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Phylogeny and Morphological Evolution of the Amblystegiaceae (Bryopsida)

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To circumscribe the moss family Amblystegiaceae, we performed a broad-scale analysis of *trn*L–*trn*F spacer sequence data for 168 species of the Hypnales and 11 species of the Hookeriales and additional analyses of *trn*L–*trn*F and *atp*B–*rbc*L (chloroplast DNA), one nuclear region, the internal transcribed spacers of 18S–26S rDNA, and 68 morphological characters for a reduced data set of 54 species of Hypnales. The traditionally circumscribed Amblystegiaceae are polyphyletic and include the Amblystegiaceae *s. str.* and the Calliergonaceae fam. nov., plus several taxa closely related to other Hypnalean families. Generic relationships within the redefined Amblystegiaceae were investigated by analyzing data from the three DNA regions and morphology as used in the broader analysis. Reconstruction of morphological evolution was evaluated using maximum-parsimony and maximum-likelihood. Numerous independent character-state transitions implied by the phylogeny suggest that morphological characters that have traditionally been used to delineate the Amblystegiaceae are homoplastic. Sporophytic traits, which are generally given primacy over gametophytic traits in moss classification, are more labile than previously thought, and many characters that are related to sporophyte specializations are strongly correlated with habitat conditions. The evolution of several gametophyte features previously thought to be reliable for delineating the family are also strongly correlated with habitat. These observations help to explain the instability of the Amblystegiaceae in previous taxonomic and phylogenetic analyses based on morphology. © 2002 Elsevier Science (USA)

INTRODUCTION

With approximately 10,000 species, the mosses (Division Bryophyta are one of the most speciose groups of land plants (Buck and Goffinet, 2000). The true mosses, class Bryopsida, include so-called acrocarpous mosses that produce terminal archegonia and are generally sparcely branched. Pleurocarpous mosses, in contrast, form archegonia laterally and are extensively branched. Cladocarpous taxa are morphologically intermediate in that archegonia are terminal on short, lateral branches. The pleurocarpous mosses consist of two orders, the Hookeriales and Hypnales, and form a monophyletic group derived from a cladocarpous or acrocarpous ancestor (Buck *et al.*, 2000a; DeLuna *et al.*, 1999, 2000; Newton *et al.*, 2000a).

Within the Hypnales, relationships among genera and families remain poorly resolved. Only a few molecular studies have sampled extensively within the Hypnales (Buck *et al.*, 2000a,b; DeLuna *et al.*, 1999, 2000; Newton *et al.*, 2000b). Those studies provided mostly unresolved phylogenies due to a general lack of sequence divergence among pleurocarpous clades. In terms of molecular diversity, the pleurocarps as a whole are comparable to some families or even genera of acrocarps. For example, Vanderpoorten *et al.* (2001) described variation in nuclear ribosomal internal transcribed spacer (ITS) sequences from three traditionally defined pleurocarpous families, while in the acrocarpous mosses, ITS sequences are often too variable to align among congeneric species (A. J. Shaw, unpublished). One interpretation of the lack of sequence divergence in the pleurocarps is that they represent a relatively recent and rapid radiation.

Cladistic analyses of relationships among pleurocarps based on morphology are complicated by high levels of homoplasy in many morphological characters (Hedenäs, 1995, 1998b). The situation is exacerbated in aquatic and semiaquatic taxa, which are notoriously variable in morphology, and the Amblystegiaceae, a family typical of moist, wet, or aquatic habitats, is a case in point (Andrews, 1957). Both species and genera of the Amblystegiaceae are distinguished by gametophytic characters such as plant size, leaf orientation, costa prominence, the presence or absence of multistratose regions on the leaves, the presence or absence of paraphyllia (small but multicellular, often leaf-like

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structures borne on the stem), and leaf cell shape (see, e.g., Crum and Anderson, 1981; Ochyra *et al.*, 1991; Buck and Goffinet, 2000). Some of these characters are precisely those that appear to be environmentally plastic (Loeske, 1907, 1928; Mönkemeyer, 1906, 1909; Hedenäs, 1996). Unrelated aquatic mosses, especially those growing in rheophytic habitats, often share a suite of morphological characters (Vitt and Glime, 1984), and Vanderpoorten *et al.* (2001) questioned the use of possibly convergent adaptations for defining species and genera of the Amblystegiaceae. The sporophytes of the Amblystegiaceae are similar in many features to those of other families, including the Thuidiaceae and the temperate members of the Hypnaceae (Fleischer, 1904–1923; Nishimura *et al.*, 1984; Hedenäs, 1989b, 1995, 1998a), and offer limited taxonomic assistance. Circumscription of the Amblystegiaceae has therefore been unstable and controversal (e.g., Crum and Anderson, 1981; Ochyra *et al.*, 1991; Hedenäs, 1995; Buck and Goffinet, 2000).

The goals of this study were (1) to circumscribe a monophyletic Amblystegiaceae, necessarily in the context of incomplete information about broader relationships among pleurocarps, (2) to study morphological evolution within the redefined family, and (3) to infer the extent to which morphological evolution in the Amblystegiaceae is correlated with habitat shifts.

MATERIAL AND METHODS

Taxon sampling. Phylogenetic analyses were undertaken on three data sets. In a first attempt to redefine the Amblystegiaceae in the context of the Hypnales we assembled a data set of 179 pleurocarp sequences, of which 127 were obtained from Buck *et al.* (2000a), and 52 were produced for the purpose of this study (Table 1). Eleven Hookerialean species (*Adelothecium bogotense, Leskeodon cubensis, Hookeria acutifolia, Lepidopilum surinamense, L. scabrisetum, Leucomium strumosum, Pilotrichum fendleri, Ptychomnion aciculare, Glyphothecium sciuroides, Garovaglia elegans,* and *Crossomitrium rotundifolium*), also available from Buck *et al.* (2000a), were used as outgroups. Within the Hypnales, the sampling included 168 taxa, with a special emphasis on the Amblystegiaceae in a broad sense (e.g., sensu Kanda, 1975, 1976; Vitt, 1984), including the genera *Sanionia, Drepanocladus, Calliergonella, Amblystegium, Hygroamblystegium, Leptodictyum, Campylophyllum, Campylium, Campyliadelphus, Cratoneuron, Palustriella, Cratoneuropsis, Hypnobartlettia, Hamatocaulis, Scorpidium, Tomentypnum, Donrichardsia, Calliergon, Straminergon, Conardia, Hygrohypnum, Warnstorfia, Platydictya, Serpoleskea, Loeskypnum, Hamatocaulis,* and *Limbella.*

In a second attempt to circumscribe the Amblystegiaceae within the Hypnales, sequences from the *trnL–trnF* region, the *atpB–rbcL* spacer, the internal transcribed spacers of 18S–26S rDNA (ITS), and morphological characters were used. This analysis included 54 taxa, including most of the genera ever included in the Amblystegiaceae (see above), plus representative members of Hypnalean families whose potential relationships with the Amblystegiaceae are suggested by their morphology (Hedenäs, 1995, 1998a,b; Ochyra and Vanderpoorten, 1999). These include the Thuidiaceae *s. l. (Anomodon, Thuidium, Haplocladium, Abietinella, Helodium*), Hypnaceae *s. l.* (*Hypnum, Rhytidium, Caribaeohypnum, Ptilium*), Brachytheciaceae (*Platyhypnidium*), Leskeaceae (*Leskea*), and Fabroniaceae (*Anacamptodon*). *Neckera pennata* and *N. douglasii,* two members of the suborder Neckerineae (Brotherus, 1925; De Luna *et al.*, 2000), were used as outgroups.

In our third set of analyses, generic relationships within the redefined Amblystegiaceae were then investigated. This analysis, including 22 taxa, utilized data from the same three DNA regions used in the broader analysis. In this sample of more closely related species, realignment of ITS sequences was possible because positional homology was less problematic; *Anomodon* and *Thuidium,* two taxa sister to the redefined Amblystegiaceae, were used as outgroups.

Molecular protocols. DNA was extracted from 1–10 mg of shoot apices or undehisced, mature capsules from herbarium collections less than 20 years old (Table 1). The nuclear DNA sequences included the 3′ end of the 18S rDNA gene, both ITS1 and ITS2 spacer regions, the 5.8S rDNA gene, and the 5′ end of the 26S rDNA gene. The chloroplast sequences included the *trn*L gene (two exons and an intron), a spacer separating the *trn*L and *trn*F genes, approximately 50 bp of the *trn*F gene, and the *atp*B–*rbc*L intergenic spacer. Extractions, PCR amplifications, and sequencing were conducted according to the protocols described by Shaw (2000) for ITS and Buck *et al.* (2000a) for *trn*L–*trn*F. The *atp*B–*rbc*L intergenic spacer was sequenced using the primers designed by Chiang *et al.* (1998). Contigs were constructed from single-stranded forward and reverse sequences using Sequencher 3.0 (Gene Codes Corp.). Sequences were aligned manually. Gaps were inserted where necessary to preserve positional homology. Regions for which positional homology were questionable were excluded from the analysis.

Phylogenetic analyses. In all analyses, each data partition (i.e., the individual genomic regions) was analyzed under equally weighted maximum-parsimony (MP) using heuristic searches with 1000 random addition replicates and TBR branch swapping after exclusion of uninformative characters. For those analyses saving more than 25,000 equally parsimonious trees, a maximum of 20 trees per replicate were saved and only the best trees were swapped to avoid the problem of

