

## Molecular Phylogeny of the Suborder Leucodontineae (Musci; Leucodontales) Inferred from *rbcl* Sequence Data

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The phylogeny of the Leucodontaceae and its allies (pleurocarpous Diplolepidae) was inferred from chloroplast-coded *rbcl* sequence data (1324 bp on average). Our analyses indicate (1) that *Antitrichia* is distantly related to the other members of Leucodontaceae and should be excluded from the family, (2) that Cryphaeaceae form a monophyletic clade, not with Anomodontaceae and Leptodontaceae, but with Leucodontaceae, refuting the placement of Leucodontaceae and Cryphaeaceae in different superfamilies, (3) that *Forsstroemia*, variously classified in Leucodontaceae, Cryphaeaceae or Leptodontaceae, forms a monophyletic clade with *Neckera* (Neckeraceae), and (4) that the presumed monophyly of *Anomodon* and that of Anomodontaceae are not supported.

**Key words:** Anomodontaceae — Cryphaeaceae — Leucodontaceae — Leucodontineae — Molecular phylogeny — *rbcl*

In recent years much evidence has accumulated on the phylogeny of mosses as a result of the development of molecular techniques, especially from DNA sequence data. Initially, sequence data were used to explore deep diversification of bryophyte lineage (Capesius 1995, Hedderston *et al.* 1996, Krantz *et al.* 1995, Mishler *et al.* 1992, Waters *et al.* 1992). Later, DNA sequencing was applied to infer lower-level relationships, such as the delimitation of orders, families and subfamilies (Arikawa and Higuchi 1999, Cox and Hedderston 1999, Goffinet *et al.* 1998, Lewis *et al.* 1997, Tsubota *et al.* 1999). Although there still exist some serious conflicts between the results presented by different researchers using sequence data sets from the same gene (for example, see Bopp and Capesius 1998, and Vitt *et al.* 1998), it has been widely recognized that molecular data are the most convincing in order to reconstruct bryophyte phylogeny.

Members of the Anomodontaceae, Cryphaeaceae, Le-

ptodontaceae and Leucodontaceae are mostly epiphytes growing on tree trunks and branches (sometime on boulders). They have common morphological characteristics, such as smooth, rhomboidal lamina cells, well-developed alar cells, a single costa, erect capsules of the sporophytes, reduced endostome and hydrocastique movement of the outer peristome teeth. Therefore these four families are usually classified in the suborder Leucodontineae (Brotherus 1925, Fleischer 1906, Vitt 1984, Buck and Vitt 1986). Recently, Buck and Vitt (1986) proposed a new classification system that recognized three superfamilies (Cryphaeacanae, Leucodontacanae, and Cyrtopodacanae) in the suborder Leucodontineae. According to them, the Cryphaeaceae, Leptodontaceae, and Anomodontaceae are classified into the Cryphaeacanae, and the Leucodontacanae includes the Hedwigiaceae and Leucodontaceae. On the contrary, De Luna (1995) and Vitt *et al.* (1998) excluded the Hedwigiaceae to the different order Orthotrichales on the basis of morphological and *rbcl* sequence data, respectively. We follow their opinion and did not include the Hedwigiaceae in our present analyses.

The Leucodontaceae and Cryphaeaceae have long been recognized as being closely related to each other (Brotherus 1925, Manuel 1974, 1982). Their fully immersed capsules among perichaetial leaves, autoicous sexuality, and well-developed single costa can distinguish the Cryphaeaceae from the Leucodontaceae. The sole exception is *Forsstroemia*; most species of which have short and forked costae (rarely single or almost absent) and some have shortly exerted capsules (Akiyama 1988b). Consequently, some researchers classified this genus into the Cryphaeaceae (Brotherus 1925, Manuel 1974, 1982, Noguchi 1989), and others into the Leucodontaceae (Vitt 1984, Akiyama 1994). In addition, *Forsstroemia* was recently placed in the Leptodontaceae on the basis of its general resemblance to the genus *Leptodon* (Buck 1980, Stark 1987, Buck and Vitt 1986). To determine the systematic position of *Forsstroemia* is fundamental to defining the familial delimitation of both the Cryphaeaceae and Leucodontaceae and also in resolving the inter-relationships between the two families.

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There have been several concepts for the systematic position of *Anomodon* and its related genera, *Haplohymerium*, *Herpetineuron*, and *Miyabea*. In traditional classifications, they were included in the Thuidiaceae in their own subfamily Anomodontoideae (Watanabe 1972). Recently, they were classified as a separate family, Anomodontaceae, among the superfamily Cryphaeacanae (Buck and Vitt 1986). In contrast, Noguchi (1991) advocated placing those genera in the Leskeaceae of the Hypnales, but without giving his reasons. In addition, based on cladistic analyses of morphological features, Granzow-de la Cerda (1992, 1997) indicated that *Haplohymerium* was nested within *Anomodon*, implying that the latter was paraphyletic.

The main purposes of this study are to determine, from *rbcL* sequence data, possible inter-relationships between members of the Leucodontacanae and Cryphaeacanae, and to provide additional evidence to those systematic issues that might be difficult to resolve utilizing morphological characteristics alone.

