

## Molecular phylogeny of hypnobryalean mosses as inferred from a large-scale dataset of chloroplast *rbcL*, with special reference to the Hypnaceae and possibly related families<sup>1</sup>

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Phylogenetic relationships within the hypnobryalean mosses (i.e., the Hypnales, Leucodontales, and Hookeriales) have been the focus of much attention in recent years. Here we present phylogenetic inferences on this large clade, and especially on the Hypnaceae and possibly related families, based on maximum likelihood analysis of 181 *rbcL* sequences. Our study corroborates that (1) the Hypnales (s. str. [= sensu Vitt 1984]) and Leucodontales are each not monophyletic entities. The Hypnales and Leucodontales together comprise a well supported sister clade to the Hookeriales; (2) the Sematophyllaceae (s. lat. [= sensu Tsubota et al. 2000, 2001a, b]) and Plagiotheciaceae (s. str. [= sensu present]) are each resolved as monophyletic groups, while no particular clade accommodates all members of the Hypnaceae and Cryphaeaceae; and (3) the Hypnaceae as well as its type genus *Hypnum* itself were polyphyletic. These results do not concur with the systems of Vitt (1984) and Buck and Vitt (1986), who suggested that the groups with a single costa would have diverged from the hypnalean ancestor at an early evolutionary stage, followed by the groups with a double costa (see also Tsubota et al. 1999; Buck et al. 2000). Our results from likelihood analyses of a large *rbcL* data set confirm the suggestion by smaller data sets from multiple loci first by De Luna et al. (2000) and then Buck et al. (2000) that there is no evidence for supporting the Hypnales (s. str. [= sensu Vitt 1984]) and Leucodontales. A detailed reconsideration of ordinal classification and familial classification within the Hypnidae is now necessary.

Key Words: Hypnales, Leucodontales, Hookeriales, hypnobryalean mosses, molecular phylogeny, *rbcL*, large data set, Hypnaceae, Sematophyllaceae, Plagiotheciaceae, Amblystegiaceae

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## Introduction

The hypnobryalean mosses, including the orders Hypnales, Leucodontales and Hookeriales, are one of the most diversified moss groups. Attention has been paid to the classification of the hypnobryalean mosses since Vitt (1984) provided a new system for the Bryopsida. Within the pleurocarps he suggested that "a double costa group" as represented by the Hypnaceae, Sematophyllaceae and Entodontaceae, is divergent from a group with a single costa, which can be regarded as the most recent evolutionary stage of the order Hypnales (see also Tsubota et al. 1999; Buck et al. 2000). Subsequently, Buck and Vitt (1986) constructed another classification for hypnobryalean mosses based on morphological and ecological data.

In recent years, phylogenetic relationships within the hypnobryalean mosses have been the focus of much attention at several taxonomic levels (Arikawa & Higuchi 1999, 2002; Buck et al. 2000; De Luna & coworkers 1999, 2000; Maeda et al. 2000; Tsubota & coworkers 1999, 2000, 2001a, b; Vanderpoorten et al. 2002). The relationships within the hypnobryalean mosses have been evaluated by both morphology and molecular studies (Hedenäs 1995; De Luna et al. 1999). Hedenäs (1995, 1996) showed the monophyly of the Hookeriales–Sematophyllaceae and discovered the Leucodontales as a grade taxon based on morphological data. De Luna et al. (1999) clarified the homology of pleurocarpy and the monophyly of all hypnobryalean pleurocarpous mosses (the Leucodontales, Hypnales and Hookeriales), based on a combined *rbcL* sequences and morphology data set. Further details were recently revealed by De Luna et al. (2000) and Buck et al. (2000) who proposed new phylogenetic relationships within these pleurocarps. These works suggested that the Leucodontales and Hypnales as previously understood are not monophyletic. Families from these two orders are mixed in a single large clade sister to the hookerialean lineage.

The objectives of this study are: 1) to construct a phylogenetic tree for hypnobryalean mosses based on large-scale data set; 2) to test the monophyly of the three orders (Hypnales, Leucodon-

tales and Hookeriales) for corroboration of the phylogenetic hypothesis of De Luna et al. (2000) and Buck et al. (2000); 3) to clarify the familial relationships, especially between the Hypnaceae and related families, such as the Sematophyllaceae and Plagiotheciaceae, within the hypnobryalean mosses, given that the Leucodontales and Hypnales are not monophyletic; and 4) to trace in the phylogeny the transitions of the costa from single to double, one of the important characters for the familial classification of the hypnobryalean mosses.

Recent, molecular phylogenetic analyses for pleurocarpous mosses were based on *rbcL* sequences along with other data sets, such as *atpB*, *trnL-F* and *rps4*. At the present time, the resources of *rbcL* sequences are the most comprehensive sampling for the hypnalean mosses. Alignment of the *rbcL* sequences is also easy because of their distinction as a protein-coding region in the codon positions. So, we present here phylogenetic inferences for the three orders Hypnales, Leucodontales and Hookeriales, based on the maximum-likelihood (ML) analyses of 181 sequences of the chloroplast ribulose 1,5-bisphosphate carboxylase/oxygenase large subunit (*rbcL*), together with recent information on the molecular phylogeny of hypnobryalean mosses, and discuss the implications of our findings on the Hypnaceae and their related families.

## Materials and methods

Phylogenetic analyses based on the *rbcL* gene sequences were basically performed following Tsubota and coworkers (1999, 2000, 2001a, b). The method consists of two steps: 1) obtaining sequence data (DNA extraction, PCR amplification and DNA sequencing, or DNA database homology search); and 2) data analyses.

### Sources and Plant Materials

A total of 181 moss species were examined for the analyses as shown in Table 1 and in Appendix A. The latter contains voucher information and DNA Database Accession Nos. Voucher specimens sequenced in the present study were deposited in TNS, HYO, or HIRO. Some sequences used for the analyses were also obtained from the DNA database.

Table 1. List of species and families investigated for *rbcL* gene with the accession number and voucher or reference of the sequences. Treatment of families in this table follows Vitt (1984) with some modifications by Buck and Vitt (1986). Further information for the sequences obtained in the present study is shown in Appendix A.

Order/Family/Species	Accession No.	References/Origin
<b>Hypnales (s. str.)</b>		
<b>Hypnodendraceae</b>		
<i>Hypnodendron menziesii</i> (Hook.) Paris	AF231093	Mishler et al. (only in DNA database)
<b>Pleuroziopsidaceae</b>		
<i>Pleuroziopsis ruthenica</i> (Weinm.) Kindb. ex Britt.	AB024683	Arikawa and Higuchi (1999)
<b>Fontinalaceae</b>		
<i>Fontinalis antipyretica</i> Hedw.	AB050949	Tsubota et al. (2001a)
<i>Fontinalis antipyretica</i> Hedw.	AJ275183	Cox et al. (2000)
<i>Fontinalis dalecarlica</i> Bruch & Schimp.	AF231074	De Luna et al. (2000)
<b>Echinodiaceae</b>		
<i>Echinodium umbrosum</i> (Mitt.) A.Jaeger	AF233568	De Luna et al. (2000)
<i>Sciaromium tricostatum</i> (Sull.) Mitt.	AB024677	Arikawa & Higuchi (1999)
<b>Thuidiaceae</b>		
<i>Abietinella abietina</i> (Hedw.) M.Fleisch.	AF005519	Goffinet et al. (1998)
<i>Thuidium delicatulum</i> (Hedw.) Schimp.	AF158177	De Luna et al. (2000)
<i>Thuidium pristocalyx</i> (Müll.Hal.) A.Jaeger	AB071416	Tsubota et al. (2001b)
<i>Thuidium recognitum</i> (Hedw.) Lindb.	AB019476	Akiyama et al. (2000; only in DNA database); Tsubota et al. (2001a)
<b>Leskeaceae</b>		
<i>Boulaya mittenii</i> (Broth.) Cardot	AB024963	Tsubota et al. (1999)
<i>Duthiella speciosissima</i> Broth. ex Cardot	AB019467	Akiyama et al. (2000; only in DNA database); this study
<i>Miyabea fruticella</i> (Mitt.) Broth.	AB019475	Maeda et al. (2000)
<i>Okamuraea hakoniensis</i> (Mitt.) Broth.	AB019477	Maeda et al. (2000)
<b>Amblystegiaceae</b>		
<i>Calliergonella cuspidata</i> (Hedw.) Loeske	AB024678	Arikawa & Higuchi (1999)
<i>Cratoneuron filicinum</i> (Hedw.) Spruce	AB095270	this study
<i>Drepanocladus aduncus</i> (Hedw.) Warnst.	AB024681	Arikawa & Higuchi (1999)
<i>Hygroamblystegium tenax</i> (Hedw.) Jenn.	AF233565	De Luna et al. (2000)
<b>Lembophyllaceae</b>		
<i>Dolichomitriopsis diversiformis</i> (Mitt.) Nog.	AB019465	Akiyama et al. (2000; only in DNA database); this study
<i>Lembophyllum divulsum</i> (Hook.f. & Wilson) Lindb.	AF233570	De Luna et al. (2000)
<b>Stereophyllaceae</b>		
<i>Entodontopsis leucostega</i> (Brid.) W.R.Buck & Ireland	AB024635	Arikawa & Higuchi (1999)
<i>Stereophyllum radiculosum</i> (Hook.) Mitt.	AB024637	Arikawa & Higuchi (1999)
<b>Fabroniaceae</b>		
<i>Anacamptodon splachnoides</i> (Froel. ex Brid.) Brid.	AF231077	De Luna et al. (2000)
<i>Helicodontium capillare</i> (Hedw.) A.Jaeger	AF233571	De Luna et al. (2000)
<b>Brachytheciaceae</b>		
<i>Brachythecium plumosum</i> (Hedw.) Schimp.	AF233566	De Luna et al. (2000)
<i>Brachythecium rivulare</i> Schimp.	AB024674	Arikawa & Higuchi (1999)
<i>Brachythecium salebrosum</i> (Hoffm. ex F.Weber & D.Mohr) Schimp.	AF158176	De Luna et al. (2000)
<i>Myuroclada maximowiczii</i> (Borszcz.) Steere & Schof.	AB029389	Tsubota et al. (1999)
<i>Platyhypnidium riparioides</i> (Hedw.) Dix.	AB029385	Tsubota et al. (1999)
<i>Pseudoscleropodium purum</i> (Hedw.) M.Fleisch.	AF233567	De Luna et al. (2000)
<i>Rhynchostegium pallidifolium</i> (Mitt.) A.Jaeger	AB024944	Tsubota et al. (1999)
<i>Tomentypnum nitens</i> (Hedw.) Loeske	AB024676	Arikawa and Higuchi (1999)
<b>Entodontaceae</b>		
<i>Entodon challengerii</i> (Paris) Cardot	AB050993	Tsubota et al. (2001a)
<i>Entodon luridus</i> (Griff.) A.Jaeger	AB050994	Tsubota et al. (2001a)
<i>Entodon myurus</i> (Hook.) Hampe	AB024640	Arikawa & Higuchi (1999)
<i>Entodon rubicundus</i> (Mitt.) A.Jaeger & Sauerb.	AB029386	Tsubota et al. (1999)
<i>Entodon scabridens</i> Lindb.	AB050995	Tsubota et al. (2001a)
<b>Sematophyllaceae</b>		
<i>Acanthorrhynchium papillatum</i> (Harv.) M.Fleisch.	AB051224	Tsubota et al. (2001a)
<i>Acroporium pungens</i> (Hedw.) Broth.	AF233572	De Luna et al. (2000)
<i>Acroporium stramineum</i> (Reinw. & Hornsch.) M.Fleisch.	AB051225	Tsubota et al. (2001a)

Table 1. continued.