TABLE 1

Taxon Sampling and GenBank Accession Numbers

Species	Voucher or reference	*trn*L–*trn*F GenBank Accession No.	*atp*B–*rbc*L GenBank Accession No.	ITS GenBank Accession No.
Abietinella abietina (Hedw.) Fleisch.	Allen 19816	AY009850	AF322308	AY009802
Amblystegium fluviatile (Hedw.) B, S. & G.	Allen 16372	AY009822	AF322324	AF168154
Amblystegium humile (P. Beauv.) Crundw.	Buck 15943	AY009823	AF322359	AF168165
Amblystegium serpens (Hedw.) B., S. & G.	Schofield 106313	AY009827	AF322326	AF168152
Amblystegium tenax (Hedw.) C. Jens. I	Schofield and Belland 94941	AY980024	AF322360	AF168164
Amblystegium tenax (Hedw.) C. Jens. II	Vanderpoorten 4195	AY009820	AF322361	AF168156
Amblystegium tenax (Hedw.) C. Jens. III	Vanderpoorten 4263	AY009821	AF322327	AF168157
Amblystegium varium (Hedw.) Lindb.	Anderson 27620	AY009825	AF322328	AF168159
Anacamptodon splachnoides (Brid.) Brid.	Schofield *et al.* 96592	AY009816	AF322336	AY009810
Anomodon attenuatus (Hedw.) Hueb.	Schofield 106622	AY009851	AF322309	AF168133
Calliergon cordifolium (Hedw.) Kindb	Ireland 24198	AY009836	AF322341	AF168146
Calliergon giganteum (Schimp). Kindb.	Schofield *et al.* 93733	AY009834	AF322342	AF168144)
Calliergonella cuspidata (Hedw.) Loeske	Schofield 100768	AY009859	AF322310	AF168145
Calliergonella lindbergii (Mitt.) Hedenäs	Anderson 27099b	AF315069	AF322315	AY009813
Campyliadelphus chrysophyllus (Brid.) Kanda	Anderson 26799	AY009831	AF322355	AF168150
Campylium stellatum (Hedw.) C. Jens.	Schofield 105981	AY009832	AF322354	AF168151
Campylophyllum halleri (Sw. ex Hedw.) Fleisch.	Schofield *et al.* 93995	AY009853	AF322311	AF168134
Caribaeohypnum polypterum (Mitt.) Ando & Higuchi	Allen 11570	AY009846	AF322353	AY009799
Conardia compacta (C. Muell.) Robins.	Risk 10846	AY009865	AF322312	AY009806
Cratoneuron filicinum (Hedw.) Spruce	Lewis 87262	AY009817	AF322332	AY009812
Cratoneuron filicinum var. *atrovirens* (Brid.) Ochyra	Vanderpoorten 769	AY009826	AF322362	AF168155
Cratoneuropsis relaxa (Hook. & Wils.) Fleich.	Stech *et al.*, 1999	AF098525	AF322337	AF152388–AF152391
Donrichardsia macroneuron (Grout) Crum & Anderson	Redfearn 27208	AY009848	AF322323	AF167350
Drepanocladus aduncus (Hedw.) Warnst.	Schofield 104541	AY009828	AF322331	AF180949
Drepanocladus sendtneri (Schimp. ex H. Muell.) Warnst.	Hedenäs B35345 (S)	AY009818	AF322338	AY009811
Drepanocladus sordidus (C. Muell.) Hedenäs	Hedenäs B39576 (S)	AY009868	AF322333	AY009792
Gradsteinia andicola Ochyra *et al.*	Stech and Frahm 2000	—	—	AF230992–AF231007
Hamatocaulis vernicosus (Mitt.) Hedenäs	Hedenäs and Janssens B16968 (S)	AY009819	AF322343	AF315073
Haplocladium virginianum (Brid.) Broth.	Buck 32482 (NY)	AF161133	AF322305	AF168160
Helodium blandowii (Web. & Mohr) Warnst.	Schofield 108637	AY009852	AF322313	AY009803
Hygrohypnum luridum (Hedw.) Jenn.	Schofield *et al.* 104900	AY009862	AF322339	AF168137
Hygrohypnum montanum (Lindb.) Broth.	Schofield *et al.* 92572	AY009863	AF322320	AY009804
Hygrohypnum ochraceum (Wils.) Loeske	Piercey-Normore NF57	AY009861	AF322345	AF168138
Hygrohypnum smithii (Sw.) Broth.	Schofield 104556	AY009856	AF322306	AF168139
Hypnobartlettia fontana Ochyra	Stech *et al.*, 1999	AF098524	AF322340	AF152386–AF152389
Hypnum pallescens (Hedw.) P. Beauv.	Schofield 106643	AY009844	AF322314	AY009796
Leptodictyum riparium (Schimp.) Warnst.	Bowers and Haynes 15869	AY009830	AF322325	AF168163
Leskea gracilescens Hedw.	Buck 30102 (NY)	AF161135	AF322356	AF176277
Limbella pachyloma (Mont.) C. Muell.	Matteri & Schiavone 4846 (S)	—	—	AY009814
Neckera douglasii Hook.	Schofield 107766	AF315070	AF322358	AY009808
Neckera pennata Hedw.	Shaw 9354	AF315072	AF322357	AY009809
Palustriella falcata (Brid.) Hedenäs	Schofield and Godfrey 97864	AY009829	AF322330	AF168158
Platydictya jungermannioides (Brid.) Crum	Schofield *et al.* 101911	AY009857	AF322307	AF168162
Platygyrium repens (Brid.) Schimp.	Buck 33448 (NY)	AF161131	AF322317	AY009798
Platyhypnidium riparioides (Hedw.) Dix.	Stech and Frahm, 1999	—	—	—
Pseudo-calliergon trifarium (Web. & Mohr) Loeske	Schofield and Spence 84225	AY009835	AF322329	AY009793
Pseudo-calliergon turgescens (Jens.) Loeske	Schofield *et al.* 92508	AY009843	AF322335	AY009794
Ptilium crista-castrensis (Hedw.) De Not.	Schofield 108705	AY009847	AF322316	AY009800
Rhytidium rugosum (Hedw.) Kindb.	Schofield and Godfrey 98103	AY009849	AF322318	AY009801
Sanionia uncinata (Hedw.) Loeske	Schofield 95255	AY009860	AF322321	AF168148
Scorpidium revolvens (Sw. ex Anonymo) Rubers	Schofield *et al.* 99517	AY009841	AF322363	AF168140
Scorpidium scorpioides (Hedw.) Limpr.	Hedenäs 20461 (S)	AY009791	AF322348	AY009790
Serpoleskea confervoides (Brid.) Kartt.	Allen and Risk 4761	AY009858	AF322334	AF168142
Straminergon stramineum (Kindb.) Hedenäs	Fagerstén 5092	AY009833	AF322349	AF168143
Thuidium delicatulum (Hedw.) B., S. & G.	Buck 32594 (NY)	AF161132	AF322322	AF176278
Tomentypnum falcifolium (Ren. ex Nich.) Tuom.	Schofield and Hedderson 94870	AY009855	AF322351	AF168136
Tomentypnum nitens (Hedw.) Loeske	Schofield 103470	AY009854	AF322352	AF168161
Warnstorfia exannulata (B., S. & G.) Loeske	Schofield and Talbot 99701	AY009839	AF322344	AF315074
Warnstorfia fluitans (Hedw.) Warnst.	Belland and Schofield 17963	AY009838	AF322350	AF168149

Note. All vouchers for which sequences were generated during the course of this study are deposited at DUKE unless otherwise noted.

local, suboptimal trees. Support for clades was assessed using the bootstrap analysis (Felsenstein, 1985). Bootstrap analyses were conducted with 300 replicates using simple taxon addition, except for each of the *trn*L–*trn*F and *atp*B–*rbc*L partitions, for which "fast" bootstraps (i.e., without branch swapping) with 10,000 replicates were run due to time constraints. The decision to combine the partitions was taken after comparing both the topologies and the support for the branches as assessed by bootstrap.

Morphological and ecological characters. Sixty-eight morphological characters were scored, including 42 gametophytic and 26 sporophytic characters (Appendixes 1 and 2). Wherever possible, morphological characters were scored from the same specimens used in the molecular analyses. Some, however, lacked sporophytes, and so morphological characters were also scored in specimens cited by Hedenäs (1987b, 1989a,c, 1990, 1993, 1997a, 1998a). The selection and definition of morphological characters were based on previous analyses by Hedenäs to make this work comparable with his work based solely on morphology. The molecular data set provides an independent test of relationships, and the evolution of morphological characters hypothesized to be informative in previous analyses can be assessed in the context of molecular evidence.

The investigated species were also scored for their occurrence in the three most important habitats for the Amblystegiaceae (swamps, running waters, and epiphytic) (Appendix 2), based on our personal field experience and a review of the literature (Crum and Anderson, 1981). A species was scored "1" if it can occur in a habitat and "0" if it never occurs in such a habitat. Swamps were defined as permanently wet habitats where plants are mostly emergent, but can sometimes be completely submerged; running water included flowing streams and rivers. Epiphytism was defined as the occurrence of the taxa on living trees.

Reconstruction of ancestral character-states. Ancestral character-states were reconstructed for all the morphological characters by choosing those estimates requiring the smallest number of state changes through time (maximum-parsimony; see Maddison and Maddison, 1992).

The probabilities of alternative ancestral states were quantified using maximum-likelihood (ML) methods (Schluter *et al.*, 1997; Pagel, 1999). In these analyses, the ML substitution model best fitting the data was chosen, after exclusion of the morphological characters, by calculating the likelihood of a neighbor-joining tree under 48 different nested likelihood models as implemented by Modeltest 3.0 (Posada and Crandall, 1998). For the circumscription of the Amblystegiaceae, the selected model maximizing the likelihood of the neighbor-joining tree ($-\ln L = 10352.3496$) was a general time-reversible model (Rodriguez *et al.*, 1990) with a gamma distribution to model rate heterogeneity among sites and the following settings: rate matrix, R [A-C] = 0.9443; R [A-G] = 1.9496; R [A-T] = 0.3409; R [C-G] = 1.7275; R [C-T] = 2.3731; R [G-T] = 1.0000; base frequencies, A = 0.3052; C = 0.1887; G = 0.2002; T = 0.3059; proportion of invariable sites (I) = 0.4127; Γ distribution shape parameter = 0.6777. For phylogenetic analyses within the Amblystegiaceae, the selected model maximizing the likelihood of the neighbor-joining tree ($-\ln L = 5433.4473$) was the Hasegawa *et al.* (1985) model plus gamma with the following settings: Ti/tv ratio = 1.1870; base frequencies, A = 0.2854, C = 0.2121, G = 0.2135, T = 0.2890; Γ distribution shape parameter = 0.2846. Branch lengths on one of the most parsimonious trees were calculated under the assumptions of the selected model with fixed parameter estimates as described above.

Branch lengths were used as proxies for time, and therefore the molecular clock was enforced, assuming constant transition rates along the branches. Using branch lengths in this manner as proxies for time, it was possible to interpret the likelihood of morphological evolution along different branches in relation to time. Failing to enforce the clock would require an unrealistic assumption for the analyses: that rates of morphological and molecular evolution, heterogeneous across the tree, are correlated with one another.

One of the most parsimonious trees from the phylogenetic analyses was used for reconstructing ancestral states of the morphological characters using a ML model implemented by Discrete 4.0 (Pagel, 1999). Although the morphological characters were included in the formulation of this tree, our analyses permit investigations of morphological evolution in the context of total (morphological and molecular) evidence. Models allowing different rates of forward (α) and backward (β) transitions were compared to single-parameter models, i.e., models with identical transition rates. For each character, the likelihood ratio, $LR = -2\ln[L(\alpha = \beta)/L(\alpha, \beta)]$, where L represents the likelihood of the observed data, was calculated, and a χ^2 approximation with a 0.05 level of confidence and one degree of freedom was employed for testing significant improvement with the two-parameter model. When the two-parameter model did not significantly increase the fit over the one-parameter model, the latter was used for reconstructing the ancestral character-states (Pagel, 1999; Mooers and Schluter, 1999). Transition rates were calculated for each character and then fixed for assessing the likelihood of each node at a state 0 or 1. Support for ML estimates was assessed by calculating the ratio of the likelihood of the two estimates (0 or 1 at that node), with a ratio of at least 7:1 considered significant (Schluter *et al.*, 1997). Because this approach does not allow for the analysis of ancestral character-states at polytomies, or the inclusion of missing data, taxa were

deleted as necessary from the tree, and branch lengths were adapted by simple addition of the original branch lengths for each character. Multistate characters were transformed into additive binary characters for the ancestral state reconstruction under ML, as described by Wiley *et al.* (1991).

Testing correlated evolution. Hypotheses of correlated evolution in two characters were tested by comparing the fit of two different models to the observed data. First, the method fits a model to the data in which the two characters are treated as evolving independently. For a trait that can take only two values (0,1), two rates must be estimated, one for transitions from "0" to "1" and the other for transitions from "1" to "0." These parameters are sufficient to characterize the evolution of traits in isolation from one another. Four parameters are required for two traits evolving independently. The goodness-of-fit of this model is then compared to that of a more complicated model in which the characters evolve in a correlated fashion. The model of correlated or dependent trait evolution considers the four possible states that two binary characters can jointly adopt (0,0; 0,1; 1,0; 1,1). It then allows one of the variables to change state on any branch of the tree, yielding eight possible transitions to be estimated. The model of correlated change is justified as a representation of the data if it fits the data significantly better then the model of independent change (Pagel, 1994). This is assessed by a likelihood ration test as implemented by Discrete 4.0, LR = $-\ln[L(I)/L(D)]$, where $L(I)$ and $L(D)$ are the likelihoods of the data under a four-parameter independent model of evolution and an eight-parameter dependent model of evolution, respectively. The likelihood ratios are conventionally multiplied by two to form the likelihood ratio statistic. No transformations were excluded *a priori;* hence, no transition rate was set to 0.

When the independent model can be considered a subcase of the dependent model, the likelihood ratio distribution is asymptotic to that of a χ^2 distribution with four degrees of freedom. The validity of this assumption, however, varies as a function of the size of the data set and the value of the transition rates (Pagel, 1994). Hence a Monte Carlo procedure was also used to obtain the null distribution by finding the maximum-likelihood of α and β (independent model) applied to the observed data. These parameters were then fixed and used to evolve the two characters along the branches of the phylogenetic tree. The new set of simulated data was analyzed with the model of correlated change and the model of independence, and the likelihood ratio of the two models was found. After having assessed the computational time needed for 25, 50, 100, and 150 iterations, we ran the procedure 50 times. The hypothesis of correlated evolution was accepted when no more than 5% of the simulated LRS were greater than the observed LR.

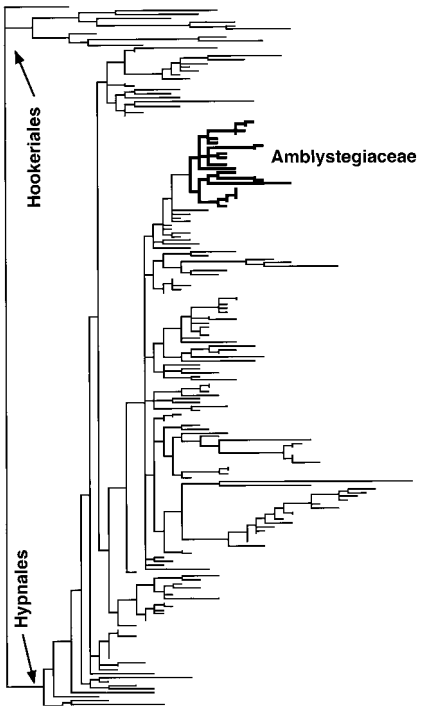


FIG. 1. Simplified taxonomic representation of one of the most parsimonious trees (L = 725, CI = 0.29, RI = 0.67) from the analysis including 179 pleurocarps. Eleven species of the Hookeriales were included as outgroups.