## Materials and Methods

### Plant materials

Leaves of 25 species (26 samples) were collected from the field or obtained from dried herbarium specimens (Table 1). Fresh materials were dried with silica gel. Two samples of *Pilotrichopsis dentata* were used from two different localities to determine potential variation within a single species. Voucher specimens are deposited at the following three herbaria: Hiruzen Institute, Okayama Science University (HIRU) - *Okamuraea hakoniensis* (Mitt.) Besch.; Instituto de Ecología (XAL) - *Neckera urnigera* C. Muell.; and Museum of Nature and Human Activities (HYO)-all remaining species, as indicated in Table 1.

According to Buck and Vitt (1986), 22 of the species used in this study belong to the suborder Leucodontineae. Since *Leptodon*, classified in the Leptodontaceae with *Forsstroemia*, is believed to have a close affinity to the Neckeraceae (Brotherus 1925), for comparison we also included *Neckera urnigera* in the present analyses. We also included *Antitrichia*, tentatively placed in the Leucodontaceae, although Buck and Vitt (1986) included only three genera, *Leucodon*, *Felipponea* and *Dozya* in this family.

*Okamuraea* was used as an outgroup in the present analyses. Rhorer (1985) once suggested its close relationship to the Leucodontaceae on the basis of shared morphological characteristics with leucodontaceous members, such as reduced endostome, presence of a defined alar region in the leaves, and rhomboidal lamina cells. The genus, however, is usually classified in the Leskeaceae (Noguchi 1991) of the Hypnales on the basis of long seta, more or less asymmetrical, inclined capsules, and developed peristome teeth with dense papillae. Our preliminary analyses using *rbcL* sequences of other taxa in the Hypnales and Bryales also suggest its affinity to the Hypnales.

### *rbcL* gene sequencing

Dried plants were cleaned under a dissecting microscope,

and then total DNA was isolated from 20 mg of leaves using the CTAB method (Doyle and Doyle 1987).

Partial fragments of *rbcL* were amplified by the polymerase chain reaction (PCR) with two synthetic primers F26 and R1351 (Table 2). The amplification was performed in a 25  $\mu$ l reaction volume containing ca. 5 ng total DNA, 5 pmol of each primer, 0.1 mM of each dNTP, 50 mM KCl, 3 mM MgCl<sub>2</sub>, 10 mM Tris-HCl (pH 8.3), and 0.75 unit of *Taq* DNA polymerase. The amplification procedures consist of an initial five cycles of denaturation at 94C for one min, annealing at 55C for one min, extension at 72C for 2 min, followed by 20 cycles of 96C for 10 sec, 55C for 10 sec, 72C for 45 sec, in the Program Temp Control System PC-800 (Astec, Japan). Using the first amplified solution (1  $\mu$ l) and the second PCR primers in Table 2, sequencing fragments were obtained by a semi- or nested-PCR reaction (25 cycles of 96C for 10 sec, 55C for 10 sec and 72C for 30 sec). These fragments were purified with the GENECLEAN kit (BIO 101). Each fragment was sequenced from both directions using a forward primer ACACAGGAAACAGCTATGACC and a reverse primer CGTTGTAAAACGACGGCCAGT, the Dye Terminator Cycle Sequencing FS Kit and ABI PRISM 310 Genetic Analyzer (Perkin Elmer).

### Phylogenetic analyses

Phylogenetic analyses with 26 *rbcL* sequences representing 25 taxa were carried out by the parsimony method using PAUP 3.1 (Swofford 1993). To test the phylogenetic signals in the data set, the  $g_i$  statistic of distribution of 100,000 random trees was calculated (Hills and Huelsenbeck, 1992). The most parsimonious trees were constructed using heuristic searches with random sampling (100 replicates) and MULPARS option. Tree statistics, such as the consistency index (CI of Kluge and Farris 1969) and the retention index (RI of Farris 1989, Archie 1989), were also estimated. Bootstrap analysis was carried out with 1000 replicates. The number of additional steps required to shift a particular genus or family into a monophyletic group was calculated by CON-STRAIN analysis, using an equal character weighting data set.

Using Kimura's 2-parameter distance, a neighbor-joining tree was constructed and a bootstrapping test was carried out for 10,000 replicates with the program packages of MEGA ver. 1.02 (Kumar *et al.* 1993).

## Results

We determined 1,324 nucleotides from 26 accessions of *rbcL* sequences (Table 1). Ambiguous sites in some species were treated as an "unknown character" and not used in the analyses. There were no deletions or insertions in this region in all taxa examined. Of the 1,324 sites, 175 (13%) were variable and 84 are potentially phylogenetically informative. They were distributed evenly throughout the total sequence but about 71% of variable sites were at the 3rd codon position (125 sites). The transition/transversion ratio was 2 : 5, biased to transitions. The skewness index  $g_i$  of 100,000 random trees was -0.77, which implied the pres-

Table 1. Taxa included in the analyses. Classification mostly followed Buck and Vitt (1986) with some modifications.

