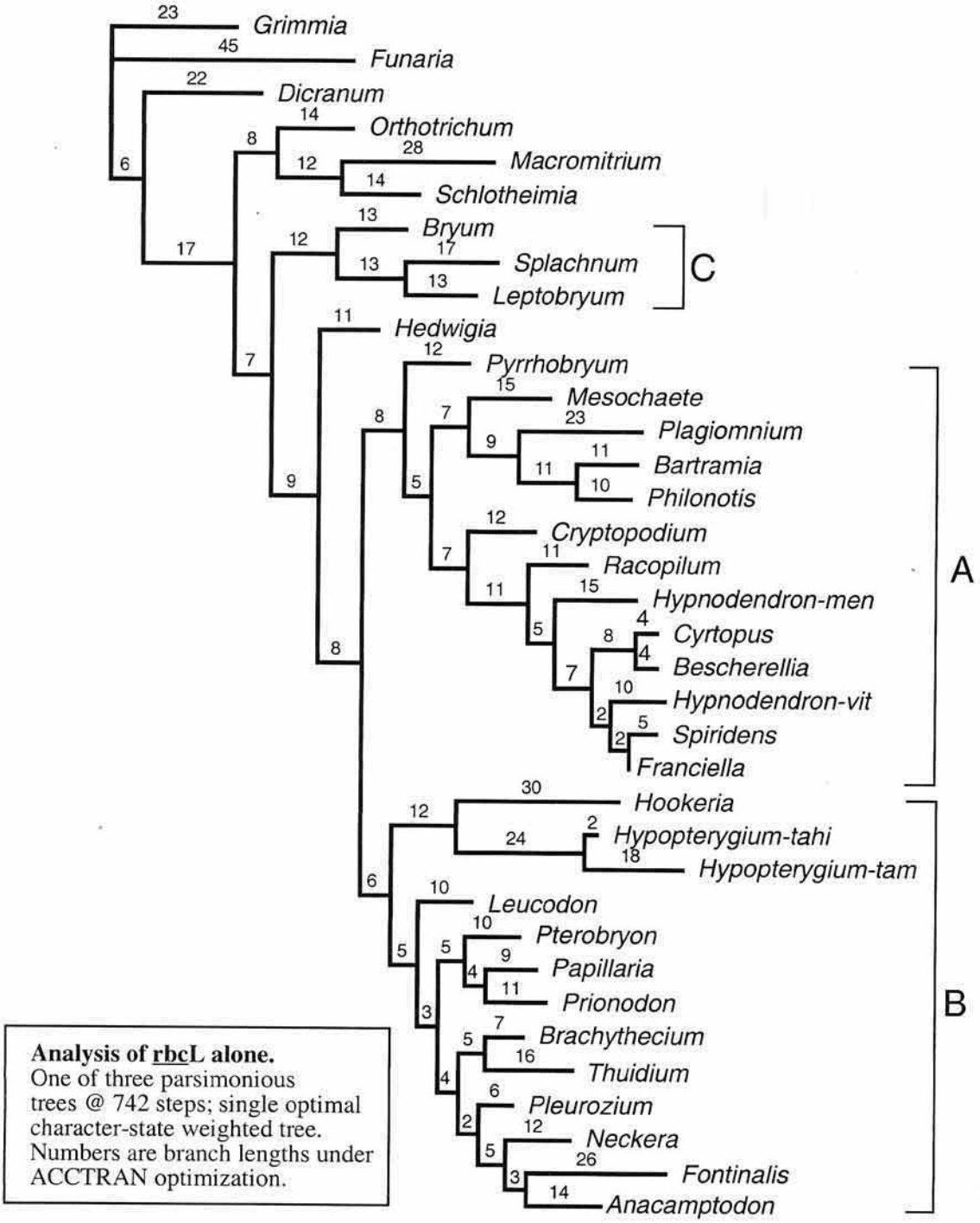

FIGURE 2. One tree selected from the set of three equally most parsimonious trees found in a heuristic search using the *rbc*L data alone. This is also the single most parsimonious tree found in a heuristic search applying character-state weighting. Numbers above branches are branch lengths (ACCTRAN), indicative of the number of character state changes (unambiguous and ambiguous).

[D + E] and the Hedwigiaceae is sister to the large clade incorporating the Rhizogoniaceae and the bryalean and hypnobryalean pleurocarps. The third important clade (F) includes acrocarpous taxa traditionally classified in the Bryales (Bartramiaceae, Bryaceae) and the Splachnaceae; this clade is sister to the group [[[D + E] + *Pyrrhobryum*] + *Hedwigia*] (Fig. 3).

The combined data matrix has 28 taxa, a subset of taxa in the morphological and molecular matrices. Nevertheless, this sample of families in the Bryales, Leucodontales, Hookeriales, and Hypnales suggests some details of phylogenetic patterns in the pleurocarps. The three main clades in the combined tree (D, E, F) are stable under different combinations of particular exemplar species (results not shown), when single ingroup taxa are excluded, and with the exclusion of distant (Dicranales) or adjacent outgroups (Orthotrichales). Decay indices and the number of character changes on particular branches suggest that the combined tree has several robust branches that might survive further sampling of taxa and characters. Estimated levels of clade support on the strict consensus tree (Fig. 3) range from one to five or more, suggesting at least three robust clades relevant to the origin of pleurocarpy. Clade D (di = 4) includes representatives of the Hookeriales, Leucodontales, and Hypnales. This group is supported by 19 character state changes (Fig. 4). The second clade with a high decay index (di = 5+) is clade E, grouping the pleurocarpous Bryales (Cyrtopodaceae, Spiridentaceae, Hypnodendraceae, and Racopilaceae). This group is supported by 18 character state changes (Fig. 4). The third robust group is a large clade including clades D and E, as well as *Pyrrhobryum* (di = 3, 12 character state changes, Fig. 4). The bryalean acrocarps (Clade F) and the monophyletic group including all the bryalean and hypnobryalean pleurocarps [D + E] have good character support (18 changes each, Fig. 4), nevertheless, both clades decay quickly as longer trees are examined (di = 1).

Excluding uninformative characters, the consistency index (CI) was only slightly higher in the combined data set (CI = 0.373) than in the *rbc*L (CI = 0.352), suggesting similar levels of homoplasy. Measures of the amount of synapomorphy in data sets, the retention index (RI), and the rescaled consistency index (RC), are only slightly higher in the *rbc*L analysis (RI = 0.527, RC = 0.193) than in the combined tree (RI = 0.444, RC = 0.165). Observed differences in these values do not seem significant when placed in the wider context of other phylogenetic studies. We examined cladistic analyses with the same number of taxa among those reviewed by Sanderson and Donoghue (1989), who found that homoplasy increases as the number of taxa increases. Comparisons show similar levels of homoplasy in the molecular data set (38 taxa, CI = 0.366) and the morphological data set (39 taxa, CI = 0.328, Newton & De Luna 1999), which would be expected if there are no real differences in phylogenetic pattern between the two data sets. Neither of these indices from our separate analyses nor the CI of the combined analysis (28 taxa, CI = 0.373) were different from indices observed for the same number of taxa in other studies reviewed by Sanderson and Donoghue (1989). These patterns of variation in homoplasy suggest that phylogenetic signal (homology) is equivalent in our different data sets.