Order/Family/Species	Accession No.	References/Origin
<i>Aptychella glomeratopropagulifera</i> (Toyama) Seki	AB051217	Tsubota et al. (2001a)
<i>Heterophyllum affine</i> (Hook.) M. Fleisch.	AB051218	Tsubota et al. (2001a)
<i>Heterophyllum nematosum</i> Broth. ex P.de la Varde & Thér.	AB029391	Tsubota et al. (1999)
<i>Isocladiaella surcularis</i> (Dixon) B.C.Tan & Mohamed	AB039784 [as <i>Neacroporium flagelliferum</i> ]	Tsubota et al. (2000)
<i>Mastopoma pulchella</i> (Herzog) ined.	AB071410 [as <i>Trismegistia pulchella</i> ]	Tsubota et al. (2001b)
<i>Mastopoma subfiliferum</i> Horik. & Ando	AB071411	Tsubota et al. (2001b)
<i>Mastopoma uncinifolium</i> (Broth.) Broth.	AB071412	Tsubota et al. (2001b)
<i>Meiothecium microcarpum</i> (Hook.) Mitt.	AB051223	Tsubota et al. (2001a)
<i>Papillidiopsis macrosticta</i> (Broth. & Paris) W.R.Buck & B.C.Tan	AB051220 [as <i>Rhaphidostichum macrostictum</i> ]	Tsubota et al. (2001a)
<i>Pseudotrismegistia undulata</i> (Broth. & M.Yasuda) H.Akya. & Tsubota	AB051229 [as <i>Trismegistia undulata</i> ]	Tsubota et al. (2001a)
<i>Pylaisiadelpha complanata</i> (Reimers & Sakurai) W.R.Buck	AB039785 [as <i>Brotherella complanata</i> ]	Tsubota et al. (2000)
<i>Pylaisiadelpha fauriei</i> (Besch. ex Cardot) W.R.Buck	AB039786 [as <i>Brotherella fauriei</i> ]	Tsubota et al. (2000)
<i>Pylaisiadelpha henonii</i> (Duby) W.R.Buck	AB029167 [as <i>Brotherella henonii</i> ]	Tsubota et al. (1999)
<i>Pylaisiadelpha herbacea</i> (Sakurai) W.R.Buck	AB039787 [as <i>Brotherella herbacea</i> ]	Tsubota et al. (2000)
<i>Pylaisiadelpha recurvans</i> (Michx.) W.R.Buck	L13475 [as <i>Brotherella recurvans</i> ]	Manhart (1994)
<i>Pylaisiadelpha tenuirostris</i> (Bruch & Schimp. ex Sull.) W.R.Buck	AB024641	Arikawa & Higuchi (1999)
<i>Pylaisiadelpha yokohamae</i> (Broth.) W.R.Buck	AB039789 [as <i>Pylaisiadelpha tenuirostris</i> ]	Tsubota et al. (2000)
<i>Sematophyllum pulchellum</i> (Cardot) Broth.	AB071413	Tsubota et al. (2001b)
<i>Sematophyllum subhumile</i> subsp. <i>japonicum</i> (Broth.) Seki	AB039675	Tsubota et al. (2000)
<i>Taxithelium planum</i> (Brid.) Mitt.	AF233573	De Luna et al. (2000)
<i>Trichosteleum papillosum</i> (Hornsch.) A.Jaeger	AF233574	De Luna et al. (2000)
<i>Trichosteleum stissophyllum</i> (Hampe & Müll.Hal.) A.Jaeger	AB051226	Tsubota et al. (2001a)
<i>Trismegistia</i> aff. <i>calderensis</i> (Sull.) Broth.	AB071414	Tsubota et al. (2001b)
<i>Trismegistia korthalsii</i> (Dozy & Molk.) Broth.	AB051227	Tsubota et al. (2001a)
<i>Trismegistia plicata</i> (ms)	AB051228	Tsubota et al. (2001a)
<i>Wijkia concavifolia</i> (Cardot) H.A.Crum	AB095271	this study
<i>Wijkia deflexifolia</i> (Mitt. ex Renaud & Cardot) H.A.Crum	AB051221	Tsubota et al. (2001a)
<i>Wijkia hornsuschuchii</i> (M.Fleisch.) H.A.Crum	AB029383	Tsubota et al. (1999)
<i>Wijkia nakanishikii</i> (Broth.) H.A.Crum	AB051222	Tsubota et al. (2001a)
Hylacomiaceae		
<i>Hylacomiastrum pyrenaicum</i> (Spruce) M.Fleisch.	AB024660	Arikawa & Higuchi (1999)
<i>Hylacomium splendens</i> (Hedw.) Schimp.	AB024662	Arikawa & Higuchi (1999)
<i>Loeskeobryum cavifolium</i> (Sande Lac.) M.Fleisch. ex Broth.	AB024658	Arikawa & Higuchi (1999)
<i>Neodolichomitra yunnanensis</i> (Besch.) T.J.Kop.	AB024671	Arikawa & Higuchi (1999)
<i>Pleurozium schreberi</i> (Willd. ex Brid.) Mitt.	AB024664	Arikawa & Higuchi (1999)
<i>Pleurozium schreberi</i> (Willd. ex Brid.) Mitt.	AF231076	Mishler et al. (only in DNA database)
<i>Rhytidiadelphus japonicus</i> (Reimers) T.J.Kop.	AB039788	Tsubota et al. (2000)
<i>Rhytidiadelphus loreus</i> (Hedw.) Warnst.	AB024666	Arikawa & Higuchi (1999)
<i>Rhytidiadelphus squarrosus</i> (Hedw.) Warnst.	AB024667	Arikawa & Higuchi (1999)
<i>Rhytidiadelphus triquetrus</i> (Hedw.) Warnst.	AB024668	Arikawa & Higuchi (1999)
Plagiotheciaceae		
<i>Isopterygopsis muelleriana</i> (Schimp.) Z.Iwats.	AB134942	Arikawa & Higuchi (2002)
<i>Plagiothecium denticulatum</i> (Hedw.) Schimp.	AB024623	Arikawa & Higuchi (1999)
<i>Plagiothecium draytonii</i> (Sull.) E.B.Bartram	AB024625	Arikawa & Higuchi (1999)
<i>Plagiothecium eurphyllum</i> (Cardot & Thér.) Z.Iwats.	AB024626	Arikawa & Higuchi (1999)
<i>Plagiothecium neckeroideum</i> Bruch & Schimp.	AB024630	Arikawa & Higuchi (1999)
<i>Plagiothecium nemorale</i> (Mitt.) A.Jaeger	AB029387	Tsubota et al. (1999)
<i>Plagiothecium undulatum</i> (Hedw.) Schimp.	AB024634	Arikawa & Higuchi (1999)
Hypnaceae		
<i>Ctenidium molluscum</i> (Hedw.) Mitt.	AB024657	Arikawa & Higuchi (1999)
<i>Glossadelphus ogatae</i> Broth. & M.Yasuda	AB050950	Tsubota et al. (2001a)
<i>Gollania ruginosa</i> (Mitt.) Broth.	AB094341	this study
<i>Gollania splendens</i> (Lisiba) Nog.	AB094340	this study
<i>Herzogiella perrobusta</i> (Broth.) Z.Iwats.	AB134944	Arikawa & Higuchi (2002)
<i>Hypnum cupressiforme</i> Hedw.	AB039674	Tsubota et al. (2000)

Table 1. continued.

