Phylogenetic study of *Grimmia* (Grimmiaceae) based on plastid DNA sequences (*trn*L-*trn*F and *rps*4) and on morphological characters

Anne Streiff

Conservatoire et Jardin botaniques de la Ville de Genève,
Ch. de l'Impératrice 1, CH-1292 Chambésy, Switzerland
University of Lausanne, DEE, CH-1015 Lausanne, Switzerland
e-mail: anne.streiff@ville-ge.ch

Abstract. This work investigates the phylogenetic relationships within *Grimmia* Hedw. using 33 species of *Grimmia* and ten outgroup species from the Funariidae and the Dicranidae using a combination of two molecular markers and 52 morphological and anatomical characters. Plastid (*trn*L-*trn*F and *rps*4) DNA sequences were used to reconstruct the molecular phylogeny of *Grimmia*. The 33 chosen *Grimmia* species represented the majority of those found in Europe and Asia. An analysis using *rps*4 and *trn*L-*trn*F with six outgroup species supported the monophyly of the Grimmiaceae. The combined analysis of both plastid markers and morphological characters also resolved the Grimmiaceae as monophyletic. The results indicate that *Grimmia*, as currently defined, is paraphyletic. Two main clades were present, one that contained the species traditionally placed in the subgenus *Rhabdogrimmia* Limpr. and one that contained the remaining *Grimmia* species.

Keywords. *Grimmia*, molecular characters, morphology, paraphyly, phylogeny, *rps*4, *trn*L-*trn*F.

◆ ◆ ◆

The genus *Grimmia* Hedw. belongs to a monophyletic group of mosses called the Haplolepidae (or Dicranidae). *Grimmia* contains 71 recognized species from about 800 published names (Muñoz & Pando 2000). *Grimmia* is principally defined by plants with a dark color; cushion or tufted growth form; lanceolate leaves that are tapering to the apex and generally possessing hair-points; guide-cells basal in leaf cross-section; capsules generally symmetrical, ovate to cylindrical; peristome teeth divided to insertion and entire or irregularly perforate (Crum & Anderson 1981; Maier & Geissler 1995; Muñoz 1998a; Nyholm 1998). Species of *Grimmia* are relatively well studied (Deguchi 1979; Greven 1995, 2003; Maier 2002a, b; Maier & Geissler 1995, Muñoz 1998a, b, 1999;), providing a good foundation for phylogenetic research. A review of the subgeneric classification of the genus *Grimmia* is presented in Streiff (2005).

***Distribution and Ecology of* Grimmia.** *Grimmia* species are found on every continent, in both temperate and polar areas, and in the mountains of the tropics (Churchill 1981). The combined regions of central Europe, the Mediterranean and the Himalayas contain the largest number of *Grimmia* species and represent one of the diversity centers of the genus, the second being North America (Muñoz & Pando 2000). *Grimmia* species are saxicolous and are mostly found growing on non-calcareous substrates (Loeske 1913)

 0007-2745/$1.35/0

with a few species tolerating calcareous substrates. *Grimmia* species mostly grow in sunny, open areas (photophilous), with only two species (*Grimmia incurva* Brid. and *G. torquata* Drumm.) known to prefer shaded conditions (Loeske 1913). *Grimmia* species are xerothermophilous and react rapidly to rewetting (Loeske 1913).

***Phylogenetic Studies concerning* Grimmia.** Churchill (1981) published a cladistic study of the Grimmiaceae using 19 morphological characters taken from taxa representing different genera and subgenera of the family. His results suggested the paraphyly of the genus *Grimmia*. Cao and Vitt (1986) did two separate cladistic studies on *Grimmia* and *Schistidium*. These authors considered 22 species of *Grimmia* and were interested in examining the distribution of Chinese species within the different subgenera described in the literature. No conclusion could be drawn on the monophyly of *Grimmia* from the latter study because no outgroup taxa were included in the analyses. Tsubota et al. (2003) published a phylogenetic study of the Grimmiales based on the plastid DNA *rbc*L marker. They included 24 species belonging to the Grimmiales of which 13 were from the Grimmiaceae (seven *Grimmia* species). The resulting phylogenetic tree positioned the Grimmiaceae sister to the Ptychomitriaceae. These two families constituted a monophyletic lineage, which corresponded to the Grimmiales. The genus *Grimmia* was found to be paraphyletic but no conclusion could be drawn about the relationships between the *Grimmia* species and the species of the other Grimmiaceae because of the low statistical support for the basal branches of the trees. A study using 42 *Grimmia* species, and 52 morphological and anatomical characters, partially confirmed the earlier suggestion of Churchill (1981), that the Grimmiaceae was monophyletic, and that *Grimmia* was paraphyletic (Streiff 2005). As in previous studies based on morphological-anatomical characters, statistical support for the branches of the resulting cladograms was low.

The objective of this study was to assess the paraphyly of the genus *Grimmia* (Churchill 1981; Streiff 2005; Tsubota et al. 2003) by sampling 33 species of *Grimmia*, outgroup species from the Funariidae and the Dicranidae, using plastid DNA markers and morphological-anatomical data. Two plastid markers were chosen for this analysis: *rps*4 (*ribosomal protein subunit* 4) and the *trn*L-*trn*F region (partial tRNA-*Leu* gene, *trn*L intron, *trn*L spacer) and 52 morphological and anatomical characters.

Materials and Methods

Materials. Thirty-three species of *Grimmia* were used. Selected taxa principally represented those growing in central Europe, the Mediterranean and the Himalayas. In **Table 1**, species distributions are given according to Muñoz and Pando (2000) and Maier (2002a, b). Samples were taken from the herbarium of Conservatoire et Jardin botaniques de la Ville de Genève (G) and from the personal collection of the author (HB. STREIFF). Species nomenclature is presented in **Tables 1 and 2**, and all author names are listed within these two Tables.