The skewness test showed that the *rbc*L mean random tree-length (x = 1156 steps, sd = 21.74) and the combined data mean random tree-length (x = 1549 steps, sd = 26.37) are 414 and 387 steps longer than the most parsimonious *rbc*L and combined trees, respectively. In both cases, the length distribution of a random sample of 10,000 trees has a left skewed frequency curve (g1 = −0.5594 for the *rbc*L data, g1 = −0.4668 for the combined data). These values suggest that both data sets (*rbc*L alone, and combined) are consistent with one or very few phylogenetic hypotheses in the left tail of their corresponding curves, and are thus highly correlated with history.

Discussion

Methodological issues.—A current debate on "taxonomic congruence" vs. "total evidence" (De Queiroz et al. 1995; Kluge & Wolf 1993) focuses on how different types of data (morphological, fossils, molecular) should be analysed and integrated, either by comparing trees, or by combining data. The methodological and theoretical assumptions that should be considered include asymmetry in the size of the data sets (number of characters and states), reliability of molecular data, homoplasy in morphological data, different evolutionary models of character change in the different character sets, different tree resolutions, and different consensus techniques. Empirical analyses have shown the weakness of some of these assumptions. For example, molecular data is commonly perceived as being better than morphological data at revealing homology and monophyletic groups. However, a review of cladistic studies found that levels of homoplasy are equivalent in morphological and molecular data sets (Sanderson & Donoghue 1989). In addition, simulation studies have revealed a positive relationship between levels of homoplasy and the number of taxa in a data matrix (Klassen et al. 1991).

Two strong arguments in favor of the use of a

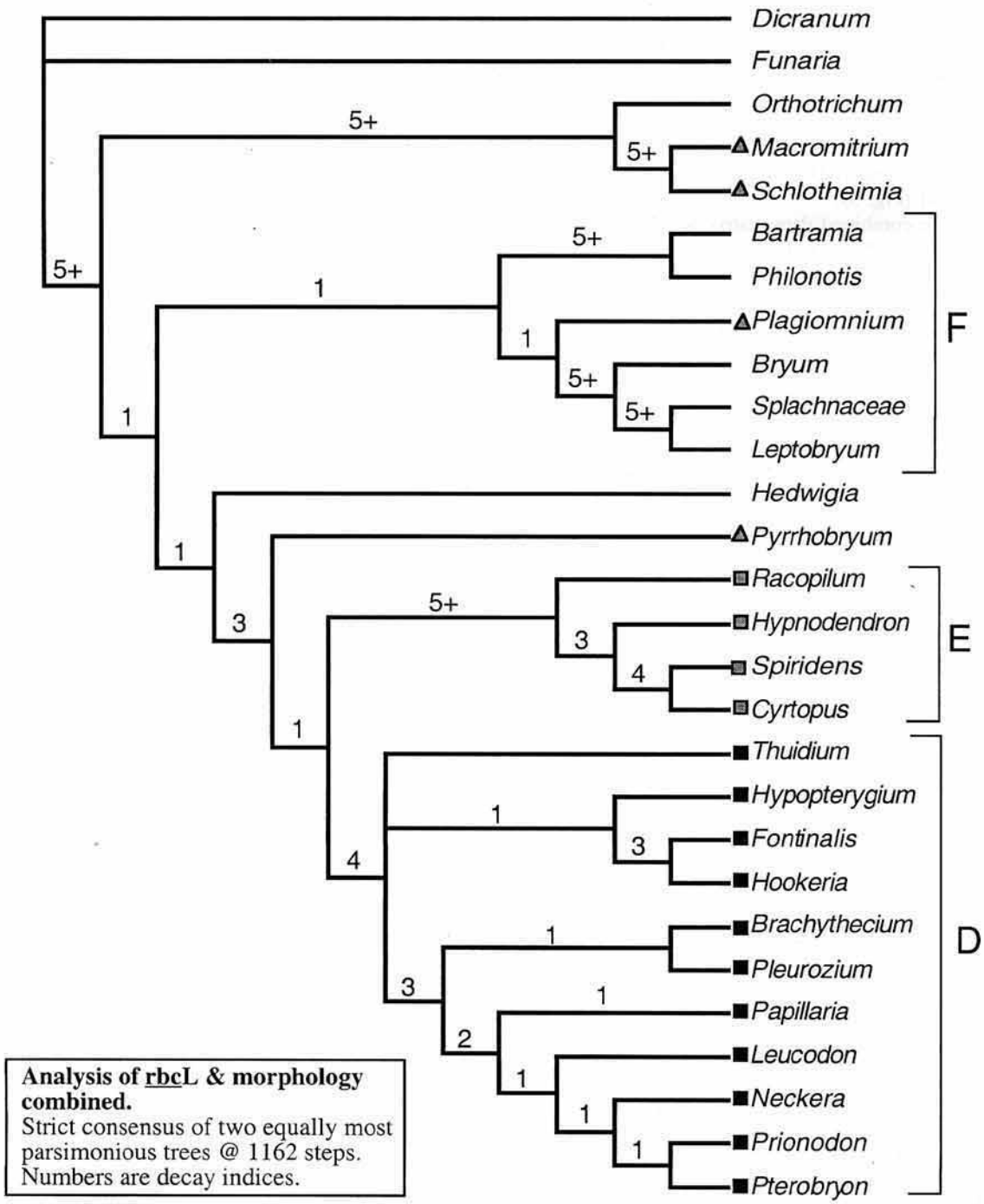

FIGURE 3. Phylogenetic relationships of diplolepidous pleurocarp bryalean (E) and hypnobryalean (D) mosses. Strict consensus of two equally most parsimonious trees (1162 steps, CI = 0.373, RI = 0.444) found in a heuristic search using the combined *rbc*L and morphology data for 28 exemplar species. The tree was rooted with *Dicranum* and *Funaria*. Numbers above the branches are estimated values of the decay index. Squares and triangles as in Figure 1.

