

Molecular phylogeny of the Grimmiales (Musci) based on chloroplast *rbcL* sequences¹

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Familial circumscriptions of the Grimmiales and their allies, including the Grimmiaceae, the Ptychomitriaceae and the Seligeriaceae, have been repeatedly discussed. Here we present phylogenetic inferences on the Grimmiaceae and their allies based on maximum likelihood analyses of chloroplast *rbcL* gene sequences. Our study shows that (1) the Grimmiaceae (s. lat.) with the inclusion of the genera *Campylostelium* and *Ptychomitrium* is monophyletic; (2) *Glyphomitrium* has a close affinity to *Arctoa* of the Dicranales; (3) the Seligeriaceae form a distinct clade sister to the Grimmiaceae (s. lat.); (4) the Grimmiales (s. lat.) with the inclusion of the Seligeriaceae are monophyletic when the Drummondiaceae, the Scouleriaceae and the Wardiaceae are omitted; and (5) the Drummondiaceae–Scouleriaceae clade appears at the basal position of the haplolepidous moss group Dicranidae, adjacent to the Bryoxiphiaeae. Since the monophyly of the Grimmiales (s. lat.) including members of the Seligeriaceae is well supported, we propose here that the order can be treated in a wide sense, and includes families which have in common the *Grimmia/Seligeria* type peristome.

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The order Grimmiales is one of the basal members of the haplolepidous mosses, accommodating the two families Grimmiaceae and Ptychomitriaceae. The order Seligerales, accommodating the family Seligeriaceae, is also one of the basal orders. Circumscription and taxonomic position of the families Grimmiaceae, Ptychomitriaceae, Seligeriaceae and their allies have been repeatedly discussed. After the first description of the Grimmiaceae as an independent family by Arnott (1825), many workers variously proposed their systems on the Grimmiaceae and their allies (Table 1). Brotherus (1901–1902) placed the

Grimmiaceae (as Grimmieae) and Ptychomitriaceae (as Ptychomitrieae) in the Grimmiales (as Grimmiaceae) in the first edition of “Die natürlichen Pflanzenfamilien”. Subsequently, Brotherus (1924–1925) transferred the Ptychomitriaceae to the Isobryales. Nyholm (1954–1960) placed the Ptychomitriaceae in the Eubryales, in which are included the families primarily with a diplolepidous peristome: Bryaceae, Mniaceae, Aulacomniaceae, Meesiaceae, Timmiaceae, Catoscopiaceae, Bartramiaceae, Orthotrichaceae, and Hedwigiaeae. Noguchi (1954) discussed the relationship between *Ptychomitrium*, *Grimmia* and *Racomitrium* based on leaf structures, especially ornamentation of leaf cells, and placed *Ptychomitrium* in the Grimmiaceae. Noguchi (1974) suggested

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that *Racomitrium* is an intermediate genus between *Ptychomitrium* and *Grimmia* on the basis of the leaf cells and exothelial cell development. Deguchi (1979) treated *Ptychomitrium* as a member of the Grimmiaceae emphasizing the morphological and anatomical affinity of calyptra, leaf cells and seta twisting. Vitt (1984), and Buck and Goffinet (2000) treated the Ptychomitriaceae as an independent family of the Grimmiaceae. In recent years, several molecular phylogenetic works relevant to the phylogeny of haplolepidous mosses have been carried out (La Farge & coworkers 2000, 2002; Goffinet *et al.* 2001), showing the monophyly of the Grimmiales and Seligeriales, although the numbers of OTUs for these orders seem to be not enough to clarify and discuss their phylogenetic positions within the system.

The objectives of this study were: (1) to construct a phylogenetic tree for the Grimmiaceae, the Seligeriaceae and their allies based on maximum likelihood criteria; (2) to test the monophyly of the families and clarify the familial and generic relationships. We present here phylogenetic inferences for the families previously treated in the Grimmiales and Seligeriales, based on the maximum-likelihood (ML) analyses of 90 sequences of the chloroplast ribulose 1,5-bisphosphate carboxylase/oxygenase large subunit (*rbcL*), together with recent information on the molecular phylogeny of grimmialean mosses, and discuss the implications of our findings on the Grimmiaceae and their related families.

Materials and methods

Phylogenetic analyses based on the *rbcL* gene sequences were performed following the methods of Tsubota and coworkers (1999, 2001a, 2002). The method consists of two steps: (1) determining sequence data (DNA extraction, PCR amplification and DNA sequencing, or DNA database homology search); and (2) data analyses.

DNA Extraction, PCR Amplification and DNA Sequencing

Total DNA's were extracted from fresh samples or dried 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 segments for *rbcL* gene, and direct sequence analyses were carried out using standard conditions according to the manufacturer's instructions. Reactions were performed in a thermal cycler (Thermal Cycler 9600, Perkin Elmer) with PCR amplification kits and synthetic primers (Tsubota *et al.* 2001b). Direct DNA sequence analysis of the PCR products was performed using ABI or Amersham kits according to the manufacturer's instructions. The sequences were electrophoresed on automated sequencers (ABI 373A and ABI PRISM 310), and analyzed on the data-analysis systems. 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 Tsubota and coworkers (1999, 2000, 2001a, b).

Sources and Analyzed Data

Data set—90 *rbcL* gene sequences for 86 moss species were examined for the analyses (Table 2, with DDBJ/EMBL/GenBank Accession Nos.). The *rbcL* gene sequences of the grimmialean and seligerialean mosses were manually aligned with 71 moss sequences registered in the DNA database. Outgroup taxa were selected based on the phylogenetic hypothesis proposed by La Farge *et al.* (2000) and Tsubota *et al.* (in ms., and the abstract of the MO Symposium 2003).

Alignment—Manual alignment was carried out for the dataset against a complete *rbcL* sequence of *Leucobryum scabrum* (AB029388) with an editor program and our original program, Sclean 2.87 (Tsubota 1998–2003, in our http sites, see Appendix B). Undetermined sites, gaps, and regions not clearly alignable for all sequences were excluded from the analyses. Among sequences aligned, identical sequences are represented by a single sequence, and an 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; Fitch 1971), and the maximum-likelihood (ML; 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, see Appendix B).

Distance analysis—A NJ tree was constructed with NucML and NJdist in MOLPHY using the 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 NucML in MOLPHY and fastDNAml 1.2.2 (Olsen *et al.* 1994). In ML analyses, the best-conformed transition/transversion (Ts/Tv) parameter was estimated by NucML according to calculations with the HKY85 model employing the “-topt” option parameter as 2.093 (as 4.185 in the NucML notational system). ML trees were constructed with the local rearrangement search from the NJ tree by NucML and NJdist. In analyses by fastDNAml, ML trees were searched with the global rearrangements options. The Ts/Tv parameter was then used as

Table 1. Classifications of the Grimmiaceae, Ptychomitriaceae and Seligeriaceae, or parts of the family, and possibly related genera. The table shows the distribution of families and genera that have been considered to belonging to the Grimmiales and Seligerales at one time or another and included in the present analysis.

| Bruch & Schimper (1837–1846) | Schimper (1876) | Limprecht (1885–1889) | Brotherus (1901–1902) | Fleischer (1915–1923) |
|---------------------------------|-----------------------|--|------------------------|-----------------------|
| Seligeriaceae | Seligeriaceae | Seligeriaceae | Dicranaceae | Dicraales |
| <i>Seligeria</i> | <i>Seligeriae</i> | <i>Seligeria</i> | <i>Seligeriae</i> | <i>Seligeriaceae</i> |
| <i>Blindia</i> | <i>Seligeria</i> | <i>Blindia</i> | <i>Seligeria</i> | <i>Seligeria</i> |
| <i>Campylostelium</i> | <i>Blindiae</i> | | <i>Blindia</i> | <i>Blindia</i> |
| Orthotrichaceae | Brachydontea | Campylosteliaceae | Grimmiaceae | Grimmiales |
| <i>Ptychomitrium</i> | <i>Campylostelium</i> | <i>Brachydontium</i> | <i>Ptychomitriæ</i> | <i>Grimmiaceæ</i> |
| <i>Glyphomitrium</i> | | <i>Campylostelium</i> | <i>Glyphomitrium</i> | <i>Scoleuria</i> |
| <i>Coscinodon</i> | Grimmiaceae | Grimmiaceae | <i>Ptychomitrium</i> | <i>Coscinodon</i> |
| Grimmiaceae | Grimmiæ | Grimmiæ | <i>Euglyphomitrium</i> | <i>Schistidium</i> |
| <i>Schistidium</i> | <i>Grimmia</i> | <i>Cinclidontæ</i> | <i>Campylostelium</i> | <i>Grimmia</i> |
| <i>Grimmia</i> | <i>Racomitrium</i> | <i>Cinclidotus</i> (<i>Scoleuria</i>) | <i>Scouleriae</i> | <i>Racomitrium</i> |
| <i>Racomitrium</i> | Ptychomitriæ | Grimmieæ | <i>Scouleria</i> | |
| | <i>Coscinodon</i> | <i>Schistidium</i> | Grimmieæ | Isobryales |
| | <i>Ptychomitrium</i> | <i>Coscinodon</i> | <i>Coscinodon</i> | Erpodiaeæ |
| | <i>Glyphomitrium</i> | <i>Grimmia</i> | <i>Grimmia</i> | <i>Erpodium</i> |
| | | <i>Racomitrium</i> | <i>Grimmia</i> | <i>Venturiella</i> |
| | | Ptychomitriæ | <i>Schistidium</i> | Orthotrichaceæ |
| | | <i>Brachysteleum</i> | <i>Racomitrium</i> | <i>Glyphomitrium</i> |
| | | (<i>Ptychomitrium</i>) | | <i>Campylostelium</i> |
| | | (<i>Glyphomitrium</i>) | | |
| | | (<i>Campylostelium</i>) | | |