RESULTS

Phylogenetic analyses: circumscription of the Amblystegiaceae. The *trn*L–*trn*F data matrix of 168 Hypnalean taxa and 11 Hookerialean outgroup taxa included 123 informative characters. The maximum-parsimony analysis was stopped after saving 40,000 equally parsimonious trees of 725 steps (CI = 0.29, RI = 0.67). Within the Hypnales, relationships were almost totally unresolved in the consensus tree (not shown). One of the most parsimonious trees (Fig. 1) included an unsupported clade containing *Amblystegium serpens* (the type of the Amblystegiaceae). In this tree, *A. serpens* was resolved with representatives of several other genera traditionally included in the Amblystegiaceae: *Leptodictyum, Campylium, Campylophyllum, Cratoneuron, Palustriella, Drepanocladus s. str., Hypnobartlettia, Cratoneuropsis, Hygrohypnum luridum, Serpoleskea, Anacamptodon,* and *Pseudo-calliergon.* Although not definitive, this broad-scale analysis showed that *trn*L–*trn*F sequences were consistent with the existence of a core group of genera, including *Amblystegium,* which form a monophyletic group.

In a second attempt to resolve a monophyletic Amblystegiaceae for subsequent analyses, a data set was

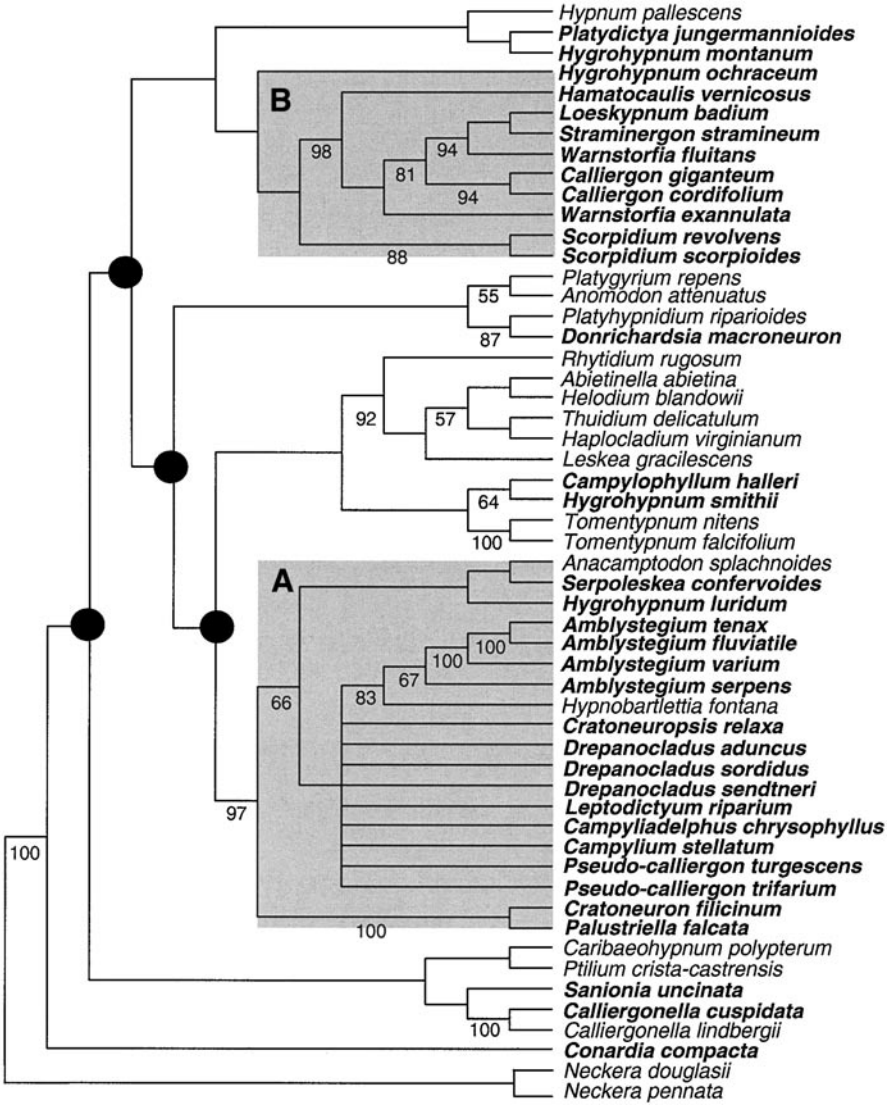


FIG. 2. Strict consensus tree of five equally parsimonious trees (L = 1383, CI = 0.38, RI = 0.58) for 54 taxa of the Hypnales from the combined molecular and morphological partitions, using *Neckera* spp. as outgroups. Numbers below the branches are the bootstrap support percentages >50%. Taxa traditionally included within the Amblystegiaceae appear in boldface. A, Amblystegiaceae s str.; B, Calliergonaceae. ●, Node resolved only when morphology is combined with the molecular partitions.

compiled with sequences from two cpDNA regions, rDNA (ITS), and morphological characters. Separate analyses of the *trn*L–*trn*F, *atp*B–*rbc*L, and morphological partitions for the data set including 54 Hypnalean taxa each resulted in almost unresolved strict consensus topologies where the few resolved clades were also present in the ITS consensus topology. No clades that were resolved with >50% bootstrap support in separate analyses were in conflict across data partitions. The partitions were therefore combined. The combined analysis included a total of 371 informative characters, among which 171 derived from ITS, 50 from *trn*L–*trn*F, 83 from *atp*B–*rbc*L, and 67 from morphology. The combined analysis produced five equally parsimonious trees of 1383 steps. In the strict consensus, two main clades of taxa previously aligned within the Amblystegiaceae were resolved with strong bootstrap support (Fig. 2). Clade (A), with 97% bootstrap support, included *Amblystegium*, *Leptodictyum*, *Campylium*, *Campyliadelphus*, *Cratoneuron*, *Palustriella*, *Drepanocladus s. str.*, *Hypnobartlettia*, *Cratoneuropsis*, *Hygrohypnum luridum*, *Serpoleskea confervoides*, *Anacamptodon*, and *Pseudo-calliergon*. Because this clade included *Amblystegium*, it constituted the "core" Amblystegiaceae and includes most of the same taxa resolved as Amblystegiaceae in the first analysis (*trn*L–*trn*F alone). Clade B included the *Calliergon–Warnstorfia* complex, *Hamatocaulis*, *Scorpidium*, and *Hygrohypnum ochraceum*. The complex of genera including *Calliergon–Warnstorfia* had 98% bootstrap support, but the inclusion of *Scorpidium*

and *Hygrohypnum* was without support. Relationships among the major clades were resolved in this analysis only when morphological characters were combined with the molecular data (see the bold nodes in Fig. 2).

A number of genera traditionally aligned within the Amblystegiaceae, such as *Sanionia, Calliergonella, Platydictya, Hygrohypnum, Campylophyllum,* and *Conardia,* did not belong to clades A or B and exhibited relationships with members of different Hypnalean families. *Limbella pachyloma* and *Gradsteinia andicola,* two species often included in the Amblystegiaceae, were not included in this analysis because cpDNA sequences could not be obtained. However, these taxa were included in an analysis of ITS plus morphological characters (not shown). *Limbella* occurred on a fairly long branch between clade B and the *Thuidium–Leskea* complex, whereas *Gradsteinia* was nested within clade A with *Cratoneuropsis* and *Drepanocladus s. str.*

Phylogenetic analyses within the Amblystegiaceae. Based on the previous analyses, a core clade of Amblystegiaceae, which included the type species of *Amblystegium, A. serpens,* and a number of taxa traditionally allied with the Amblystegiaceae, was identified for further analyses. Morphology, *atp*B–*rbc*L, and *trn*L–*trn*F each provided strict consensus trees with very low resolution when analyzed separately. The ITS consensus tree conflicted with the consensus tree resulting from the combined chloroplast sequences in one aspect. In the ITS partition, population II of *Amblystegium tenax* grouped with *Cratoneuron filicinum.* In the cpDNA partition, population II of *A. tenax* grouped with the other populations of *Amblystegium.* This population apart, the data sets yielded compatible topologies. Population II of *A. tenax* was therefore deleted from the analysis prior to combining partitions. The combined analysis included 207 informative characters; 76 from ITS, 57 from *trn*L–*trn*F, 33 from *atp*B–*rbc*L, and 41 morphological characters. This combined analysis produced eight equally parsimonious trees of 412 steps (CI = 0.59, RI = 0.71).

In the strict consensus (Fig. 3), *Cratoneuron* and *Palustriella* formed a clade with 76% bootstrap support that was sister to the remaining Amblystegiaceae (74% support). *Leptodictyum, Campylium, Campyliadelphus, Serpoleskea, Anacamptodon,* and *Hygrohypnum luridum* formed a clade with 82% bootstrap support. This group was sister to a clade including *Amblystegium, Hypnobartlettia, Cratoneuropsis, Drepanocladus,* and *Pseudo-calliergon* with 81% bootstrap support. *Serpoleskea, Anacamptodon,* and *Hygrohypnum luridum* formed a clade with 70% bootstrap support, which was sister to a clade with 97% bootstrap support including *Campyliadelphus, Campylium,* and *Leptodictyum,* the last two forming a clade with 92% bootstrap support. *Drepanocladus* and *Pseudo-calliergon* constituted a clade supported at 73%, which was sister to *Hypnobartlettia* and *Amblystegium.* No relationships were supported within the *Drepanocladus–Pseudo-calliergon* complex. *Hypnobartlettia* was sister to *Amblystegium,* a genus forming a clade supported at 93%. *Amblystegium serpens* and *A. varium* formed a basal node, and were sister to *A. humile, A. tenax,* and *A. fluviatile,* which formed an unresolved clade supported at 94%.

Reconstruction of ancestral character-states. The tree on which we reconstructed morphological character evolution was not fully resolved but is sufficient, with well-supported clades, to make inferences about character evolution at the generic level. For ML reconstructions, employing a model allowing different rates of forward and backward transitions did not significantly improve the fit over a one-parameter model. Consequently, a model where both rates were constrained to be identical was used, as recommended by Schluter *et al.* (1997), Mooers and Schluter (1999), and Pagel (1999).

In terms of circumscribing the Amblystegiaceae (Fig. 4), reconstruction of character evolution using MP failed to identify a single morphological synapomorphy. Two characters [alar cells size (25) and pseudoparaphyllium shape (30)] were identified as synapomorphies for the Calliergonaceae (clade B in Fig. 4) based on MP. (Alar cells are leaf cells at the basal margins; pseudoparapyllia are tiny structures associated with branch primordia.) ML ancestral character-state reconstructions were identical to those obtained using MP for the shape of the alar cells (25). For pseudoparaphyllium shape (30), synapomorphies at deeper nodes (arrows, Fig. 5) could not be definitely attributed under ML, because the reconstruction of the ancestral character-states was too uncertain. Figure 5 (compared to Fig. 4) shows how MP reconstructions can mask ambiguities that are more evident using ML, which are sensitive to the problem of determining along which internode the change in state actually occurred.

MP reconstructions for all parsimony-informative characters within the Amblystegiaceae are shown in Fig. 6. Using MP and ML models to reconstruct ancestral character-states provided identical results for the synapomorphies 10, 26–28, 65, and 67 and for all the autapomorphies. For a number of other characters (1, 7, 16, 17, 23–25, 30, 31, 33, 36, and 61), the reconstruction of the ancestral states was too uncertain for defining synapomorphies, with estimates significant only at the most near-terminal nodes. One such character-state, short laminal cells (16), was synapomorphic for *Amblystegium* and the clade including *Serpoleskea* and *Anacamptodon* under MP (arrows in Fig. 6). Under ML, the condition of the ancestors was too uncertain for the synapomorphy to be defined (Fig. 7). Finally, for