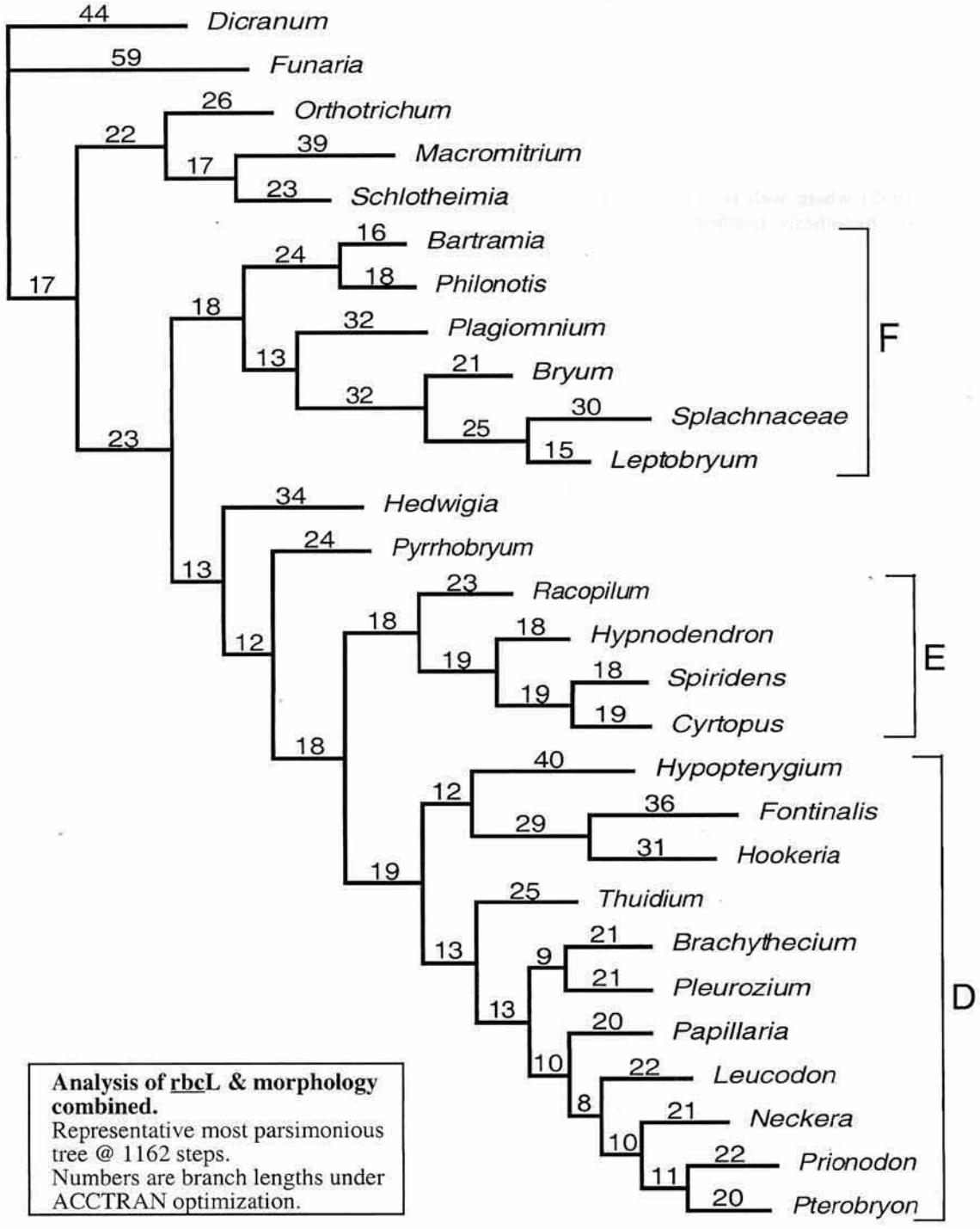

FIGURE 4. One tree selected from the set of two equally most parsimonious trees found in a heuristic search using the combined *rbc*L and morphology data. Numbers above branches are branch lengths (ACCTRAN), indicative of the number of character state changes (unambiguous and ambiguous).

combination of several kinds of data in an analysis are that they are likely to be independently evolving and that some will have phylogenetic signal at one level while others will be useful at other levels. The advantages of combining data sets are revealed in several recent cladistic studies (Doyle et al. 1994; Eernise & Kluge 1993; Mishler et al. 1994; Pryer et al. 1995) where well resolved and robust phylogenetic hypothesis resulted from the character congruence present in the combined data matrices. In the present study, the morphological and molecular data sets were each analyzed separately, and then as a combined data set, in preference to comparing trees from the separate analyses using consensus techniques, since the most parsimonious explanation of all characters is expected to be derived from combined data analysis. The combined data set, including characters of different rates of state change and fixation relative to the rate of lineage divergence, is expected to reveal phylogenetic history better than a consensus tree based on the trees from analyses of separate data sets.

An evaluation of the data matrix, the most parsimonious tree(s), and the relative robustness of clades within a cladogram are required before accepting a cladogram (fundamental or consensus) as a phylogenetic hypothesis. Different measures are now commonly used to assess the phylogenetic structure of the data matrix, to evaluate the stability and structure of an entire tree, and to examine the support of particular clades within a tree. The "permutation tail probability" test (PTP, Faith & Cranston 1991), the skewness of tree-length frequency distribution (g1, Huelsenbeck 1991), the "total support" tests (ts, Källersjö et al. 1992), and the "total support index" (tsi, Bremer 1994) attempt to estimate data structure and tree stability as a whole. Another set of related procedures aim to examine the support for particular clades within a single cladogram. Relative measures of group support include random resampling statistics (such as the "bootstrap" and "jackknife" frequencies, Cracraft & Helm-Bychowsky 1991; Felsenstein 1985; Sanderson 1989, 1995), the frequency distribution of specific clades among most parsimonious trees (hierarchic signal, Naylor 1992), the examination of clades in each strict consensus of one-step successively suboptimal trees (decay or branch support index, Bremer 1994; Mishler et al. 1991), and evaluations of successive character removals (clade stability index, Davis 1993).

Concerns have been raised that not all of these quantitative estimations are equally useful (Källersjö et al. 1992) or that they may be misapplied in systematics (Hillis 1995). In fact, Carpenter (1992) has argued that interpretations of group frequencies from randomization procedures (particularly the PTP test, and bootstrap percentiles) represent a misapplication of statistics in phylogenetic systematics. Additionally, Kluge and Wolf (1993) pointed out that assumptions underlying procedures for random resampling techniques and PTP tests are unrealistic; they concluded that such estimators are applicable, but only under empirical conditions that are rarely met, and are therefore of limited use. In contrast to these problematic statistical procedures, it seems to us that parsimony and character based indices, such as the decay or branch support index, are promising means for assessing the reliability of phylogenetic signal. Consequently, these were emphasised in assessing support in our analyses. A phylogenetic hypothesis based on the combined data and with robust and weak clades identified is presented as a framework to interpret the evolution of pleurocarpy among mosses with diplolepidous alternate peristomes.