\*Samples were obtained from dried herbarium specimens

Taxa	Voucher specimens	Accession number
<b>Leucodontaceae</b>		
<i>Antitrichia formosana</i> Nog.	Taiwan. Nanto Co., Mt. Howan-shan <i>Akiyama et al. 188</i> (HYO).	AB019445
<i>Dozya japonica</i> Sande Lac.	Japan. Nara Pref., Mt. Odaigahara <i>Akiyama 14180</i> (HYO).	AB019446
<i>Felipponea esquirolii</i> (Ther.) Akiyama	China. Yunnan, Ridatsu <i>Akiyama et al. 1168</i> (HYO).	AB019447
<i>Leucodon nipponicus</i> Nog.	Japan. Hyogo Pref., Mikazuki-cho <i>Akiyama 14220</i> (HYO).	AB019451
<i>Leucodon sapporensis</i> Besch.	Japan. Okayama Pref., Nishiwakura-mura <i>Akiyama 14201</i> (HYO).	AB019452
<i>Leucodon atrovirens</i> Nog.	Japan., Nara Pref., Mt. Odaigahara <i>Akiyama 14181</i> (HYO).	AB019453
* <i>Leucodon secundus</i> (Harv.) Mitt. var. <i>secundus</i> (Harv.) Akiyama.	China. Tibet Prov., Motou, <i>Su Y.-g s.n.</i> (KUN).	AB019454
<i>Leucodon sohayakiensis</i> Akiyama	Japan. Kochi Pref., Mt. Ishidate <i>Akiyama 14351</i> (HYO).	AB019455
<i>Leucodon temperatus</i> Akiyama	Taiwan. Nanto Co., Mt. Howan-shan <i>Akiyama et al. 154</i> (HYO).	AB019456
<b>Cryphaeaceae</b>		
<i>Cryphaea sinensis</i> Bartr.	Taiwan. Nanto Co., Tzuen <i>Akiyama et al. 153</i> (HYO).	AB019457
<i>Cyrtodontopsis obtusifolia</i> (Nog.) Nog.	Japan. Wakayama Pref., Hikigawa-cho, <i>Doi June 27, 1998</i> (HYO).	AB019458
<i>Pilotrichopsis dendata</i> (Mitt.) Besch.	China. Guanshi Prov., Napo <i>Akiyama et al. 1341</i> (HYO).	AB019459
<i>Pilotrichopsis dendata</i> (Mitt.) Besch.	Japan. Hyogo Pref., Mikazuki-cho, <i>Akiyama 14228</i> (HYO).	AB019460
<b>Leptodontaceae</b>		
<i>Forsstroemia trichomitra</i> (Hedw.) Lindb.	Japan. Hyogo Pref., Mikazuki-cho, Onaru <i>Akiyama 14241</i> (HYO).	AB019448
<i>Forsstroemia neckeroides</i> Broth.	Japan. Tokyo Pref., Okutama-cho, Nippara <i>Akiyama 13322</i> (HYO).	AB019449
<i>Forsstroemia japonica</i> (Besch.) Par.	Japan. Hyogo Pref., Oya-cho, Tentaki <i>Akiyama 14281</i> (HYO).	AB019450
<b>Anomodontaceae</b>		
<i>Anomodon abbreviatus</i> Mitt.	Japan. Tokyo Pref., Okutama-cho, Nippara <i>Akiyama 13313</i> (HYO).	AB019468
<i>Anomodon giraldii</i> C. Muell.	Japan. Okayama Pref., Nishiwakura-mura, Wakasugi <i>Akiyama 114207</i> (HYO).	AB019469
<i>Anomodon rugelii</i> (C. Muell.) Keissl.	Japan. Okayama Pref., Nishiwakura-mura, Wakasugi <i>Akiyama 14207</i> (HYO).	AB019470
<i>Anomodon minor</i> (Hedw.) Lindb.	Japan. Hyogo Pref., Mikazuki, Onaru <i>Akiyama 14226</i> (HYO).	AB019471
<i>Haplohymenium longinerve</i> (Broth.) Broth.	Japan. Nara Pref., Mt. Odaigahara <i>Akiyama 14191</i> (HYO).	AB019472
<i>Haplohymenium pseudo-triste</i> (C. Muell.) Broth.	Japan. Hyogo Pref., Oya-cho <i>Akiyama 14290</i> (HYO).	AB019473
<i>Herpetineuron toccocae</i> (Sull. & Lesq.) Card.	Japan. Wakayama Pref., Kushimoto-cho, Oshima <i>Akiyama 13969</i> (HYO).	AB019474
<i>Miyabea fruticella</i> (Mitt.) Broth.	Japan. Hyogo Pref., Oya-cho, Tentaki <i>Akiyama 14294</i> (HYO).	AB019475
<b>Neckeraceae</b>		
<i>Neckera urnigera</i> C. Muell.	Mexico. Veracruz, Jardin Botanico Claveijero <i>De Luna 2235</i> (XAL).	AF158173
<b>Leskeaceae</b>		
* <i>Okamuraea hakoniensis</i> (Mitt.) Besch.	Japan. Kochi Pref., Otoyo-cho, Kajigamori <i>Tanaka 134</i> (HIRU).	AB019477

Table 2. Primer sequences used for PCR amplification. The primer numbers correspond with 3' end of annealing positions on the *Marchantia polymorpha rbcL* gene (X04465) from the start codon. F: forward primer, R: reverse primer.