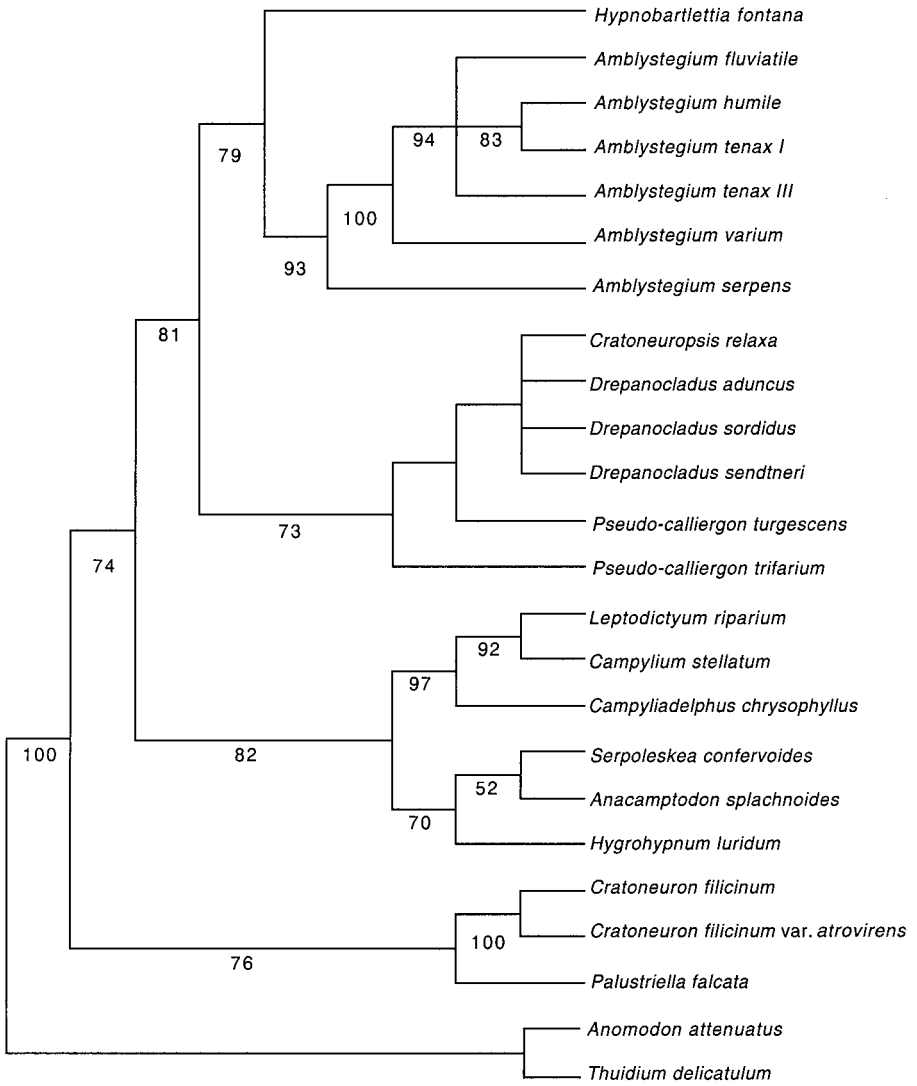
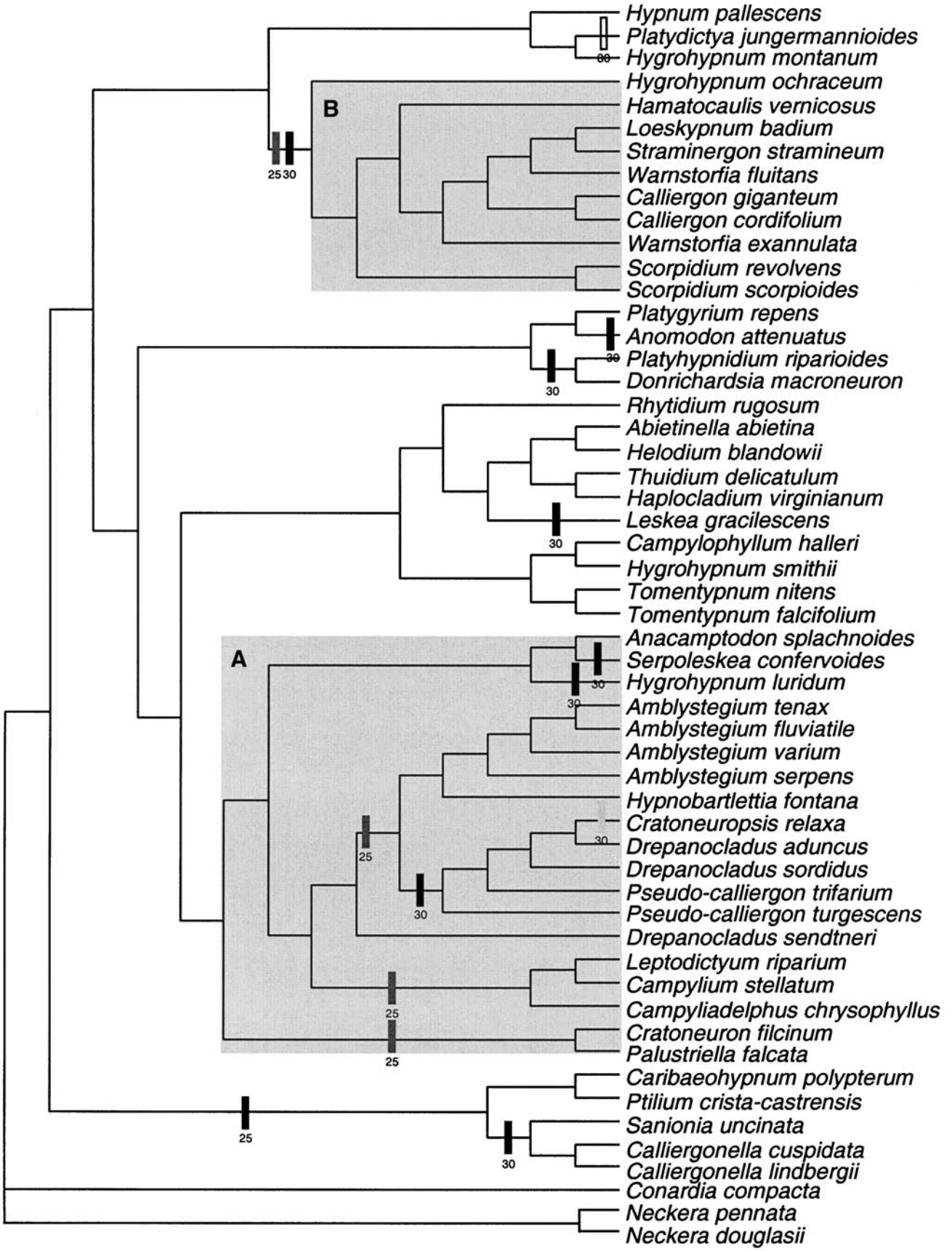


FIG. 3. Strict consensus tree of eight equally parsimonious trees (L = 412, CI = 0.59, RI = 0.71) for 22 taxa of Amblystegiaceae from the combined molecular and morphological partitions using *Anomodon* and *Thuidium* as outgroups. Numbers below the branches are the bootstrap support percentages >50%.

two characters, MP and ML reconstruction provided conflicting results: under MP, the ancestral state of the sexual condition (Fig. 6, character 1) of the Amblystegiaceae was dioicous; hence the monoicous condition of *Amblystegium* was synapomorphic. Under ML, conversely (Fig. 7), the ancestors of the *Amblystegium* complex and of the *Drepanocladus–Pseudocalliergon* complex were estimated, though with considerable ambiguity, to be monoicous; hence the dioicous condition of *Drepanocladus–Pseudo-calliergon* was synapomorphic for that clade. In the same way, the possession of numerous axillary hairs per leaf axil (33) was the ancestral condition of the Amblystegiaceae under MP (Fig. 6); hence the possession of single hair per leaf axil was a synapomorphy of *Amblystegium*. Under ML, the plesiomorphic state is 1- (2) axillary hairs per leaf axil (Fig. 7); hence the possession of numerous axillary hairs per leaf axil was synapomorphic for the *Drepanocladus–Pseudocalliergon* complex.

FIG. 4. MP reconstruction of morphological ancestral character-states on one of the five most parsimonious trees from the analysis of 54 taxa of the Hypnales using *Neckera* spp. as outgroups. Morphological characters that are synapomorphic for clades A and B are represented. ▮▮▨, Synapomorphic transitions from a state 1 to a state 2, from a state 0 to a state 1, and from a state 1 to a state 0, respectively. Numbers of the characters refer to those given in Appendix 1.


Hypnum pallescens
Platydictya jungermannioides
Hygrohypnum montanum
Hygrohypnum ochraceum
Hamatocaulis vernicosus
Loeskypnum badium
Straminergon stramineum
Warnstorfia fluitans
Calliergon giganteum
Calliergon cordifolium
Warnstorfia exannulata
Scorpidium revolvens
Scorpidium scorpioides
Platygyrium repens
Anomodon attenuatus
Platyhypnidium riparioides
Donrichardsia macroneuron
Rhytidium rugosum
Abietinella abietina
Helodium blandowii
Thuidium delicatulum
Haplocladium virginianum
Leskea gracilescens
Campylophyllum halleri
Hygrohypnum smithii
Tomentypnum nitens
Tomentypnum falcifolium
Anacamptodon splachnoides
Serpoleskea confervoides
Hygrohypnum luridum
Amblystegium tenax
Amblystegium fluviatile
Amblystegium varium
Amblystegium serpens
Hypnobartlettia fontana
Cratoneuropsis relaxa
Drepanocladus aduncus
Drepanocladus sordidus
Pseudo-calliergon trifarium
Pseudo-calliergon turgescens
Drepanocladus sendtneri
Leptodictyum riparium
Campylium stellatum
Campyliadelphus chrysophyllus
Cratoneuron filcinum
Palustriella falcata
Caribaeohypnum polypterum
Ptilium crista-castrensis
Sanionia uncinata
Calliergonella cuspidata
Calliergonella lindbergii
Conardia compacta
Neckera pennata
Neckera douglasii
A
B
25
30

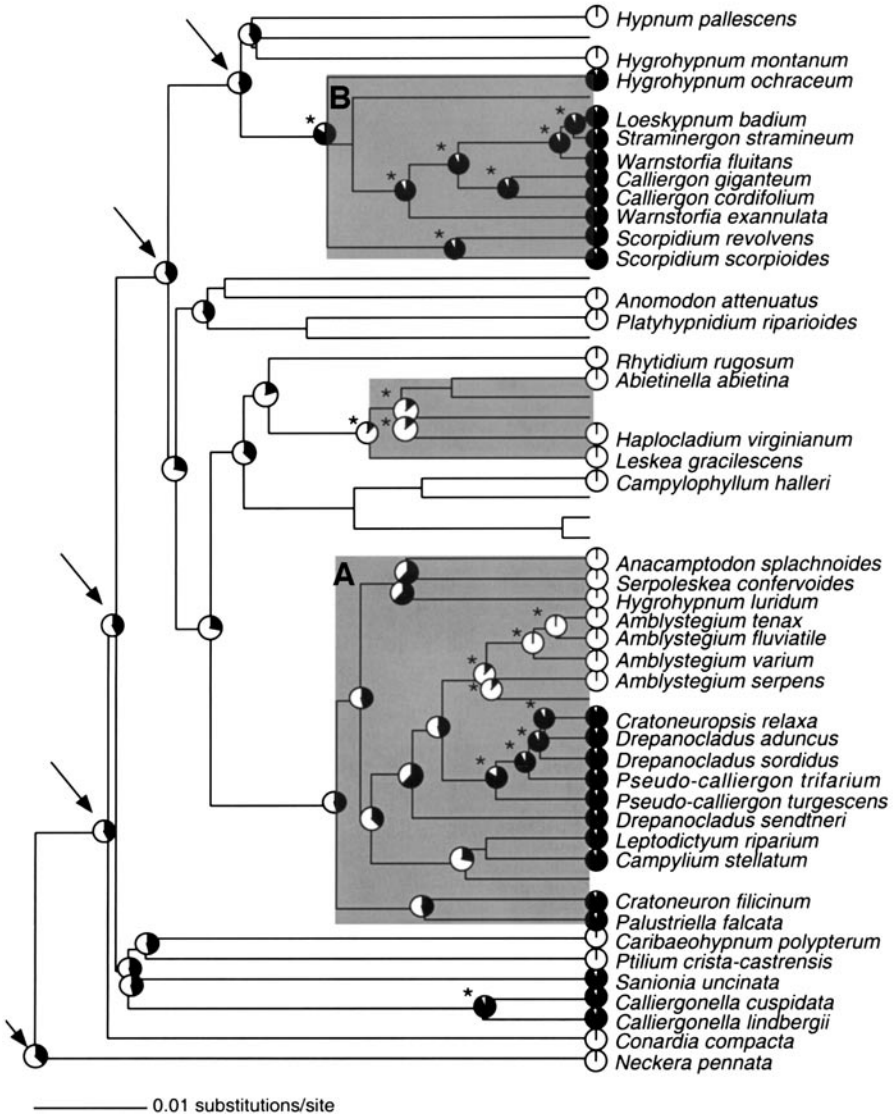
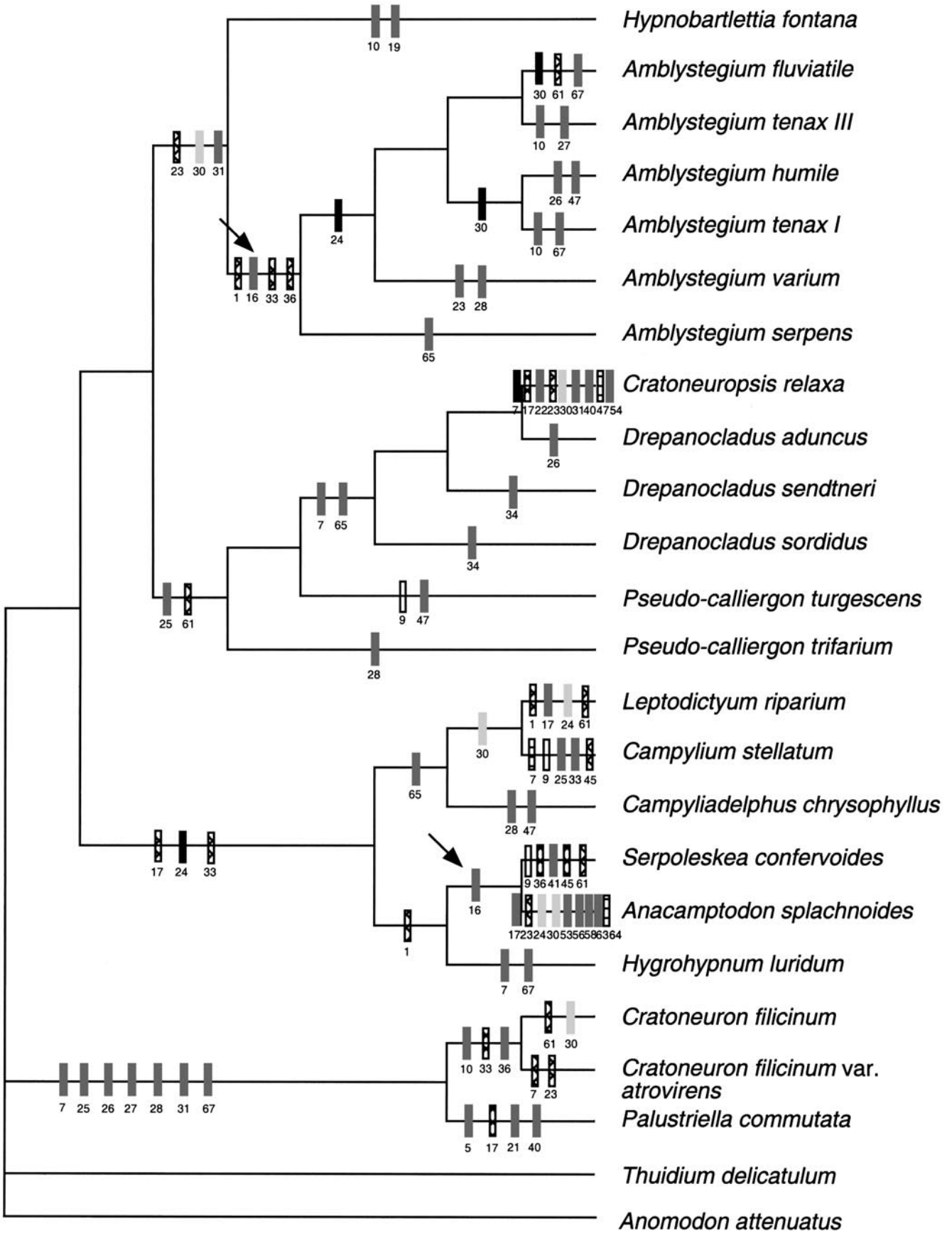