Order/Family/Species	Accession No.	References/Origin
<i>Hypnum lindbergii</i> Mitt.	AB029390	Tsubota et al. (1999)
<i>Hypnum plumaeforme</i> Wilson	AB029384	Tsubota et al. (1999)
<i>Hypnum tristo-viride</i> (Broth.) Par.	AB024656	Tsubota et al. (1999)
<i>Isopterygium tenerum</i> (Sw.) Mitt.	AF233569	De Luna et al. (2000)
<i>Isopterygium vineale</i> E.B.Bartram	AB024650	Arikawa & Higuchi (1999)
<i>Orthothecium rufescens</i> (Dicks. ex Brid.) Schimp.	AB050951	Tsubota et al. (2001a)
<i>Pylaisia intricata</i> (Hedw.) Schimp.	AB024642 [as <i>Pylaisiella intricata</i> ]	Arikawa & Higuchi (1999)
<i>Platygyrium repens</i> (Brid.) Schimp.	AB024645 [as <i>Pylaisiella polyantha</i> ]	Arikawa & Higuchi (1999)
<i>Taxiphyllum aomoriense</i> (Besch.) Z.Iwats.	AB024648	Arikawa & Higuchi (1999)
<b>Leucodontales</b>		
<b>Climaciaceae</b>		
<i>Climacium dendroides</i> (Hedw.) F.Weber & D.Mohr	AB019442	Akiyama et al. (2000; only in DNA database); this study
<i>Climacium japonicum</i> Lindb.	AB019443	Akiyama et al. (2000; only in DNA database); this study
<b>Neckeraceae</b>		
<i>Bissetia lingulata</i> (Mitt.) Broth.	AB094789	this study
<i>Homaliadelphus targionianus</i> (Mitt.) Dixon & P. de la Varde	AB094792	this study
<i>Homaliodendron scalpellifolium</i> (Mitt.) M.Fleisch.	AB094788	this study
<i>Neckera urnigera</i> Müll.Hal.	AF158173	Maeda et al. (2000)
<i>Neckeropsis nitidula</i> (Mitt.) M. Fleisch.	AB094790	this study
<i>Pinnatella ambigua</i> (Bosch & Sande Lac.) M.Fleisch.	AB094787	this study
<i>Thamnobryum subseriatum</i> (Mitt. ex Sande Lac.) B.C.Tan	AB094791 [as <i>Thamnobryum sandei</i> ]	this study
<b>Cyrtopodaceae</b>		
<i>Bescherellia brevifolia</i> Hampe	AJ275184	Cox et al. (2000)
<i>Bescherellia elegantissima</i> Duby	AF231097	De Luna et al. (2000)
<i>Cyrtopus setosus</i> (Hedw.) Hook.f.	AF231096	Mishler et al. (only in DNA database)
<b>Cryphaeaceae</b>		
<i>Cryphaea sinensis</i> E.B.Bartram	AB019457	Maeda et al. (2000)
<i>Cyrtodontopsis obtusifolia</i> (Nog.) Nog.	AB019458	Maeda et al. (2000)
<i>Dozya japonica</i> Sande Lac.	AB019446	Maeda et al. (2000)
<i>Pilotrichopsis dentata</i> (Mitt.) Besch.	AB019460	Maeda et al. (2000)
<b>Anomodontaceae</b>		
<i>Anomodon abbreviatus</i> Mitt.	AB019468	Maeda et al. (2000)
<i>Anomodon giraldii</i> Müll.Hal.	AB019469	Maeda et al. (2000)
<i>Anomodon minor</i> (Hedw.) Lindb.	AB019471	Maeda et al. (2000)
<i>Anomodon rugelii</i> (Müll.Hal.) Keissl.	AB019470	Maeda et al. (2000)
<i>Haplophymenium longinerve</i> (Broth.) Broth.	AB019472	Maeda et al. (2000)
<i>Haplophymenium pseudotriste</i> (Müll.Hal.) Broth.	AB019473	Maeda et al. (2000)
<i>Herpetineuron toccocae</i> (Sull. & Lesq.) Cardot	AB019474	Maeda et al. (2000)
<b>Hedwigiaceae</b>		
<i>Hedwigia ciliata</i> (Hedw.) P.Beauv.	AF005517	Goffinet et al. (1998)
<i>Rhacocarpus purpurascens</i> (Brid.) Paris	AJ275171	Cox et al. (2000)
<b>Leucodontaceae</b>		
<i>Antitrichia formosana</i> Nog.	AB019445	Maeda et al. (2000)
<i>Felipponea esquirolii</i> (Thér.) H.Akiy.	AB019447	Maeda et al. (2000)
<i>Forsstroemia japonica</i> (Besch.) Paris	AB019450	Maeda et al. (2000)
<i>Forsstroemia neckeroides</i> Broth.	AB019449	Maeda et al. (2000)
<i>Forsstroemia trichomitria</i> (Hedw.) Lindb.	AB019448	Maeda et al. (2000)
<i>Leucodon atrovirens</i> Nog.	AB019453	Maeda et al. (2000)
<i>Leucodon julaceus</i> (Hedw.) Sull.	AF231075	Mishler et al. (only in DNA database)
<i>Leucodon nipponicus</i> Nog.	AB019451	Maeda et al. (2000)
<i>Leucodon sapporensis</i> Besch.	AB019452	Maeda et al. (2000)
<i>Leucodon sciuroides</i> (Hedw.) Schwägr.	AB095988	this study
<i>Leucodon secundus</i> (Harv.) Mitt.	AB019454	Maeda et al. (2000)
<i>Leucodon sohayakiensis</i> H.Akiy.	AB019455	Maeda et al. (2000)
<i>Leucodon temperatus</i> H.Akiy.	AB019456	Maeda et al. (2000)
<b>Ptychomniaceae</b>		
<i>Ptychomnion aciculare</i> (Brid.) Mitt.	AF233576	De Luna et al. (2000)
<b>Myuriaceae</b>		
<i>Eumyrium sinicum</i> (Mitt.) Nog.	AB019463	Akiyama et al. (2000; only in DNA database); this study
<i>Myurium hochstetteri</i> (Schimp.) Kindb.	AF233575	De Luna et al. (2000)

Table 1. continued.

Order/Family/Species	Accession No.	References/Origin
<b>Wardiaceae</b>		
<i>Wardia hygrometrica</i> Harv. & Hook.	AJ275170	Cox et al. (2000)
<b>Prionodontaceae</b>		
<i>Prionodon densus</i> (Sw. ex Hedw.) Müll.Hal.	AF158174	De Luna et al. (2000)
<i>Taiwanobryum speciosum</i> Nog.	AB019466	Akiyama et al. (2000; only in DNA database); this study
<b>Pterobryaceae</b>		
<i>Pterobryon arbuscula</i> Mitt.	AB019461	Maeda et al. (2000)
<i>Pterobryon densum</i> Hornsch.	AF158175	De Luna et al. (2000)
<i>Pterobryopsis orientalis</i> subsp. <i>yuennanensis</i> (Broth.) Nog.	AB019462	Maeda et al. (2000)
<i>Trachyloma indicum</i> Mitt.	AB019464	Akiyama et al. (2000; only in DNA database); this study
<b>Trachypodaceae</b>		
<i>Trachypodopsis auriculata</i> (Mitt.) M.Fleisch.	AB024682	Arikawa & Higuchi (1999)
<i>Trachypus bicolor</i> Reinw. & Hornsch.	AF233577	De Luna et al. (2000)
<b>Meteoriaceae</b>		
<i>Papillaria deppei</i> (Hornsch. ex Müll.Hal.) A.Jaeger	AF158172	De Luna et al. (2000)
<b>Hookeriales</b>		
<b>Daltoniaceae</b>		
<i>Lepidopilum surinamense</i> Müll.Hal.	AF233578	De Luna et al. (2000)
<b>Hookeriaceae</b>		
<i>Hookeria acutifolia</i> Hook. & Grev.	AF158170	De Luna et al. (2000)
<b>Hypopterygiaceae</b>		
<i>Hypopterygium tamarisci</i> (Sw.) Brid. ex Müll.Hal.	AF158171	De Luna et al. (2000)
<i>Hypopterygium tahitense</i> Ångström	AF231095	Mishler et al. (only in DNA database)
<b>Bryales</b>		
<b>Bryaceae</b>		
<i>Mielichhoferia elongata</i> (Hoppe & Hornsch.) Nees & Hornsch.	AF232693	Cox et al. (2000)
<i>Orthodontium lineare</i> Schwägr.	AJ275174	Cox et al. (2000)
<i>Pohlia cruda</i> (Hedw.) Lindb.	AJ275175	Cox et al. (2000)
<b>Leptostomataceae</b>		
<i>Leptostomum macrocarpum</i> (Hedw.) Bach.Pyl.	AJ275178	Cox et al. (2000)
<b>Mniaceae</b>		
<i>Mnium thomsonii</i> Schimp.	AF005518	Goffinet et al. (1998)
<i>Plagiomnium cuspidatum</i> (Hedw.) T.J.Kop.	U87082 [as <i>Mnium cuspidatum</i> ]	Lewis et al. (1997)
<i>Plagiomnium japonicum</i> (Lindb.) T.J.Kop.	AB050992	Tsubota et al. (2001a)
<b>Rhizogoniaceae</b>		
<i>Pyrrhobryum vallis-gratiae</i> (Hampe ex Müll.Hal.) Manuel	AJ275179	Cox et al. (2000)
<b>Bartramiaceae</b>		
<i>Bartramia pomiformis</i> Hedw.	AB024620	Arikawa & Higuchi (1999)
<i>Leiomela bartramioides</i> (Hook.) Paris	AF478238	Magombo (2002)
<i>Philonotis andina</i> (Mitt.) A.Jaeger	AF478240	Magombo (2002)
<b>Aulacomniaceae</b>		
<i>Aulacomnium turgidum</i> (Wahlenb.) Schwägr.	AJ275180	Cox et al. (2000)
<b>Racopilaceae</b>		
<i>Racopilum convolutaceum</i> (Müll.Hal.) Reichardt	AF231094	Mishler et al. (only in DNA database)
<b>Outgroup taxa</b>		
<b>Erpodiaceae</b>		
<i>Venturiella sinensis</i> (Vent.) Müll.Hal.	AF005546	Goffinet et al. (1998)
<b>Splachnaceae</b>		
<i>Tayloria lingulata</i> (Dicks.) Lindb.	AF005515	Goffinet et al. (1998)
<b>Orthotrichaceae</b>		
<i>Drummondia obtusifolia</i> Müll.Hal.	AF232697	Cox et al. (2000)
<i>Macromitrium incurvifolium</i> (Hook. & Grev.) Schwägr.	AF005528	Goffinet et al. (1998)
<i>Macromitrium incurvifolium</i> (Hook. & Grev.) Schwägr.	AF226802	Wheeler et al. (only in DNA database)
<i>Ulota lutea</i> (Hook.f. & Wilson) Mitt.	AF005540	Goffinet et al. (1998)
<i>Zygodon reinwardtii</i> (Hornsch.) A.Braun	AF005533	Goffinet et al. (1998)
<b>Leucobryaceae</b>		
<i>Leucobryum scabrum</i> Sande Lac.	AB029388	Tsubota et al. (1999)
<b>Encalyptaceae</b>		
<i>Encalypta procera</i> Bruch	AF005548	Goffinet et al. (1998)
<b>Funariaceae</b>		
<i>Funaria hygrometrica</i> Hedw.	AF005513	Goffinet et al. (1998)
<i>Physcomitrella patens</i> (Hedw.) Bruch & Schimp.	X74156	Kruse et al. (1995)

### *DNA Extraction, PCR Amplification and DNA Sequencing*

Total DNA's were extracted from fresh samples or herbarium specimens by modifications of the CTAB method (Murray & Thompson 1980; Arikawa & Higuchi 1999; Tsubota et al. 2000) or phenol-chloroform method (Tsubota et al. 1999). PCR amplifications of *rbcL* gene segments and direct sequence analyses were carried out using standard conditions according to the manufacturers instructions. The reactions were performed in the thermal cyclers with PCR amplification kits and synthetic primers. The sequences were electrophoresed on automated sequencers, and analyzed on the data-analysis system. The sequences obtained in the present study were submitted to the DDBJ/EMBL/GenBank International Nucleotide Sequence Database Collaboration.

Details and further information for DNA extracting protocols, amplification primers, PCR protocols and those of sequencing reactions are described previously in Arikawa and Higuchi (1999), De Luna et al. (1999), Maeda et al. (2000), and Tsubota and coworkers (1999, 2000, 2001a, b).

### *Data Analyses*

*Data set*—A total of 181 *rbcL* gene sequences were examined (Table 1, with DDBJ/EMBL/GenBank accession numbers). This data set contains nine sequences obtained from our laboratory and 172 registered sequences after BLAST searches in the NCBI homepage for the DNA database. The matrix includes 100 species (102 OTUs) of the Hypnales, 51 species of Leucodontales, 4 species of Hookeriales, and others as outgroups, to make a survey for the phylogenetic relationships between the hypnobryalean mosses. *Funaria hygrometrica* and *Physcomitrella patens* were used as the most distant outgroups.

*Alignment*—Manual alignment was carried out for the 181 sequences against those of *Physcomitrella patens* and *Leucobryum scabrum* with an editor program and our original program, Sclean 2.81 (by Tsubota 1998–2002, in our http site, see Appendix B). The other original programs written in Perl and C were also applied for the data processing. Undetermined sites, gaps, and regions not clearly alignable for all sequences were excluded from the analyses. Among sequences

aligned, those belonging to the same sequence are represented by a single sequence, and any unalignable sequence was omitted from the data sets.