First PCR primers	
F26	TGTCACCACAAACAGAGACTAAAGC
R1351	GCAGCAGCTAGTTCCGGGCTCCA
Second PCR primers	
F30	ACACAGGAAACAGCTATGACCTGTCACCACAAACAGAGACTAAAGCAGGT
R496	CGTTGTAAAACGACGGCCAGTACATCCTAATAAGGGACGACC
F461	ACACAGGAAACAGCTATGACCAACTTTCCAAGGTCCGCCTCATGG
R967	CGTTGTAAAACGACGGCCAGTCCAGCGTGAATATGATCTCC
F891	ACACAGGAAACAGCTATGACCCTTACATTACCGCGCAATG
R1346	CGTTGTAAAACGACGGCCAGTGCAGCAGCTAGTTCCGGGCTCCATTTAG

ence of a strong phylogenetic signal in our data set.

The number of pairwise nucleotide differences ranged from two sites (between *Leucodon nipponicus* and *L. sapporensis*) to 53 sites (between *Forsstroemia trichomitria* and *L. atrovirens* or *Cyrtodontopsis obtusifolia*) (Table 3). In addition, there were four site differences between two samples collected from geographically distant localities in *Pilotrichopsis dentata*.

Parsimony analysis yielded 1,217 most parsimonious trees (length=282, CI=0.652, RI=0.678) and their strict consensus tree is presented in Fig. 1. The topology of the neighbor-joining tree (Fig. 2) agreed with that of the strict consensus tree. Three clades, indicated as A, B and C in both Figs. 1 and 2, were separated at the base. Three taxa, *Antitrichia formosana*, *Miyabea fruticella* and *Anomodon rugelii*, did not form a distinct clade.

The clade A consists of the Cryphaeaceae and Leucodontaceae, excluding *Antitrichia*. In the neighbor-joining tree (Fig. 2), three genera of the Leucodontaceae, *Leucodon*, *Felipponea* and *Dozya*, formed a subclade, as additionally did six species of *Leucodon*, though the bootstrap value was low (46% and 17%). The remaining three genera of the Cryphaeaceae, *Cryphaea*, *Cyrtodontopsis* and *Pilotrichopsis*, did not form a monophyletic clade, but the former two genera joined with bootstrap value 65% in Fig. 1. Within the genus *Leucodon*, the numbers of nucleotide differences between sequences were ranging 2-17.

The clade B includes the members of the Anomodontaceae, except for *Miyabea*, *Anomodon rugelii* and *A. giraldii*. In this clade of the neighbor-joining tree (Fig. 2), *Herpetineuron* was located basally and *Haplohymenium* terminally. Among *Haplohymenium pseudo-triste*, *A. abbreviatus* and *A. minor*, the numbers of different nucleotides were noticeably low (6-9; Table 3).

The clade C consists of three species of the genus *Forsstroemia* and *Neckera urnigera*, which formed a monophyletic subclade, and *Anomodon giraldii*. Though this clade was supported with high bootstrap values, the branch length of the neighbor-joining tree (Fig. 2) and the dissimilarities of nucleotides (Table 3) obviously suggest the relatively distant relationship of *A. giraldii* with the other four taxa.

In the neighbor-joining and strict consensus trees, members of Leucodontaceae, Anomodontaceae, and those of

*Anomodon* did not form monophyletic clades, respectively. Constraint analysis revealed that five additional steps were required to force the monophyly of the Leucodontaceae. Constraining the tree to force monophyly of *Anomodon* or the Anomodontaceae required additional eight steps or 11 steps, respectively.

## Discussion

### *Leucodontaceae* and *Leucodon*

The delimitation of the Leucodontaceae has been one of the most controversial issues in the classification of pleurocarpous mosses (Brotherus 1925, Manuel 1974, Buck 1980, Buck and Vitt 1986, for review see Akiyama 1994). It is because the members of the Leucodontaceae show much variation in morphology, such as the number and length of costae, the shape of lamina cells, and peristome morphology. Genera such as *Antitrichia*, *Bestia*, *Dendroalsia*, *Leucodontopsis*, *Pterogonium* are now excluded from the Leucodontaceae (Akiyama 1994), and thus the family includes only a small number of genera (*Leucodon*, *Felipponea* and *Dozya* in Buck and Vitt 1986; *Leucodon*, *Felipponea*, *Forsstroemia*, *Alsia*, *Pseudocryphaea*, and *Dozya* in Akiyama 1994).

Akiyama (1994) has previously pointed out the distant relationship of *Antitrichia* to the Leucodontaceae on the basis of its xerocastique peristome movement, pinnate branching in ascending branches, and linear lamina cells, all of which are much different from those found in other members of the Leucodontaceae. In the *rbcL* trees, *Antitrichia formosana* separates from the clade A that includes members of the Leucodontaceae and Cryphaeaceae. Additional five steps are required to make *Antitrichia* and the other members of the Leucodontaceae monophyletic. This result supports conclusions based on morphological dissimilarities.