Complementarity of data sets.—The present phylogenetic study revealed some of the advantages of separate and combined analyses of data sets. Morphological and molecular characters initially appeared to be discordant because each data set, when analyzed separately, resolved clades at different levels of inclusivity. For example, the *rbc*L sequences resolved a robust group of acrocarpous plus pleurocarpous bryales (Fig. 1. Clade A, di = 3). However, the morphological characters alone did not find this clade, while the combined data set split the Clade A taxa into two separate clades. Part of the apparent discordance here might be due to the artifact of smaller taxon sampling in the combined data set. Since only 28 taxa had both morphology and *rbc*L sequences, the combined data set included only these taxa. A search for the most parsimonious trees was conducted using *rbc*L sequences alone for only these 28 taxa (results not shown). Clade A was not recovered in this reduced *rbc*L tree presumably due to insufficient taxon sampling. It still remains to be explored whether an expanded matrix in which more taxa have both morphological and *rbc*L data will yield a phylogeny congruent with the *rbc*L data alone.

The evaluation of results from the independent analyses of *rbc*L (present study) and morphology (Newton & De Luna 1999) indicates that the two different types of data are complementary, as is also shown by the combined tree (Fig. 3). For example, the combined analysis found a well resolved and robust clade of hypnobryalean pleurocarps (Fig. 3, Clade D, di = 4) although the *rbc*L data, for the few taxa sampled, found only a weak group (Fig. 1, Clade B, di = 1) while the larger number of taxa in the morphological data formed a well resolved but weak clade (Newton & De Luna 1999, Fig. 2, clade B, di = 2). Thus, cladistic analyses of

combined data from *rbc*L sequences and morphology has more explanatory power in terms of levels of clade support and topological resolution than analysis of each data set alone. Under the present conditions of taxon sampling, and by virtue of character congruence, the combined analyses of *rbc*L and morphology provide a strong phylogenetic hypothesis for the monophyly of the pleurocarps.

Monophyly and phylogenetic relationships of hypnobryalean pleurocarpous mosses.—Our results show that pleurocarpous mosses currently classified in the Hypnales, Leucodontales, and Hookeriales together form a monophyletic group, either using morphology (Newton & De Luna 1999) and *rbc*L data sets alone (clade B, Fig. 1), or using the combined data (clade D, Fig. 3). At this grouping level, there are no contradictions between the data sets, although there are differences in internal resolution, branching order, and branch support. The monophyly of our representatives from the Hypnales, Hookeriales, and Leucodontales (clade D, Fig. 3) agrees with the large scale classification scheme of Buck and Vitt (1986). Previously, Koponen (1979) had developed a hypothesis in which all families in these three Orders were included in a single group, the Hypnobryales. Recently, Hedenäs (1994) concluded that the pleurocarps (Hypnales, Leucodontales, and Hookeriales) formed a monophyletic group. His study sampled eight putative representatives of these orders (Table 1, Hedenäs, 1994) and his analyses recovered a clade containing five of those exemplars (*Cyclodictyon, Homalia, Thamnobryum, Brachythecium,* and *Duthiella,* Figs. 7–8, Hedenäs 1994), together with *Hypopterygium.* His remaining exemplars (*Hypnodendron, Pterobryella,* and *Ptychomnion*) were distributed across several lineages with representatives of the bryalean pleurocarps and the Orthotrichales. Analyses reported here are based on a different combination of exemplars and on a different application of the cladistic approach (see Newton & De Luna 1999) as compared to that used in the analyses of Hedenäs (1994). However, despite our disagreements in criteria for selection of morphological characters, taxon sampling, and different search strategies for most parsimonious trees, the concurrence of our results indicates that there is sufficient historical signal in the observable similarities of the pleurocarpous mosses to strongly support an explicit hypothesis of monophyly of the Hypnales, Hookeriales, and Leucodontales (clade B, Fig. 1; clade D, Fig. 3.)

Phylogenetic relationships of the bryalean and hypnobryalean pleurocarps.—The analyses of the combined data shows that the phylogenetic position of the pleurocarps traditionally classified in the Bryales (clade A, Fig. 1; clade E, Fig. 3) seems to be as sister group to the other pleurocarps in the Leucodontales, Hypnales, and Hookeriales. This sister group relationship between clade E and D in the combined analyses is weakly supported (di = 1, Fig. 3). Confirmation of the monophyly of pleurocarps in clades E & D will require additional exemplars as well as more morphological and molecular data. Nevertheless, our results suggest that the hypothesis that all the pleurocarps in the diplolepidous mosses form a monophyletic group (clade D + E, Fig. 3) should be taken into consideration for further studies. The sister group of this pleurocarp clade appears to be the Rhizogoniaceae (here represented by *Pyrrhobryum*). The large monophyletic group that includes all exemplars from clades E and D plus *Pyrrhobryum* is well supported (di = 3, Fig. 3). In addition, our study reveals the key position of the Hedwigiaceae as potential outgroup for future analyses of the Rhizogoniaceae and the clade grouping the bryalean and hypnobryalean pleurocarpous mosses (Fig. 3).

Our results contradict earlier hypotheses (Buck & Vitt 1986; Hedenäs 1994) that the bryalean and hypnobryalean pleurocarps do not form a monophyletic group (Fig. 5). Hedenäs (1994) proposed that the set of pleurocarps from the Bryales do not belong in the monophyletic group that included representatives of the Leucodontales, Hypnales, and Hookeriales (Fig. 5). He sampled 12 bryalean pleurocarps (eight exemplars from the Rhizogoniaceae, two of the Spiridentaceae, and one each of the Racopilaceae and Hypopterigiaceae), which were placed by his analyses in a series of paraphyletic lineages that included the exemplars of the Orthotrichales and the Bartramiales. Thus, his conclusions concerning potential outgroups for the hypnobryalean pleurocarps, and his further analyses using those outgroups (Hedenäs 1995) are to be regarded with caution.