| Dixon (1924) | Brotherus (1924–1925) | Jones (1933) in Grout, & Grout (1936) | Nyholm (1954–1960) | Churchill (1981) |
|-----------------------|-----------------------|--|----------------------|-------------------------|
| Dicranaceae | Dicraales | Bryales | Dicraales | Grimmiaceæ |
| <i>Seligeriæ</i> | <i>Seligeriaceæ</i> | <i>Seligeriaceæ</i> | <i>Seligeriæ</i> | <i>Grimmoideæ</i> |
| <i>Seligeria</i> | <i>Seligeria</i> | <i>Seligeria</i> | <i>Blindia</i> | <i>Grimmia</i> |
| Dicraneæ | <i>Blindia</i> | <i>Blindia</i> | <i>Seligeria</i> | <i>Schistidium</i> |
| <i>Blindia</i> | | | <i>Brachydontium</i> | <i>Coscinodontoideæ</i> |
| Grimmiaceæ | Grimmiales | Grimmiaceæ | Grimmiales | <i>Coscinodon</i> |
| <i>Grimmia</i> | Grimmiæ | Grimmiæ | Grimmiæ | Ptychomitriodeæ |
| <i>Racomitrium</i> | Scolelioideæ | <i>Glyphomitrium</i> | <i>Grimmia</i> | <i>Racomitrium</i> |
| <i>Coscinodon</i> | <i>Scoleuria</i> | <i>Grimmia</i> | <i>Schistidium</i> | <i>Campylostelium</i> |
| <i>Ptychomitrium</i> | Grimmoideæ | <i>Scoleuria</i> | <i>Coscinodon</i> | <i>Ptychomitrium</i> |
| <i>Glyphomitrium</i> | <i>Coscinodon</i> | <i>Scoleuria</i> | <i>Grimmia</i> | Scouleriaceæ |
| <i>Campylostelium</i> | <i>Grimmia</i> | Ptychomitriæ | <i>Racomitrium</i> | <i>Scoleuria</i> |
| | <i>Schistidium</i> | <i>Campylostelium</i> | Eubryales | |
| | <i>Racomitrium</i> | <i>Ptychomitrium</i> | <i>Ptychomitriæ</i> | |
| | | <i>Racomitrium</i> | <i>Glyphomitrium</i> | |
| | | | <i>Ptychomitrium</i> | |
| Isobryales | | | | |
| Ptychomitriaceæ | | | | |
| <i>Campylostelium</i> | | | | |
| <i>Glyphomitrium</i> | | | | |
| <i>Ptychomitrium</i> | | | | |

Table 1. Continued.

| Vitt (1984) | Noguchi et al. (1987–1988) | Iwatsuki (1991) Iwatsuki et al. (2001) | Buck & Goffinet (2000) |
|-------------------------|-------------------------------|---|-------------------------|
| Orthotrichineae | Seligeriaceae | Dicranales | Grimmiales |
| Erpodiaceae | <i>Blindia</i> | Seligeriaceae | Grimmiaceae |
| <i>Aulacopilum</i> | <i>Seligeria</i> | <i>Blindia</i> | <i>Aligrimmia</i> |
| <i>Erpodium</i> | <i>Brachydontium</i> | <i>Seligeria</i> | <i>Coscinodon</i> |
| <i>Venturiella</i> | | <i>Brachydontium</i> | <i>Coscinodontella</i> |
| <i>Wildia</i> | | | <i>Dryptodon</i> |
| | | | <i>Grimmia</i> |
| Leucodontineae | | Grimmiales | <i>Indusiella</i> |
| Wardiaceae | <i>Grimmia</i> | Grimmiaceae | <i>Jaffueliobryum</i> |
| <i>Wardia</i> | <i>Racomitrium</i> | <i>Grimmia</i> | <i>Leucoperchaetum</i> |
| | <i>Ptychomitrium</i> | <i>Schistidium</i> | <i>Racomitrium</i> |
| | <i>Campylostelium</i> | <i>Coscinodon</i> | <i>Schistidium</i> |
| Seligeriinae | | <i>Racomitrium</i> | |
| Seligeriaceae | Erpodiaceae | <i>Ptychomitrium</i> | Ptychomitriaceae |
| <i>Blindia</i> | | <i>Campylostelium</i> | <i>Campylostelium</i> |
| <i>Brachydontium</i> | <i>Venturiella</i> | | <i>Glyphomitrium</i> |
| <i>Hymenolomopsis</i> | <i>Aulacopilum</i> | | <i>Ptychomitriopsis</i> |
| <i>Seligeria</i> | <i>Glyphomitrium</i> | Erpodiaceae | <i>Ptychomitrium</i> |
| <i>Stylostegium</i> | | <i>Glyphomitrium</i> | Scouleriaceae |
| <i>Trochobryum</i> | | <i>Venturiella</i> | <i>Scouleria</i> |
| <i>Verrucidens</i> | | <i>Aulacopilum</i> | <i>Tridontium</i> |
| | | | Drummondiaeae |
| Grimmiineae | | | <i>Drummondia</i> |
| Ptychomitriaceae | | | |
| <i>Campylostegium</i> | | | Seligeriales |
| <i>Glyphomitrium</i> | | | Seligeriaceae |
| <i>Ptychomitriopsis</i> | | | <i>Blindia</i> |
| <i>Ptychomitrium</i> | | | <i>Brachydontium</i> |
| Grimmiaceae | | | <i>Hymenolomopsis</i> |
| <i>Aligrimmia</i> | | | <i>Seligeria</i> |
| <i>Coscinodon</i> | | | <i>Trochobryum</i> |
| <i>Coscinodontella</i> | | | Wardiaceae |
| <i>Dryptodon</i> | | | <i>Wardia</i> |
| <i>Grimmia</i> | | | |
| <i>Hydrogrimmia</i> | | | |
| <i>Indusiella</i> | | | |
| <i>Jaffueliobryum</i> | | | |
| <i>Pseudohypophila</i> | | | |
| <i>Racomitrium</i> | | | |
| <i>Schistidium</i> | | | |
| <i>Scouleria</i> | | | |

2.093 for the analyses by fastDNAml.

Tree comparison with log-likelihood and several tests—Tree comparison with the ML criteria was carried out by NucML to evaluate the resulting trees. Several measures were used estimating the log-likelihood of different tree topologies, the standard errors (SEs) of the difference in log-likelihood (Kishino & Hasegawa 1989), and the bootstrap probability estimated by the RELL (resampling of estimated log-likelihood of sites) method (Kishino et al. 1990; Hasegawa & Kishino 1994). The program package CONSEL 0.1f (Shimodaira & Hasegawa 2001) was also used to calculate the *p*-values of the confidence for the bifurcating candidate topologies using several testing pro-

cedures: the approximately unbiased (AU) test (Shimodaira 2000, 2002) using the multiscale bootstrap technique; and the Bayesian posterior probability (PP) calculated by the Bayesian information criterion (BIC) approximation (Schwarz 1978; Hasegawa & Kishino 1989).

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

Table 2. List of species and families investigated for *rbcL* gene with the accession number and voucher or reference of the sequences. Treatment of families follows Buck and Goffinet (2000). Further information for the sequences obtained in the present study is shown in Appendix A