FIG. 5. ML ancestral character-state reconstruction of pseudoparaphyllium shape (30) on one of the five most parsimonious trees resulting from the analysis of 54 taxa of the Hypnales, using *Neckera* spp. as outgroup, with branch lengths calculated via ML and equal forward and backward transition rates ($\alpha = \beta = 28.437$) (see text for details concerning the substitution models). Pie diagrams indicate the relative degrees of support for alternative character-states (1 in white and 2 in black), with a ratio of at least 7:1 considered significant (*). Arrows indicate ambiguous character-state reconstructions for the ancestors of the Calliergonaceae, making it impossible to determine where the change of state actually occurred.

Correlations between morphological evolution and habitat shifts. Tests were conducted of correlated evolution of all morphological characters and mating system, with habitat shifts. Changes in habitat were significantly correlated with changes in a number of morphological characters and mating system (Table 2, Fig. 8). Morphological features that are correlated with different habitats include both sporophyte and gametophyte characters, and there is no indication that one generation is more or less labile than the other.

FIG. 6. MP reconstruction of morphological ancestral character-states on one of the eight most parsimonious trees from the analysis of 22 taxa of the Amblystegiaceae using *Anomodon* and *Thuidium* as outgroups. Arrows indicate the synapomorphy "short laminal cells" (16) for *Amblystegium* and the clade including *Serpoleskea* and *Anacamptodon*. ▮▮▮▨▯, Synapomorphic transitions from a state 1 to a state 2, from a state 0 to a state 1, from a state 2 to a state 1, from a state 1 to a state 0, and from a state 0 to a state 2, respectively. Numbers of the characters refer to those given in Appendix 1.


Hypnobartlettia fontana
Amblystegium fluviatile
Amblystegium tenax III
Amblystegium humile
Amblystegium tenax I
Amblystegium varium
Amblystegium serpens
Cratoneuropsis relaxa
Drepanocladus aduncus
Drepanocladus sendtneri
Drepanocladus sordidus
Pseudo-calliergon turgescens
Pseudo-calliergon trifarium
Leptodictyum riparium
Campylium stellatum
Campyliadelphus chrysophyllus
Serpoleskea confervoides
Anacamptodon splachnoides
Hygrohypnum luridum
Cratoneuron filicinum
Cratoneuron filicinum var. atrovirens
Palustriella commutata
Thuidium delicatulum
Anomodon attenuatus

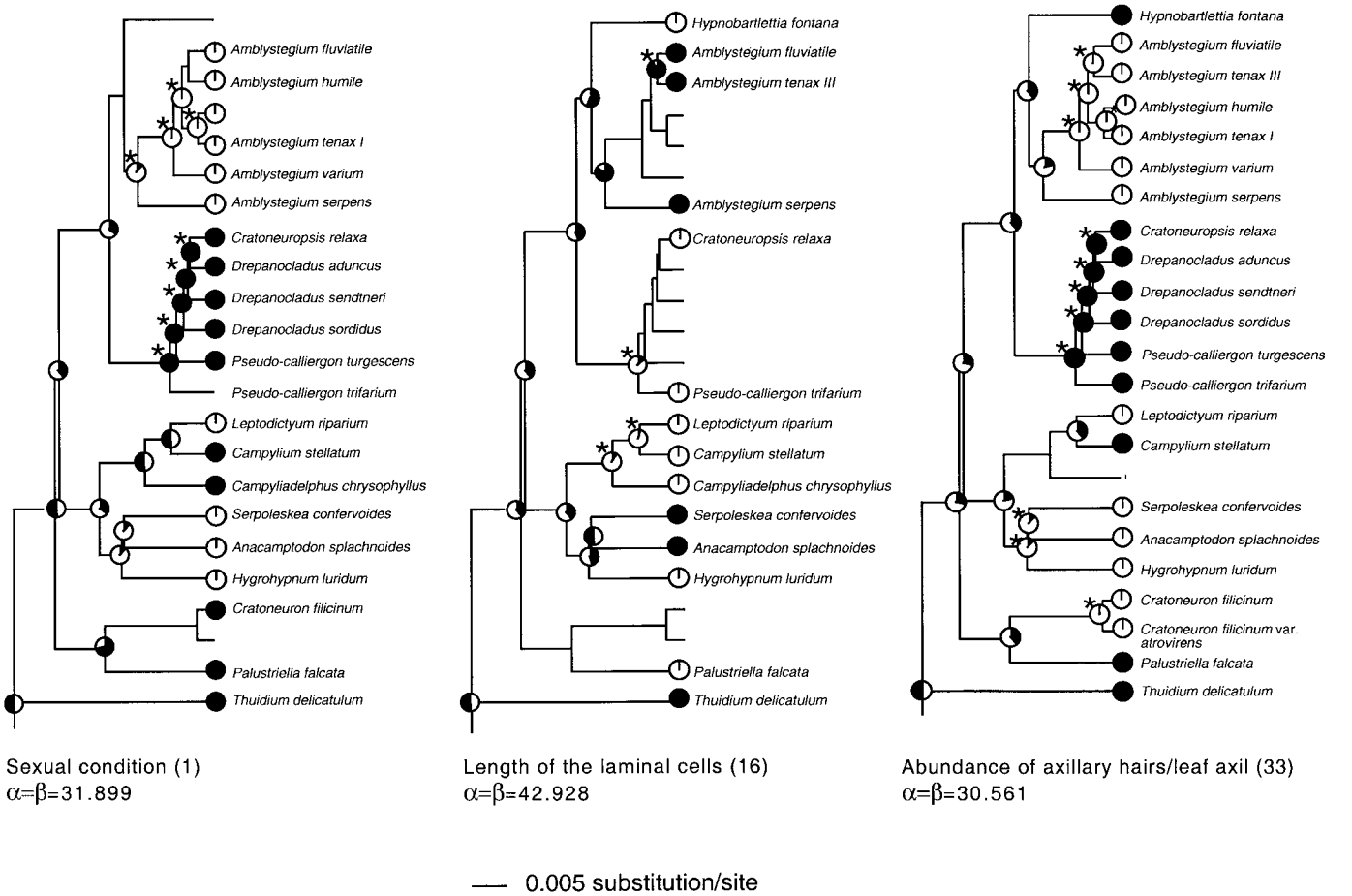


FIG. 7. ML ancestral character-state reconstruction of morphological characters on one of the eight most parsimonious trees resulting from the analysis of 22 taxa of the Amblystegiaceae using *Anomodon* and *Thuidium* as outgroups, with branch lengths calculated via ML and equal forward and backward transition rates (see text for details concerning the substitution models). Pie diagrams indicate the relative degrees of support for alternative character-states (0 in white and 1 in black), with a ratio of at least 7:1 considered significant (*).

DISCUSSION

The results of the present study are incompatible with a monophyletic interpretation of the Amblystegiaceae as traditionally circumscribed by Roth (1899), Fleicher (1920), (Kanda 1975, 1976), and Vitt (1984) and clearly indicate, together with recent morphological phylogenetic analyses (Hedenäs, 1998a), that the Amblystegiaceae are polyphyletic. The analyses presented in this paper provided two well-supported clades that include taxa traditionally included in the Amblystegiaceae. The Amblystegiaceae *s. str.* include *Leptodictyum, Campylium, Campylophyllum, Cratoneuron, Palustriella, Drepanocladus s. str., Hypnobartlettia, Cratoneuropsis, Hygrohypnum luridum, Serpoleskea, Anacamptodon, Pseudocalliergon,* and, tentatively, *Gradsteinia andicola.* The Calliergonaceae stat. et comb. nov. include the *Calliergon–Warnstorfia* complex, *Hamatocaulis, Scorpidium,* and *Hygrohypnum ochraceum.*

The taxonomic implications of these analyses and the presentation of a new system of classification of the family with the appropriate nomenclatural changes are presented in a separate paper (A. Vanderpoorten *et al.*, 2002).

Reconstruction of ancestral character-states. Exploration of morphological character evolution indicates that the interpretation of ancestral character-states is more complicated than parsimony analyses might suggest. ML ancestral character-state reconstructions were identical to those obtained using MP most often when character-state changes were estimated to be rare. The different reconstructions can be attributed to the assumptions used by the two methods, with MP minimizing the total number of character-state changes and ML taking branch length into account and thus favoring changes along long branches (Schluter *et al.*, 1997; Cunningham, 1999). For example, the ancestral mating system condition in the Amblystegiaceae level under MP is dioicous; hence a

TABLE 2

Significant Log-Likelihood Ratio Tests of Correlations between the Morphological Characters and the Occurrence of Species in Epiphytic, Rheophilous, and Swampy Habitats

Characters	Correlations
Swampy habitats	
Sexual condition	2.951
Mid-leaf cells length	4.222
Inflation of the alar cells	12.490
Pseudoparaphyllium shape	5.393
Differentiation of outer layer of stem cells	5.166
Capsule stance	5.446
Lid shape	4.190
Ornamentation of exostome	3.478
Height of basal membrane of endostome	4.373
Endostome cilia length	3.911
Epiphytic habitats	
Sexual condition	3.125
Leaf stance	3.149
Mid-leaf cells length	4.064
Porosity of the basal laminal cells	3.679
Inflation of the alar cells	5.182
Leaf decurrence	2.640
Pseudoparaphyllium shape	4.056
Plication of inner perichaetial leaves	2.900
Capsule stance	6.776
Lid shape	6.202
Exostome specialization	6.570
Height of basal membrane of endostome	5.261
Endostome cilia length	7.284
Rheophilous habitats	
Thickness of leaf lamina	3.286
Axillary hairs insertion	3.005

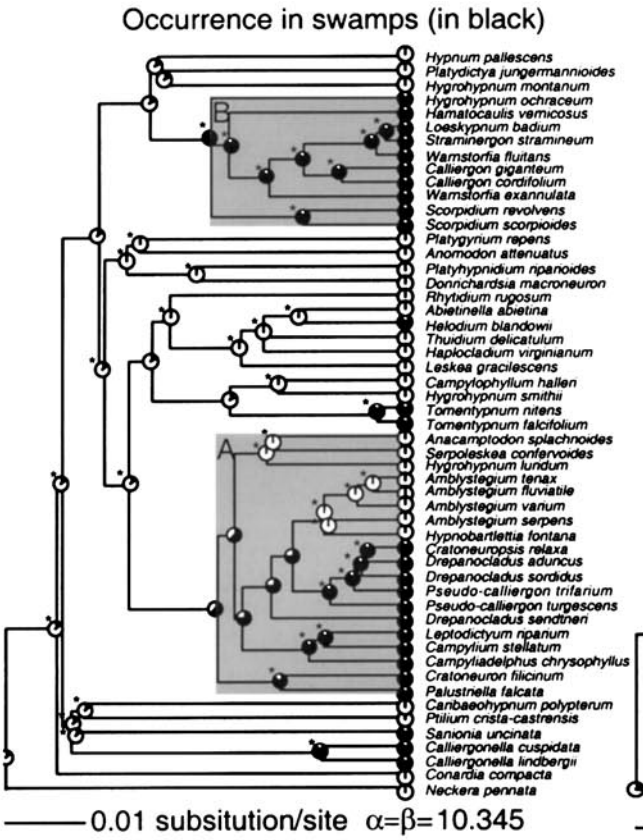
monoicous condition is synapomorphic for *Amblystegium*. Under ML, the plesiomorphic state is monoicous, and a dioicous condition is synapomorphic for the *Drepanocladus–Pseudo-calliergon* and *Cratoneuron–Palustriella* complexes because more time is available for change along the branches leading to both complexes than to the *Amblystegium* complex. ML ancestral character-state estimates for most internal nodes were ambiguous and approached significance only at a few near-terminal nodes. This observation can be interpreted as evidence of a high rate of evolution in particular characters, such that character-state transformations could occur along any branch (Cunningham, 1999).