Gametophyte material was selected for extraction with a binocular microscope and forceps, to limit the presence of contaminants such as fungi, algae, lichens and/or other mosses in the sample. Samples were than reverified taxonomically to check that they were not mixed collections. The samples for DNA extraction were up to six years old. In certain species (e.g., *G. ovalis*) the sequences *trn*L-*trn*F and *rps*4 could not be obtained from the same sample, because of their poor DNA quality.

The classification system used here follows Buck and Goffinet (2000). Most of the outgroup sequences were taken from GenBank (Goffinet & Cox 2000; Goffinet et al. 2001; La Farge et al. 2000). The GenBank accession numbers for the sequences newly presented in this study are listed in **Table 1** and outgroup sequences taken from GenBank are in **Table 2**.

DNA Extraction, PCR Amplification and Sequencing. Green parts of the sample gametophyte were selected (ca. 20 mg per sample) and ground into a powder with liquid nitrogen. DNA extraction was based on the CTAB method (Doyle & Doyle 1987). DNA was made soluble in 30 µl of TE8 buffer (Tris-HCl 10 mM, EDTA 1mM, pH 8.0).

The *trn*L-*trn*F and *rps*4 sequences were amplified using PCR (Polymerase Chain Reaction). Primers $trnL_{(UAA)}$ and $trnF_{(GAA)}$ (Taberlet et al. 1991) were used to amplify the *trn*L-*trn*F region. The *rps*4 segment was amplified with the primers *rps*5 and *trn*S (Souza-Chies et al. 1997). The 50 µl-reactions were

Table 1. List of specimens used for DNA extraction, species distribution after Muñoz and Pando (2000), with * taken from Maier (2002a, b) (N.Am. = North America, S.Am = South America, Afr. = Africa, Eur. = Europe, As. = Asia, Oc. = Oceania, Ant. = Antarctica, Cosm. = cosmopolitan), collector, herbarium number, herbarium of origin (G = Conservatoire et Jardin botaniques de la Ville de Genève, STREIFF = personal herbarium), country of origin (CH = Switzerland, D = Germany, A = Austria, FL = Liechtenstein, F = France, I = Italy, U.S.A. = United States of America), and accession number (AN) in GenBank for *trn*L-*trn*F and *rps*4.

Taxon	Species repartition	Herbarium number	*Country*	*trn*L-*trn*F (AN)	*rps*4 (AN)
Ingroup species					
Grimmia alpestris (F. Weber & D. Mohr) Schleich.	N.Am., Eur., As.	*Maier s.n.* (G)	CH	AJ847887	AJ845237
Grimmia anodon Bruch & Schimp.	N.Am., S.Am., Eur., Afr., As.	*Maier s.n.* (STREIFF)	CH	AJ847859	AJ845209
Grimmia anomala Hampe *ex* Schimp.	N.Am., Eur., As.	*Maier 11762* (G)	CH	AJ847860	AJ845210
Grimmia austrofunalis Müll. Hal.	S.Am., Afr., Oc.	*Heinrichs 4133* (G)	Bolivia	AJ847861	
		Price 1342 (G)	Bolivia		AJ845211
Grimmia caespiticia (Brid.) Jur.	N.Am., Eur., As.	*Maier s.n.* (G)	CH	AJ847862	
		Maier s.n. (G)	CH		AJ845212
Grimmia crinita Brid.	Eur., Afr., As.	*Lübenau s.n.* (G)	Syria	AJ847863	
		Maier s.n. (G)	CH		AJ845213
Grimmia decipiens (Schultz) Lindb.	Eur., Afr., As.	*Düll s.n.* (G)	D	AJ847865	AJ845215
**Grimmia dissimulata* E. Maier	Eur., As.	*Maier 10489* (G)	CH	AJ847866	
		Lübenau s.n. (G)	Greece		AJ845216
Grimmia donniana Sm.	N.Am., S.Am., Eur., As.	*Maier 11207 (G)*	CH	AJ847867	AJ845217
Grimmia elatior Bals.-Criv. & De Not.	N.Am., Eur., Afr., As.	*Streiff 50* (STREIFF)	CH	AJ847868	
		Streiff 54 (STREIFF)	CH		AJ845218
Grimmia elongata Kaulf.	N.Am., S.Am., Eur., Afr., As.	*Dürhammer s.n. (G)*	*A*	AJ847869	AJ845219
Grimmia funalis (Schwägr.) Bruch & Schimp.	N.Am., Eur., Afr., As.	*Maier s.n.* (G)	CH	AJ847870	AJ845220
Grimmia fuscolutea Hook.	Cosm. (exc. Oc.)	*Long 24065* (G)	China	AJ847871	AJ845221
Grimmia hartmanii Schimp.	N.Am., Eur., As.	*Streiff 11* (STREIFF)	CH	AJ847872	
		Maier s.n. (G)	CH		AJ845222
Grimmia incurva Schwägr.	N.Am., Eur., Afr., As.	*Maier 11596* (G)	CH	AJ847873	
		Bertram s.n. (G)	CH		AJ845223
**Grimmia khasiana* Mitt.	N.Am., As.	*Lübenau s.n.* (G)	U.S.A.	AJ847874	AJ845224
Grimmia laevigata (Brid.) Brid.	Cosm. (exc. Ant.)	*Streiff 81* (STREIFF)	CH	AJ847875	AJ845225
Grimmia lisae De Not.	N.Am., Eur., Afr., As.	*Vittoz 88* (STREIFF)	CH	AJ847876	AJ845226
Grimmia longirostris Hook.	Cosm. (exc. Ant.)	*Senn s.n.* (G)	FL	AJ847877	AJ845227
**Grimmia meridionalis* (Müll. Hal.) E. Maier	Eur.	*Streiff 40* (STREIFF)	F	AJ847878	AJ845228
Grimmia montana Bruch & Schimp.	N.Am., Eur., Afr.	*Maier s.n.* (G)	CH	AJ847879	
		Maier s.n. (G)	CH		AJ845229
Grimmia muehlenbeckii Schimp.	N.Am., Eur., As.	*Streiff 53* (G)	CH	AJ847880	AJ845230
Grimmia orbicularis Wilson	Cosm. (exc. S.Am.)	*Maier s.n.* (G)	CH	AJ847881	AJ845231
Grimmia ovalis (Hedw.) Lindb.	N.Am., Eur., Afr., As.	*Maier s.n.* (G)	CH	AJ847882	
		Vittoz 89 (STREIFF)	I		AJ845232
Grimmia pilifera P. Beauv.	N.Am., Eur., As.	*Lübenau s.n.* (G)	U.S.A.	AJ847883	
		Price 1789 (G)	U.S.A.		AJ845233
Grimmia plagiopodia Hedw.	Cosm. (exc. Afr.)	*Skrzypczak* (G)	F	AJ847884	AJ845234
Grimmia pulvinata (Hedw.) Sm.	Cosm. (exc. Ant.)	*Maier s.n.* (STREIFF)	CH	AJ847885	AJ845235
Grimmia ramondii (DC.) Margad.	N.Am., Eur., As.	*Maier 11228* (G)	CH	AJ847864	
		Maier 11748 (G)	CH		AJ845214
**Grimmia sessitana* De Not.	Eur., As.	*Vittoz 85* (STREIFF)	CH	AJ847886	AJ845236
Grimmia tergestina Bruch & Schimp.	N.Am., S.Am., Eur.,	*Maier 11433* (G)	CH	AJ847888	