The present *rbcL* data reconfirmed the close relationship among three genera of the Leucodontaceae *sensu* Buck and Vitt (1986), though the monophyly of the family lacks the bootstrap support (Fig. 2). From a morphological point of view, *Leucodon* and *Felipponea* are closely related to each other. They are characterized by rhomboidal, smooth lamina cells, a well-developed alar region, total absence of

Table 3. Number of different nucleotides between *rbxL* sequences.

OTUs number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	
1. <i>Antitrichia formosana</i> (LEU)	25																										
2. <i>Leucodon nipponicus</i> (LEU)	27	2																									
3. <i>Leucodon sapporensis</i> (LEU)	22	7	9																								
4. <i>Leucodon securidus</i> (LEU)	24	5	7	4																							
5. <i>Leucodon temperatus</i> (LEU)	32	15	17	16	14																						
6. <i>Leucodon atrovirens</i> (LEU)	27	10	12	11	9	11																					
7. <i>Leucodon sohayakiensis</i> (LEU)	31	14	16	14	15	23	16																				
8. <i>Dozya japonica</i> (LEU)	28	14	16	12	13	21	16	20																			
9. <i>Felipponea esquilonii</i> (LEU)	27	16	18	15	15	25	18	24	23																		
10. <i>Cryphaea sinensis</i> (CRY)	33	22	24	21	23	33	26	28	27	22																	
11. <i>Cryptodontopsis obtusifolia</i> (CRY)	23	14	16	13	15	23	16	15	17	20	20																
12. <i>Pilotrichopsis dentata</i> (Japan) (CRY)	27	18	20	17	19	27	20	19	21	24	22	4															
13. <i>Pilotrichopsis dentata</i> (China) (CRY)	36	31	33	31	30	36	33	35	35	36	42	32	36														
14. <i>Miyabea fruticella</i> (ANO)	32	31	33	29	28	38	33	35	33	36	41	32	34	40													
15. <i>Herpetineuron tococcae</i> (ANO)	25	22	24	20	19	31	24	28	24	23	29	21	25	29	21												
16. <i>Haplohymerium longineve</i> (ANO)	29	24	26	24	21	33	26	32	28	25	33	25	29	31	21	6											
17. <i>Haplohymerium pseudo-triste</i> (ANO)	26	27	29	25	24	35	29	33	27	28	34	26	30	32	26	9	13										
18. <i>Anomodon abbreviatus</i> (ANO)	28	25	27	23	22	34	27	31	27	22	30	26	30	34	26	7	11	14									
19. <i>Anomodon minor</i> (ANO)	24	20	20	18	19	27	20	24	22	23	29	17	19	29	31	14	20	17	21								
20. <i>Anomodon rugelii</i> (ANO)	37	35	37	33	32	44	37	41	39	36	42	34	38	44	42	29	33	30	32	29							
21. <i>Anomodon giraldii</i> (ANO)	46	46	48	44	45	53	48	48	46	49	53	43	47	48	49	40	44	43	43	42	41						
22. <i>Forsstroemia trichomitria</i> (LEP)	39	35	37	33	32	44	37	39	37	38	44	34	38	40	42	29	33	32	32	29	30	17					
23. <i>Forsstroemia dendroides</i> (LEP)	40	36	38	34	33	45	38	40	38	39	45	35	39	41	45	32	36	35	35	32	29	18	7				
24. <i>Forsstroemia japonica</i> (LEP)	44	40	42	40	41	51	44	44	42	45	49	39	43	45	49	36	40	39	39	38	39	22	13	14			
25. <i>Neckera umigera</i> (NEK)	34	34	34	32	33	41	36	40	34	37	41	33	33	40	40	31	35	30	36	28	41	52	43	42	46		
26. <i>Okamuraea hakoniensis</i> (LES)																											

Abbreviation in parentheses: LEU; Leucodontaceae, CRY; Cryphaeaceae, ANO; Anomodontaceae, LEP; Leptodontaceae, NEK; Neckeraaceae, LES; Leskeaceae.