| Class/Order/Family/Species | Accession No. | References/Origin |
|---|--|---------------------------|
| Bryopsida | | |
| Grimmiaceae | | |
| <i>Grimmiaceae (s. str.)</i> | | |
| <i>Coscinodon cribrosus</i> (Hedw.) Spruce | AB125575 | This study |
| <i>Grimmia apiculata</i> Hornsch. | AB125576 | This study |
| <i>Grimmia elongata</i> Kaulf. | AB125577 | This study |
| <i>Grimmia laevigata</i> (Brid.) Brid. | AF231081 | Mishler et al. (Database) |
| <i>Grimmia laevigata</i> (Brid.) Brid. | AF478230 | Magombo (2003) |
| <i>Grimmia orbicularis</i> Bruch | AB125578 | This study |
| <i>Grimmia pilifera</i> P.Beauv. | AB125579 | This study |
| <i>Grimmia pulvinata</i> (Hedw.) Sm. | AB125580 | This study |
| <i>Racomitrium fasciculare</i> (Hedw.) Brid. | AF231082 | Mishler et al. (Database) |
| <i>Racomitrium fasciculare</i> var. <i>atroviride</i> Cardot | AB125581 | This study |
| <i>Racomitrium japonicum</i> Dozy & Molk. | AB125582 | This study |
| <i>Schistidium apocarpum</i> (Hedw.) Bruch & Schimp. | AF231065 [as <i>Grimmia apocarpa</i>] | Mishler et al. (Database) |
| <i>Schistidium strictum</i> (Turner) Loeske ex Martensson | AB125583 | This study |
| Ptychomitriaceae | | |
| <i>Campylostelium saxicola</i> (F.Weber & D.Mohr) Bruch & Schimp. | AB125584 | This study |
| <i>Glyphomitrium humillimum</i> (Mitt.) Cardot | AB125585 | This study |
| <i>Glyphomitrium minutissimum</i> (S.Okamura) Broth. | AB125586 | This study |
| <i>Ptychomitrium drummondii</i> (Wilson) Sull. & Lesq. | AF226821 | Wheeler et al. (Database) |
| <i>Ptychomitrium gardneri</i> Lesq. | AF005549 | Goffinet et al. (1998) |
| <i>Ptychomitrium gardneri</i> Lesq. | AF231313 | La Farge et al. (2000) |
| <i>Ptychomitrium dentatum</i> (Mitt.) A.Jaeger | AB125587 | This study |
| <i>Ptychomitrium wilsonii</i> Sull. & Lesq. | AB125588 | This study |
| Scouleriaceae | | |
| <i>Scouleria aquatica</i> Hook. | AF226822 | Wheeler et al. (Database) |
| Drummondiaeae | | |
| <i>Drummondia obtusifolia</i> Müll.Hal. | AF232697 | Cox et al. (2000) |
| <i>Drummondia prorepens</i> (Hedw.) E.Britton | AF005542 | Goffinet et al. (1998) |
| Seligeriales | | |
| Seligeriaceae | | |
| <i>Blindia acuta</i> (Hedw.) Bruch & Schimp. | AF226817 | Wheeler et al. (Database) |
| <i>Blindia magellanica</i> Schimp. | AF478232 | Magombo (2003) |
| <i>Blindia japonica</i> Broth. | AB125590 | This study |
| <i>Seligeria austriaca</i> T.Schauer | AB125589 | This study |
| Wardiaceae | | |
| <i>Wardia hygrometrica</i> Harv. & Hook. | AJ275170 | Cox et al. (2000) |
| Dicranales | | |
| Bryoxiphiaeae | | |
| <i>Bryoxiphium norvegicum</i> (Brid.) Mitt. | AF231294 | La Farge et al. (2000) |
| Fissidentaceae | | |
| <i>Fissidens dubius</i> P.Beauv. | AF231303 | La Farge et al. (2000) |
| Dicranaceae | | |
| <i>Arctoa falcata</i> (Hedw.) Loeske | AF231292 | La Farge et al. (2000) |
| <i>Arctoa fulvella</i> (Dicks.) Bruch & Schimp. | AF231293 | La Farge et al. (2000) |
| <i>Brothera leana</i> (Sull.) Müll.Hal. | AB122033 | Oguri et al. (2003) |
| <i>Campylopus argyrocaulon</i> (Müll. Hal.) Broth. | AF231315 | La Farge et al. (2000) |
| <i>Cynodontium jenneri</i> (Schimp.) Stirn. | AF231318 | La Farge et al. (2000) |
| <i>Dicranella heteromalla</i> (Hedw.) Schimp. | AF231296 | La Farge et al. (2000) |
| <i>Dicranodontium denudatum</i> (Brid.) E.Britton | AB122034 | Oguri et al. (2003) |
| <i>Dicranoweisia cirrata</i> (Hedw.) Lindb. | AF231297 | La Farge et al. (2000) |
| <i>Dicranum scoparium</i> Hedw. | AF478226 | Magombo (2003) |
| <i>Kiaeria blyttii</i> (Bruch & Schimp.) Broth. | AF231306 | La Farge et al. (2000) |
| <i>Leucoloma rutenbergii</i> (Müll.Hal.) C.H.Wright | AF231308 | La Farge et al. (2000) |
| <i>Octolepharum albidum</i> Hedw. | AF226795 | Wheeler et al. (Database) |
| <i>Oncophorus wahlenbergii</i> Brid. | AF231310 | La Farge et al. (2000) |

Table 2. continued.

| Class/Order/Family/Species | Accession No. | References/Origin |
|--|---------------|-------------------------------|
| Dicranaceae <i>Orthodicranum fulvum</i> (Hook.) G.Roth | AF231311 | La Farge et al. (2000) |
| Dicnemonaceae <i>Eucamptodon muelleri</i> Hampe & Müll.Hal. | AF231319 | La Farge et al. (2000) |
| Leucobryaceae <i>Leucobryum scabrum</i> Sande Lac. | AB029388 | Tsubota et al. (1999) |
| Ditrichaceae <i>Ditrichum pallidum</i> (Hedw.) Hampe | AF231302 | La Farge et al. (2000) |
| <i>Pleuridium acuminatum</i> Lindb. | AF231312 | La Farge et al. (2000) |
| Rhabdoweisiaceae <i>Rhabdoweisia crenulata</i> (Mitt.) H.Jameson | AF005544 | Goffinet et al. (1998) |
| Erpodiaceae <i>Aulacopilum hodgkinsoniae</i> (Hampe & Müll.Hal.) Broth. | AF005545 | Goffinet et al. (1998) |
| <i>Venturiella sinensis</i> (Vent.) Müll.Hal. | AB125591 | This study |
| Pottiales | | |
| Pottiaceae <i>Barbula convoluta</i> Hedw. | AF478225 | Magombo (2003) |
| <i>Pottia intermedia</i> (Turner) Fürnr. | AB125592 | This study |
| <i>Pseudosymblepharis schimperiana</i> (Paris) H.A.Crum | AF226805 | Wheeler et al. (Database) |
| <i>Tortula obtusissima</i> (Müll.Hal.) Mitt. | AF226823 | Wheeler et al. (Database) |
| <i>Tortula ruralis</i> (Hedw.) P.Gaertn., B.Mey. & Scherb. | AJ275169 | Cox et al. (2000) |
| Calymperaceae <i>Calymperes afzelii</i> Sw. | AF226789 | Wheeler et al. (Database) |
| <i>Leucophanes albescens</i> Müll.Hal. | AF226798 | Wheeler et al. (Database) |
| <i>Mitthyridium obtusifolium</i> (Lindb.) H.Rob. | AF226777 | Wheeler et al. (Database) |
| <i>Syrrhopodon leprieurii</i> Mont. | AF226783 | Wheeler et al. (Database) |
| Orthotrichales | | |
| Orthotrichaceae <i>Amphidium lapponicum</i> (Hedw.) Schimp. | AF005543 | Goffinet et al. (1998) |
| <i>Bryodixonia perichaetialis</i> Sainsbury | AF005541 | Goffinet et al. (1998) |
| <i>Cardiotella quinquefaria</i> (Hornschr.) Vitt | AF005523 | Goffinet et al. (1998) |
| <i>Groutiella apiculata</i> (Hook.) H.A.Crum & Steere | AF005527 | Goffinet et al. (1998) |
| <i>Macrocomita tenuis</i> (Hook. & Grev.) Vitt | AF005524 | Goffinet et al. (1998) |
| <i>Macromitrium incurvifolium</i> (Hook. & Grev.) Schwägr. | AF005528 | Goffinet et al. (1998) |
| <i>Orthotrichum obtusifolium</i> Brid. | AF005537 | Goffinet et al. (1998) |
| <i>Schlotheimia brownii</i> Schwägr. | AF005522 | Goffinet et al. (1998) |
| <i>Uleastrum palmicola</i> (Müll.Hal.) R.H.Zander | AF005547 | Goffinet et al. (1998) |
| <i>Ulota lutea</i> (Hook.f. & Wilson) Mitt. | AF005540 | Goffinet et al. (1998) |
| <i>Zygodon intermedius</i> Bruch & Schimp. | AF005532 | Goffinet et al. (1998) |
| Hedwigiales | | |
| Hedwigiaceae <i>Hedwigia ciliata</i> (Hedw.) P.Beauv. | AF478234 | Magombo (2003) |
| <i>Hedwigia ciliata</i> (Hedw.) P.Beauv. | AF005517 | Goffinet et al. (1998) |
| Bryales | | |
| Bartramiaceae <i>Bartramia halleriana</i> Hedw. | AF231090 | De Luna et al. (2000) |
| Bryaceae <i>Bryum capillare</i> Hedw. | AY163027 | Pedersen & Hedenäs (Dababase) |
| Mniaceae <i>Plagiomnium japonicum</i> (Lindb.) T.J.Kop. | AB050992 | Tsubota et al. (2001a) |
| Rhizogonales | | |
| Hypnodendraceae <i>Hypnodendron menziesii</i> (Hook.) Paris | AF231093 | Mishler et al. (Database) |
| Hookerales | | |
| Daltoniaceae <i>Benitotania elimbata</i> H.Akiy., T.Yamag. & Suleiman | AB103351 | Akiyama et al. (2003) |
| Hypnales | | |
| Hypnaceae <i>Hypnum plumaeforme</i> Wilson | AB029384 | Tsubota et al. (1999) |
| Leucodontaceae <i>Dozya japonica</i> Sande Lac. | AB125593 | This study |

Table 2. continued.

| Class/Order/Family/Species | Accession No. | References/Origin |
|--|---|-----------------------------|
| Outgroup taxa | | |
| Funariales | | |
| Funariaceae | | |
| <i>Funaria hygrometrica</i> Hedw. | AF005513 | Goffinet et al. (1998) |
| <i>Physcomitrella patens</i> (Hedw.) Bruch & Schimp. | AB066207 | Sugita & Sugiura (Database) |
| Encalyptales | | |
| Encalyptaceae | | |
| <i>Encalypta streptocarpa</i> Hedw. | AF478239 | Magombo (2003) |
| Timmiales | | |
| Timmiaceae | | |
| <i>Timmia austriaca</i> Hedw. | AJ275185 | Cox et al. (2000) |
| <i>Timmia megapolitana</i> Hedw. | AF478242 | Magombo (2003) |
| Diphysciales | | |
| Diphysciaceae | | |
| <i>Diphyscium fulvifolium</i> Mitt. | AF478222 | Magombo (2003) |
| <i>Diphyscium lorifolium</i> (Cardot) Magombo, Zacharia Lekodi | AF478213 [as <i>Theriotia lorifolia</i>] | Magombo (2003) |
| Polytrichopsida | | |
| Tetraphidales | | |
| Buxbaumiaceae | | |
| <i>Buxbaumia piperi</i> Best | AF478211 | Magombo (2003) |
| Polytrichales | | |
| Polytrichaceae | | |
| <i>Polytrichadelphus aristatus</i> (Hampe) Mitt. | AF478207 | Magombo (2003) |

Consensus tree—50% majority-rule consensus trees for the topologies with high-ranking log-likelihood values and passing the AU or PP test were also computed by PAUP*.