Table 1. Continued

Taxon	Species repartition	Herbarium number	*Country*	*trn*L-*trn*F (AN)	*rps*4 (AN)
	As.	*Maier s.n.* (G)	CH		AJ845238
Grimmia torquata Hook. *ex* Drumm.	N.Am., Eur.	*Maier s.n.* (G)	CH	AJ847889	AJ845239
Grimmia trichophylla Grev.	Cosm. (exc. Ant.)	*Maier 11476* (G)	D	AJ847890	AJ845240
Grimmia unicolor Hook.	N.Am., Eur., Afr., As.	*Streiff 82* (STREIFF)	CH	AJ847891	AJ845241
Outgroup species					
Coscinodon cribrosus (Hedw.) Spruce		*Maier s.n.* (STREIFF)	CH	AJ847855	AJ845205
Ditrichum flexicaule (Schwägr.) Hampe		*Price 2309* (G)	F	AJ847854	AJ845204
Funaria hygrometrica Hedw.		*Price 2258* (G)	CH	AJ847853	AJ845203
Hydrogrimmia mollis (Bruch & Schimp.) Loeske		*Streiff 18* (STREIFF)	CH	AJ847856	AJ845206
Racomitrium aciculare (Hedw.) Brid.		*Maier s.n.* (G)	CH	AJ847857	AJ845207
Schistidium apocarpum (Hedw.) Bruch & Schimp. *s.l.*		*Streiff 46* (STREIFF)	CH	AJ847858	
		Maier s.n. (G)	CH		AJ845208

composed of 0.2 µl AmpliTaq® DNA Polymerase (Applied Biosystems) (5 U/µl), 5 µl $MgCl_2$ (2.5 mM), 5 µl 10× buffer PCR, 5 µl dNTP (2mM each), 0.5 µl BSA (0.5%), and 0.5 µl of each primer (100 mM). Sterilized, distilled water was added to the solution to bring it up to 49 µl and 1 µl of extraction product was added. DNA fragments were amplifed in a thermocycler with the following programs: trnL-trnF [2 min 94°C (45 sec 94°C, 30 sec 50°C, 45 sec 72°C) × 35, 5 min 72°C, hold 4°C] and rps4 [2 min 94°C (1 min 94°C, 1 min 52°C, 3 min 72°C) × 35, 7 min 72°C, hold 4°C]. PCR products were purified with Prep-A-Gene™ DNA purification kit from Bio-Rad® and eluted in 13 µl of TE8 buffer. Sequencing was done on an automatic sequencer (ABI Prism™ 377 DNA Sequencer) with Applied Biosystems protocol (ABI Prism® BigDye™ Terminator Cycle Sequencing Ready Reaction Kits).

Sequence analysis. For each plastid region, forward (5'–3') and reverse (3'–5') sequences were assembled with CAP and ALIGNN (http://www.infobiogen.fr). A BLAST search (http://www.ncbi.nlm.nih.gov; Altschul et al., 1990) was performed to check that the consensus sequences of *trn*L-*trn*F and *rps*4 were from mosses. Sequences were aligned manually using Se-Al v2.0a8 (Rambault 2001), and exported to Paup 4.0b10 (Swofford 2002). Unresolved regions and ambiguities were excluded from matrices

Table 2. List of outgroup sequences (AN = GenBank accession number) and references.