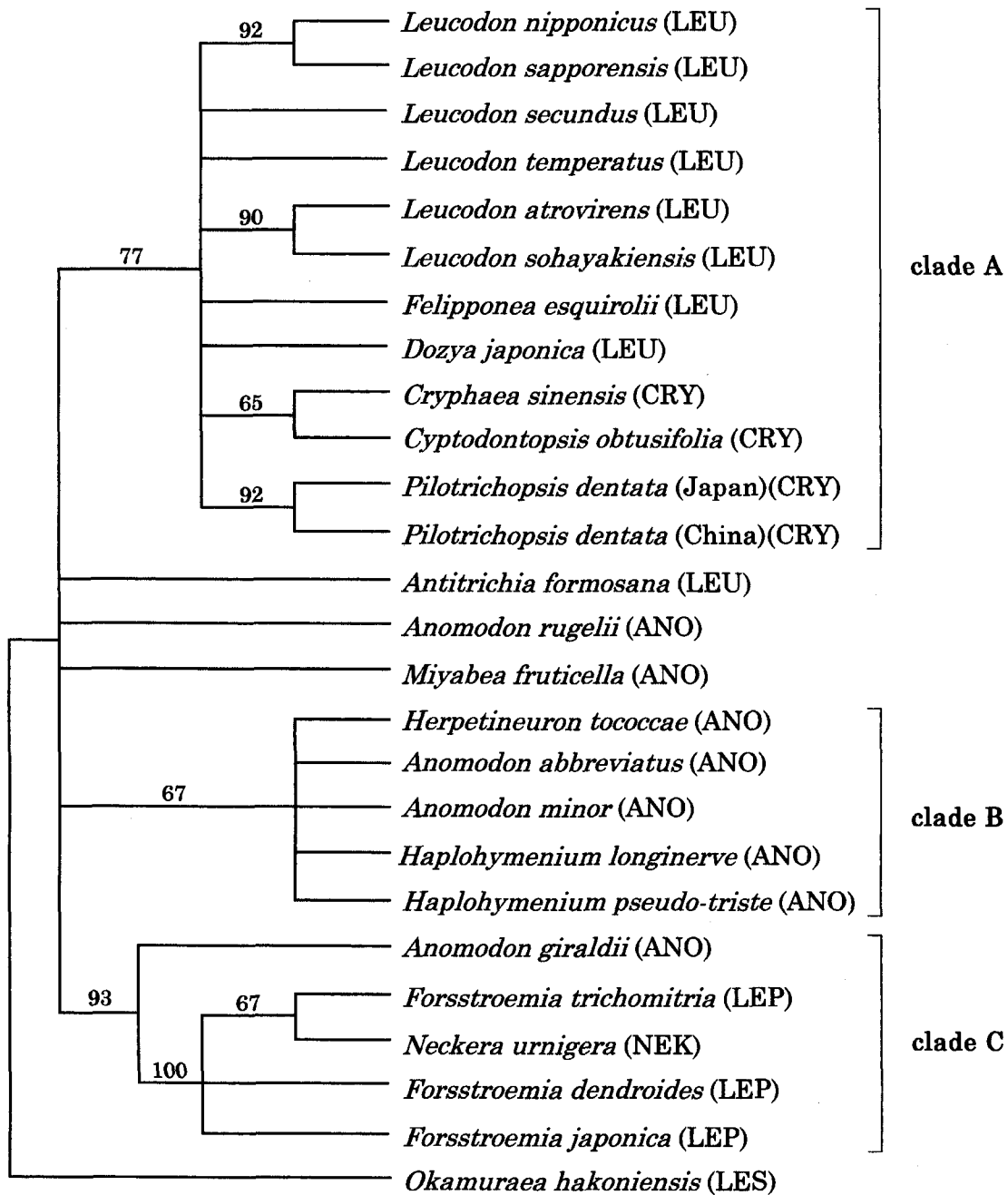


Fig. 1. Strict consensus of 1,217 most parsimonious trees based on the *rbcL* sequences data from 25 taxa in the suborder Leucodontineae (length=282, CI=0.652, RI=0.678). Numbers beside branches are bootstrap values (shown only those over 60% in 1,000 replicates). Abbreviation in parentheses: LEU; Leucodontaceae, CRY; Cryphaeaceae, ANO; Anomodontaceae, LEP; Leptodontaceae, NEK; Neckeraaceae, LES; Leskeaceae.

costa in the leaves, a white-colored peristome, and a reduced endostome (Akiyama 1988a, b). On the other hand, *Dozya* has a number of unique features in the Leucodontaceae, such as a single costa reaching nearly to the leaf apex and presence of the parastome. Thus, the genus had been regarded as distantly related to other members of the Leucodontaceae (Buck and Vitt 1986, Akiyama 1987, 1994). Our present results, however, could

not resolve the inter-relationships between these three genera.

#### Familial affinity of *Forsstroemia*

*Forsstroemia* can be characterized by rhomboidal lamina cell, differentiation of an alar region having quadrate cells, smooth exostome teeth, and a reduced endostome. However, it shows considerable variation in morphology among