Systematic implications in the Bryales.—The monophyly and classification of the Bryales remains ambiguous. Several alternative groupings have previously been proposed for the bryalean acrocarpous mosses (see Fig. 5). In early classifications, this group included 13 families and was given either the rank of order (Bryales, Vitt 1982, p. 314) or suborder (Bryineae, Vitt 1984, p. 746). In a preliminary cladogram discussing the classification of the Mniaceae and it's relatives, Koponen (1979, fig. 4) separated some families (including the Bryaceae and Mniaceae) in the Eubryales and others (such as Bartramiaceae) in the Bartramiales, based on spore morphology, leaf areolation, and costa structure. Later, Buck and Vitt (1986) recognized two suborders within the order Bryales: the Bryineae and the Rhizogoniineae. The bryalean acrocarpous mosses were classified in a single group,

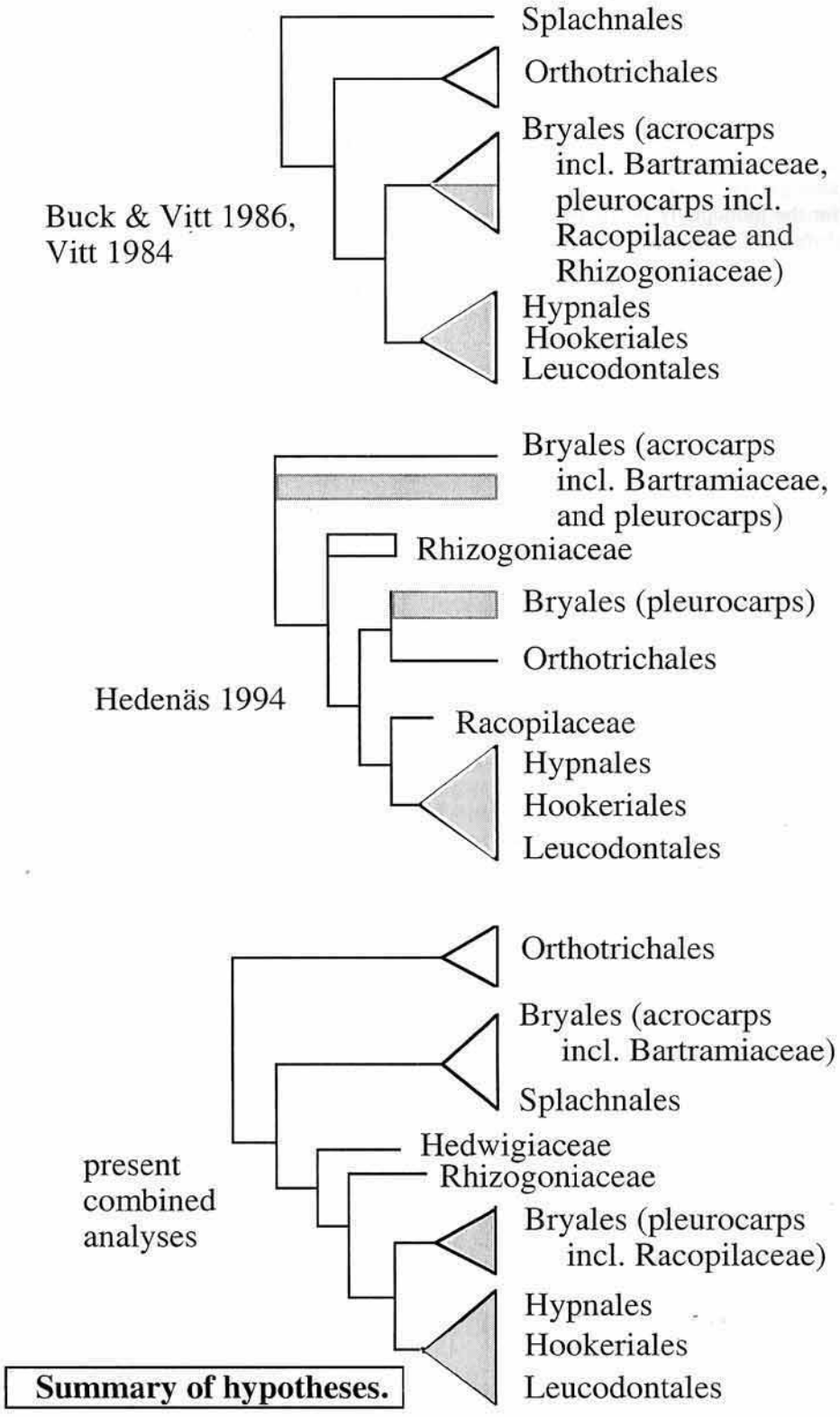

FIGURE 5. Summary of alternative hypotheses of phylogenetic relationships among bryalean and hypnobryalean pleurocarp diplolepidous mosses. Gray zones are taxonomic placement of pleurocarpic mosses.

the suborder Bryineae, without recognizing Koponen's two subgroups (Buck & Vitt 1986). The Rhizogoniineae were differentiated as being pleurocarpous and consisted of the Rhizogoniaceae and Spiridentaceae, together with three families recently transferred from other orders. These were the Racopilaceae (previously in the Hypnales *sensu* Vitt 1984), Hypopterygiaceae (transferred from the Hookeriineae *sensu* Vitt 1984), and Helicophyllaceae (from the Orthotrichineae *sensu* Vitt 1984). In his study of the basal pleurocarpous mosses Hedenäs (1994) dispersed the pleurocarpous and acrocarpous members of the Bryales, together with the exemplars from the Orthotrichales and Bartramiaceae, across several clades and paraphyletic groups basal to the hypnobryales (see Fig. 5).

The phylogenetic analysis by Withey (1996*a*) based on *rbc*L data indicated that the Bryales (*sensu* Buck & Vitt 1986) might be paraphyletic. Our present studies increased the sampling of the hypnobryalean pleurocarps and added a morphological data set. It now seems clear from Withey's (1996*a*) and our present *rbc*L and combined analyses that neither of the proposed groupings of 13 families in the Bryales *sensu* Vitt (1982; suborder Bryineae *sensu* Vitt 1984), or the 16 families in the Bryales *sensu* Buck and Vitt (1986) can be regarded as monophyletic (Fig. 5).

Our phylogenetic hypothesis based on the combined data set is presented here as the best supported summary of our current state of knowledge for the diplolepidous pleurocarp mosses (Fig. 5). We use this cladogram as the only appropriate basis for interpreting the evolution of characters associated with pleurocarpy (Newton & De Luna 1999). However, it must be clear that the internal outline of phylogenetic relationships among pleurocarp mosses still remains inconclusive with the data, taxon sampling, and cladistic analyses at hand. As more exemplars are added and data sets from different gene sequences become available, further cladistic analyses should be designed to identify other potential outgroups for the pleurocarps. With an improved understanding of the outgroup taxa, an attempt can be made to elaborate on the phylogenetic relationships within the ingroup of the pleurocarpous families.