Results

Obtained sequence data

New *rbcL* sequences were determined for 19 species: **Coscinodon humilis*, **Grimmia apiculata*, **Gr. elongata*, **Gr. orbicularis*, **Gr. pilifera*, *Gr. pulvinata*, **Racomitrium fasciculare* var. *atroviride*, **R. japonicum*, **Schistidium strictum*, *Campylostelium saxicola*, **Glyphomitrium humillimum*, **Gl. minutissimum*, **Ptychomitrium dentatum*, *Pt. wilsonii*, **Seligeria austriaca*, *Blindia japonica*, **Venturiella sinensis*, *Pottia intermedia*, and **Dozya japonica*, for which 14 the species with the asterisk were shown as an entire sequence 1,428 bp long. The obtained sequences were submitted to the DNA database under the serial DNA Database Accession Nos. as shown in Table 2 and Appendix A, the latter containing voucher information. The voucher specimens of the sequenced material in the present study are deposited in HIRO.

Sequence alignment

The data matrix was prepared for phylogenetic analyses by manual alignment of the sequences. For the analyses, a total of 1,169 sites (81.9% in 1,428 bp of the entire sequence) of 90 *rbcL* sequences were used. Nucleotide frequencies from the data set were A = 29.4%, C = 16.9%, G = 21.0%, and T = 32.7%. In the sites of the aligned *rbcL* sequences, 395 sites (= 33.8% in 1,169 sites) were variable.

Phylogenetic analyses

The data matrix was prepared for analyses of phylogenetic relationships of the grimmialean and seligerialean mosses, including other haplolepidous mosses. A total of 692 topologies, of which only 11 tree topologies were bifurcate, were obtained from three analyses: 1 ML and 1 NJ topologies by NucML; 1 ML by fastDNAml; and 689 MP by PAUPRat over PAUP*4. More detailed topologies were searched through the obtained trees using log-likelihood measure and several tests (Table 3). HKY85 model was used as the estimate model with the transition/transversion parameter estimated from the data set for the ML tree as 4.287. The best-supported ML tree with the highest likelihood value is shown in Fig. 1. The log-likelihood value for the ML tree was -12066.0 ± 554.4 . Two 50% majority-rule con-

Table 3. Comparison of log-likelihood scores among the 692 resulting topologies for *rbcL* sequences in the grimmialean mosses with the HKY85 model (Hasegawa *et al.* 1985). The log-likelihood values were calculated by NucML in MOLPHY 2.3b3 (Adachi & Hasegawa 1996), and all the *p*-values by CONSEL 0.1f (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 \pm their standard errors (SE). The *p*-values that are not significant at $\alpha = 0.05$ are emphasized in bold type. Topologies with asterisks in the AU and PP columns were used for the consensus trees (values are superimposed on the ML tree in Fig. 1).

| Rank | $\Delta L \pm SE$ | <i>p</i> -Values | | | Method | Program | Note |
|--------------------------------------|-------------------|------------------|---------------|--------------|--------|----------------|-------------|
| | | AU | PP | BP | | | |
| 1 | <-12066.0> | 0.727* | 0.355* | 0.076 | ML | fastDNAML | ML (Fig. 1) |
| 2 | -0.5 ± 37.2 | 0.655* | 0.212* | 0.022 | MP | PAUPRat | |
| 3 | -1.9 ± 27.5 | 0.793* | 0.055* | 0.008 | MP | PAUPRat | |
| 4 | -1.9 ± 27.5 | 0.794* | 0.054* | 0.000 | MP | PAUPRat | |
| 5 | -1.9 ± 40.3 | 0.756* | 0.054* | 0.010 | MP | PAUPRat | |
| 6 | -1.9 ± 40.3 | 0.759* | 0.054* | 0.002 | MP | PAUPRat | |
| 7 | -1.9 ± 35.7 | 0.575* | 0.052* | 0.009 | MP | PAUPRat | |
| 8 | -2.5 ± 39.0 | 0.691* | 0.029 | 0.005 | MP | PAUPRat | |
| 9 | -2.5 ± 28.4 | 0.720* | 0.028 | 0.016 | MP | PAUPRat | |
| 10 | -3.2 ± 39.7 | 0.506* | 0.015 | 0.005 | MP | PAUPRat | |
| 11 | -3.4 ± 37.5 | 0.642* | 0.012 | 0.011 | MP | PAUPRat | |
| 12 | -3.8 ± 41.2 | 0.597* | 0.008 | 0.009 | MP | PAUPRat | |
| 13 | -3.8 ± 40.0 | 0.542* | 0.008 | 0.004 | MP | PAUPRat | |
| 14 | -4.1 ± 39.2 | 0.609* | 0.006 | 0.011 | MP | PAUPRat | |
| 15 | -4.2 ± 39.6 | 0.488* | 0.005 | 0.006 | MP | PAUPRat | |
| 16 | -4.4 ± 40.0 | 0.491* | 0.004 | 0.000 | MP | PAUPRat | |
| 17 | -4.4 ± 29.3 | 0.702* | 0.004 | 0.012 | MP | PAUPRat | |
| 18 | -4.5 ± 38.2 | 0.520* | 0.004 | 0.008 | MP | PAUPRat | |
| 19 | -4.5 ± 40.1 | 0.585* | 0.004 | 0.016 | MP | PAUPRat | |
| 20 | -4.5 ± 40.6 | 0.633* | 0.004 | 0.006 | MP | PAUPRat | |
| : | : | : | : | : | : | : | : |
| 688 | -56.6 ± 48.0 | 0.156* | 0.000 | 0.002 | MP | PAUPRat | |
| 689 | -56.9 ± 47.4 | 0.083* | 0.000 | 0.002 | MP | PAUPRat | |
| 690 | -67.0 ± 42.9 | 0.123* | 0.000 | 0.001 | MP | PAUPRat | |
| 691 | -77.7 ± 42.2 | 0.062* | 0.000 | 0.001 | MP | PAUPRat | |
| 692 | -80.5 ± 32.9 | 0.079* | 0.000 | 0.000 | NJ | NucML + NJdist | |
| No. of topologies for consensus tree | | 665 | 7 | | | | |

Note.—AU: approximate unbiased test (Shimodaira 2000, 2002) using the multiscale bootstrap technique, PP: Bayesian posterior probability calculated by the Bayesian information criterion (BIC) approximation (Schwarz 1978; Hasegawa & Kishino 1989), BP: bootstrap selection probability of Felsenstein (1985) estimated by the RELL resampling method (Kishino *et al.* 1990; Hasegawa & Kishino 1994).

sensus trees were also obtained for the 7 and 656 topologies with high-ranking log-likelihood values that passed the AU or PP test, respectively; and the values for percentage of supported topologies for each branch were superimposed in Fig. 1.

Three large clades corresponding to the Dicranidae, Bryidae, and Funariaceae–Encalyptaceae, members of the Funariidae, were resolved with high support (LBP/AU/PP = 99/100/100 [99%

LBP value in ML tree, 100% support in the consensus tree for the topologies passing the AU test, 100% support in the consensus tree for the topologies passing the PP test], 100/100/100, 99/100/100, respectively). Within the Dicranidae clade, nine subclades were resolved: the Grimmiales clade corresponding to the Grimmiales (s. lat.) with inclusion of the Seligeriaceae (100/100/100); the Pottiaceae–Erpodiaceae clade (96/