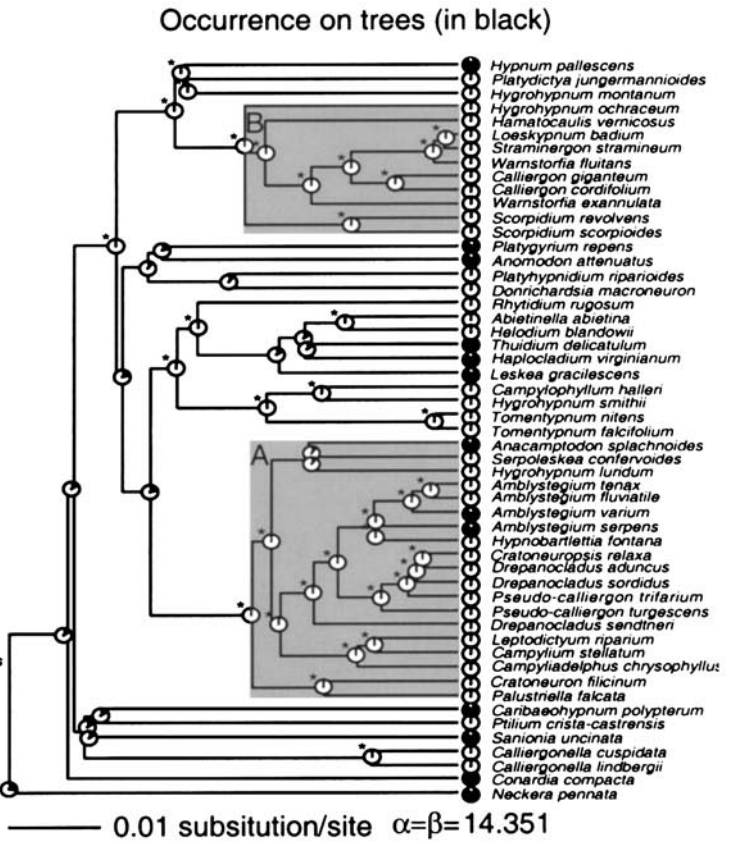
The inclusion of *Anacamptodon* in the Amblystegiaceae, supported by recent analyses of relationships among pleurocarps using cpDNA genes (Buck *et al.*, 2000b), indicates that sporophytic traits are more labile in the family than previously thought. Indeed, the straight capsule, rostrate lid, reflexed peristome teeth when dry, and low endostome membrane of the taxon strongly contrast with the long, arcuate, cylindrical capsules with a conical lid, erect peristome teeth, and high endostome membrane of the other family members. Buck and Crum (1990) and Buck (1991) observed similar suites of character-states in various epiphytic lineages of pleurocarpous mosses. The large order of pleurocarpous mosses, Leucodontales, defined essentially by sporophyte characters that are associated with epiphytic habitats, has been shown to be polyphyletic and has recently been abandoned (Buck *et al.*, 2000a). Similarly, within the Hookeriales, Whittemore and Allen (1989), Tan and Robinson (1990), and Hedenäs (1997b) have interpreted peristome specializations associated with epiphytism as convergences. Among pleurocarps, it is possible that characters related to sporophyte specializations are among the most homoplastic and possibly correlated with habitat conditions (Buck, 1991).

Habitat radiation and morphological divergence. The approach adopted here seeks repeated, phylogenetically independent examples of support for the same character-state transitions that are correlated with changes of habitat. However, this comparative method does not allow separation of true adaptation from correlated evolution resulting from other factors such as pleiotropy or genetic linkage. Evolution in a particular morphological character that is not actually adaptive may be correlated with habitat shifts because it is linked with adaptive characters. This approach does, however, allow predictions about the evolution of morphological traits as a function of habitat conditions (Martins, 2000).

The ancestral ecological condition of the Amblystegiaceae and Calliergonaceae is occurrence in swampy habitats. Morphological features correlated with a transition to a swampy habitat in the ancestors of modern Amblystegiaceae and Calliergonaceae (outgroups are all terrestrial) include dioicism, elongate mid-leaf cells, inflated alar cells, broad pseudoparaphyllia, arcuate capsules, conical lids, high basal endostomial membrane, and long cilia. Experiments by Zastrow (1934) demonstrated that enlarged, thin-walled alar cells were directly induced by habitat and that the indistinct alar cells of *Calliergon giganteum* and *C. cordifolium* became inflated when grown on humid soil (analogous to swampy conditions in this study). One possible explanation of the observed enlargement of alar cells would be to permit rapid water uptake at the leaf base; they therefore should be expected not in an obligate limnophilous species but in emergent plants (Vitt and Glime, 1984).

Several derived taxa of Amblystegiaceae have not retained the ancestral “marsh” phenotype as they colonized alternative habitats. As a consequence, the family displays substantial morphological heterogeneity. For example, the morphologies of *Anacamptodon splachnoides, Serpoleskea confervoides,* and *Hygrohypnum luridum* contrast with those typically encountered in taxa of swampy habitats. *Hygrohypnum luri-*


Occurrence in swamps (in black)
Hypnum pallescens
Platydictya jungermannioides
Hygrohypnum montanum
Hygrohypnum ochraceum
Hamatocaulis vernicosus
Loeskypnum badium
Straminergon stramineum
Warnstorfia fluitans
Calliergon giganteum
Calliergon cordifolium
Warnstorfia exannulata
Scorpidium revolvens
Scorpidium scorpioides
Platygyrium repens
Anomodon attenuatus
Platyhypnidium riparioides
Donnichardsia macroneuron
Rhytidium rugosum
Abietinella abietina
Helodium blandowii
Thuidium delicatulum
Haplocladium virginianum
Leskea gracilescens
Campylophyllum halleri
Hygrohypnum smithii
Tomentypnum nitens
Tomentypnum falcifolium
Anacamptodon splachnoides
Serpoleskea confervoides
Hygrohypnum lundum
Amblystegium tenax
Amblystegium fluviatile
Amblystegium varium
Amblystegium serpens
Hypnobartlettia fontana
Cratoneuropsis relaxa
Drepanocladus aduncus
Drepanocladus sordidus
Pseudo-calliergon trifarium
Pseudo-calliergon turgescens
Drepanocladus sendtneri
Leptodictyum riparium
Campylium stellatum
Campyliadelphus chrysophyllus
Cratoneuron filicinum
Palustriella falcata
Caribaeohypnum polypterum
Ptilium crista-castrensis
Sanionia uncinata
Calliergonella cuspidata
Calliergonella lindbergii
Conardia compacta
Neckera pennata
A
B
0.01 subsitution/site α=β=10.345


Occurrence on trees (in black)
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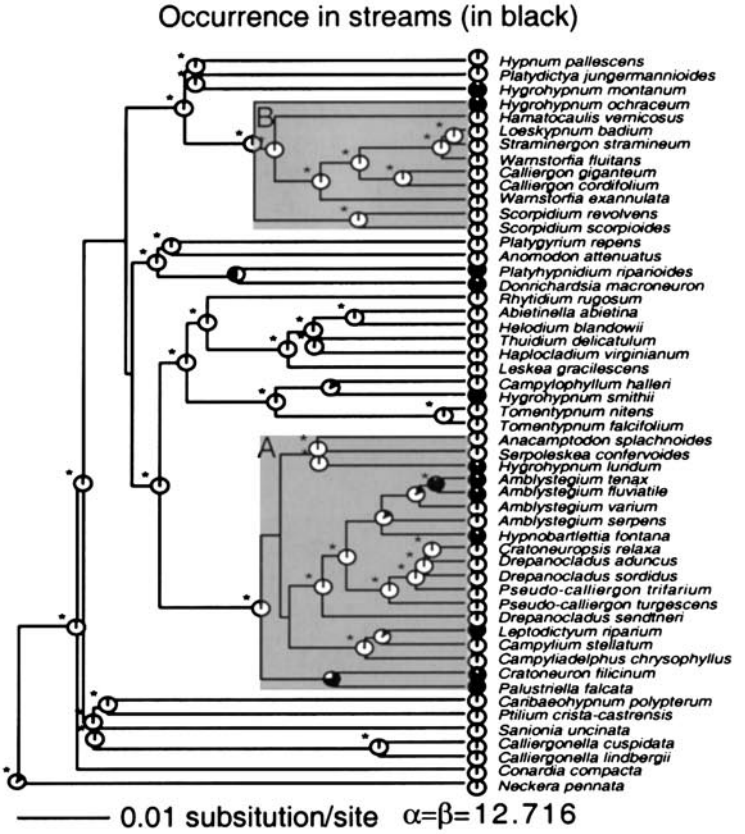
Platydictya jungermannioides
Hygrohypnum montanum
Hygrohypnum ochraceum
Hamatocaulis vernicosus
Loeskypnum badium
Straminergon stramineum
Warnstorfia fluitans
Calliergon giganteum
Calliergon cordifolium
Warnstorfia exannulata
Scorpidium revolvens
Scorpidium scorpioides
Platygyrium repens
Anomodon attenuatus
Platyhypnidium riparioides
Donnichardsia macroneuron
Rhytidium rugosum
Abietinella abietina
Helodium blandowii
Thuidium delicatulum
Haplocladium virginianum
Leskea gracilescens
Campylophyllum halleri
Hygrohypnum smithii
Tomentypnum nitens
Tomentypnum falcifolium
Anacamptodon splachnoides
Serpoleskea confervoides
Hygrohypnum lundum
Amblystegium tenax
Amblystegium fluviatile
Amblystegium varium
Amblystegium serpens
Hypnobartlettia fontana
Cratoneuropsis relaxa
Drepanocladus aduncus
Drepanocladus sordidus
Pseudo-calliergon trifarium
Pseudo-calliergon turgescens
Drepanocladus sendtneri
Leptodictyum riparium
Campylium stellatum
Campyliadelphus chrysophyllus
Cratoneuron filicinum
Palustriella falcata
Caribaeohypnum polypterum
Ptilium crista-castrensis
Sanionia uncinata
Calliergonella cuspidata
Calliergonella lindbergii
Conardia compacta
Neckera pennata
A
B
0.01 subsitution/site α=β=14.351


Occurrence in streams (in black)
Hypnum pallescens
Platydictya jungermannioides
Hygrohypnum montanum
Hygrohypnum ochraceum
Hamatocaulis vernicosus
Loeskypnum badium
Straminergon stramineum
Warnstorfia fluitans
Calliergon giganteum
Calliergon cordifolium
Warnstorfia exannulata
Scorpidium revolvens
Scorpidium scorpioides
Platygyrium repens
Anomodon attenuatus
Platyhypnidium riparioides
Donnichardsia macroneuron
Rhytidium rugosum
Abietinella abietina
Helodium blandowii
Thuidium delicatulum
Haplocladium virginianum
Leskea gracilescens
Campylophyllum halleri
Hygrohypnum smithii
Tomentypnum nitens
Tomentypnum falcifolium
Anacamptodon splachnoides
Serpoleskea confervoides
Hygrohypnum lundum
Amblystegium tenax
Amblystegium fluviatile
Amblystegium varium
Amblystegium serpens
Hypnobartlettia fontana
Cratoneuropsis relaxa
Drepanocladus aduncus
Drepanocladus sordidus
Pseudo-calliergon trifarium
Pseudo-calliergon turgescens
Drepanocladus sendtneri
Leptodictyum riparium
Campylium stellatum
Campyliadelphus chrysophyllus
Cratoneuron filicinum
Palustriella falcata
Caribaeohypnum polypterum
Ptilium crista-castrensis
Sanionia uncinata
Calliergonella cuspidata
Calliergonella lindbergii
Conardia compacta
Neckera pennata
A
B
0.01 subsitution/site α=β=12.716

dum has retained some gametophyte features from its swamp-dwelling ancestor, including elongate laminal cells, inflated alar cells, and broad paraphyllia, despite its rheophilous preferences. The retention of these characters may be due to "phylogenetic time-lag," that is, by the retention of traits in the descendant before they are eliminated by selection or other processes (Proctor, 1984; Thiers, 1988).