Directions for further research.—The present study suggests directions for further phylogenetic research. A detailed cladistic analysis of the collection of families placed in the Bryales (*sensu* Buck and Vitt 1986) is critical to resolve the relationships of these taxa, in particular the question of whether clade A (Fig. 1) can be recognized as a taxonomic group separate from clade C (Fig. 1), or whether the two subgroups referable to the Bartramiales and Eubryales (*sensu* Koponen 1979) should be recognized. The possible existence of these two subgroups needs to be evaluated further. The two groups revealed by our combined analysis (clades F and E, Fig. 3) place the bryalean acrocarps that we sampled with the Bartramiaceae, and seems to reinforce Koponen's (1979) recognition of two subgroups, with the Bartramiales as sister to the Eubryales. However, the results of our *rbc*L analyses recover a different relationship, splitting the bryalean acrocarps into two groups. Here, the clade that includes the Bartramiaceae is sister to the bryalean pleurocarps, and the clade that includes *Bryum* is sister to *Splachnum* and *Leptobryum* (Fig. 1). Both the *rbc*L and the combined data analyses place the Splachnales with a subset of mainly acrocarpous bryalean mosses (Fig. 5).

Our sampling of the Bryales (*sensu* Buck & Vitt 1986) is incomplete. Seven families traditionally classified in this order, the Leptostomataceae, Mitteniaceae, Schistostegaceae, Timmiaceae, Aulacomniaceae, Meesiaceae, and Catoscopiaceae, still need to be investigated for morphological and *rbc*L sequence data and to be analyzed with formal cladistic methods. The phylogenetic position of these families remains to be tested to determine their relationships with other mosses in the Bryales (*sensu* Buck and Vitt 1986). The inclusion of these seven families will also help to explore the relationship of the Splachnales to the bryalean acrocarpous mosses (Fig. 5).

The Rhizogoniaceae seem to hold a critical position in the understanding of the pleurocarpous taxa, and genera placed in this family need both extensive sampling of sequence data and critical analysis of the morphological characters, particularly those related to pleurocarpy.

Conclusions

The data at hand and present cladistic analyses suggest a phylogenetic hypothesis in which the bryalean pleurocarps are a sister group of the hypnobryalean pleurocarps. Both clades are well supported according to the number of character changes (18 and 19, respectively) and both clades appear to be relatively robust, as suggested by decay indices of four or more. The combined analyses of *rbc*L sequences and morphology provide the strongest phylogenetic hypothesis for the monophyly of bryalean and hypnobryalean pleurocarps. This hypothesis is not conclusive, but the cladogram in Figure 3 is the best current summary of the large scale relationships of these diplolepidous taxa. It is offered here as an inferential basis for a phylogenetic interpretation of the origin of pleurocarpy (see Newton & De Luna 1999). It is also a suitable starting point for more detailed investigations into the

morphology and ontogeny of special character systems, which will undoubtedly provide better resolution of phylogenetic relationships at the ordinal level.

Our present study shows the value of *rbc*L gene sequences as a promising source of informative characters for phylogenetic analyses of the pleurocarps. Besides allowing the recognition of two main clades (bryalean and hypnobryalean pleurocarps), this gene also seems to have resolution for terminal taxa. The observed pattern of variation in the *rbc*L gene makes it an excellent candidate for phylogenetic studies at the family level and above within the pleurocarps. However, there does not seem to be any justification to claim that molecular data are superior compared to morphology as a historical marker in the case of pleurocarp mosses. Neither is there an indication that levels of homoplasy in morphological data are higher than in sequence data for the pleurocarps. Thus, a phylogeny based only on molecules should not be used as a basis for changes in the classification. In our future studies within the pleurocarp mosses, we plan to use the *rbc*L gene in combination with morphological data for phylogenetic analyses of the main groups of families.

In view of empirical and methodological advances, previous interpretations of morphological features such as branching systems need to be reevaluated, before we can make any further progress in our understanding of the origin of pleurocarpy. The work of Catherine La Farge-England (1996) has been essential here in highlighting crucial elements of pleurocarpy. In such interpretations, it must be evident that a phylogenetic approach is necessary to analyze the growing body of morphological and molecular data and to test inferences about homology and monophyletic groups. As additional data become available, it is the formal application of cladistic analyses to the full range of data (morphology plus DNA sequences) that will allow a more accurate explanation of phylogenetic relationships within the pleurocarp mosses.

Acknowledgments

We appreciate the support of a grant from CONACYT of México (PACIME # 1837P-N9507) to Dolores González and Efraín De Luna for gene sequencing. Computer equipment and software for present cladistic analyses were provided by a previous grant from CONACYT (PACIME # 1860-N9212. This research was also supported by NSF grant #BSR-9107484 to Brent Mishler and Rytas Vilgalys. Attendance at the 1997 (Montreal) and 1999 (Xalapa) symposia and workshops on "Phylogeny within the major bryophyte clades" was possible thanks to the support from the Green Plant Phylogeny Research Coordination Group (funding provided by the DOE/NSF/USDA Panel on Collaborative Research in Plant Biology, USDA grant 94-37105-0713, PIs: Mark A. Buchheim, Brent D. Mishler, and Russell L. Chapman). The Smithsonian Institution financed field work in Guyana to AEN. Field work in México and the completion of this paper was possible through support from the Instituto de Ecología, AC, Xalapa (acc. # 902-14). We thank Malcolm Sargent for providing plant material from his bryophyte culture collection. Bernard Goffinet kindly shared the *rbc*L sequences for *Macromitrium* and *Schlotheimia.* The authors are grateful to Terry Hedderson for useful discussions and two anonymous reviewers for valuable comments on the manuscript.

Literature Cited

Albert, V. A., B. D. Mishler & M. W. Chase. 1992. Character-state weighting for restriction site data in phylogenetic reconstruction, with an example from chloroplast DNA, pp. 369–403. *In* P. Soltis, D. Soltis and J. Doyle (eds.), Molecular Systematics of Plants. Chapman and Hall.

———, M. W. Chase & B. D. Mishler. 1993. Character-state weighting for cladistic analysis of protein-coding DNA sequences. Annals Missouri Botanical Garden 80: 752–766.

Bopp, S. L. & I. Capesius. 1995. New aspects of the systematics of bryophytes. Naturwissenschaften 82: 193–194.

Bremer, K. 1994. Branch support and tree stability. Cladistics 10: 295–304.

Buck, W. R. & D. H. Vitt. 1986. Suggestions for a new familial classification of pleurocarpous mosses. Taxon 35: 21–60.

Carpenter, J. M. 1992. Random cladistics. Cladistics 8: 147–153.

Cox C. J. & T. A. Hedderson. 1999. Phylogenetic relationships among the ciliate arthrodontous mosses: evidence from chloroplast and nuclear DNA sequences. Plant Systematics and Evolution 215: 119–139.

Cracraft, J. & K. Helm-Bychowski. 1991. Parsimony and phylogenetic inference using DNA sequences: some methodological strategies, pp. 184–220. *In* M. M. Miyamoto & J. Cracraft (eds.), Phylogenetic Analysis of DNA sequences. Oxford University Press, NY.