### *Analysis Methods*

Trees were constructed by the following three methods: the neighbor-joining (NJ) (Saitou & Nei 1987), the maximum-parsimony (MP) method (Fitch 1971), and the maximum-likelihood (ML) method (Felsenstein 1981). The trees obtained by the three methods were appraised by the log-likelihood measure with NucML in MOLPHY 2.3b3 (Adachi & Hasegawa 1996).

*Distance analysis*—A NJ tree was constructed by NucML and NJdist in MOLPHY version 2.3b3 package (Adachi & Hasegawa 1996, see Appendix B) using HKY85 model (Hasegawa et al. 1985) for the distance estimations.

*Parsimony analysis*—MP trees were constructed by PAUPRat (Sikes & Lewis 2001), which is a tool to implement the Parsimony Ratchet searches (Nixon 1999) with PAUP\*, over PAUP\* 4.0b10 (Swofford 2002). In analyses by PAUPRat, MP trees were searched using the Parsimony Ratchet search strategy in twenty 200 iteration runs.

*Maximum likelihood analysis*—ML trees were constructed with the NucML in MOLPHY version 2.3b3 package (Adachi & Hasegawa 1996). For the ML analyses, HKY85 model (Hasegawa et al. 1985) was used as the estimate model. ML trees were constructed with the local rearrangement search from the NJ tree by NucML and NJdist 1.2.5 (Adachi & Hasegawa 1996); and MP trees by PAUPRat. Best-conformed transition/transversion (Ts/Tv) parameters were estimated based on calculations with the HKY85 model by NucML employing the “-topt” option parameter.

*Tree comparison with log-likelihood and several tests*—Tree comparison with the ML criteria by NucML was carried out to evaluate the resulting trees. Several measures were used estimating the log-likelihood of different tree topologies, standard errors (SEs) of the difference in log-likelihood (Kishino & Hasegawa 1989), and the bootstrap probability estimated by the RELI (resampling of estimated log-likelihood of sites) method (Kishino et al. 1990; Hasegawa & Kishino 1994).

The program package CONSEL 0.1e (Shimo-

daira & Hasegawa 2001) was also used to calculate the *p*-values of the confidence for the bifurcating candidate topologies using several testing procedures: the Approximately Unbiased (AU) test (Shimodaira 2000, 2002) using the multiscale bootstrap technique; the Kishino-Hasegawa (KH) test (Kishino & Hasegawa 1989); and the Shimodaira-Hasegawa (SH) test (Shimodaira & Hasegawa 1999; Goldman et al. 2000).

**Bootstrap test**—Using NucML, a bootstrapping test with local bootstrap probabilities (LBPs; in %) (Adachi & Hasegawa 1996) was applied for the best ML topology. LBP is a relative bootstrap frequency obtained from topology search by local rearrangements of NucML. The value was comparable with a value of Felsenstein's (1985) bootstrap probability, and it is a little larger than the standard bootstrap probability.

**Consensus tree**—A strict consensus tree for the bifurcating topologies with high-ranking log-likelihood values that passed the AU test was also computed by PAUP\*.

## Results

### Obtained sequence data

New *rbcL* sequences were obtained for eleven species: *Bissetia lingulata*, *Cratoneuron filicinum*, *Gollania ruginosa*, *G. splendens*, *Homalidadelphus targinonianus*, *Homaliodendron scalpellifolium*, *Leucodon sciuroides*, *Neckeropsis nitidula*, *Pinnatella ambigua*, *Thamnobryum subseriatum*, and *Wijkia concavifolia*. For *Wijkia concavifolia* an entire sequence 1,428 bp long was obtained. The sequences have been submitted to the DNA database under the serial accession numbers as shown Table 1.

### Sequence alignment

The data matrix was prepared for phylogenetic analyses by manual alignment of the sequences. The data sets were submitted to a 5% chi-square test by TREE-PUZZLE 5.0 (Strimmer & Haeseler 1996), and all the data passed the test. For the analyses, a total of 1,092 sites of 181 *rbcL* sequences were used. Nucleotide frequencies estimated from the data set were A = 29.2%, C = 17.5%, G = 22.2%, and T = 31.1%. In the sites of the aligned *rbcL* sequences, 417 sites (= 38.2% in 1,092 sites) were variable.

### Phylogenetic analyses

The data matrix was prepared for analyses of phylogenetic relationships of the hypnobryalean mosses. A total of 2,223 topologies, of which only 7 tree topologies were bifurcate, were obtained from four analyses: 1 ML and 1 NJ topologies by NucML; and 2,221 MP by PAUPRat over PAUP\*. On the 7 bifurcating topologies, local rearrangement searches were constructed for the obtained topologies. A total of 12 bifurcating topologies were used for the following analysis. More detailed topologies were searched through the obtained trees using log-likelihood measure and several tests (Table 2). HKY85 model was used as the estimate model with the transition/transversion parameter estimated from data set for the ML tree as 3.39. The highest likelihood tree of hypnobryalean mosses was obtained, and shown in Fig. 1. The Log-likelihood value for the ML tree was  $-15445.8 \pm 794.6$ . The LBPs for the clades were comparatively low values (41–100%). Because each topology did not gain a decided advantage over other topologies, a 50% majority-rule consensus tree of high-ranking trees was also obtained, and presented in Fig. 2. This tree also has a tendency toward the ML tree.

The ML and consensus trees form notable clades: (1) two major clades are shown in Fig. 1 [the combined Hypnales (s. str. [= sensu Vitt 1984])–Leucodontales clade (87% LBP value in ML tree, 82% support in consensus tree); and the Hookeriales clade (64% LBP, 73%)] in the hypnobryalean clade (96% LBP, 100%); and (2) 48 basic clades [Sematophyllaceae clade (96% LBP, 100%); *Entodon* clade (96% LBP, 100%); *Entodon rubicundus* clade; *Thuidium* clade (100% LBP, 100%); *Abietinella abietina* clade; *Isopterygiopsis*–*Orthothecium* clade (94% LBP, 100%); *Boulaya mittenii* clade; Hypnaceae clade (71% LBP, 55%); *Leucodon*–*Pterobryon*–*Cryphaea* clade (89% LBP, 55%); *Anomodon rugelii* clade; *Herzogiella perrobusta* clade; *Isopterygium vineale* clade; *Prionodon densus* clade; *Antitrichia formosana* clade; *Rhytidiadelphus*–*Hylocomium*–*Loeskeobryum*–*Ctenidium* clade (89% LBP, 100%); *Myurium*–*Sciaromium* clade (88% LBP, 100%); *Trachypus*–*Papillaria*–*Trachypodopsis*–*Duthiella* clade (89% LBP, 100%); *Plagiothecium* clade (98% LBP, 100%);



Table 2. Comparison of log-likelihood scores among the 12 resulting bifurcating topologies among the hypnobryalean mosses for *rbcL* sequences with the HKY85 model (Hasegawa et al. 1985). The log-likelihood values were calculated by NucML (Adachi & Hasegawa 1996), and all the *p*-values by CONSEL (Shimodaira & Hasegawa 2001) from 10,000 repetitions. The log-likelihood values of the highest likelihood trees are given in angle brackets, and the differences in log-likelihood of alternative trees from that of the ML tree are shown with their standard errors (SE) following  $\pm$ . The *p*-values that are not significant at  $\alpha = 0.05$  are emphasized in bold type. Topologies with asterisks in the AU column were used for the consensus tree (Fig. 2).

Rank	$\Delta l \pm SE$		<i>p</i> -Values				Method	Program	Note
			AU	BP	KH	SH			
1	<-15445.8>	ML (Fig. 1)	<b>0.699*</b>	<b>0.239</b>	<b>0.654</b>	<b>0.957</b>	ML	PAUPRat + NucML	LRS from MP-1
2	-9.9 $\pm$ 26.7		<b>0.564*</b>	<b>0.080</b>	<b>0.346</b>	<b>0.895</b>	ML	PAUPRat + NucML	LRS from MP-2
3	-12.7 $\pm$ 28.0		<b>0.435*</b>	<b>0.092</b>	<b>0.305</b>	<b>0.858</b>	MP	PAUPRat (MP-1)	
4	-16.5 $\pm$ 47.3		<b>0.516*</b>	<b>0.115</b>	<b>0.358</b>	<b>0.846</b>	ML	PAUPRat + NucML	LRS from MP-4
5	-16.9 $\pm$ 30.7		<b>0.433*</b>	<b>0.055</b>	<b>0.290</b>	<b>0.812</b>	ML	PAUPRat + NucML	LRS from MP-3
6	-20.9 $\pm$ 54.7		<b>0.488*</b>	<b>0.146</b>	<b>0.348</b>	<b>0.779</b>	ML	PAUPRat + NucML	LRS from MP-5
7	-22.5 $\pm$ 39.6		<b>0.373*</b>	0.048	<b>0.283</b>	<b>0.795</b>	MP	PAUPRat (MP-2)	
8	-23.0 $\pm$ 38.8		<b>0.360*</b>	0.045	<b>0.277</b>	<b>0.774</b>	MP	PAUPRat (MP-3)	
9	-27.1 $\pm$ 47.6	ME	<b>0.297*</b>	<b>0.057</b>	<b>0.276</b>	<b>0.730</b>	MP	PAUPRat (MP-4)	
10	-40.4 $\pm$ 61.4		<b>0.264*</b>	<b>0.078</b>	<b>0.253</b>	<b>0.601</b>	MP	PAUPRat (MP-5)	
11	-54.9 $\pm$ 60.3		<b>0.192*</b>	0.045	<b>0.179</b>	<b>0.481</b>	ML	NucML	LRS from NJ-1
12	-160.5 $\pm$ 80.2		0.003	0.000	0.030	0.046	NJ	NucML + NJdist (NJ-1)	

Note. — AU: Approximate unbiased test (Shimodaira 2000, 2002), BP: the bootstrap selection probability of Felsenstein (1985) estimated by the RELL resampling method (Kishino et al. 1990; Hasegawa & Kishino 1994), KH: Kishino-Hasegawa test (Kishino & Hasegawa 1989), SH: Shimodaira-Hasegawa test (Shimodaira & Hasegawa 1999; Goldman et al. 2000); and LRS: the local rearrangement search (Adachi & Hasegawa 1996) by NucML.

*Pleurozium-Hylocomiastrum* clade (82% LBP, 82%); Brachytheciaceae clade (66% LBP, 100%); Climaciaceae clade (99% LBP, 100%); *Pleuroziopsis* clade; Stereophyllaceae clade (100% LBP, 100%); *Neodolichomitra yunnanensis* clade; *Tomentypnum nitens* clade; Amblystegiaceae clade (99% LBP, 100%); *Hypnum cupressiforme* clade; *Trachyloma indicum* clade; Neckeraceae-*Forsstroemia* clade (86% LBP, 100%); Lembophyllaceae clade (82% LBP, 100%); *Taxiphyllum-Glossadelphus-Miyabea-Bissetia-Homaliadelphus* clade (90% LBP, 73%); Anomodontaceae clade (70% LBP, 100%); Fontinalaceae clade (100% LBP, 100%); Hookeriales clade (64% LBP, 73%); *Leptostomum macrocarpum* clade; *Bartramia pomiformis* clade; Cyrtopodaceae clade (100% LBP, 100%); *Hypnodendron menziesii* clade; *Racopilum convolutaceum* clade; *Pyrrobryum vallis-gratiae* clade; *Aulacomnium turgidum* clade; *Orthodontium lineare* clade; *Philonotis-Leiomela* clade (97% LBP, 100%); *Mielichhoferia elongate* clade; *Pholia cruda* clade; Mniaceae clade (100% LBP, 100%); Hedwigiaceae clade (100% LBP, 100%); and *Wardia hygrometrica* clade].