Anacamptodon splachnoides has the most atypical phenotype of the Amblystegiaceae *s. str.* Gametophytically, the species resembles a large *Amblystegium* (Crum and Anderson, 1981), whereas sporophytically it is a typical epiphytic species with straight capsules, reflexed peristome teeth when dry, low endostomial membrane, and a rostrate lid. Therefore, *Anacamptodon* has previously been classified in Hypnalean families such as the Fabroniaceae (Crum and Anderson, 1981). The atypical phenotypic traits of *Anacamptodon* for the Amblystegiaceae are precisely those whose evolution are strongly correlated with habitat. Similar examples of diverging suites of gametophytic and sporophytic characters have been documented in bryophytes (Buck, 1991), and there can be little doubt that to a large extent gametophytes and sporophytes can evolve independently (Rohrer, 1988; Shaw and Beer, 1997). Different evolutionary pressures act upon each generation because each has different roles in the life of the organism. For instance, particular gametophyte features of rheophilous mosses include thick leaf laminae, strong costae, and rhizoids (Vitt and Glime, 1984), yet sporophytically these mosses are assumed to have retained the features of their terrestrial ancestors (Vitt, 1981). These species produce sporophytes above the water level; hence the gametophyte is mostly submersed, whereas the sporophyte is mostly emergent. Such evolutionary trends also exist in aquatic angiosperms, which have flowers pollinated by wind or insects above the water surface, with aquatic dispersal of pollen only in some highly derived genera (Cox, 1988; Cox and Knox, 1988; Les, 1988).

CONCLUSIONS

Sporophyte features, which are most often given primacy over gametophyte traits in moss classification (e.g., Fleischer, 1904–1923; Brotherus, 1925; Vitt, 1984), are more labile in pleurocarpous taxa than previously thought, with many sporophytic specializations correlated with habitat. However, the evolution of a number of gametophyte features such as presence–absence of a costa, cell shape, stem anatomy, and axillary hair morphology, thought to be "reliable when speculating on relationships at the family level" (Buck, 1991, p. 181), are also strongly correlated with habitat. The use of these characters for taxonomic circumscription has consequently led to the delineation of polyphyletic families such as the Donrichardsiaceae based on polystratose laminae (Ochyra, 1985), the Campyliaceae based on elongate laminal cells, differentiated alar cells, and mainly single costa (Buck *et al.*, 2000b; Buck and Goffinet, 2000), and the Cratoneuraceae based on the presence of foliose paraphyllia and differentiated alar cells (Ochyra, 1989; Buck and Goffinet, 2000).

The instability of the taxonomic circumscription of the Amblystegiaceae in phylogenetic analyses based on morphology can be explained by the evolutionary lability of characters correlated with habitat (contrast, e.g., Crum and Anderson, 1981; Ochyra *et al.*, 1991; Hedenäs, 1995; Buck and Goffinet, 2000). Indeed, we were not able to detect any single morphological synapomorphy defining the recircumscribed Amblystegiaceae *s. str.* In addition to their taxonomic implications, correlations between morphological characters and habitats demonstrated in this paper suggest functional hypotheses about traits that can be tested in an experimental context.

APPENDIX 1

Morphological Characters

1. Species monoicous (0) or dioicous (1). *Conardia compacta*, which may be both monoicous and dioicous, was coded as polymorphic.
2. Plants distichously (0) or radially (1) branched.
3. Plants without (0) or with (1) secondary red coloration.
4. Stem leaves varying around ovate, oblong, triangular, or lanceolate (0) or broadly cordate to broadly triangular (1).
5. Stem leaves smooth (0) or longitudinally plicate (1). The second state is presumably due to differences in width between the ad- and abaxial sides of the leaf lamina cells. If along the transverse axis of the leaf there is an alternation between cells having wider adthan abaxial sides and wider ab- than adaxial sides, respectively, the result should be longitudinally plicate leaves. (Transversely undulate leaves occur only in two of the outgroup species.)

FIG. 8. ML ancestral character-state reconstruction of ecological character-states on one of the five most parsimonious trees resulting from the analysis of 54 taxa of the Hypnales using *Neckera* spp. as outgroups, with branch lengths calculated via ML and equal forward and backward transition rates (see text for details concerning the substitution models). Pie diagrams indicate the relative degrees of support for alternative character-states (0 in white and 1 in black), with a ratio of at least 7:1 considered significant (*). A, Amblystegiaceae *s. str.*; B, Calliergonaceae.

6. Stem leaves when moist ± evenly arranged around the stem (0) or mostly distinctly complanate to subcomplanate, at least in their basal portion (1).

7. Stem leaves when moist straight (0), at least sometimes distinctly falcate-secund (1), or ± squarrose (2). Most species that have distinctly falcate-secund leaves can sometimes have straight leaves. Campyliadelphus chrysophyllus, which can have all states of this character, was coded as polymorphic.

8. Curved leaves evenly curved ± throughout their length (0) or from a straight or much less curved basal part suddenly curved in their upper part (1). State 1 is described in more detail in Hedenäs (1989a).

9. Stem leaf costa short, reaching <1/3 way up leaf and double, sometimes single, or absent (0) in at least some leaves ending more than 1/3 way up leaf and double, or sometimes forked shortly above insertion (1), or single and long (2). The difficulties of classifying the leaf costas of pleurocarpous mosses into different states were discussed by Hedenäs (1995). Within the present group of species, the interpretation of long costas that are forked shortly above the leaf base caused the greatest problems. Because every state between double and forked shortly above the insertion seem to occur, these are here interpreted as double and long costas (1).

10. Stem leaf costa when single and long always ending below leaf apex (0) or sometimes or always percurrent to excurrent (1). Species with double and/or short costas were coded as unknown for this character.

11. Stem leaf costa on back smooth (0), with prorate cell ends (1) or dentate (2).

12. Lower stem leaf margin and/or costa on back without (0) or (at least sometimes) with paraphyllia (1). In species lacking paraphyllia, this character was coded as unknown.

13. Stem leaves without (0) or with (1) a limbidium of 2–8 layers of cells that are usually slightly longer than the lamina cells further in.

14. Stem leaf margin in upper part entire to denticulate (0) or strongly denticulate to dentate (1).

15. Stem leaf margin plane or occasionally narrowly inflexed, at most narrowly recurved close to the insertion (0) or in most studied leaves recurved in at least lower 1/3 (1). In *Helodium blandowii,* the margin is often plane near the insertion, but frequently recurved almost throughout the rest of the margin. It is interpreted as belonging to state 1.

16. Mid-leaf cells of stem leaves elongate (0) or short (1). Cell elongation was estimated by dividing the maximum cell length with the minimum cell width, and the minimum cell length with the maximum cell width (exceptional deviating cells excluded). To be considered elongate the maximum length/minimum width ratio should exceed 10 and the minimum length/maximum width ratio should exceed 2. If both ratios are lower, the cells are considered short, and when one ratio fits the definition of short and the other elongate cells, the species was coded as variable. Although this may not completely reflect the distribution of cell elongation, because, for example, the longest cells may, e.g., not always include the narrowest ones, it is here taken as a reasonable estimate.

17. Mid-leaf cells of stem leaves narrow (0) or wide (1). Cells were considered wide when the narrowest cells were ≥6 μm wide and the widest cells were ≥10 μm wide. If only one of these limits was exceeded, the species was coded as variable.

18. Cells in the central and upper parts of the stem leaves without mammillae (0) or with mammillae on both sides (1). If only some cells were mammillose, the cells were interpreted as having mammillae.

19. Leaf lamina, except possible limbidium, unistratose (0) or at least bi- to multistratose (1).

20. Cells in the central and upper parts of the stem leaves without papillae (0) or with papillose on both sides (1). Projections were considered papillae either when they were clear projections from a plane wall (i.e., not bulging, as seen in transverse section) or if the projections were situated on mammillae when they were not only gradually more strongly thickened central wall portions.

21. Cells in the central and upper parts of the stem leaf lamina not prorate (0) or distally prorate (1). The special wall thickenings in the cell ends of Donrichardsia (Crum and Anderson, 1979: cf. their Fig. 2 where the upper leaf lamina cells are shown in scanning electron microscope photos) are not interpreted as homologous with prorate cells (where the entire cell end projects).

22. Basal lamina cells of stem leaves about as long as or shorter than median lamina cells (0) or longer than median lamina cells (1).

23. Basal lamina cells of stem leaves eporose (0) or porose (1). The absence or presence of pores in the walls of the leaf cells is a somewhat ambiguous character, because pores are much more easily seen in specimens or species with thick cell walls than in those with thin walls. Hence, in the mid-leaf cells it is often difficult to judge whether pores are present. The cells which are usually most strongly incrassate (if the cells are incrassate at all) are those in the basal part of the leaves, and for this reason the degree of porosity was judged in the basal cells. When the cells were only slightly and indistinctly porose or when they were sometimes eporose and sometimes slightly porose, they were classified as variable.

24. Alar group consisting of few (1–10) strongly differentiated cells (0), cells numerous and grouped triangular to transversely triangular (1), cells numerous

and grouped isodiametric, quadrate, ovate, or rectangular and extending shortly up along basal leaf margin (2), or cells numerous and grouped ovate, elongate ovate, or elongate rectangular (rarely broadly triangular) and extending up along leaf margin at least 10% of leaf length (3). When the alar cells were not or were very indistinctly and diffusely differentiated from the rest of the basal leaf cells this and the following three characters were coded as unknown.

25. Entire alar group consisting of noninflated, transversely rectangular, quadrate, shortly rectangular or near insertion, rectangular to shortly linear cells (0) or basal alar cells inflated, linear to shortly rectangular, rarely with a few cells quadrate (1) (cf. character 25).

26. Basal alar cells slightly to strongly incrassate (0) or thin-walled (1) (cf. character 25).

27. Alar cell ontogeny of "aduncus-kind" (0) or of "*exannulata*-kind" (Hedenäs, 1987a) (1).

28. Leaves not or hardly (with <4 small cells) decurrent (0) or decurrent (1).

29. Branch leaves similar to stem leaves or smaller and/or narrower (0) or markedly different in both shape and size (1).

30. Pseudoparaphyllia absent (0), present, at least some of the outer ones filamentose to narrowly triangular or narrowly ovate (1), or present, all pseudoparaphyllia broader, triangular or ovate to suborbicular, often very irregular in outline (2). (See Hedenäs, 1995, for additional discussion.)

31. Paraphyllia absent (0) or linear-lanceolate or ovate paraphyllia present (at least in some specimens) (1). These kinds of paraphyllia are frequently inserted in transverse or oblique rows and usually consist of linear, smooth cells that are more or less tapering in their ends.

32. Paraphyllia absent (0), or uniseriate, or in basal part sometimes 2-3-(4)-seriate and frequently branched paraphyllia present (1). These kinds of paraphyllia are inserted on spread points all over the stem and usually consist of transversely rectangular, quadrate or rectangular, frequently papillose or prorate cells with square walls.

33. Axillary hairs at most 1–2 in each leaf axil (0) or with 3 or more (up to 12) hairs per axil (1). Species having 1–3 or 2–3 hairs per axil were interpreted as variable with regard to this character, whereas species having 2-4(-6) hairs per axil were considered to belong to state (1).

34. Axillary hairs ± strictly axillary or inserted on stem at most 0.5 cells above axil (0), some or all inserted on the stem 0.5–1.5 cells above axil (1), or some inserted on other places on stem, sometimes in scattered axils of paraphyllia (2).

35. Axillary hairs with 1 or 1–2 upper cells (0), or with 1–4, 1–5, 1–6, 1–7, or 2–several (up to 9) upper cells (1). See Hedenäs, 1990, for additional discussion. Species having 1–3 upper cells were coded as polymorphic.

36. Axillary hairs narrow (smallest width at most 7 μm, widest width at most 9 μm) (0) or wide (minimum width >7 μm, maximum width >9 μm) (1). If only one of these limits was exceeded, the species was coded as polymorphic.

37. Stem-borne rhizoids inserted predominantly at or just below leaf costa insertion (0), scattered on stem (1), or axillary (2). *Tomentypnum falcifolium* seems to have only leaf-borne rhizoids (cf. Vitt and Hamilton, 1975) and is coded as unknown for the present character.

38. Leaf-borne rhizoids inserted only at the most basal part of the abaxial costa, just above its insertion (0), or in addition high up on the leaf costa and/or lamina (1).