	AN trnL-trnF	AN rps4	Reference
Coscinodon cribrosus (Hedw.) Spruce	AJ847855	AJ845205	Streiff, this paper
Dicranum muehlenbeckii Bruch & Schimp.	AF231245	AF231276	La Farge et al., 2000
Ditrichum flexicaule (Schwägr.) Hampe	AJ847854	AJ845204	Streiff, this paper
Drummondia obtusifolia Müll.Hal.	AF229895	AF223038	Goffinet & Cox, 2000
Funaria hygrometrica Hedw.	AJ847853	AJ845203	Streiff, this paper
Hydrogrimmia mollis (Bruch & Schimp.) Loeske	AJ847856	AJ845206	Streiff, this paper
Ptychomitrium gardneri Lesq.	AF231258	AF231290	La Farge et al., 2000
Racomitrium aciculare (Hedw.) Brid.	AJ847857	AJ845207	Streiff, this paper
Schistidium apocarpum s.l.	AJ847858	AJ845208	Streiff, this paper
Scouleria aquatica Hook.	AF231179		La Farge et al., 2000
Scouleria aquatica Hook.		AF306984	Goffinet et al., 2001

for the analyses. Insertions/deletions were excluded, but were coded (0, 1) in case they were informative (Simmons & Ochoterena 2000). The intraspecific variability were studied in some Grimmia species and it varied from 0% to 1% for both sequences considered.

Morphological and anatomical characters. The list of the specimens and the 52 morphological-anatomical characters and character states used in the morphological-anatomical study are described and coded in Streiff (2005).

Phylogenetic analyses. According to the Akaike Information Criterion (AIC, Akaike 1974), Hasegawa-Kishino-Yano model (HKY, Hasegawa et al. 1985) plus Gamma distributed rate heterogeneity (Yang 1994) was chosen for *trn*L-*trn*F and the General Time-Reversible model (GTR, Rodriguez et al. 1990) plus Gamma distributed rate heterogeneity was chosen for *rps*4 as the models that best fitted the data by Modeltest v.3.7 (Posada & Crandall 1998). The *rps*4 and *trn*L-*trn*F datasets were analysed separately, together, and combined with the morphological/anatomical dataset. The primer annealing sites of *rps*4 sequences were excluded from analysis when present. The sequences *trn*L-*trn*F and *rps*4 were combined for 43 ingroup and outgroup taxa. Bayesian analyses were conducted using MrBayes 3.1 (Huelsenbeck & Ronquist 2002). Four analyses were simultaneously run for 5,000,000 generations, sampling every 1,000 trees and recording branch lengths. The 2,500 first trees were removed for the "burn-in" phase. The remaining 2,501 trees were combined. Each of the four runs was analyzed independently and the four consensus trees were compared to verify the stability of the tree topologies and of the posterior probabilities.

Maximum parsimony (MP) analyses were performed using Paup 4.0b10 (Swofford 2002) with 100 replicates (stepwise random taxon addition) using "Tree Bisection and Reconnection" (TBR) branch-swapping. All the equally most-parsimonious trees were saved. Branch support was calculated using 1,000 bootstrap replicates with the same options as for the heuristic search. Gaps were treated as missing data, and characters as unordered. The *rps*4 and *trn*L-*trn*F datasets were analyzed independently, combined together and with the morphological dataset. In the analysis of molecular and morphological characters combined, the data set was successively weighted as a function of the Rescaled Consistency Index (RC), and the same search protocol was repeated. The weighting was done to give more importance to stable characters and to minimize the impact of homoplasy on the phylogenetic reconstruction as shown in literature (e.g., Hassanin et al. 1998). The weighting methods have been critized for their subjectivity and for their circularity, respectively (e.g., Neff 1986; Philippe et al. 1996). The main changes in morphological character states were mapped onto the consensus tree using MacClade 3.08a (Maddison & Maddison 1999).

RESULTS

Values from the heuristic searches of the four analyses are presented in **Table 3**. The analyses based on *rps*4 (not shown) and *trn*L-*trn*F (not shown) are mostly congruent, except for the position of *Grimmia incurva*. This taxon is close to the *Racomitrium* species in the analyses based on *rps*4 and belongs to the clade "*Grimmia*" in the analyses based on *trn*L-*trn*F. Trees obtained with *trn*L-*trn*F are less structured than those obtained with *rps*4. The combined plastid DNA analyses (**Fig. 1**) and the morphological and molecular combined analyses (**Fig. 2**) have the same topology as that seen in the *rps*4 analysis.

rps4 and trnL-trnF combined. The trees obtained

Table 3. Values for the four analyses (*rps*4, *trn*L-*trn*F, *rps*4 and *trn*L-*trn*F combined, and molecular and morphological data combined (simple and 2× RC-reweighted)). CI = Consistency Index; RI = Retention Index; RC = Rescaled Consistency Index.

Analysis	No. characters	Informative characters	mp-trees obtained	Length	CI	RI	RC
*rps*4	533	70 (13%)	9100	266	0.684	0.737	0.504
*trn*L-*trn*F	432	72 (17%)	89	224	0.674	0.738	0.498
*rps*4 + *trn*L-*trn*F	965	130 (13%)	285	469	0.680	0.713	0.485
Molecular + morphology	1017	176 (17%)	24	747	0.513	0.608	0.312
	Reweight (2×)		1		0.863	0.871	0.752

Figure 1. Phylogenetic tree found by Bayesian distances (model used for *rps*4: GTR + G; model used for *trn*L-*trn*F: HKY + G; 5,000,000 generations, sampling every 1,000 trees, recording branch lengths, 2,500 first trees burn-in) with 43 species and *rps*4 and *trn*L-*trn*F combined, Bayesian support (>0.90) above branches. Principal nodes: I–VI.