Crosby, M. R. 1980. The diversity and relationships of mosses, pp. 115–129. *In* R. J. Taylor & A. E. Leviton (eds.), The Mosses of North America. Pacific Division, AAAS, California Academy of Science, San Francisco.

Davis, J. I. 1993. Character removal as a means for assessing stability of clades. Cladistics 9: 201–210.

De Luna, E. 1992. Developmental and systematic studies in the Hedwigiaceae (Musci). Ph.D. Dissertation. Duke University, Durham, NC.

———. 1995. The circumscription and phylogenetic relationships of the Hedwigiaceae (Musci). Systematic Botany 20: 347–373.

De Queiroz, A., M. J. Donoghue & J. Kim. 1995. Separate versus combined analysis of phylogenetic evidence. Annual Review of Ecology and Systematics 26: 657–681.

DOYLE, J. J. & J. L. DOYLE. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin 19: 11–15.

DOYLE, J. A., M. J. DONOGHUE & E. A. ZIMMER. 1994. Integration of morphological and ribosomal RNA data on the origin of angiosperms. Annals of the Missouri Botanical Garden 81: 419–450.

EERNISE, D. J. & A. G. KLUGE. 1993. Taxonomic congruence versus total evidence, and amniote phylogeny inferred from fossils, molecules and morphology. Molecular Biology and Evolution 10: 1170–1195.

FAITH, D. P. & P. S. CRANSTON. 1991. Could a cladogram this short have arisen by chance alone?: On permutation tests for cladistic structure. Cladistics 7: 1–28.

FELSENSTEIN, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39: 783–791.

GRANZOW DE LA CERDA, I. 1990. Aplicación del método cladístico a un caso real: Análisis filogenético de *Anomodon* Hook & Tayl. (Musci). Jardín Botánico de Madrid 47: 305–325.

HEDENÄS, L. 1994. The basal pleurocarpous diplolepidous mosses—a cladistic approach. THE BRYOLOGIST 97: 225–243.

———. 1995. Higher taxonomic level relationships among diplolepidous pleurocarpous mosses—a cladistic overview. Journal of Bryology 18: 723–781.

———. 1996*a*. A cladistic evaluation of relationships between the Hookeriales, the Sematophyllaceae and some other taxa. Lindbergia 21: 49–82.

———. 1996*b*. A cladistic overview of the Hookeriales. Lindbergia 21: 107–143.

HILLIS, D. 1995. Approaches for assessing phylogenetic accuracy. Systematic Biology 44: 3–16.

HUELSENBECK, J. P. 1991. Tree-length distribution skewness: an indicator of phylogenetic information. Systematic Zoology 40: 257–270.

HYVÖNEN, J. & J. ENROTH. 1994. Cladistic analysis of the genus *Pinnatella* (Neckeraceae, Musci). THE BRYOLOGIST 97: 305–312.

KÄLLERSJÖ, M., J. S. FARRIS, A. G. KLUGE & C. BULT. 1992. Skewness and permutation. Cladistics 8: 275–287.

KLASSEN, G. J., R. D. MOOI & A. LOCKE. 1991. Consistency indices and random data. Systematic Zoology 40: 446–457.

KLUGE, A. G. & A. J. WOLF. 1993. Cladistics: What's in a word. Cladistics 9: 183–199.

KOPONEN, T. 1979. Generic and family concepts in the Mniaceae. Nova Hedwigia 71: 249–259.

LA FARGE-ENGLAND, C. 1996. Growth form, branching pattern, and perichaetial position in mosses: cladocarpy and pleurocarpy redefined. THE BRYOLOGIST 99: 170–186.

MADDISON, D. R. 1991. The discovery and importance of multiple islands of most-parsimonious trees. Systematic Zoology 40: 315–328.

MEUSEL, H. 1935. Wuchsformen und Wuchstypen der europaischen Laubmoose. Nova Acta Leopoldina Neue Folge 3: 123–277.

MISHLER, B. D. & E. DE LUNA. 1991. The use of ontogenetic data in phylogenetic analyses of mosses. Advances in Bryology 4: 121–167.

———, M. J. DONOGHUE & V. A. ALBERT. 1991. The decay index as a measure of relative robustness within a cladogram. Abstract, Annual meeting, Willi Hennig Society. Toronto, Canada.

———, L. A. LEWIS, M. A. BUCHHEIM, K. S. RENZAGLIA, D. J. GARBARY, C. F. DELWICHE, F. W. ZECHMAN, T. S. KANTZ & R. L. CHAPMAN. 1994. Phylogenetic relationships of the "green algae" and "bryophytes". Annals of the Missouri Botanical Garden 81: 451–483.

———, P. THRALL, J. S. H. JR., E. DE LUNA & R. VILGALYS. 1992. A molecular approach to the phylogeny of bryophytes: cladistic analysis of chloroplast-encoded 16S and 23S ribosomal RNA genes. THE BRYOLOGIST 95: 172–180.

NAYLOR, G. J. P. 1992. Plotting frequency distributions of phylogenetic groupings found among sets of most parsimonious trees. Cladistics 8: 161–164.

NEWTON, A. 1993. Phylogenetic systematics of the tropical moss genus *Pireella*. Ph.D. Dissertation. Duke University, Durham, NC.

——— & E. DE LUNA. 1999. A survey of morphological characters for phylogenetic study of the transition to pleurocarpy. THE BRYOLOGIST 102: 651–682.

PRYER, K. M., A. R. SMITH & J. E. SKOG. 1995. Phylogenetic relationships of extant ferns based on evidence from morphology and *rbc*L sequences. American Fern Journal 85: 205–282.

ROBINSON, H. 1971. A revised classification for the orders and families of mosses. Phytologia 21: 289–293.

SANDERSON, M. J. 1989. Confidence limits on phylogenies: The bootstrap revisited. Cladistics 5: 113–129.

———. 1995. Objections to bootstrapping phylogenies: A critique. Systematic Biology 44: 299–320.

——— & M. J. DONOGHUE. 1989. Patterns of variation in levels of homoplasy. Evolution 43: 1781–1795.

SCHOFIELD, W. B. & C. HÉBANT. 1984. The morphology and anatomy of the moss gametophore, pp. 627–657. *In* R. M. Schuster (ed.), New Manual of Bryology. The Hattori Botanical Laboratory, Nichinan, Japan.

SWOFFORD, D. L. 1993. PAUP (v. 3.1.1): Phylogenetic analysis using parsimony. Illinois Natural History Survey, Champaign.

VITT, D. H. 1982. Bryopsida, pp. 307–336. *In* S. P. Parker (ed.), Synopsis and Classification of Living Organisms. Vol. 1. McGraw-Hill, NY.