Our study corroborates that 1) the Hypnales (s. str. [= sensu Vitt 1984]) and Leucodontales are not monophyletic entities, while the Hookeriales with the inclusion of the Ptychomniaceae are; 2) the combined Hypnales (s. str. [= sensu Vitt 1984]) and Leucodontales comprise a well supported sister clade to the Hookeriales; and 3) the families Entodontaceae (s. str. [= sensu Vitt 1984; Tsubota et al. 2000]), Fontinalaceae, Sematophyllaceae (s. lat. [= sensu Tsubota & co-workers 2000, 2001a, b]), Thuidiaceae (s. str. [= sensu Tsubota et al. 2000]) and Plagiotheciaceae (s. str. [= sensu present]) are resolved as monophyletic groups. Members of the Amblystegiaceae, Hypnaceae, and Leucodontaceae are dispersed among several clades.

## Discussion

### 1. Ordinal relationships

Our phylogenetic analysis with *rbcL* large-scale data set did not recover either the monophyly of the order Hypnales (s. str. [= sensu Vitt 1984]), or the monophyly of the Leucodontales; although the analysis recovered the monophyly of the

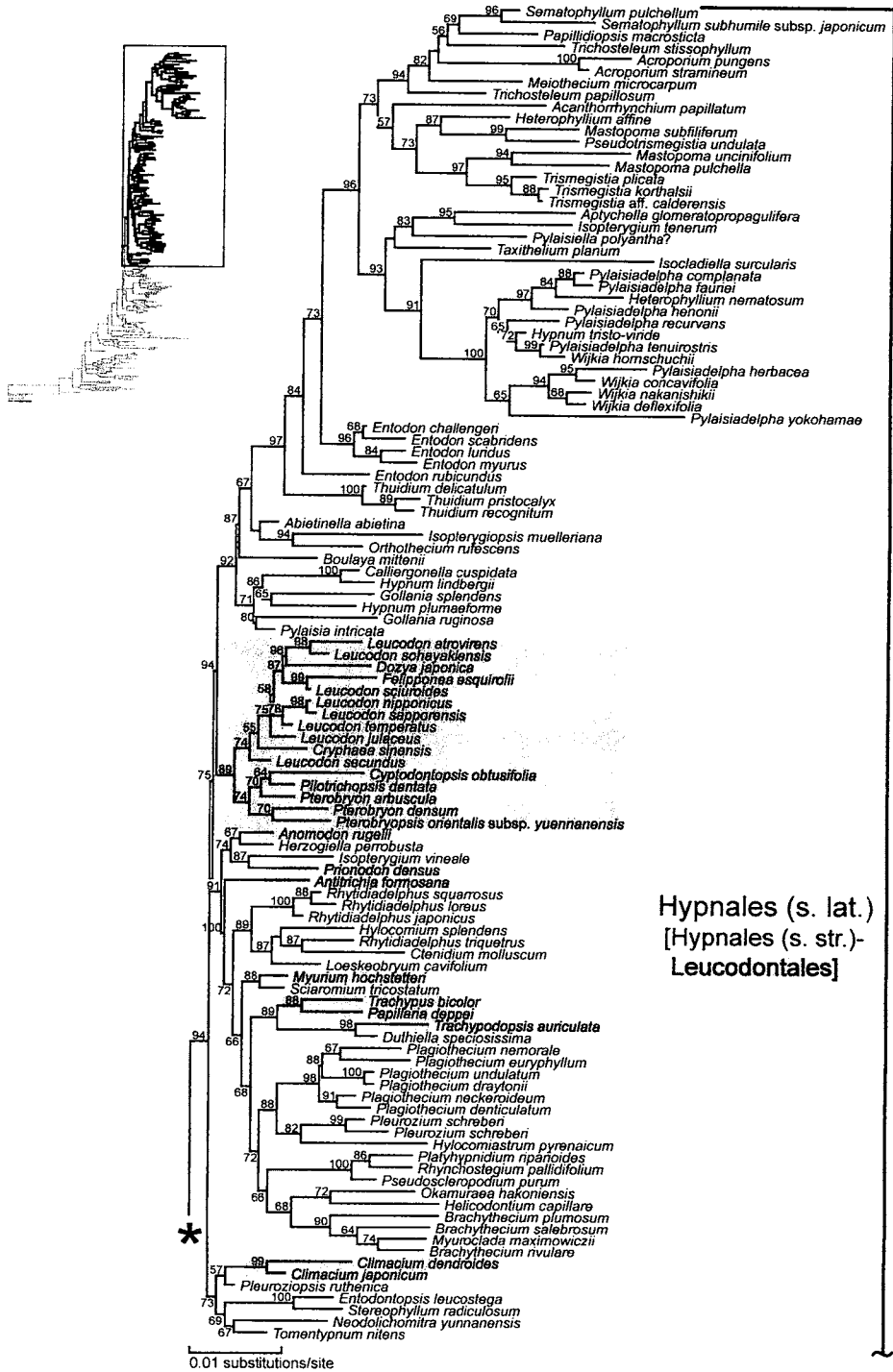
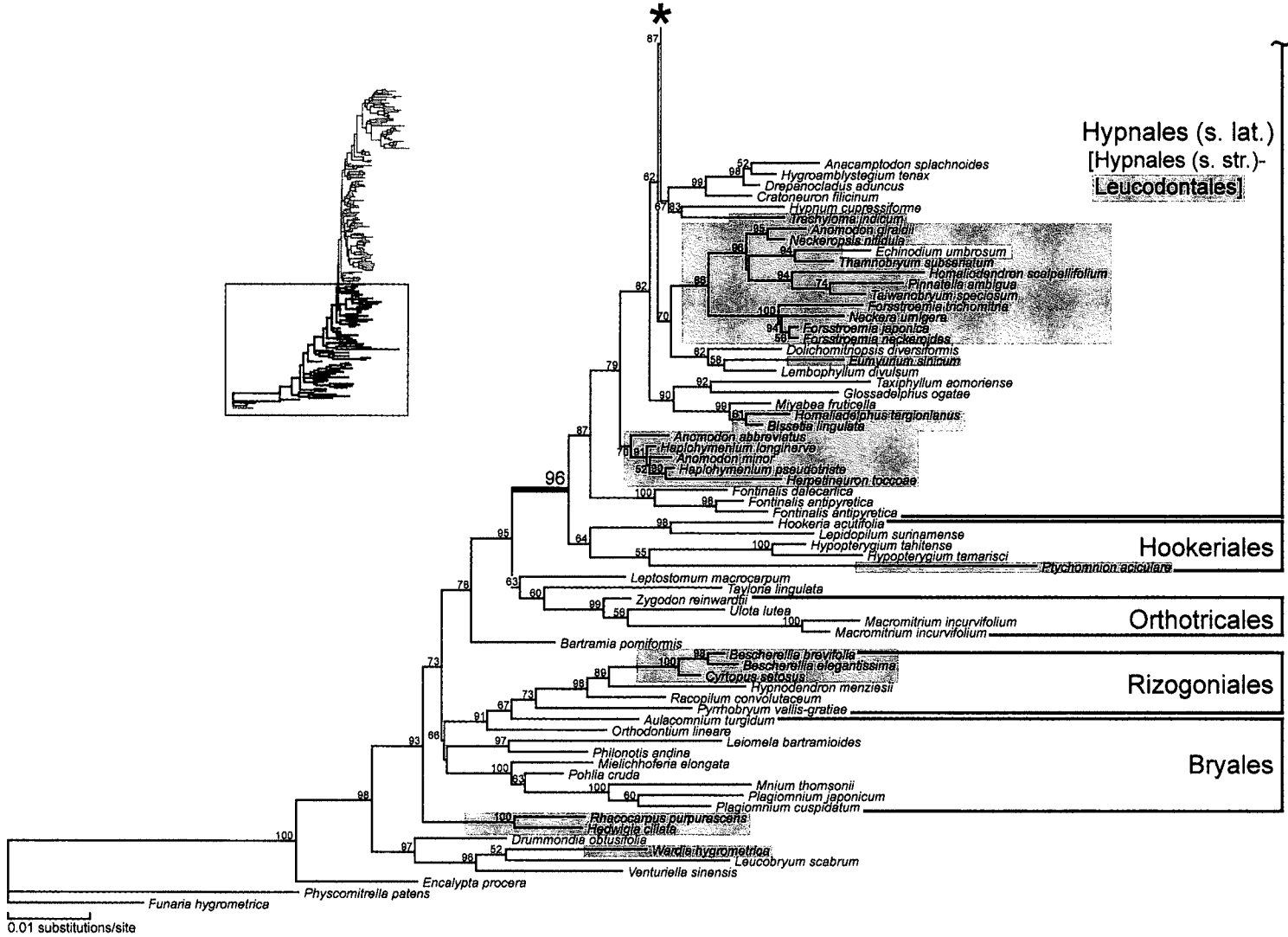


Fig. 1. The highest likelihood tree for the aligned 1,092 bp of the 181 *rbcL* gene sequences (HKY85 model;  $\alpha/\beta = 3.39$ ;  $\ln L = -15445.8 \pm 794.6$  by NucML); and the major clades: the Hypnales (s. lat.) clade [= the combined Hypnales (s. str. [= sensu Vitt 1984])–Leucodontales clade], and the Hookeriales clade. The horizontal length of each branch is proportional to the estimated number of nucleotide substitutions. The root is arbitrarily placed on the branch leading to the *Funaria hygrometrica* and *Physcomitrella patens*. Local bootstrap probabilities (LBP; %) more than 50% are shown above or near branches. Shaded boxes identify the traditional Leucodontales.

Fig. 1. continued.