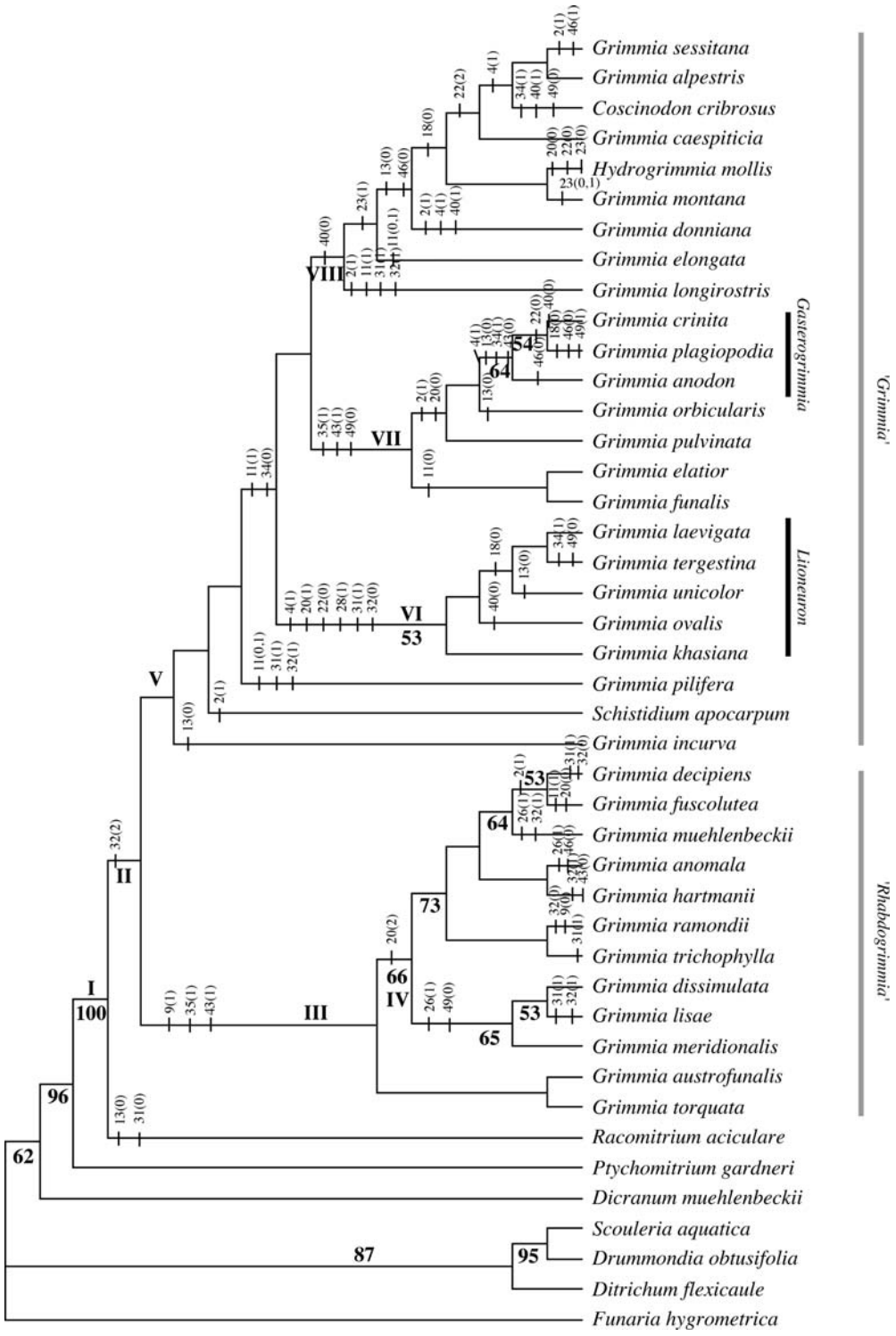

Figure 2. Tree obtained with two successive RC-weightings using *rps*4, *trn*L-*trn*F and morphological characters. Bootstrap values (> 50%) shown above branches. Important changes in character states are placed on the tree. In bracket: character state. I–VIII: important nodes.

from the Bayesian (**Fig. 1**) and the MP- (not shown) analyses have small differences in their topologies. The main differences are the positions of *Racomitrium aciculare* and *G. incurva*. In the MP-consensus tree, these two species form a small clade differentiated from the two clades: "*Rhabdogrimmia*" and "*Grimmia*." Furthermore, *G. torquata* has an unresolved position at the base of the Grimmiaceae clade. The two clades "*Rhabdogrimmia*" and "*Grimmia*" are supported with bootstraps of 67% and 72%, respectively.

In the Bayesian analysis, the tree is rooted by *Funaria hygrometrica* (**Fig. 1**). The clade containing the members of the Grimmiaceae is well supported (node II, posterior probability of 1.00) and two clades are distinct within the Grimmiaceae. The first of these corresponds to the "*Rhabdogrimmia*" clade (node III) and contains species traditionally included in the subgenus *Rhabdogrimmia* Limpr. Three species remain unresolved at the base of this clade: *Racomitrium aciculare*, *Grimmia australis* and *G. torquata*. The remaining species (node V, posterior probability of 0.99) are well differentiated and their branches have high posterior probabilities (between 0.94 and 1.00). The second clade "*Grimmia*" contains the remaining *Grimmia* species and the three genera *Coscinodon*, *Schistidium* and *Hydrogrimmia*. *Grimmia incurva* is basal to the clade "*Grimmia*" (node IV). The remaining species form a well-supported clade (node VI, posterior probability of 1.00), but one that is not well structured and the majority of the species form a polytomy. Three small subclades are present which lack support.