39. Rhizoids unbranched to moderately strongly branched (0) or ± strongly branched and often tomentum-forming (1). The degree of rhizoid branching was subjectively scored into the categories unbranched to slightly branched, moderately strongly branched, and strongly branched. Species with rhizoids that vary from unbranched to strongly branched were interpreted as polymorphic, whereas species with moderately strongly to strongly branched rhizoids were interpreted as being strongly branched.

40. Rhizoids smooth (0), at least sometimes warty-papillose (1), or granular-papillose (2). The last state occurs only in *Platydictya* among the included species.

41. Stem with (0) or without (1) central strand.

42. Outer layer of stem cells undifferentiated (0) or with a well differentiated hyalodermis of inflated and thin-walled cells (1). *Warnstorfia exannulata* has an epidermis that is transitional between a normal one and a hyalodermis, and this species was coded as variable.

43. Inner perichaetial leaves having straight and erect basal parts with patent to recurved upper portion (0) or entirely more or less straight and erect (sometimes flexuose to erecto-patent in upper portion) (1).

44. Inner perichaetial leaves smooth (0) or plicate (1).

45. Costa of inner perichaetial leaves double or single and short, reaching at most 45% way up leaf (0) or single and long (sometimes only in some leaves) (1). Since the appearance of the costa in the inner perichaetial leaves is often not the same as in the stem leaves, this is treated as a separate character.

46. Margin of inner perichaetial leaf acumen denticulate to entire (0) or dentate to strongly denticulate (1).

47. Margin of inner perichaetial leaf shoulder denticulate to entire (0), dentate to strongly denticulate (sometimes only with scattered, irregular teeth) (1), or at least sometimes with cilia present (2).

48. Upper lamina cells of perichaetial leaves smooth (0) or prorate on back (1).

49. Vaginula and sometimes lower calyptra with (0) or without (1) paraphyses.

50. Calyptra in upper part 2-4-stratose (0) or 5-11-stratose (1). This character was studied in the part of the calyptra which was not filled by the theca (in cucullate calyptras in the part above the split).

51. Capsule cylindrical and straight (0), cylindrical and curved (1), or ovate (2). The swollen capsules in *Calliergonella cuspidata* and *C. lindbergii* are interpreted as a special case of state 1. In species with erect capsules and specialized peristomes, it was considered impossible to hypothesize primary homology with the shapes of taxa with perfect peristomes.

52. Capsules when dry not constricted below mouth (0) or distinctly constricted (1).

53. Capsules (inclined to) horizontal or cernuous (0) or erect (to inclined) (1). This character relates to the orientation of the capsule, not its curvature.

54. Exothecial cells with ± evenly thickened walls (0) or distinctly collenchymatous (1).

55. Stomata round-pored (0) or long-pored (1).

56. Lid conical (0) or rostrate (1).

57. Annulus separating (0) or separating annulus absent (1). In some species, such as *Anomodon attenuatus, Hygrohypnum luridum,* and *Platygyrium repens,* one or a few rows of small cells are differentiated and sometimes partly irregularly separating. These cases probably represent cases intermediate between a developed separating annulus and the lack of such a structure, and the three species were coded as variable for this character.

58. Exostome unspecialized to slightly specialized (0) or strongly specialized (or lacking) (1). Specialized exostomes are usually narrower and/or shorter than unspecialized exostomes and, at least in more strongly specialized ones, commonly inserted below the rim of the capsule. The degree of specialization was judged by the number of characters which are still present in the exostomes. Slightly specialized exostomes still have most characters left, but one can observe, i.a., distinct differences in shape (narrow teeth) or in the development of the border (gets narrower or disappears) as compared with perfect exostomes. Strongly specialized exostomes have few characters (if any) that can still be homologized with those found in the unspecialized exostomes. Characters Nos. 59–62 could often not be judged in taxa with strongly specialized exostomes. Among the studied taxa, we have found no possibility to see with certainty whether the specializations in species with specialized exostomes are homologous. To include the degree of exostome specialization, but to avoid putting undue weight on specialized exostomes, these are treated as if having the same state (1) of the present character, but the different character losses are not treated individually in the analyses.

59. Exostome in lower half red, orange, orange-brown, or red-brown (i.e., with red or orange colors) (0), (pale yellow), yellow, yellow-brown, or brownish yellow (i.e., with yellow colors) (1), or whitish yellow (2). The character was observed in recently dehisced capsules and was studied in reflected light on dry material. It is common that the exostome teeth are paler in their upper part than closer to their base or that the pigment (or structure, if the color is structural) that is present in the tooth base is absent above. Thus, the color was judged in the lower part of the teeth.

60. Lower outside of exostome cross-striolate (0) or in large parts reticulate (appearing dotted when studied in the light microscope) (1).

61. Exostome margin entire to slightly dentate (0) or dentate (1). It was suggested to be of importance in separating some taxa within the Amblystegiaceae by Tuomikoski and Koponen (1979).

62. Exostome border not widened at the zone of transition in outer peristomial layer pattern (0) or widened (1).

63. Basal membrane of endostome high (35% or more of total endostome height in peristomes with developed processes) (0) or low (<35% of total endostome height) (1).

64. Endostome cilia well developed, the longest ones at least 75% as long as the processes (0), cilia short (25–74% as long as processes) (1) or vestigial to absent (at most 24% as long as processes) (2).

65. Cilia nodose (0) or at least some cilia appendiculate in their upper portions (1).

66. Average distance between horizontal walls of the primary peristomial layer of the endostome in basal membrane up to 8 μm (minimum value for the species) and 9 μm (maximum value) (0) or >8 μm and >9 μm, respectively (1). This measure is the average of the distances between the horizontal walls in the basal membrane (cf. Hedenäs, 1995: Fig. 3).

67. Spores small (smallest diameter <13.0 μm, largest diameter <20.0 μm) (0) or large (smallest diameter at least 13.0 μm, largest diameter at least 20.0 μm) (1).

68. Spore maturation time during winter half-year (0) or summer half-year (1). The problems with using spore maturation time as a character when dealing with a mixture of taxa from different climatic regions were pointed out by Hedenäs (1994). In the present analyses, we are only including the spore maturation time (when known) for taxa occurring in areas with more distinctly seasonal climates, i.e., mainly outside the tropical areas, because the seasonality is difficult to compare between tropical and more northern or southern areas.

APPENDIX 2

Matrix of the 68 Morphological Characters and 3 Ecological Characters Scored

			abc
Hypnum	*pallescens*	000000100.1.0010000010120001010000.00000001101000011.0.1001011000101	010
Platygyrium	*repens*	1000000.0.0.001010000012.00101000011000010.....00.01001.11.....00	010
Caribaeohypnum	*polypterum*	100010101.0.001000000012100.01001111....001.0010011.1100.0100.12.11.	010
Ptilium	*crista-castrensis*	100010101.0000.000000012110101101111..0000110000011100.00000.1000100	000
Platyhypnidium	*riparioides*	0000000.200.0000000000.200000200101100000000000000020000100000000000110	001
Donrichardsia	*macroneuron*	.000000.200.00000010001....002001001000000.....	001
Rhytidium	*rugosum*	10001010201.011010001013000101001011000000111100011100 1.001001001101	000
Abietinella	*abietina*	1001100.20110011.101010....1110110.1000000111120010001010000110001.0	000
Anomodon	*attenuatus*	10000.0.201.00011001011300.1020010110000100010 0.00.01101.1.0...2.000	010
Thuidium	*delicatulum*	1001100.20110011.101010....1110110.1000000111120010001010000110001.0	010
Helodium	*blandowii*	0000100.2021001..10111.....101.112110010101110110.110010001011000001	100
Haplocladium	*virginianum*	0001000.201101010101010300.11101.01.00.100111010011100110010..000.01	010
Leskea	*gracilescens*	0000100.20000011.100010300.1021010.10000001010000..0101001.0..12.10.	010
Campylophyllum	*halleri*	000..02.0.000010000010120001010.01.00010011.0000.1100100010.1000001	000
Tomentypnum	*nitens*	1000100.200.00000000001....0010010110110001110000 11.0010001101000111	100
Tomentypnum	*falcifolium*	10001010200.00000000001....00100.011.110001110000.1.00100011010001.1	100
Hygrohypnum	*smithii*	0000000.200.0000.00000.2.0010100101100000001110000.11001000100.010011	001
Platydictya	*jungermannioides*	10000.0.0.0.0001.000001....0000000002.0210100110000.1010002000110001	000
Sanionia	*uncinata*	00001010200.000000001011110002001011000001111001 0.110010001011000101	110
Hygrohypnum	*ochraceum*	100000101.0.000000000012110102000011000001111010 0.110010001001.00001	101
Hygrohypnum	*montanum*	000000101.3.000000001012000101000 01.....10111111001100100010010100 01	001
Conardia	*compacta*	.00000102.0.0000.000001100.10100.0110111001010000011.01.00110.110.11	010
Neckera	*douglasii*	1000010.0.00010.000000120000020012110000 10.000000..01001111...12.110	010
Neckera	*pennata*	0000010.0.00000..000001200010110111100001000000000.010.11110..12..10	010
Calliergonella	*cuspidata*	1000010.0.0.000000000011110102001111000001110000011100100101001111	100
Calliergonella	*lindbergii*	100001100.0.000000000011110002001010000001110000011100100010110011101	100
Hamatocaulis	*vernicosus*	10101011200.00000000001....00200.011000010111000011100100011010001.1	100
Loeskhypnum	*badium*	1.000010200.000010000011001002000 0.111000010101010110010101111000111	100
Straminergon	*stramineum*	1.00000.200.0000.00000121.110200000011000010111 01.1100101011110001.1	100
Calliergon	*giganteum*	11..000.200.000010000011111102001111100001010001.1100101011.1000111	100
Calliergon	*cordifolium*	0100000.200.00001000001111110200.1111100001010101.1100101011110001.1	100
Warnstorfia	*fluitans*	01100010200.0000.000001111100200001111000010 10001.110010101111000111	100
Warnstorfia	*exannulata*	11100010200.0000000000111.100200.0.111000.1010101.1.0010101111000.11	100
Scorpidium	*revolvens*	00100011200.00000000001011.00200101100000111100 00.110010001101000111	100
Scorpidium	*scorpioides*	101000111-0-0000.000001011-00200101100000111100 00.110010001101000..1	100
Anacamptodon	*splachnoides*	0000000.200.000110000001000001000001000000101000 01..1011111...12..01	000
Hygrohypnum	*luridum*	00000010.00.000000000012000002000011000000111000011100 10.01011000011	001
Amblystegium	*tenax I*	0000000.2100000.1000000200000210000000000001110000.110010001011000.11	001
Amblystegium	*tenax II*	.000000.210000011000000 2001.01100000000000.....	001
Amblystegium	*tenax III*	000000.210000021000000 2001.01100000000000.....	001
Amblystegium	*fluviatile*	0000000.200000011000000200000210000000000001110000.110.1000100100.011	001
Amblystegium	*varium*	0000000.2000000.1000001200010110000000000001110000.110010001011000101	010
Amblysetgium	*serpens*	0000000.200.000110000001000001000000000000011100000110010010.1001.01	010
Amblystegium	*humile*	0000000.200.000.1000000..100021000000000.01110100.1100100010.1000001	100
Hypnobartlettia	*fontana*	000000.21000000.010000....001101011....00.....	001
Cratoneuropsis	*relaxa*	1000002.200000000000101100.0110100.00.1001110200.1101100010.1001101	100
Drepanocladus	*aduncus*	10000010200.000..000001111000200100100000011 0000.110010001001001001	100
Drepanocladus	*sordidus*	10000010200-000..00000.11.0002001101----001110000111001000100 1001-01	100
Leptodictyum	*riparium*	0000010.200.0000100000110.00010000110000001.10000.110010001001001101	100
Campyliadelphus	*chrysophyllus*	100000.02000000000000012.0010200.01100.000111010011100100010011001.01	101
Campylium	*stellatum*	100.002.0.0.0000000000121.000100101.00.0001100000.110010001011001101	100
Pseudo-calliergon	*turgescens*	1000000.0.0.000.1000001110000200100 1....00111010011100100010010000 01	100
Pseudo-calliergon	*trifarium*	1.00000.200.0000.000001110010200100100000011100001110010001001000.01	100
Drepanocladus	*sendtneri*	10000010200.000..00000111.000200110100000001110000.110010001001001.01	100
Serpoleskea	*confervoides*	0000000.0.0.0001000000.200000200000000000101.000000110010001001000001	000
Cratoneuron	*filicinum*	100.00102100000..00000.1111101100000000100011101 00.110010001001000111	100
Cratoneuron	*filicinum* var. *atrovirens*	000000.2100000.10000001111.02100000000000.....	001
Palustriella	*falcata*	1000101020000.00000010111111021010.100110011102001110110001011000111	101

Note. See Appendix 1 for the labels of the morphological characters; a, swamps; b, epiphytic; c, running waters; ., missing data or variable character.

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