ML tree LBP/AU/PP

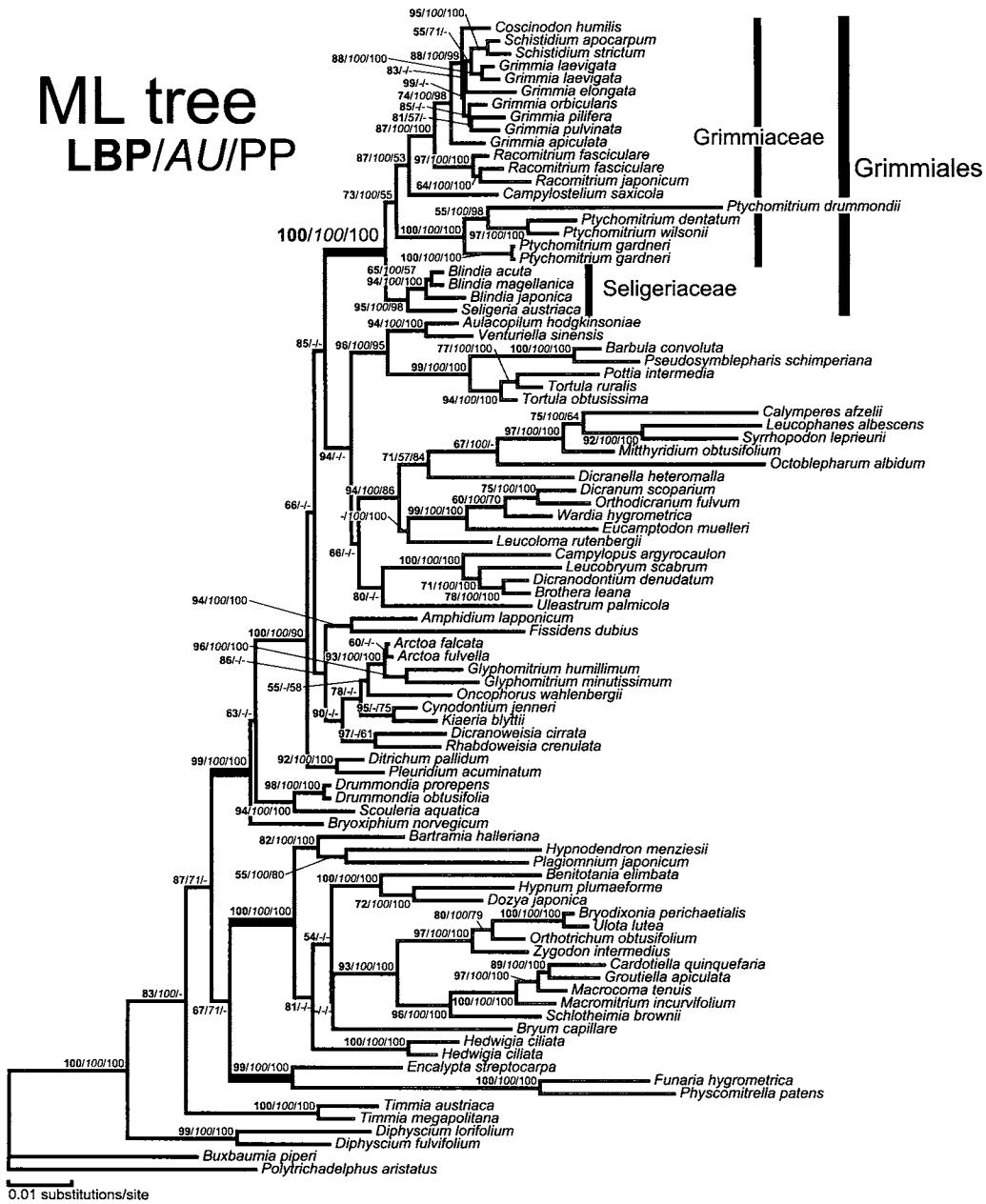


Fig. 1. The best-supported ML tree with the highest likelihood value for the aligned 1,169 bp of the 90 *rbcL* gene sequences (HKY85 model [Hasegawa *et al.* 1985]; $2\alpha/\beta = 4.287$; $\ln L = -12066.0 \pm 554.4$ by NucML); and the clades within the Grimmiales (s. lat. [= sensu present]) clade. 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 *Polytrichadelphus aristatus* following Magombo (2003) and Tsubota *et al.* (in ms.). Local bootstrap probabilities more than 50%, and the values of percentage of supported topologies that passed the AU or PP test for each branch are shown on or near branches (LBP/AU/PP; in %)

100/95); the Dicranales clade with inclusion of the Wardiaceae (66/-/-); the Fissidentaceae–*Amphidium* clade (94/100/100); the Rhabdoweisiaeae clade (90/-/-); the Ditrichaceae clade (92/100/100); the Drummondiaclae–Scouleriaeae clade (94/100/100); and the Bryoxiphiaeae clade. The Dicranales clade and Rhabdoweisiaeae clade were supported with rather low values. Within the Grimmiales clade, four small clades were resolved: the *Grimmia*–*Coscinodon*–*Schistidium*–*Racomitrium* clade (87/100/100), the *Campylostelium* clade, the *Ptychomitrium* clade (100/100/100), and the *Seligeria*–*Blindia* clade (95/100/98).

Discussion

1. Ordinal relationships

The present study suggests the alignment of the Grimmiales (s. lat. [= sensu La Farge *et al.* (2000); sensu present]) as a resolved monophyletic group within the Dicranidae, consisting of two families: the Grimmiaceae (s. lat. [= sensu present]) with inclusion of members previously considered as mosses belonging to the Ptychomtriaceae, and the Seligeriaceae; except for the genera *Glyphomitrium*, *Drummondia*, *Scouleria* and *Wardia*. No affinity was shown between the members of the Grimmiales (s. lat.) and members of the Pottiaceae, nor with *Venturiella*.

Grimmiales

Circumscription of the Grimmiales has been variously transformed (Table 1). Brotherus (1924–1925) and Nyholm (1954–1960) [also Fleischer (1915–1923)] treated the Grimmiales with the narrowest sense only with inclusion of the Grimmiaceae. Buck and Goffinet (2000) classified the Grimmiales with the widest sense with the inclusion of not only families traditionally placed in the Grimmiales but also the Scouleriaceae and Drummondiaclae. Noguchi *et al.* (1987–1988), Iwatsuki (1991) and Deguchi (in Iwatsuki *et al.* 2001) placed the Grimmiaceae (s. lat.) with the inclusion of *Campylostelium* and *Ptychomitrium*. Our results support the treatment by Noguchi *et al.* (1987–1988), Iwatsuki (1991) and Deguchi in Iwatsuki *et al.* (2001).

Drummondia Hook. is characterized by very reduced peristome teeth, and traditionally placed in

the Orthotrichaceae. Vitt (1982) established the monogeneric subfamily Drummondioideae in the Orthotrichaceae, and Goffinet (in Buck & Goffinet 2000) transferred it to the Grimmiales as the new family Drummondiaclae. The Scouleriaceae consist of two genera *Scouleria* and *Tridonium*, and traditionally classified in the Grimmiales. Buck and Goffinet (2000) treated the four families Grimmiaceae, Ptychomtriaceae, Scouleriaceae and Drummondiaclae in the Grimmiales. In the present study, the ML tree resolves *Scouleria* and *Drummondia* as a basal lineage within the Dicranidae, supporting the suggestion by La Farge *et al.* (2000). Our results support the placement of the Drummondiaclae as an independent family but do not support the placement of the family in the Grimmiales, suggesting a newly placement in an independent order with the Scouleriaceae.

Seligeriales

The Seligeriales accommodates only the single family Seligeriaceae, composed of three genera *Seligeria*, *Blindia* and *Brachydontium*. The order is characterized by small plant size and degenerate peristomal teeth. Historically, the Seligeriaceae (and some workers separated *Brachydontium* as an independent family) were placed in the Dicranales. Vitt (1984) segregated the Seligeriaceae into the suborder Seligeriineae (Schimp.) Vitt., describing differences in alar cells between the Seligeriineae [= Seligeriales] and the Grimmiiineae [= Grimmiales]. Many workers have treated them as the distinct order Seligeriales (e.g., Vitt 1984 [as suborder Seligeriineae], Buck & Goffinet 2000).

Edwards (1979) proposed a grouping of genera with *Seligeria* type peristome teeth, i.e., *Seligeria*, *Blindia*, *Glyphomitrium* and *Dicranoweisia*. Goffinet *et al.* (1998) also suggested that “though the monophyly of a group of taxa sharing the *Seligeria*-type peristome has not been critically examined, the nearly identical peristomes of *Rachithecium* (Rachitheciaceae), *Glyphomitrium* (Ptychomtriaceae), and *Blindia* (Seligeriaceae) may be seen as an indication of close phylogenetic relationships, despite gametophytic differences.” La Farge *et al.* (2000) made a phylogenetic study on the haplolepidous mosses and

suggested the inclusion of the Seligeriaceae represented by *Blindia acuta*, Grimmiaceae, and Ptychomitriaceae in the Grimmiales (s. lat.), a conclusion we support, and the formation of one of basal lineage of *Scouleria aquatica* within the Dicranidae, although the number of treated OTUs were not enough to clarify the phylogenetic relationship of the Grimmiales. In the present study, the Seligeriaceae form a well supported sister group to the Grimmiaceae (s. lat.), although *Rachithecium*, *Glyphomitrium*, and *Dicranoweisia* do not.

Buck and Goffinet (2000) classified the Wardiales as well as the Seligeriaceae in the Seligeriales. Our results would indicate the placement of only the Seligeriaceae in the Seligeriales and suggest the placement of the Wardiaceae in the Dicraňales near the Dicranaceae. Tsubota *et al.* (in ms., and the abstract of the MO Symposium 2003) also support the monophyly of the Grimmiales (s. lat.) based on a large scale data set of *rbcL* sequences.

Cavers (1910–1911) classified the mosses on the basis of the peristome conformation, dividing the Haplolepidoideae into five subdivisions, and only the Grimmiaceae and Seligeriaceae were assigned to the fifth group Platycranoideae. Our study supports this, and these facts suggest that haplolepidous peristomes of the Grimmiales and Seligeriales are similar to each other with some modifications. Since the monophyly of the Grimmiales (s. lat.) was well supported, including *Ptychomitrium*, *Campylostelium* and members of the Seligeriales within the Dicranidae, we propose here that we could treat the order in a wider sense, to include the *Grimmia/Seligeria* type peristome.