Ptychomitrium gardneri is basal to the clade containing the Grimmiaceae species and this relationship (node I) is supported by a posterior probability of 1.00.

Molecular and morphological data combined. The trees obtained with Bayesian (not shown) and MP-analyses (**Fig. 2**) have the same topologies except for two differences. The position of *Racomitrium aciculare* is basal with respect to the "*Rhabdogrimmia*" clade in the Bayesian analysis (relationship not supported with posterior probability) whereas this species is basal with respect to the Grimmiaceae clade in the MP-analysis (no bootstrap support). The second difference is the presence of two small clades containing *G. anodon, G. plagiopodia, G. crinita* and *G. elatior*, *G. funalis*, *G. orbicularis*, *G. pulvinata* in the Bayesian tree. These taxa form a unique clade in the MP-analysis.

In the MP-analysis, the CI (Consistency Index) and RI (Retention Index) stabilized after three rounds of successive weighting and one tree was obtained (**Fig. 2**). Primary information was given by molecular characters whereas the morphological characters structured the terminal branches of the tree.

The consensus tree obtained with the MP-analysis (**Fig. 2**) contained a main clade (node I) composed of all the Grimmiaceae species included in the analysis and supported by 100% bootstrap. Two clades were present ("*Rhabdogrimmia*" and "*Grimmia*," node II) as seen in the previous analysis. Clade "*Rhabdogrimmia*" (node III) was composed of 12 species that belong to the subgenus *Rhabdogrimmia*. The clade "*Grimmia*" (node V) contained the remaining species of *Grimmia*, *Coscinodon*, *Hydrogrimmia* and *Schistidium*. The basal branch of clade "*Rhabdogrimmia*" was not supported (node III) but the species of this clade had three morphological characters in common: the presence of gemmae, at least in apical leaves (9, except in *G. ramondii*), setae curved (35) and furrowed capsules (43). The presence of gemmae was the only synapomorphy of this clade, the setae curved and the furrowed capsules are found in some species of the "*Grimmia*" clade. The remaining branches of this clade had better, albeit low, statistical supports (bootstraps of 66%, 73%, 64% and 65%, respectively).

Clade "*Grimmia*" (node V) was statistically not supported, however three subclades were present within it. The first subclade, "*Litoneuron*" (node VI) contained *G. khasiana*, *G. laevigata*, *G. tergestina*, *G. unicolor* and *G. ovalis*, species that generally have been included in subgenus *Litoneuron* I. Hagen. This subclade was poorly supported (bootstrap 53%), but six morphological characters were present in each species of the subclade: leaves well developed in the upper part of the stem only and forming an apical tuft (4), laminae bistratose at mid-leaf (20), leaves canaliculate (22), costae poorly developed from mid-leaf (28), four guide-cells present in mid-leaf (31), and more than six guide-cells present at leaf insertion (32). The characters of bistratose leaf laminae (20) and poorly-developed costae (28) are present only in this subclade.

A second subclade (node VII) contained *G. anodon*, *G. plagiopodia* and *G. crinita*, species traditionally included in the subgenus *Gasterogrimmia* Schimp., and *G. orbicularis, G. pulvinata*, *G. elatior* and *G. funalis*, which have generally been associated with the subgenus *Rhabdogrimmia*. This subclade was defined by the following characters: setae curved (35), a character also present in the "*Rhabdogrimmia*" clade, and peristome teeth inserted at the capsule mouth (49, except in *G. plagiopodia* where teeth were inserted below the mouth, and in *G. anodon* which lacks a peristome). The four basal species, *G. elatior*, *G. funalis*, *G. orbicularis* and *G. pulvinata*, also possessed furrowed capsules (43) as do the species in the clade "*Rhabdogrimmia*." *Grimmia anodon*, *G. plagiopodia* and *G. crinita* formed a clade equivalent to that of "*Gasterogrimmia*" as defined in the literature. They had leaves forming an apical tuft (4), basal leaf cells uniform (13) and setae short (34).

The last subclade (node VIII) contained the remaining *Grimmia*, *Hydrogrimmia* and *Coscinodon* taxa. This subclade contained species with cucullate calyptrae (40, except *G. donniana* and *C. cribrosus*). *Coscinodon cribrosus*, *G. sessitana*, *G. alpestris* and *G. caespiticia* have leaves that form a W-shape in transverse section (22).

Discussion

***Grimmiaceae and the monophyly of* Grimmia.** In this study, the Grimmiaceae are monophyletic. The main clade containing the Grimmiaceae species is well supported in both analyses. The Ptychomitriaceae (represented here by *Ptychomitrium*) are sister to the Grimmiaceae. These two families belong to the Grimmiales as shown by Tsubota et al. (2003) with *rbc*L, Hedderson et al. (2004) with *rps*4 and Goffinet and Buck (2004) with DNA sequence data. Furthermore, as seen in these three articles, this present analysis shows that *Scouleria* and *Drummondia* do not belong to the Grimmiales as proposed previously in literature (Buck & Goffinet 2000), and the true relationships of these two genera warrant further investigation.