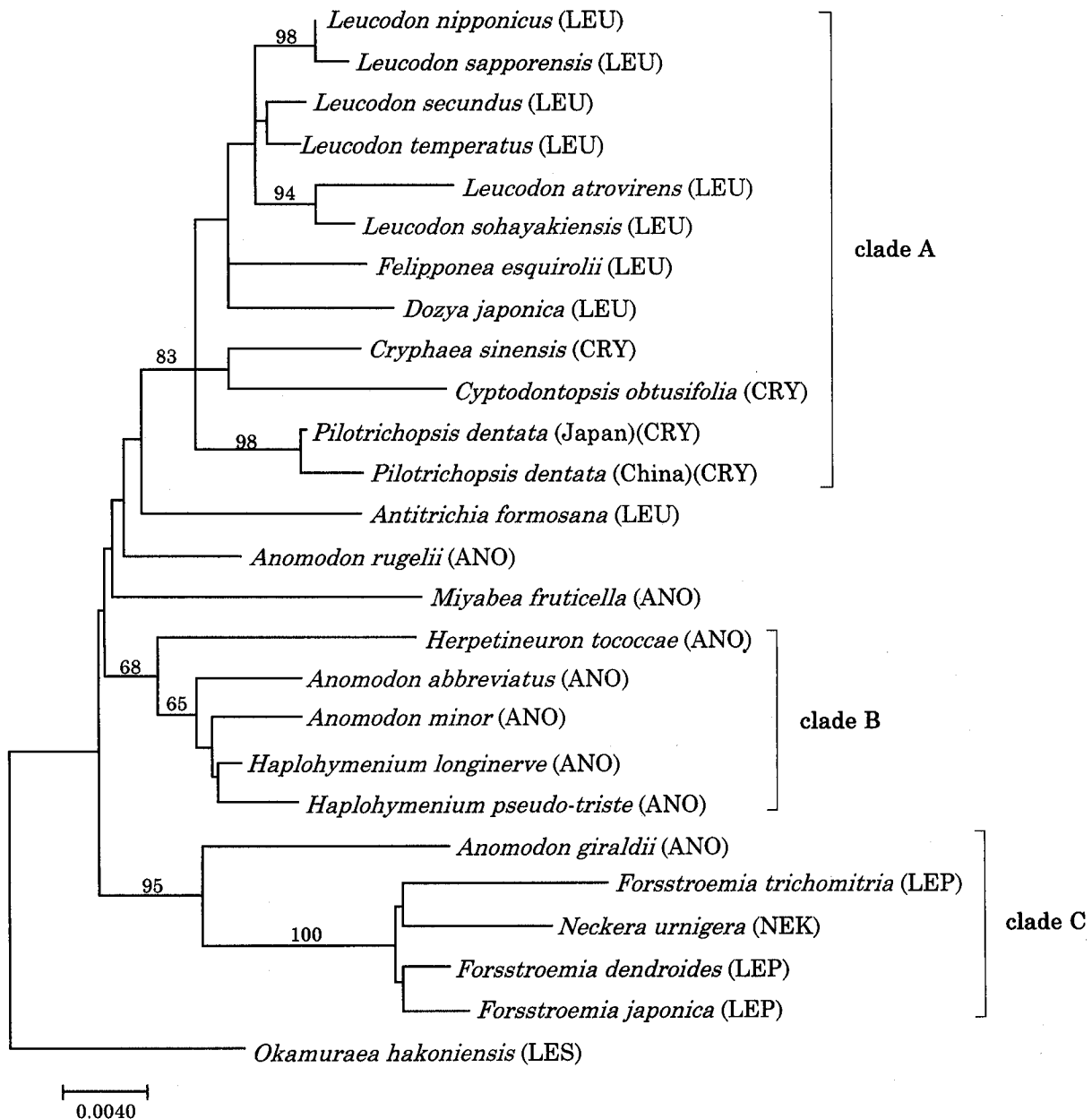


Fig. 2. Neighbor joining tree based on the *rbcL* sequences from 25 taxa in the suborder Leucodontineae by Kimura's 2-parameter distance. Numbers beside branches are bootstrap values (shown only those over 60% in 10,000 replicates). Abbreviations of family names in parentheses are presented in Fig. 1

species; some are characterized by leaves with a single costa and deeply immersed capsules among the perichaetial leaves, while others have a very short, sometimes scarcely developed, double-forked costa and more or less exerted capsules. Species having the former features show a morphology characteristic of the Cryphaeaceae, while species with the latter features show a morphology characteristic of the Leucodontaceae, and this is the reason why the genus has been variously classified both in the Cryphaeaceae (Brotherus 1925, Manuel 1982) or Leucodontaceae (Vitt 1984, Akiyama 1988a, 1994). In addition, Buck (1980) proposed to classify *Forsstroemia* in the

Leptodontaceae, and several authors have followed his treatment (Buck and Vitt 1986, Stark 1987).

Present *rbcL* trees indicated that three species of *Forsstroemia* were closely related neither to the Leucodontaceae nor Cryphaeaceae, and thus both systems to classify the genus either in the Leucodontaceae or Cryphaeaceae were not supported. On the contrary, they formed a monophyletic clade with *Neckera urnigera* (Neckeraceae). To interpret the results, it is relevant to note that *Leptodon* (type genus of the Leptodontaceae) was once classified in the Neckeraceae (Brotherus 1925). Since we have not analyzed *rbcL* sequences of *Leptodon* itself, the systematic position of

*Forsstroemia* still remains ambiguous. Both morphological (Buck 1980) and the present molecular evidence, however, suggest that *Forsstroemia* have close affinity to the Neckeraceae or Leptodontaceae.