2. Familial and generic relationships

Grimmiaceae and Ptychomitriaceae

The familial circumscription proposed by previous systems differs in the placement of *Campylostelium*, *Coscinodon*, *Glyphomitrium* and *Racomitrium* (see Table 1). Some workers treated *Ptychomitrium* and related genera as the distinct family Ptychomitriaceae. Bruch and Schimper (1837–1846), Limprecht (1885–1889), and Nyholm (1954–1960) recognized the Ptychomitriaceae with inclusion of *Ptychomitrium* and *Glyphomitrium* having shared characters of calyptrae.

Schimper (1876), Brotherus (1901–1902; 1924–1925), Vitt (1984), Vitt *et al.* (1998), and Buck and Goffinet (2000) included *Coscinodon* and/or *Campylostelium* in the Ptychomitriaceae. Brotherus (1924–1925) included *Ptychomitrium*, *Campylostelium* and *Glyphomitrium* in the Ptychomitriaceae placing them near the Orthotrichaceae in the Isobryales. Jones (1933, in Grout 1928–41) considered there was a close relation between *Glyphomitrium* and *Grimmia* on the basis of peristome characters. Dixon (1924), Noguchi (1974), Deguchi (1979), Churchill (1981), Iwatsuki (1991), and Iwatsuki *et al.* (2001) have all treated the Grimmiaceae in the wider sense.

Noguchi (1954) reviewed Japanese *Ptychomitrium* treating it as a member of the Grimmiaceae on the basis of the shape and arrangement of leaf cells in section and suggested that the Brotherus' (1924–1925) placement of *Ptychomitrium* and *Campylostelium* in the Ptychomitriaceae may be derived from the characteristics of calyptra and of leaves, but that it seemed to be an unsatisfactory one. Noguchi (1974) also discussed the genus *Racomitrium* in relation to the genera *Grimmia* and *Ptychomitrium*, and suggested that *Grimmia* and *Racomitrium* have a close relationship to each other derived from a common ancestor on the basis of their broad and rather thin costae, consisting of almost uniform cells, and similar peristomes. He regarded *Racomitrium* as an intermediate genus between *Ptychomitrium* and *Grimmia*. Deguchi (1977) showed small male-branches of *Ptychomitrium* arising from the base of the vaginula inside the perichaetial leaf circle, and suggested this cryptoicous feature to be one of the distinct characters in the genus. Deguchi (1979) revised *Grimmia*, *Schistidium* and *Coscinodon* in Japan, and included *Ptychomitrium* in the Grimmiaceae. He showed morphological relationships among the genera in the Grimmiaceae, and suggested the close relationship between *Racomitrium* and *Ptychomitrium*, especially in the character of peristome teeth (as indicated by Noguchi 1974), calyptrae and setae, and concluded that *Grimmia* may be the most primitive genus in the family. Churchill (1981) made a cladistic analysis for the Grimmiaceae, and proposed a revision of the classification of the family. He also showed that characters, such as the divided peristome outline and presence of a basal

membrane, are apomorphic in the Grimmiaceae.

In the present study, *Grimmia*, *Schistidium* and *Coscinodon* constitute a monophyletic group; and *Racomitrium* and *Ptychomitrium* (also *Campylostelium*) paraphyletically appeared to the clade of *Grimmia*, *Schistidium* and *Coscinodon*. This suggests that the remarkable characters indicated by Noguchi (1974) and Deguchi (1979) are plesiomorphic in the Grimmiaceae (s. lat.) and include the smooth surface of the calyptra; 3–4 or more cell-layered calyptra; setae twisted to the right; peristome teeth linear-lanceolate and deeply divided into two filiform branches; and presence of a basal membrane. In the present study, the Ptychomitriaceae appear as poly- or para-phyletic entities, although some branches have low support values.

Seligeriaceae and Ptychomitriaceae

Bruch and Schimper (1846) and Schimper (1876) placed *Campylostelium* in the Seligeriaceae on the basis of its small plant size, peristome cells, cell ornamentation of the peristome teeth, and seta characters. Meanwhile, Limpricht (1885–1889), Brotherus (1901–1902; 1924–1925), and Fleischer (1915–1923) showed the affinity of *Glyphomitrium* and *Campylostelium*. *Campylostelium* appeared as sister to the *Grimmia–Schistidium–Coscinodon–Racomitrium* clade in the ML tree and PP consensus tree with 100% support, although in the AU consensus tree, the position of *Campylostelium* was ambiguous. Our results suggest that *Campylostelium* is best placed in the Grimmiaceae (s. lat.).

Glyphomitrium was traditionally classed in the Ptychomitriaceae or Grimmiaceae (see Table 1). Noguchi (1952), in his revision of the Japanese species, placed *Glyphomitrium* in the Erpodiaceae on the basis of similarity in peristome development to *Venturiella*. Crum (1972a, b) failed to see the reason for transferring the genus into the Erpodiaceae, and argued the position of the genus in the Ptychomitriaceae. Edwards (1979) also concurred with Crum's suggestion. Estébanez *et al.* (2002) made histochemical observations on the peristome of several haplolepidous mosses, and confirmed the distinctive features supporting the segregation of *Glyphomitrium* from the Ptychomitriaceae. La Farge *et al.* (2000) showed the Erpodiaceae, as represented by *Aulacopilum*

hodgkinsoniae, to have a close affinity with the Rhachitheciaceae within the Dicranidae, with a sister relationship to the Pottiales, forming a weak monophyletic clade. Our tree shows that *Glyphomitrium* has a close relation to *Arctoa* with high support (93/100/100) in the Dicranidae clade, and *Venturiella sinensis* and *Aulacopilum hodgkinsoniae* form a distinct single clade (94/100/100) sister to the Pottiaceae with strong support (96/100/95). The results do not support the inclusion of *Glyphomitrium* in the Ptychomitriaceae. These facts suggest that *Glyphomitrium* be best placed in the Dicranaceae (s. lat. [= sensu Vitt 1984]) or Rhabdoweisiaceae (sensu La Farge *et al.* 2000), not in the Erpodiaceae, Ptychomitriaceae or Grimmiaceae.

Scouleriaceae, Drummondiaaceae and Wardiaceae

Scouleria has been traditionally placed in the Grimmiaceae, although Hooker (1837) suggested the similarity between *Scouleria* and *Wardia*. Edwards (1979) suggested that the proliferation of the cell pattern of the genus is markedly similar to the *Seligeria* type peristome, although *Scouleria* has 32 irregular teeth with rudimentary prostomial layers. Churchill (1981) stated *Scouleria* in its own family, the Scouleriaceae. Churchill (1985) placed *Scouleria* within the Haplolepideae, and tentatively accepted the hypothesis that *Scouleria* be the sister group of some haplolepidous taxa, such as the Pottiales and Grimmiaceae; and related it to a higher taxonomic level, i.e. family, within the Haplolepideae excluding the Dicranales and Fissidentales. Recently, La Farge *et al.* (2000) showed the basal placement of *Scouleria* and *Drummondia* in the Dicranidae. Goffinet *et al.* (2001) also did not support the close affinity between the Grimmiaceae–Seligeriaceae and the Drummondiaaceae–Scouleriaceae based on *rps4* sequences. Buck and Goffinet (2000) placed the Scouleriaceae and Drummondiaaceae in the Grimmiaceae. In the present study, *Scouleria* appeared at the basal position of the Dicranidae forming a single clade with *Drummondia* with high support (94/100/100) near *Bryoxiphium*, not supporting placement of the two families in the Grimmiaceae. The monogeneric family Wardiaceae was placed in the Seligeriales by Buck and Goffinet (2000). *Wardia* was traditionally placed in the Orthotrichales or Leucodon-

tales. Additional sequences are needed to test and resolve the phylogenetic positions of the Scouleriaceae, Drummondiaceae and Wardiaceae.

Conclusion

Our study corroborates that (1) the Grimmiaceae (s. lat. [= sensu present]) with the inclusion of the genera *Grimmia*, *Schistidium*, *Coscinodon*, *Racomitrium*, *Campylostelium*, *Ptychomitrium* are monophyletic; (2) members of genera previously considered as belonging to the Ptychomitriaceae and represented by the genera *Ptychomitrium* and *Campylostelium*, with the exclusion of *Glyphomitrium*, did not form a single clade, and are paraphyletic to the *Grimmia*–*Schistidium*–*Coscinodon*–*Racomitrium* clade; (3) *Glyphomitrium* showed a close affinity to *Arctoa* in the Dicranales, not to the Grimmiaceae; (4) members of the Seligeriaceae, represented by *Seligeria* and *Blindia*, form a distinct clade sister to the clade of the Grimmiaceae (s. lat. [= sensu present]) including members of *Ptychomitrium* and its allied genera; (5) the Grimmiaceae (s. lat. [= sensu present]), accommodating the Grimmiaceae (s. lat.) and the Seligeriaceae, are monophyletic without members of the Drummondiaceae, the Scouleriaceae or the Wardiaceae; and (6) the clade consisting of the Drummondiaceae and Scouleriaceae appeared at the basal position of the haplolepidous moss group Dicranidae, next to the Bryoxiphiaeae. The previous taxonomical controversy regarding the Grimmiaceae is reflected in their phylogenetic relationships.