In the present study, the genus *Grimmia*, as previously circumscribed in literature (e.g., Limpricht 1890; Loeske 1913), is not monophyletic. This hypothesis is supported by the position of the representatives of the three genera *Hydrogrimmia*, *Coscinodon* and *Schistidium*, within the clade "*Grimmia*," none of which is clearly distinct from *Grimmia*. Furthermore, the taxa sampled from *Grimmia* are divided in two clades (**Figs. 1 and 2**). In a study on the Dicranidae, Hedderson et al. (2004), using the sequence *rps*4 and nine Grimmiaceae species (with three *Grimmia* species: *Grimmia curvata* [= *Dryptodon patens* (Hedw.) Brid.], *G. pulvinata* and *G. torquata*), also found that the Grimmiaceae were monophyletic and that *Grimmia* was not. The Grimmiaceae contained one clade composed of species belonging to *Schistidium*, *Coscinodon* and *Hydrogrimmia* associated with *G. pulvinata*. The two remaining *Grimmia* were basal to the Grimmiaceae clade and situated with *Racomitrium* species. The tree topology is congruent with the trees of this present study.

"Rhabdogrimmia" *and* "Grimmia" *subclades.* Two clades ("*Rhabdogrimmia*" and "*Grimmia*") are observed (**Figs. 1 and 2**). In the clade "*Rhabdogrimmia*," *G. anomala*, *G. decipiens*, *G. hartmanii*, *G. muehlenbeckii*, *G. lisae*, *G. ramondii* and *G. trichophylla* are species traditionally placed in the subgenus *Rhabdogrimmia* (e.g., Brotherus 1924; Loeske 1913). The recently described *G. dissimulata* (Maier 2002a) and *G. meridionalis*, which was first described as a variety of *G. trichophylla* (*G. trichophylla* var. *meridionalis* Müll. Hal.), also belong to *Rhabdogrimmia* based on morphology (Maier 2004). *Grimmia fuscolutea*, found close to *G. decipiens* and *G. muehlenbeckii* in the clade "*Rhabdogrimmia*," is generally placed either in the subgenus *Grimmia* (Hedw.) Schimp. (Limpricht 1890) or in the subgenus *Guembelia* (Hampe) Schimp. (Brotherus 1924). Recently, *G. fuscolutea* was placed in the subgenus *Rhabdogrimmia* (Nyholm 1998). The species *G. austrofunalis*, found in South America, Oceania and Africa, is not known from Europe (Muñoz & Pando 2000). Since the main revisions of the genus *Grimmia* have principally concerned Europe and Asia (Deguchi 1979; Greven 1995; Maier & Geissler 1995; Maier 2002a, b), this species is understudied. It has not been clearly affiliated with a particular subgenus in the literature. In this study this species is found in a basal position with respect to the subclade "*Rhabdogrimmia*." *Grimmia torquata*, in an unresolved position in the "*Rhabdogrimmia*" clade of the tree from *rps*4 and *trn*L-*trn*F analysis (**Fig. 1**) or in a basal position in the

combined analysis (**Fig. 2**), has a special status in the majority of the revisions concerning the genus *Grimmia* partly because of particular morphological traits such as very crisped leaves. *Grimmia torquata* belongs traditionally to the section *Torquatae* I. Hagen of *Rhabdogrimmia* or to subgenus *Torquatae* (I. Hagen) Loeske (e.g., Loeske 1913; Nyholm 1998). *Grimmia torquata* and the species belonging to the "*Rhabdogrimmia*" clade can produce gemmae. This character is found only in this group of species.

In the clade "*Grimmia*," the subclade containing *Gasterogrimmia* species (node VII) is also composed of four species that are generally placed in the subgenus *Rhabdogrimmia*. *Grimmia orbicularis* is generally associated with *G. pulvinata* in generic treatments. Loeske (1913) described the subgenus *Pulvinatae* Loeske, containing only these two species. He made a comment on their morphological similarities to *Rhabdogrimmia* species, but noted that the cell pattern and the hair-points looked different from those species placed in *Rhabdogrimmia*. *Grimmia funalis* has not been unanimously placed in subgenus *Rhabdogrimmia*. For example, Limpricht (1890) placed it in subgenus *Rhabdogrimmia*, Brotherus (1924) in subgenus *Guembelia* and Loeske (1913) in subgenus *Torquatae* with *G. torquata*. Finally, *G. elatior*, considered unanimously as belonging to *Rhabdogrimmia* (Brotherus 1924, Limpricht 1890, Loeske 1930; Maier 2004; Nyholm 1998), is also found in the "*Grimmia*" subclade. These four species do not produce gemmae.

Except the presence of gemmae in "*Rhabdogrimmia*," no clear morphological characters separate the two clades. Furthermore, these clades also do not show clear differences in their large-scale distribution or in their ecological characteristics. The majority of *Grimmia* species prefer siliceous rocks. In these two clades, there are some species that can grow on calcareous rocks such as *G. tergestina* from "*Grimmia*" or *G. dissimulata* from "*Rhabdogrimmia*." This preference may have appeared more than once within the genus. Most *Grimmia* species have a large geographic range and species belonging to the two subclades are found on each continent. A more detailed geographic analysis of species distribution may reveal more significant information concerning differences in fine-scale distribution patterns.

In both analyses (**Figs. 1 and 2**), a difference in the branching structures of the two clades "*Rhabdogrimmia*" and "*Grimmia*" is found. The clade "*Rhabdogrimmia*" is more internally structured than the "*Grimmia*" clade. One hypothesis explaining this phenomenom is that the "*Rhabdogrimmia*" species could have diversified earlier and contain more intraspecific variation than the "*Grimmia*" clade. In the future it will be necessary to find new molecular characters (e.g., single copy nuclear genes or faster evolving plastid loci) that permit further investigation of the phylogenetic relationships of *Grimmia* and which could be used to estimate the divergence time of the different subclades.