———. 1984. Classification of the Bryopsida, pp. 696–759. *In* R. M. Schuster (ed.), New Manual of Bryology. Hattori Botanical Laboratory, Nichinan.

WATERS, D. A., M. A. BUCHHEIM, R. A. DEWEY & R. L. CHAPMAN. 1992. Preliminary inferences of the phylogeny of bryophytes from nuclear-encoded ribosomal RNA sequences. American Journal of Botany 79: 459–466.

WITHEY, A. 1996*a*. Phylogenetic studies of the Spiridentaceae (Musci): observations of three morphological characters associated with pleurocarpy. Anales Instituto de Biología, UNAM, ser Botánica 67: 5–14.

———. 1996*b*. Systematic studies of the Spiridentaceae (Musci). Ph.D. Dissertation, Duke University, Durham, NC.

ZOMLEFER, W. B. 1993. A revision of *Rigodium* (Musci: Rigodiaceae). THE BRYOLOGIST 96: 1–72.

ms. received April 2, 1998; accepted March 17, 1999.

APPENDIX 1. Specimens used to extract DNA for the 36 exemplar taxa included in present study.

1 *Anacamptodon splachnoides* (Brid.) Brid. U.S.A. INDIANA. Portland Arch, *Sargent,* 1 June 1981
2 *Bartramia halleriana* Hedw. AUSTRALIA. VICTORIA. Mount Donna Buag, *Vitt 27824* (DUKE)
3 *Bescherellia elegantissima* Duby NEW CALEDONIA. Parc Territorial de la Riviere Bleue, *Withey 732* (DUKE)
4 *Brachythecium salebrosum* (Web. & Mohr) B.S.G. U.S.A. ILLINOIS. Urban, *Sargent,* 8 Oct. 1977.
5 *Bryum billardieri* Schwaegr. FIJI. VITI LEVU. Mt. Lomalangi, *Withey 671.* (DUKE).
6 *Cryptopodium bartramioides* (Hook.) Brid. NEW ZEALAND. SOUTH ISLAND. Fiordland National Park, *Vitt 29618* (DUKE); Wilberg Range, *Glenny 4963* (WELT).
7 *Cyrtopus setosus* (Hedw.) Hook. NEW ZEALAND. SOUTH ISLAND. Wellington, Glenny 4827 (WELT).
8 *Dicranum scoparium* Hedw. U.S.A. NORTH CAROLINA. Durham, *Mishler, Hopple & Thrall,* 9 May 1989 (DUKE)
9 *Fontinalis dalecarlica* B.S.G. U.S.A, NEW JERSEY. Delaware Gap, *Sargent,* 29 Oct 1979.
10 *Franciella spiridentoides* Thér. NEW CALEDONIA. Mt. Panié, *Withey 756* (DUKE).
11 *Funaria hygrometrica* Hedw. U.S.A. INDIANA. Busey Woods, *Sargent,* 1 June 1980.
12 *Grimmia* (=Schistidium) *apocarpa* Hedw. U.S.A. INDIANA. Portland Arch, *Sargent,* 23 March 1980
13 *Hedwigia ciliata* (Hedw.) P. Beauv. U.S.A. NORTH CAROLINA. Duke Forest, *De Luna 1751* (DUKE)
14 *Hookeria acutifolia* Hook. U.S.A. INDIANA. Portland Arch, *Sargent,* 30 Aug. 1981
15 *Hypnodendron menziesii* (Hook.) Par. NEW CALEDONIA. Parc Territorial de la Riviere Bleue, *Withey 739* (DUKE)
16 *Hypnodendron vitiense* Mitt. AUSTRALIA. NEW SOUTH WALES. Blue Mts., *Anderson 23873* (DUKE)
17 *Hypopterygium tahitense* Aongstr. COOK ISLANDS. RARATONGA. Mt. Te Ko'u, *Withey 570* (DUKE)
18 *Hypopterygium tamariscinum* (Hedw.) Brid. MEXICO. VERACRUZ. Jardín Botánico Clavijero, *De Luna 2236* (XAL)
19 *Leptobryum pyriforme* (Hedw.) B.S.G. U.S.A. INDIANA. Portland Arch, *Sargent,* 19 May 1982.
20 *Leucodon julaceus* (Hedw.) Sull. CANADA. ONTARIO. Eels Creek, *Sargent,* 5 Aug. 1989.
21 *Macromitrium longifolium* (Hook.) Brid. Sequence data from Goffinet.
22 *Mesochaete undulata* Lindb. AUSTRALIA. QUEENSLAND. Ingham vicinity, *Streimann 35245* (CBG).
23 *Neckera urnigera* C. Müll. MEXICO. VERACRUZ. Jardín Botánico Clavijero, *De Luna 2235.* (XAL)
24 *Orthotrichum pumilum* Sw. U.S.A. INDIANA. Portland Arch, *Sargent,* 30 Aug. 1981.
25 *Papillaria deppei* (Hornsch.) Jaeg. MEXICO. VERACRUZ. Jardín Botánico Clavijero, *De Luna & Newton 2267* (XAL)
26 *Philonotis nitida* Mitt. (= *P. revoluta* Bosch & Lac) FIJI. TAVEUNI. Mt. Des Voeux, *Withey 608* (DUKE)
27 *Plagiomnium cuspidatum* Hedw. U.S.A. INDIANA. Portland Arch, *Sargent,* 11 May 1980.
28 *Pleurozium schreberi* (Brid.) Mitt. U.S.A. VERMONT. Lake Fairlee, *Conklin,* 23 Oct. 1975.
29 *Prionodon densus* (Hedw.) C. Müll. MEXICO. VERACRUZ. Jardín Botánico Clavijero, *Newton & De Luna 3872* (XAL)
30 *Pterobryon densum* (Schwaegr.) Hornsch. MEXICO. VERACRUZ. Jardín Botánico Clavijero, *Newton & De Luna 3856* (XAL)
31 *Pyrrhobryum mnioides* (Hook.) Man. AUSTRALIA. VICTORIA. Marysville vicinity, *Streimann 35245* (DUKE)
32 *Racopilum convolutaceum* (C. Müll.) Reichdt. NEW ZEALAND. SOUTH ISLAND. Wellington, Haurangi Range, *Glenny 4941* (WELT)
33 *Schlotheimia brownii* Schwaegr. Sequence data from Goffinet
34 *Spiridens vieillardii* Schimp. NEW CALEDONIA. Mt Panié, *Withey 526* (DUKE)
35 *Splachnum ampullaceum* Hedw. CZECH REPUBLIC. Sumava Mountains, town of Prásily, *Sargent,* 29 Oct 1979.
36 *Thuidium delicatulum* (Hedw.) Mitt. U.S.A. NORTH CAROLINA. Linville Falls, *Newton,* 18 Nov. 1988. (DUKE).