Within the Grimmiaceae, additional data of more rapidly evolved sequences, such as *matK* and ITS or of untreated genera (e.g. *Brachydontium* and *Tridontium*), would help to clarify the relationships among genera or species. Further phylogenetic investigation on relationships among the orders within the Dicranidae is also needed.

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Literature cited

- Adachi, J. & Hasegawa, M. 1996. Computer Science Monographs, 28. MOLPHY version 2.3. Programs for molecular phylogenetics based on maximum likelihood. pp. 150. Institute of Statistical Mathematics, Tokyo.
- Akiyama, H., Tsubota, H., Yamaguchi, T. & Suleiman, M. 2003. The new genus *Benitotania* (Daltoniaceae, Bryopsida) from Mt. Kinabalu. *Bryologist* 106: 454–459.
- Arikawa, T. & Higuchi, M. 1999. Phylogenetic analysis of the Plagiotheciaceae (Musci) and its relatives based on *rbcL* gene sequences. *Cryptogamie, Bryol.* 20: 231–245.
- Arnott, W. 1825. Disposition méthodique des espèces de mousses. Paris.
- Brotherus, V. F. 1901–1902. Musci (Laubmoose). In Engler, A. & Prantl, K. (eds.), *Die natürlichen Pflanzenfamilien*, Ed. 1. Wilhelm Engelmann, Leipzig. [*Seligeria*, *Blindia* (1901); Grimmiaceae (1902)]
- 1924–1925. Musci (Laubmoose). In Engler, A. (ed.), *Die natürlichen Pflanzenfamilien*, Ed. 2, Vol. 10–11. Wilhelm Engelmann, Leipzig. [*Seligeriaceae*, *Grimmiaceae* (1924); *Ptychomitriaceae* (1925)]
- Bruch, P. & Schimper, W. P. 1837–1846. *Bryologia Europaea. Stuttgartiae.* [*Ptychomitrium*, *Glyphomitrium*, *Coscinodon* (1837); *Schistidium*, *Grimmia*, *Racomitrium* (1845); *Seligeria*, *Blindia*, *Campylostelium* (1846)]
- Buck, W. R. & Goffinet, B. 2000. Morphology and classification of mosses. In Shaw, A. J. & Goffinet, B. (eds.), *Bryophyte Biology*, pp. 71–123. Cambridge University Press, Cambridge.
- Cavers, F. 1910–1911. The inter-relationships of the bryophytes. I–XI. Republished as *New Phytologist Reprint no. 4*, pp. 1–203, 72 figs. Cambridge, 1911.
- Churchill, S. P. 1981. A phylogenetic analysis classification and synopsis of the genera of the Grimmiaceae (Musci). In Funk, V. A. & Brooks, D. R. (eds.), *Advances in Cladistics. Proceedings of the First Meeting of the Willi Hennig Society*, pp. 127–144. New York Botanical Garden, New York.
- 1985. The systematics and biogeography of

- Scouleria* Hook. (Musci: Scouleriaceae). *Lindbergia* 11: 59–71.
- Cox, C. J., Goffinet, B., Newton, A. E., Shaw, A. J. & Hedderson, T. A. J. 2000. Phylogenetic relationships among the diplolepidous-alternate mosses (Bryidae) inferred from nuclear and chloroplast DNA sequences. *Bryologist* 103: 224–241.
- Crum, H. 1972a. A taxonomic account of the Erpodiaceae. *Nova Hedwigia* 23: 201–224.
- 1972b. The dubious origin of *Glyphomitrium canadense* Mitt. *J. Bryol.* 7: 165–168.
- De Luna, E., Buck, W. R., Akiyama, H., Arikawa, T., Tsubota, H., González, D., Newton, A. E. & Shaw, A. J. 2000. Ordinal phylogeny within the hypnobryalean pleurocarpous mosses inferred from cladistic analyses of three chloroplast DNA sequence data sets: *trnL*-F, *rps4* and *rbcL*. *Bryologist* 103: 242–256.
- Deguchi, H. 1977. Small male-branches of *Ptychomitrium* (Grimmiaceae) arised from the base of vaginula inside the perichaetial leaf circle. *Misc. Bryol. Lichenol.* 7: 177–179. (In Japanese with English Figs.)
- 1979 (1978). A revision of the genera *Grimmia*, *Schistidium* and *Coscinodon* (Musci) of Japan. *J. Sci. Hiroshima Univ., ser. b, div. 2*, 16 (2): 121–256.
- Dixon, H. N. 1924. The Student's Handbook of British Mosses, Ed. 3. London. [Facsimile edition 1970, Wheldon & Wesley, London]
- Edwards, S. R. 1979. Taxonomic implications of cell patterns in haplolepidous moss peristomes. In Clarke, G. C. S. & Duckett, J. G. (eds.), *Bryophyte Systematics*, pp. 317–346. Academic Press, London.
- Estébanez, B., Tsubota, H., Yamaguchi, T. & Deguchi, H. 2002. Histochemical observations on the peristome of several haplolepidous mosses. *Hikobia* 13: 667–677.
- Felsenstein, J. 1981. Evolutionary trees from DNA sequences: a maximum likelihood approach. *J. Mol. Evol.* 17: 368–376.
- 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39: 783–791.
- Fitch, W. M. 1971. Toward defining the course of evolution: Minimum change for a specific tree topology. *Syst. Zool.* 20: 406–416.
- Fleischer, M. 1915–1923. Die Musci der Flora von Buitenzorg IV. Leiden.
- Goffinet, B., Bayer, B. J. & Vitt, D. H. 1998. Circumscription and phylogeny of the Orthotrichales (Bryopsida) inferred from *rbcL* sequence analyses. *Am. J. Bot.* 85: 1324–1337.
- , Cox, C. J., Shaw, A. J. & Hedderson, T. A. J. 2001. The Bryophyta (mosses): systematic and evolutionary inferences from an *rps4* gene (cpDNA) phylogeny. *Ann. Bot.* 87: 191–208.
- Grout, A. J. 1936. Seligeriaceae. In A. J. Grout (ed.), 1928–1941, *Moss Flora of North America, north of Mexico* Vol. 1. Published by the author, Newfane. [Facsimile edition 1972, Hafner Publishing Company, New York]
- Hasegawa, M., Kishino, H. & Yano, T. 1985. Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. *J. Mol. Evol.* 22: 160–174.
- & — 1989. Confidence limits on the maximum-likelihood estimate of the hominoid tree from mitochondrial-DNA sequences. *Evolution* 43: 672–677.
- & — 1994. Accuracies of the simple methods for estimating the bootstrap probability of a maximum likelihood tree. *Mol. Biol. Evol.* 11: 142–145.
- Hooker, W. J. 1837. *Wardia*, a new genus of mosses, discovered in Southern Africa. *Companion to the Bot. Mag.* 2: 183–184 + pl. 25.
- Iwatsuki, Z. 1991. Catalog of the mosses of Japan. Pp. 182. Hattori Bot. Lab., Nichinan.
- , Deguchi, H. & Furuki, T. 2001. *Nihon no yasei shokubutsu, Koke*. [Mosses and liverworts of Japan]. Pp. 355 + pl. 192. Heibonsha, Tokyo. (In Japanese).
- Jones, G. N. 1933. Grimmiaceae. In A. J. Grout (ed.), 1928–1941, *Moss Flora of North America, north of Mexico* Vol. 2. Published by the author, Newfane. [Facsimile edition 1972, Hafner Publishing Company, New York]
- Kishino, H. & Hasegawa, M. 1989. Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA sequence data, and the branching order in Hominoidea. *J. Mol. Evol.* 29: 170–179.
- , Miyata, T. & Hasegawa, M. 1990. Maximum likelihood inference of protein phylogeny, and the origin of chloroplasts. *J. Mol. Evol.* 31: 151–160.
- La Farge, C., Mishler, B. D., Wheeler, J. A., Wall, D. P., Johannes, K., Schaffer, S. & Shaw, A. J. 2000. Phylogenetic relationships within the haplolepidous mosses. *Bryologist* 103: 257–276.
- , Shaw, A. J. & Vitt, D. H. 2002. The