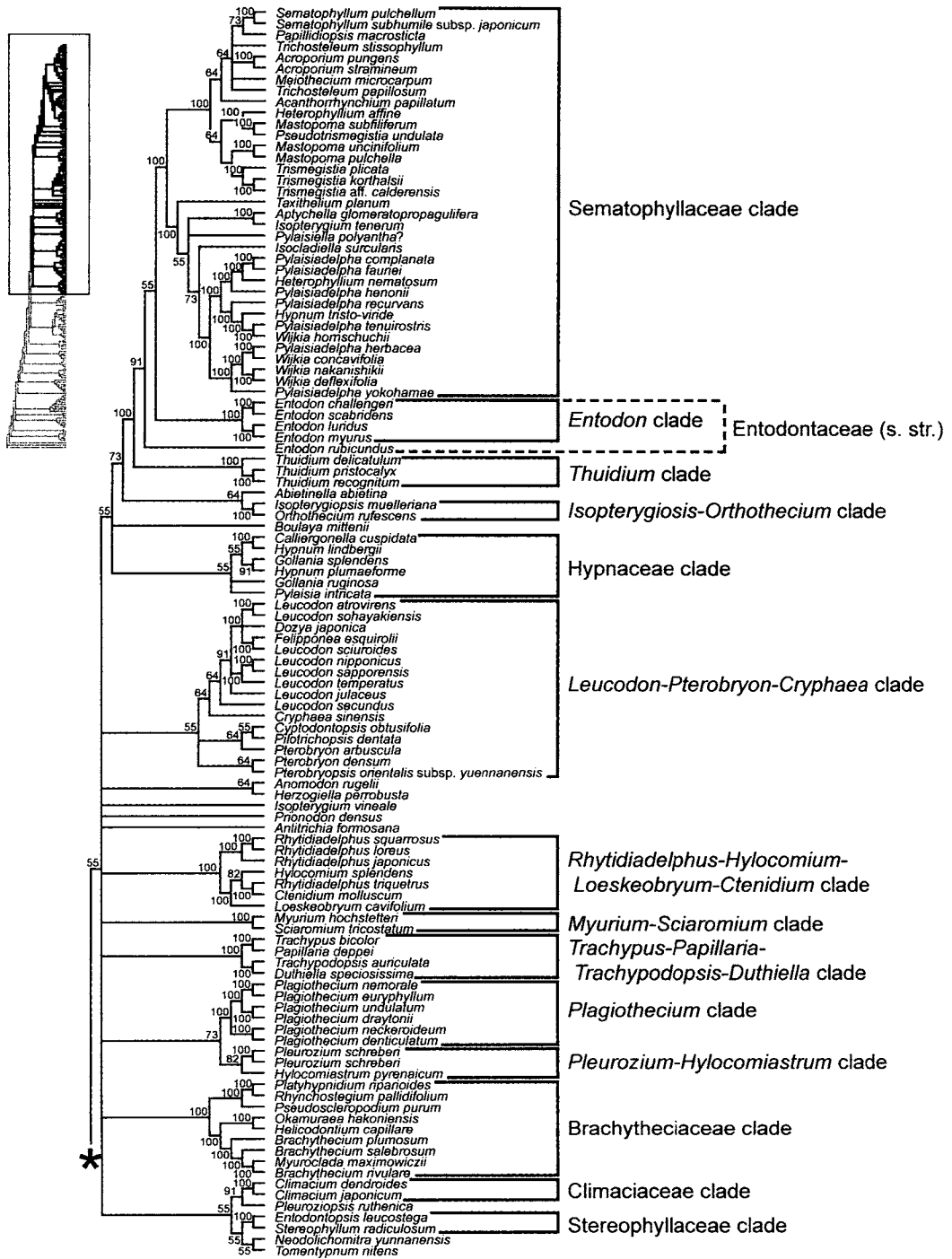


Fig. 2. The 50% majority-rule consensus tree for the 11 bifurcating topologies with high-ranking log-likelihood values that passed the AU test. The root is arbitrarily placed on the branch leading to the *Funaria hygrometrica* and *Physcomitrella patens*.

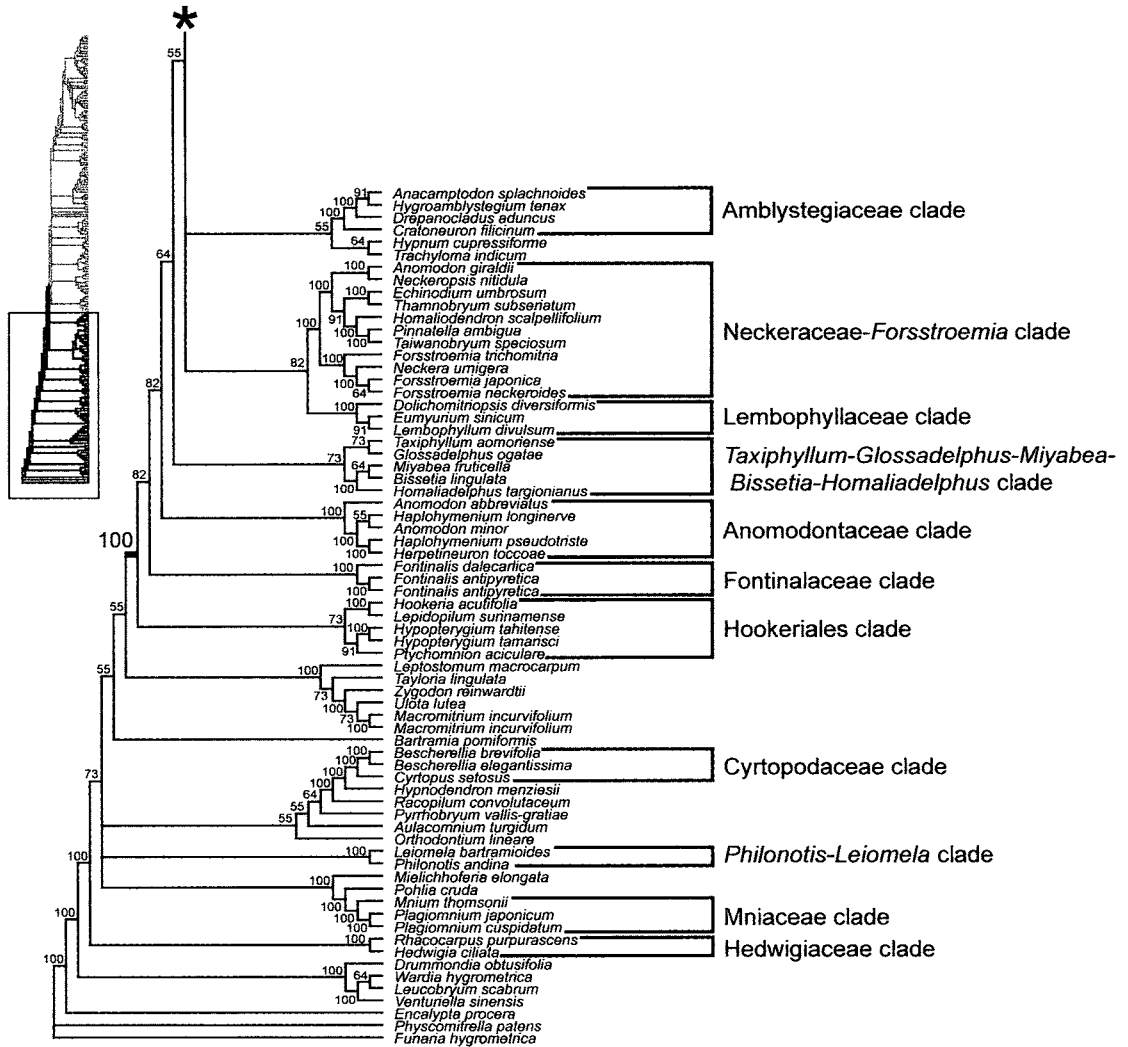


Fig. 2. continued.

Hookeriales. The combined Hypnales and Leucodontales, however, comprise a well-supported sister clade to the Hookeriales. Our results also showed the monophyly of all hypnobryalean mosses as previously discovered by De Luna and coworkers (1999, 2000), and Buck et al. (2000).

*Hypnales and Leucodontales*

Tsubota et al. (1999) showed a preliminary phylogenetic relationship of the Hypnales based on *rbcL* data set, especially revealing the polyphyly of the Hypnaceae, with 34 OTUs for the analysis. De Luna et al. (1999) suggested that monophyly of pleurocarpous mosses including

some species of the Bryales, Hookeriales, Leucodontales and Hypnales. De Luna et al. (2000) and Buck et al. (2000) revealed the non-independence of the traditional orders Hypnales and Leucodontales, and suggested the necessity to be reconsidered as a single major lineage.

In the present study with a large-scale data set, monophyly of each order Hypnales (s. str. [= sensu Vitt 1984]) or Leucodontales was not recovered under different tree search procedures. The combined Hypnales (s. str.) and Leucodontales clade was supported with 87% LBP in the ML tree and 82% support in the consensus tree, rooted by the clade of the Fontinalaceae. This com-

bined clade corresponds to the Hypnales (s. lat.) as revealed by De Luna et al. (2000) and named as the Hypniae by Buck et al. (2000). Members previously treated as the Leucodontales appeared as several clades in the combined clade. Our results of the present study based on the *rbcL* gene do not concur with the classification and phylogeny of the order Hypnales proposed by Vitt (1984), and Buck and Vitt (1986). These results also suggest that their sporophytic morphological similarities, such as development of peristomal teeth and exothecial cells, are multiple transitions to similar solutions to epiphytism in the Hypnales (s. lat.).

### *Hookeriales*

Monophyly of the Hookeriales [inc. the Ptychomniaceae, represented by *Ptychomnion aciculare*; sensu Buck et al. 2000; sensu Buck & Goffinet 2000] was supported with 55% LBP in the ML tree and 73% support in consensus tree, being sister to the combined Hypnales–Leucodontales in our phylogenetic analyses; although some of the obtained trees showed that the Ptychomniaceae appeared as a sister to the large clade including other member of the Hookeriales and the combined Hypnales–Leucodontales. More data are in need to clarify the phylogenetic position of the Ptychomniaceae.

Two major clades of the Hypnales (s. lat. [= sensu De Luna et al. 2000; sensu Buck et al. 2000]): the combined Hypnales (s. str.) and Leucodontales with the exception of the Cyrtopodaceae, and Hookeriales form a single large clade with strong support (96% LBP in the ML tree, 100% support in the consensus tree), as represented in previous works (De Luna & coworkers 1999, 2000; Buck et al. 2000).

## **2. Familial relationships, especially the Hypnaceae and possibly related families**

In the present investigation, some families, such as the Sematophyllaceae (s. lat.), Plagiotheciaceae (s. str.) and Fontinalaceae, are resolved as monophyletic groups, whereas no particular clade is recognized to accommodate members of the families Hypnaceae and Leucodontaceae. The Fontinalaceae are placed at the basal part of the combined Hypnales and Leucodontales clade.

### *Hypnaceae*

The families Hypnaceae (with ca 40 genera: Vitt 1984) are among the most diversified taxonomic groups in the pleurocarpous mosses. The familial circumscription within the Hypnales, especially between the Hypnaceae and other families, such as the Sematophyllaceae, Plagiotheciaceae, and Amblystegiaceae, has lately become a subject of special interest and been repeatedly discussed (e.g., Nishimura et al. 1984).