#### *Polyphyletic nature of Anomodontaceae and Anomodon*

Brotherus (1925) recognized six genera, *Anomodon*, *Miyabea*, *Haplohymenium*, *Herpetineuron*, *Claopodium*, and *Haplocladium* in the subfamily Anomodontoideae of the Thuidiaceae. Watanabe (1972) regarded the absence of paraphyllia and a weakly developed peristome as important characters to define the subfamily, and excluded *Claopodium* and *Haplocladium* to the subfamily Thuidioideae. The other four genera were left in the Anomodontoideae. Buck and Vitt (1986) raised the Anomodontoideae to a distinct family (Anomodontaceae), and included six genera there (*Anomodon*, *Haplohymenium*, *Herpetineuron*, *Thelia*, *Myurella*, *Lindbergia*). Short lamina cells with distinct papillae characterize all of the six genera (lamina cells of *Herpetineuron* lack papillae, but some of them are supplied with unique proration, see Akiyama 1993).

Present *rbcL* data suggest that the Anomodontaceae is polyphyletic, i.e. some *Anomodon* species formed clade B with *Haplohymenium* and *Herpetineuron*, while *A. giraldii* was included in clade C (*A. rugelii* and *Miyabea* remain unresolved). Additional 11 steps were required to make all of the members of the Anomodontaceae monophyletic.

*Miyabea* is characterized by a total lack of pseudoparaphyllia, smooth lamina cells, a short costa reaching to half the leaf length, erect capsules, and a reduced peristome. These features suggest its relationship to *Forsstroemia* (Leptodontaceae) rather than to the Anomodontaceae, where almost all authors have placed it. From the present results, the relationship of *Miyabea* to the Anomodontaceae or *Forsstroemia* remains unresolved.

Watanabe (1972) has previously pointed out the close affinity of *Haplohymenium* and *Herpetineuron* to *Anomodon*. Based on a cladistic analysis of morphological characters, Granzow-de la Cerda (1992, 1997) proposed to merge *Haplohymenium* within *Anomodon*. The present *rbcL* data suggest that both genera, *Herpetineuron* and *Haplohymenium*, have a close affinity with *Anomodon minor* and *A. abbreviatus*, and thus support their hypothesis.

Watanabe (1972) classified *Anomodon giraldii* in the subgenus *Pseudo-Anomodon* based on its unique morphological features, such as dendroid branching, an acute leaf apex, a rather high basal membrane of the endostome with distinct segments, and weakly differentiated annuli, all of which suggest distant relationship to the other members of the genus. From the cladistical analysis based on morphological features, Granzow-de la Cerda (1992, 1997) later proposed to locate the species at the most basal position among the clade of *Anomodon*. Though it is clear that the species are highly diversified within the genus, both authors still regarded *A. giraldii* as a member of *Anomodon*. In the present analyses, however, *A. giraldii* formed a cluster with *Forsstroemia* and *Neckera*, and *A. rugelii* remains unresolved in the *rbcL* trees. Additional eight steps were needed to

make all of the *Anomodon* species examined monophyletic. The genus *Anomodon* therefore appears to be polyphyletic.

#### *Relationship between Cryphaeaceae and Leucodontaceae*

Most previous authors held that the Leucodontaceae and Cryphaeaceae were closely related to each other on the basis of shared morphological features, such as short-rhomboidal lamina cells, well-developed alar regions, reduced peristome, and upright capsules (e.g. Brotherus 1925, Manuel 1982). On the other hand, noticing that the Cryphaeaceae shared with the Leptodontaceae and Anomodontaceae a single costa in leaf and an unique phenological pattern (the embryo overwinters prior to elongation of seta) in addition to the above features, Buck and Vitt (1986) pointed out close relationship among these three families and included the Cryphaeaceae in the Cryphaeacanae along with Leptodontaceae and Anomodontaceae. They considered the ecostate leaves of the Leucodontaceae to suggest its distant relationship to the Cryphaeaceae, and classified the Leucodontaceae in the different superfamily Leucodontacanae. Our *rbcL* data did not support the classification proposed by Buck and Vitt (1986) to separate the Leucodontaceae and Cryphaeaceae in different superfamilies. On the contrary, the data suggested closer relationship between the Leucodontaceae and Cryphaeaceae, if comparing with relationship between the Cryphaeaceae and Leptodontaceae or Anomodontaceae. We consider the two families to form a monophyletic group.

In contrast with morphological heterogeneity found in the Leucodontaceae as discussed in the previous section, members of the Cryphaeaceae are comparatively uniform in those features (a single and long costa, smooth and short rhomboidal lamina cells, reduced endostome, deeply immersed capsules among perichaetial leaves). Thus, the family have been regarded as a well-defined monophyletic group. In this consequence, it is rather perplexing that the three genera (*Cryphaea*, *Cyrtodontopsis*, and *Pilotrichopsis*), all of which have long been classified in the Cryphaeaceae, do not form a monophyletic clade in the present analyses. Since we could examine only a small number of members of the family, their interrelationships, especially to the Pterobryaceae, should be left for a future subject.

The *rbcL* sequence data provide us another basis to reconstruct relationships among moss families, and they have proved to be a useful tool to test hypotheses on higher-level relationships (orders and families) that have been proposed totally on the basis of morphological and anatomical comparison. Our present results also suggest that the Leucodontaceae, Anomodontaceae, and *Anomodon*, all of which have long been regarded monophyletic, represent polyphyletic assemblages.

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