- circumscription of the Dicranaceae (Bryopsida) based on the chloroplast regions *trnL-trnF* and *rps4*. *Syst. Bot.* 27: 435–452.
- Limprecht, K. G. 1885–1889. Die Laubmoose. In Rabenhorst's Kryptogamen-Flora, 4 Vols. Leipzig.
- Magombo, Z. L. K. 2003. The phylogeny of basal peristome mosses: evidence from cpDNA, and implication for peristome evolution. *Syst. Bot.* 28: 24–38.
- Murray, M. G. & Thompson, W. F. 1980. Rapid isolation of high molecular weight plant DNA. *Nuc. Acids Res.* 19: 4321–4325.
- Nixon, K. C. 1999. The Parsimony Ratchet, a new method for rapid parsimony analysis. *Cladistics* 15: 407–414.
- Noguchi, A. 1952. Musci Japonici. II. Erpodiaceae. *J. Hattori Bot. Lab.* 8: 5–17.
- 1954. Musci Japonici. IV. The genus *Ptychomitrium*. *J. Hattori Bot. Lab.* 12: 1–26.
- 1974. Musci Japonici. X. The genus *Racomitrium*. *J. Hattori Bot. Lab.* 38: 337–369.
- , Iwatsuki, Z. & Yamaguchi, T. 1987–1988. Illustrated moss flora of Japan, Parts 1 & 2., pp. 1–242 & 243–491. *Hattori Bot. Lab.*, Nichinan.
- Nyholm, E. 1954–1960. Illustrated Moss Flora of Fennoscandia. II. Musci. Fasc. 1, 2, 4. Lund. [Seligeriaceae (1954); Grimmiaceae (1956); Ptychomitiaceae (1960)]
- Oguri, E., Yamaguchi, T., Tsubota, H. & Deguchi, H. 2003. A preliminary phylogenetic study of the genus *Leucobryum* (Leucobryaceae, Musci) in Asia and the Pacific based on ITS and *rbcL* sequences. *Hikobia* 14: 45–53.
- Olsen, G. J., Matsuda, H., Hagstrom, R. & Overbeek, R. 1994. fastDNAML: A tool for construction of phylogenetic trees of DNA sequences using maximum likelihood. *Comput. Appl. Biosci.* 10: 41–48.
- Saitou, N. & Nei, M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* 4: 406–425.
- Schimper, W. P. 1876. Synopsis Muscorum europaeorum praemissa introductione de elementis bryologicis tractante, Ed. 2. pp. 886. E. Schweizerbard, Stuttgart.
- Schwarz, G. 1978. Estimating the dimension of a model. *Ann. Stat.* 6: 461–464.
- Shimodaira, H. 2000. Another calculation of the *p*-value for the problem of regions using the scaled bootstrap resamplings. *Tech. Rep. No. 2000–35*. Stanford Univ., Palo Alto, California.
- 2002. An approximately unbiased test of phylogenetic tree selection. *Syst. Biol.* 51: 492–508.
- & Hasegawa, M. 2001. CONSEL: for assessing the confidence of phylogenetic tree selection. *Bioinformatics* 17: 1246–1247.
- Sikes, D. S. & Lewis, P. O. 2001. PAUPRat: PAUP* implementation of the parsimony ratchet, beta software, version 1. Department of Ecology and Evolutionary Biology, University of Connecticut, Storrs.
- Swofford, D. L. 2002. PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4. Sinauer Associates, Sunderland, Massachusetts.
- Tsubota, H., Nakao, N., Arikawa, T., Yamaguchi, T., Higuchi, M., Deguchi, H. & Seki, T. 1999. A preliminary phylogeny of Hypnales (Musci) as inferred from chloroplast *rbcL* sequence data. *Bryol. Res.* 7: 233–248.
- , —, Yamaguchi, T., Seki, T. & Deguchi, H. 2000. Preliminary phylogenetic relationships of the genus *Brotherella* and its allied genera (Hypnales, Musci) based on chloroplast *rbcL* sequence data. *J. Hattori Bot. Lab.* 88: 79–99.
- , Akiyama, H., Yamaguchi, T. & Deguchi, H. 2001a. Molecular phylogeny of the Sematophyllaceae (Hypnales, Musci) based on chloroplast *rbcL* sequences. *J. Hattori Bot. Lab.* 90: 221–240.
- , —, — & — 2001b. Molecular phylogeny of the genus *Trismegistia* and related genera (Sematophyllaceae, Musci) based on chloroplast *rbcL* sequences. *Hikobia* 13: 529–549.
- , Arikawa, T., Akiyama, H., De Luna, E., González, D., Higuchi, M. & Deguchi, H. 2002. 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. *Hikobia* 13: 645–665.
- Vitt, D. H. 1982. Sphagnopsida and Bryopsida. In Parker, S. P. (ed), *Synopsis and classification of living Organisms*, Vol. 1, pp. 307–336. McGraw-Hill, New York.
- , 1984. Classification of the Bryopsida. In Schuster, R. M. (ed.), *New Manual of Bryology*, Vol. 2, pp. 696–759. *Hattori Bot. Lab.*, Nichinan.
- , Goffinet, B. & Hedderon, T. 1998. The ordinal classification of the mosses. Questions and answers for the 1990s. In Bates, J. W., Aston, N. W. & Duckett, J. G. (eds), *Bryology for the Twenty-first Century*, pp. 113–123. Maney and the British Bryological Society, Leeds.

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.

The sequence of information is: the name of taxon, accession number, locality and specimen number, or reference source. Grimmiaceae and its allies: *Campylostelium saxicola* (F.Weber & D.Mohr) Bruch & Schimp., AB125584, Japan, Honshu, Tottori-ken, Mt. Daisen, ca. 950 m alt., August 28, 2000, T. Yamaguchi 18851 (HIRO); *Coscinodon cribrosus* (Hedw.) Spruce, AB125575, Japan, Kyushu, Kagoshima-ken, Yakushima Isl., ca. 1,800 m alt., May 29, 2000, H. Deguchi 35024 (HIRO); *Grimmia apiculata* Hornsch., AB125576, Japan, Honshu, Nagano-ken, Mt. Shirouma, ca. 2,600 m alt., August 12, 2000, Y. Ageno 28 (HIRO); *Grimmia elongata* Kaulf., AB125577, Japan, Honshu, Nagano-ken, Mt. Shirouma, ca. 2,600 m alt., August 12, 2000, Y. Ageno 29 (HIRO); *Grimmia orbicularis* Bruch, AB125578, Spain, Madrid, Arganda Iel Rey, Dehesa, El Carrascal, 660 m alt., December 25, 1999, Y. Ageno 57 (HIRO); *Grimmia pilifera* P.Beauv., AB125579, Japan, Honshu, Hiroshima-ken, Saeki-gun, Miyajima Isl., Mt. Misen, ca. 500 m alt., April 13, 2000, Y. Ageno 19 (HIRO); *Grimmia pulvinata* (Hedw.) Sm., AB125580, Spain, Madrid, Arganda Iel Rey, Dehesa, El Carrascal, 660 m alt., December 25, 1999, Y. Ageno 56 (HIRO); *Glyphomitrium humillimum* (Mitt.) Cardot, AB125585, Japan, Kyushu, Kagoshima-ken, Makurazaki-shi, Higashi-kago, September 12, 2000, Y. Ageno 47 (HIRO); *Glyphomitrium minutissimum* (S. Okamura) Broth., AB125586, Japan, Honshu, Hiroshima-ken, Yamagata-gun, Geihoku-cho, Mt. Garyu, September 20, 1999, H. Tsubota 3385 (HIRO); *Ptychomitrium dentatum* (Mitt.) A.Jaeger, AB125587, Japan, Honshu, Hiroshima-ken, Hiroshima-shi, Nabara-kyo, 320–330 m alt., May 16, 1999, H. Tsubota 2786 (HIRO); *Ptychomitrium wilsonii* Sull. & Lesq., AB125588, Japan, Kyushu, Kagoshima-ken, Yakushima Isl., 650–700 m alt., October 17, 2000, T. Yamaguchi 19026 (HIRO); *Racomitrium fasciculare* var. *atroviride* Cardot, AB125581, Japan, Honshu, Hiroshima-ken, Hiroshima-shi, Nabara-kyo, 330–400 m alt., May 16, 1999, H. Tsubota 2788 (HIRO); *Racomitrium japonicum* Dozy & Molk., AB125582, Japan, Honshu, Hiroshima-ken, Higasi-hirosima-shi, Hiroshima University, ca. 220 m

alt., June 24, 1998, H. Tsubota 2326 (HIRO); *Schistidium strictum* (Turner) Loeske ex Martensson, AB125583, Japan, Honshu, Hiroshima-ken, Yamagata-gun, Geihoku-cho, Yahata Highlands, ca. 800 m alt., July 16, 1996, H. Deguchi 33775 (HIRO); Seligeriaceae *Seligeria austriaca* T. Schauer, AB125589, Japan, Honshu, Nagano-ken, Ina-gun, Ooshika-mura, ca. 1,570 m alt., August 21, 2000, H. Tsubota 3842 (HIRO); *Blindia japonica* Broth., AB125590, Japan, Honshu, Hiroshima-ken, Yamagata-gun, Togouchi-cho, Sandankyo; 510–600 m alt., August 23, 1996, H. Deguchi 34189 (HIRO); Erpodiaceae *Venturiella sinensis* (Venturi) Müll.Hal., AB125591, Japan, Honshu, Hiroshima-ken, Aki-gun, Kaita-cho, ca. 0 m alt., February 25, 1999, H. Tsubota 2580 (HIRO); Pottiaceae *Pottia intermedia* (Turner) Fürnr., AB125592, Japan, Honshu, Hiroshima-ken, Aki-gun, Shimokamagari-cho, April 23, 2000, H. Tsubota 3850 (HIRO), Leucodontaceae *Dozya japonica* Sande Lac., AB125593, Japan, Kyushu, Kagoshima-ken, Yakushima Isl., 1,380–1,600 m alt., October 19, 2000, H. Tsubota 4019 (HIRO).

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>>
 Sclean and our programs download site <<http://home.hiroshima-u.ac.jp/chubo/>>

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坪田博美・揚野由美・B.エステバス・山口富美夫・出口博則：葉緑体 *rbcL* 遺伝子に基づく蘚類ギボウシゴケ目の分子系統学的研究

ギボウシゴケ目とその周辺分類群について葉緑体 *rbcL* 遺伝子の配列に基づき系統解析を行った。解析の結果、ギボウシゴケ科とキヌシッポゴケ科を含むギボウシゴケ目が単系統群となった。また、サヤゴケ属はチヂレゴケ属とは近い関係ではなく、キヌシッポゴケ属と近い関係にあることが示された。