In the obtained ML and consensus trees, the family Hypnaceae, as well as its type genus *Hypnum*, proved to be non-monophyletic distributing in various positions in the trees, regardless of the inclusion of *Pylaisiadelphina* (inc. *Brotherella*) and *Wijkia* in the family, although two species of *Hypnum*, and the genera *Gollania* and *Pylaisia* form a single clade with 71% LBP support. This fact, together with the present analysis supplemented by additional taxa, corroborates the report of Tsubota et al. (1999), suggesting that the family Hypnaceae, as well as the genus *Hypnum*, is polyphyletic although the genus has been carefully revised by monographers (e.g., Ando 1986, 1995). The results of the present study based on the *rbcL* gene do not concur with the circumscription of the Hypnaceae by Nishimura et al. (1984). This also means that the taxonomic confusion in the Hypnales might be ascribed to diversify of the Hypnaceae currently understood as a large taxonomic group comprising a large number of species.

Nishimura et al. (1984) placed *Calliergonella* in the subfamily Hypnoideae of the family Hypnaceae. Hedenäs (1990) transferred *Hypnum lindbergii* Mitt. into the genus *Calliergonella* (Amblystegiaceae) based on characters including capsule structure, alar cells and pseudoparaphyllia. Ando (1995), however, disagreed with Hedenäs (1990) and retained *Hypnum lindbergii* in the genus *Hypnum*. The obtained tree shows the monophyly for the *Hypnum lindbergii*–*Calliergonella cuspidata* clade supporting by high bootstrap values (100% LBP in ML tree), although the clade changed its position according to topologies. This result was suggested by Hedenäs (1990) based on the characters including capsule structure, alar cells and pseudoparaphyllia and Tsubota et al. (1999) based on *rbcL* data; and confirmed again in the present study.

### *Sematophyllaceae*

The Sematophyllaceae is also a large taxonomic group including numerous species (with ca 50 genera: Vitt 1984), and the limits between the Hypnaceae and the Sematophyllaceae vary among taxonomists. Tan and Yu (1998, 1999) made a cladistic analysis for the morphological data, and showed the paraphyly of the Sematophyllaceae. Tsubota and coworkers (1999, 2000, 2001a, b) clarified their phylogenetic relationships in the hypnobryalean mosses, and showed the monophyly of the Sematophyllaceae (s. lat.; sensu Tsubota et al. 2000, 2001a, b). Arikawa and Higuchi (2002), however, showed the inclusion of the Entodontaceae within the clade of the Sematophyllaceae (s. lat.), although the support was not so strong.

Distinction between the Hypnaceae and Sematophyllaceae has been controversial, especially concerning the phylogenetic position of some genera, such as *Pylaisiadelpha* (inc. *Brotherella*), *Wijkia* and *Heterophyllum*. De Luna et al. (2000) also showed the rough concept of the Sematophyllaceae, although *Isopterygium tenerum* would need to be transferred from the Hypnaceae. Arikawa and Higuchi (1999) suggested *Pylaisia polyantha* reported, as *Pylaisiella polyantha*, should be considered in the Sematophyllaceae. Our result showed the monophyly of the Sematophyllaceae (s. lat.) proposed by Tsubota and coworkers (2000, 2001a, b), including the genera *Pylaisiadelpha*, *Wijkia* and *Heterophyllum*, and some species previously treated as members of the Hypnaceae: *Hypnum tristo-viride*, *Isopterygium tenerum* and *Pylaisia polyantha*, was revealed by both the most ML tree with strong support (96% LBP) and the consensus tree. We cannot support the phylogenetic position of *P. polyantha* on the basis of the data presently available. Further, it is necessary to verify the identification of the material of the species.

As previously indicated in morphological data analyses by Hedenäs (1995, 1996), phylogenetic relationships based on molecular data should be surveyed between the Sematophyllaceae and the families in the order Hookeriales. The monophyly of the Sematophyllaceae and the Hookeriales had no phylogenetic evidence in our analyses.

The present study reveals at least that the characters of well-developed alar cells and habitat are

significant for taxonomic recognition of the family Sematophyllaceae, as concluded by Tsubota et al. (2001b). Moreover, more rapid rate of nucleotide substitutions of the Sematophyllaceae (s. lat.) was different from that of the other lineage and, therefore, characteristic to this lineage. This isolation might have been correlated to the ecological requirements, as these taxa in the lineage with habitat preference for growing on tree trunks.

### *Entodontaceae*

Several discussions have been made on the relationship of the Entodontaceae and Thuidiaceae to the Hypnaceae and the Sematophyllaceae from molecular viewpoints. Mizushima (1960) and Buck (1980) published monographic works on the Entodontaceae, and they have different views with regard to the systematic position of the genus *Orthothecium*. Mizushima (1960) placed it in Entodontaceae, while Buck (1980) in the Hypnaceae. Vitt (1984), and Buck and Goffinet (2000) treated the family in a narrow sense, including only the four genera: *Entodon*, *Erythrodonium*, *Mesonodon* and *Pylaisiobryum*. The present study showed that the Entodontaceae (s. str.) confirms the exclusion of *Orthothecium* and *Pseudoscleropodium* from the family, supporting the treatment of Vitt (1984), and Buck and Goffinet (2000). *Orthothecium rufescens* formed a clade with *Isopterygiopsis muelleriana*, and *Pseudoscleropodium purum* appeared in the clade with members of the Brachytheciaceae. The tree also shows that the clade consisting of the Sematophyllaceae (s. lat. [= sensu Tsubota et al. 2000, 2001a, b]), Entodontaceae and Thuidiaceae was supported with high LBP (97% LBP in ML tree). Vitt (1984) placed the Entodontaceae close to the Sematophyllaceae on the basis of morphological and ecological characters. Our result supports the treatment of the Entodontaceae, but not the Thuidiaceae.

### *Plagiotheciaceae*

The tree showed the clade consisting of only the genus *Plagiothecium* near the clades including the Brachytheciaceae and some members of the Hylocomiaceae. Recently, Pedersen and Hedenäs (2002) showed the phylogeny of the Plagiotheciaceae based on morphological data and

two DNA regions: *rps4* and *trnL-trnF*, suggesting the family with a wide circumscription. Arikawa and Higuchi (2002) clarified the phylogenetic position of the genera *Isopterygiopsis* and *Herzogiella*, and proposed the inclusion of *Isopterygiopsis* within the Plagiotheciaceae. The present investigation did not support these previous analyses, discovering the monophyly of the Plagiotheciaceae including only the genus *Plagiothecium*.

#### *Stereophyllaceae*

Arikawa and Higuchi (1999), and the following studies (e.g., Pedersen & Hedenäs 2002; Arikawa & Higuchi 2002) clarified the exclusion of the Stereophyllaceae from the Plagiotheciaceae, and the monophyly of the Stereophyllaceae based on molecular phylogenetic analyses. The present result corroborated the previous works showing the Stereophyllaceae as a sister to the *Tomentypnum*–*Neodolichomitra* clade, although their phylogenetic position is not so stable in the Hypnales (s. lat. [= sensu De Luna et al. 2000; sensu Buck et al. 2000]) clade.

#### *Hylocomiaceae*

In the ML tree, the Hylocomiaceae appeared as two distinct clades: *Rhytidiadelphus*–*Hylocomium*–*Loeskeobryum*–*Ctenidium* clade at the basal position of the combined several clades, such as the Brachytheciaceae clade and the *Plagiothecium* clade; and *Pleurozium*–*Hylocomiastrum* clade as the sister clade to the *Plagiothecium* clade. A review of their familial circumscription is needed for the genera *Pleurozium* and *Hylocomiastrum*.

#### *Amblystegiaceae*

The phylogenetic trees in the present study discovered polyphyly of the Amblystegiaceae (s. lat.) as previously suggested by traditional taxonomists (e.g., Hedenäs 1997), showing three clades: *Cratoneuron*–*Drepanocladus*–*Hygroamblystegium*–*Anacamptodon* clade, *Calliergonella* clade, and *Tomentypnum* clade. Buck and Goffinet (2000) treated the family with a narrow sense, excluding some genera treated in the present analysis, e.g., *Calliergonella* (inc. *Hypnum lindbergii*) and *Tomentypnum*, from the family. Vanderpoorten et al. (2002) showed a phylogenetic tree based on DNA sequences and morphol-

ogy and treated the Amblystegiaceae with a narrower sense. The present tree confirms this narrower familial circumscription, placing *Anacamptodon* in the Amblystegiaceae.

#### *Brachytheciaceae*

The present study showed the monophyly of the Brachytheciaceae, including *Okamuraea* and *Helicodontium* as well as *Pseudoscleropodium* and excluding *Tomentypnum*. *Helicodontium* was previously treated in the Myriniaceae by Vitt (1984) and Buck and Goffinet (2000). Our result supports the placement of *Pseudoscleropodium* by Buck and Goffinet (2000), but not *Helicodontium*. The genera *Plagiothecium*, *Pleurozium* and *Hylocomiastrum* were placed in a common clade with the taxa of the Brachytheciaceae (excluded *Tomenthypnum nitens*) with 66% LBP in ML tree.

#### *Meteoriaceae*

Huttunen and Ignatov (pers. comm.) suggested the close relationship between the Brachytheciaceae and Meteoriaceae in their preliminary analyses. Our present analysis includes only one species of the Meteoriaceae. Further sequences of the Meteoriaceae are needed to show the relationships between the two families.

#### *Thuidiaceae and Anomodontaceae*

The Thuidiaceae (s. str. [= sensu Tsubota et al. 2000]), consisting of only the genus *Thuidium*, formed a single clade being sister to the Entodontaceae (s. str. [= sensu Vitt 1984; sensu Buck & Goffinet 2000]), as shown by Tsubota et al. (2001a). *Abietinella abietina* and *Boulaya mittenii* appeared out of, but close to, the clade of the Thuidiaceae.

The genera *Haplohymenium* and *Anomodon*, except for *A. girardii*, appeared in the clade including members of the Neckeraceae, forming a resolved clade placing the secondary basal position in the combined Hypnales–Leucodontales. Maeda et al. (2000) already clarified the polyphyly of the Anomodontaceae, showing a clade with *Haplohymenium*, *Herpetineuron* and some *Anomodon* species. These facts indicated that some genera, such as *Haplohymenium* and *Anomodon* which are sometimes treated within the Thuidiaceae, should be divided into two families:



the Thuidiaceae and Anomodontaceae, supporting the system of Maeda et al. (2000), and Buck and Goffinet (2000).

#### *Leucodontaceae, Cryphaeaceae, and Pterobryaceae*

Maeda et al. (2000) also discussed the familial affinity of *Forsstroemia*, showing relationship between the Cryphaeaceae and Leucodontaceae. As they treated only the leucodontalean mosses for their analysis, the phylogenetic position for members of the order in the pleurocarps was not so clear. In the present trees based on the large-scale data set, the traditional leucodontalean mosses form four major and some minor clades in the combined Hypnales–Leucodontales, supporting the three clades presented by Maeda et al. (2000). The members of the Leucodontaceae, except for *Forsstroemia*, Cryphaeaceae and Pterobryaceae form a clade within the clade of the Hypnales (s. lat. [= sensu De Luna et al. 2000; sensu Buck et al. 2000]). *Forsstroemia*, a member of the traditional Leucodontales, is placed in the clade near the Neckeraceae. These results did not support the appearance of the leucodontalean families in the combined Hypnales (s. str. [= sensu Vitt 1984]) and Leucodontales by Buck et al. (2000).

#### *Fabroniaceae*

The Fabroniaceae form no notable clade in the ML and consensus trees, as shown the above. Further investigations with additional taxa from other genera, such as *Fabronia*, *Schwetschkea*, and *Schwetschkeopsis* are needed.

#### *Fontinalaceae*

Although only *Fontinalis* species were included in the analysis, the Fontinalaceae appear as a single clade, at the most basal position in the Hypnales–Leucodontales clade, supporting the conclusion of Vitt (1984) and Buck & Vitt (1986). Buck & Vitt (1986) constructed a new classification for pleurocarpous mosses, recircumscribing the Hypnales (s. str.) including the Hypnodendrineae and Fontinalineae. Our analyses support the inclusion of the Fontinalineae within the Hypnales, but not the inclusion of the Hypnodendrineae. Further analysis including some species of the Fontinalaceae, especially another genus *Dichelyma*, would clarify the resolu-

tion of the Fontinalaceae.

#### *Cyrtopodaceae*

The Cyrtopodaceae were treated in the Leucodontales by Vitt (1984), and Buck and Vitt (1986). In the present investigation, the Cyrtopodaceae, represented by *Bescherellia brevifolia*, *B. elegantissima*, and *Cyrtopus setosus*, appeared in a position outside the Hypnales (s. lat. [= sensu De Luna et al. 2000; Buck et al. 2000])–Hookeriales clade, forming a notable clade (100% LBP in the ML tree) sister to the Hypnodendraceae. Buck and Goffinet (2000) treated the Cyrtopodaceae in the Rhizogoniales, and the present tree supports their placement of the family.

#### *Neckeraceae and Lembophyllaceae*

The other members of the traditional leucodontalean mosses, previously treated as the Neckeraceae, appeared as a major clade with 86% LBP support, as a sister to the Lembophyllaceae clade. In the clade, *Echinodium umbrosum* as well as *Anomodon giraldii* and *Forsstroemia* spp. were also included.

#### *Climaciaceae and Pleuroziopsidaceae*

The Climaciaceae and Pleuroziopsidaceae formed a weak clade (57% LBP, 91%) near the Stereophyllaceae. Ireland (1968) segregated *Pleuroziopsis* from the Climaciaceae into the monotypic family Pleuroziopsidaceae. Norris and Ignatov (2000) observed the stem surface anatomy in *Climacium* and *Pleuroziopsis*, and suggested that *Pleuroziopsis* should be returned to the Climaciaceae. Our trees do not conflict with their suggestion on *Pleuroziopsis*, although the value of the support is not so high.

### 3. Single costa and double costa

Vitt's system (1984) for the classification of hypnalean mosses suggests that the groups with a single costa, as represented by the Brachytheciaceae and Amblystegiaceae, would have diverged from the hypnalean ancestor at an early evolutionary stage, followed by the groups with a double costa, as represented by the Hypnaceae, Sematophyllaceae and Entodontaceae, which he regarded as the most recent evolutionary stage of the order Hypnales (s. str. [= sensu Vitt 1984]) (see also Tsubota et al. 1999; Buck et al. 2000).

In the present investigation, taxa with a double costa appear in various clades of the tree. This result suggests that a single costa is an ancestral character, and that there were multiple transitions to a double costa. Therefore, our results of the present study do not concur with the suggestion proposed by Vitt (1984), and Buck and Vitt (1986). Our analyses confirm the findings by De Luna et al. (2000) and Buck et al. (2000) that the Hypnales, together with most of the leucodontalean groups, form a monophyletic sister group to the hookerialean lineage. Results also show that the familial circumscription within the Hypnales and Leucodontales should be reconsidered.

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- Appendix A. Information on specimens from which DNA was extracted in this study.
- Here is a list of taxa whose *rbcL* sequences were utilized in this study with their accession numbers, showing their sources and voucher specimen information. Voucher specimens are kept in HIRO, HYO, or TNS. Treatment of families follows Vitt (1984) and Buck and Vitt (1986).
- The sequence of information is: the name of taxon, accession number, locality and specimen number, or reference source. Leskeaceae *Duthiella speciosissima* Broth. ex Cardot, AB019467, Japan, Shikoku, Kochi Pref., Monobe-mura, Befukyo, 950 m alt., July 27, 1998, H. Akiyama 14356 (HYO), this study; Amblystegiaceae *Cratoneuron filicinum* (Hedw.) Spruce, AB 095270, Japan, Honshu, Yamaguchi-ken, Shuhou-cho, Akiyoshi-dai, ca. 100 m alt., August 27, 1998, H. Tsubota 4773 (HIRO), this study; Lembophyllaceae *Dolichomitriopsis diversiformis* (Mitt.) Nog., AB 019465, Japan, Honshu, Hyogo Pref., Oya-cho, Tentaki, June 27, 1998, H. Akiyama 14252 (HYO), this study; Sematophyllaceae *Wijkia concavifolia* (Cardot) H.A.Crum, AB095271, Japan, Kyushu, Kagoshima-ken, Yakushima Isl., Shiratani-unsuikyo Gorge, 600–900 m alt., March 24, 2002, H. Deguchi 36051 (HIRO), this study; Hypnaceae *Gollania ruginosa* (Mitt.) Broth., AB094341, Japan, Honshu, Nagano-ken, Shimo-ina-gun, Minami-shinano-mura, Kajitani River, 670–690m alt., Oct. 19, 2000, T. Arikawa 2855 (TNS), this study; *Gollania splendens* (Ishiba) Nog., AB094340, Japan, Honshu, Chiba-ken, Sanbu-gun, Narutou-machi, ca. 5m alt., Dec. 1, 2000, coll. T. Furuki, in herb. M. Higuchi 39333 (TNS), this study; Climaciaceae *Climacium dendroides* (Hedw.) F.Weber & D.Mohr, AB019442, Japan, Honshu, Mie Pref., Nabari-shi, Nagaki, March 16, 1998, H. Akiyama 14150 (HYO), this study; *Climacium japonicum* Lindb., AB019443, Japan, Honshu, Nagano Pref., Ijima-cho, Shiojidaire, July 28, 1996, H. Akiyama 14376 (HYO), this study; Neckeraceae *Bissetia lingulata* (Mitt.) Broth., AB094789, Japan, Honshu, Nara Pref., Kamikitayama-mura, Odaigahara, 1450 m alt., May 26, 1998, H. Akiyama 14195 (HYO), this study; *Homaliadelphus targionianus* (Mitt.) Dixon & P. de la Varde, AB094792, Japan, Shikoku, Kochi Pref., Monobe-mura, Befukyo, 950 m alt., July 27, 1998, H. Akiyama 14359 (HYO), this study; *Homaliodendron scalpellifolium* (Mitt.) M.Fleisch. AB094788, Japan, Honshu, Hyogo Pref., Kamigori-cho, Onaru-keikoku, 250 m alt., June 17, 1998, H. Akiyama 14234 (HYO), this study; *Neckeropsis nitidula* (Mitt.) M.Fleisch., AB094790, Japan, Honshu, Okayama Pref., Nishiawakura-son, Wakasugi Primary Forest, 1,000 m alt., June 17, 1998, H. Akiyama 14210 (HYO), this study; *Pinnatella ambigua* (Bosch & Sande Lac.) M.Fleisch., AB094787, Taiwan, Pingung, Mt. Peitawu, 1950 m alt., August 24, 1988, H. Akiyama Taiwan-177 (HYO), this study; *Thamnobryum subseriatum* (Mitt. ex Sande Lac.) B.C.Tan, AB094791 [as *Thamnobryum sandei*], Japan, Honshu, Hyogo Pref., Kamigori-cho,

Onaru-keikoku, 250 m alt., June 17, 1991, H. Akiyama 14231 (HYO), this study; Leucodontaceae *Leucodon sciuroides* (Hedw.) Schwägr., AB095988, Japan, Honshu, Niigata Pref., Itoigawa-shi, Kotaki, 300m alt., July 28, 1994, H. Akiyama 12594 (HYO), this study; Myuriaceae *Eumyurium sinicum* (Mitt.) Nog., AB019463, Japan, Shikoku, Kochi Pref., Monobe-mura, Befukyo, July 27, 1998, H. Akiyama 14376 (HYO), this study; Prionodontaceae *Taiwanobryum speciosum* Nog., AB019466, Japan, Shikoku, Kochi Pref., Monobe-mura, Befukyo, July 27, 1998, H. Akiyama 14355 (HYO), this study; Pterobryaceae *Trachyloma indicum* Mitt., AB019464, Taiwan, Pingung, Mt. Peitawu, 1950 m alt., H. Akiyama Taiwan-50 (HYO), this study.

Appendix B. Internet resources for the phylogenetic analysis and database.

*Software*

CONSEL <<http://www.ism.ac.jp/~shimo/prog/consel/index.html>>

MOLPHY <<ftp://ftp.ism.ac.jp/pub/ISMLIB/MOLPHY/>>

PAUP\* <<http://paup.csit.fsu.edu/>>

PAUPRat <<http://viceroy.eeb.uconn.edu/paupratweb/pauprat.htm>>

TREE-PUZZLE <<http://www.tree-puzzle.de/>>

Sclean and our programs download site

<<http://home.hiroshima-u.ac.jp/chubo/>>

*Database*

DDBJ <<http://www.ddbj.nig.ac.jp/>>

Sequence data download site <[http://getentry.ddbj.nig.ac.jp/cgi-bin/get\\_entry.pl?Accession number](http://getentry.ddbj.nig.ac.jp/cgi-bin/get_entry.pl?Accession number)> (For example, a link to an entry with accession number AB095988 is <[http://getentry.ddbj.nig.ac.jp/cgi-bin/get\\_entry.pl?AB095988](http://getentry.ddbj.nig.ac.jp/cgi-bin/get_entry.pl?AB095988)>)

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坪田博美・有川智己・秋山弘之・Efrain De Luna・Dolores González・樋口正信・出口博則：葉緑体 *rbcL* 遺伝子の大量データセットに基づく腋蘚類とくにハイゴケ科とその周辺科の分子系統学的研究

ハイゴケ目・イタチゴケ目・アブラゴケ目を含む腋蘚類の系統関係を、葉緑体 *rbcL* 遺伝子の 181 の配列を含む大量データセットに基づいて解析を行った。解析の結果、ハイゴケ目とイタチゴケ目が一つの大きなクレードを形成した De Luna 他 (2000) および Buck 他 (2000) の結果を支持したが、Vitt (1984) のハイゴケ目およびイタチゴケ目それぞれの単系統性は支持されなかった。