In the present study, the different characters used support the monophyly of the Grimmiaceae with a maximum bootstrap value, but less support is present at the generic level. To approach the problem with different molecular characters, a nuclear marker, ITS (Internal Transcribed Spacer, present in multiple copies in the nucleus), has been tested in the genus *Grimmia* but the variability was too high to be included with these analyses (results unpublished). This extreme variability has been also observed in the ITS region in other moss genera such as Amblystegium (Vanderpoorten et al. 2001).

Conclusions

The Grimmiaceae are monophyletic and sister to the Ptychomitriaceae. *Grimmia*, as currently circumscribed, is not monophyletic. Two clades within the Grimmiaceae are observed: "*Rhabdogrimmia*" and "*Grimmia*." Species of both clades are not differentiated by their ecology or their distributional ranges. The production of gemmae in the species belonging to the "*Rhabdogrimmia*" clade is the main morphological difference between both clades. The weak support for some of the internal nodes in the analyses does not clearly separate these two subclades and it is not appropriate to decide upon their taxonomic status. An extension of the taxon sampling with the addition of species of the genera *Grimmia, Racomitrium*, *Schistidium* and *Coscinodon*, and the use of more variable phylogenetic markers are required to better understand the phylogenetic relationships of the family and to help in delimiting the different genera. Further analyses are in progress at the Real

Jardín Botánico of Madrid, Spain (Muñoz, pers. comm.).

ACKNOWLEDGMENTS

I thank the University of Lausanne and the Conservatoire et Jardin botaniques de la Ville de Genève for logistical support for this work, Michelle Price for her advice, and for corrections to this manuscript, Eva Maier for her help in specimen determination and our numerous discussions on *Grimmia*, Jean-François Manen for his technical assistance and helpful comments on this manuscript, Nicolas Salamin for his invaluable help with the analyses, Gwenaël Jacob, Guillaume Besnard, Bernard Goffinet and an anonymous reviewer for reviewing this manuscript, and Nicole Galland for her support and assistance.

LITERATURE CITED

Akaike, H. 1974. A new look at the statistical identification model. IEEE Transactions on Automatic Control 19: 716–723.

Altschul, S. F., W. Gish, W. Miller, E. W. Myers & D. J. Lipman. 1990. Basic Local Alignment Search Tool. Journal of Molecular Biology 215: 403–410.

Brotherus, V. F. 1924. Musci. Spezieller Teil. I. *In* A. Engler (ed.), Die näturlichen Pflanzenfamilien, ed. 2. Volume 10. Berlin, Germany.

Buck, W. R. & B. Goffinet. 2000. Morphology and classification of mosses. Pages 71–123. *In* A. J. Shaw & B. Goffinet (eds.), Bryophyte Biology. University Press, Cambridge, England.

Cao, T. & D. H. Vitt. 1986. Revision of *Grimmia* and *Schistidium* in China. Journal of the Hattori Botanical Laboratory 61: 231–247.

Churchill, S. P. 1981. A phylogenetic analysis, classification, and synopsis of the genera of the Grimmiaceae (Musci). Pages 127–144. *In* V.A Funk & D.R. Brooks (eds.), Advances in Cladistics. New York Botanic Garden, Bronx, U.S.A.

Crum, H. A. & L. E. Anderson. 1981. Mosses of Eastern Northern America. Columbia University Press, New York.

Deguchi, H. 1979. A revision of the genera *Grimmia*, *Schistidium* and *Coscinodon* (Musci) of Japan. Journal of Sciences Hiroshima University, Series B, Division 2 (Botany) 16: 121–256.

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

Goffinet, B. & W. R. Buck. 2004. Systematics of the Bryophyta (Mosses): from molecules to a revised classification. *In* B. Goffinet, V. Hollowell & R.E. Magill (eds.), Molecular systematics of bryophytes. Monographs in Systematic Botany from the Missouri Botanical Garden 98: 205–239.

——— & C. J. Cox. 2000. Phylogenetic relationships among basal-most arthrodontous mosses with special emphasis on the evolutionary significance of the Funariinae. The Bryologist 103: 212–223.

———, ———, A. J. Shaw & T. A. J. Hedderson. 2001. The Bryophyta (Mosses): Systematic and evolutionary inferences from an *rps*4 gene (cpDNA) phylogeny. Annals of Botany 87: 197–208.

Greven, H. C. 1995. *Grimmia* Hedw. (Grimmiaceae, Musci) in Europe. Backhuys Publishers, Leiden, Netherlands.

———. 2003. *Grimmias* of the world. Backhuys Publishers, Leiden, Netherlands.

Hasegawa, M., H. Kishino & T. Yano. 1985. Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. Journal of Molecular Evolution 22: 160–174.

Hassanin, A., G. Lecointre & S. Tillier. 1998. The 'evolutionary signal' of homoplasy in protein-coding gene sequences and its consequences for a priori weighting in phylogeny. Comptes Rendus de l'Académie des Sciences Paris, Sciences de la Vie 321: 611–620.

Hedderson, T. A. J., D. J. Murray, C. J. Cox & T. L. Nowell. 2004. Phylogenetic relationships of haplolepideous mosses (Dicranidae) inferred from *rps*4 gene sequences. Systematic Botany 29: 29–41.

Huelsenbeck, J. P. & F. Ronquist. 2002. MrBayes. v3.0b4.

La Farge, C., B. D. Mishler, J. A. Wheeler, D. P. Wall, K. Johannes, S. Schaffer & A. J. Shaw. 2000. Phylogenetic relationships within the haplolepideous mosses. The Bryologist 103: 257–276.

Limpricht, K. G. 1890. Die Laubmoose Deutschlands, Oesterreichs und der Schweiz. Leipzig, Germany.

Loeske, L. 1913. Die Laubmoose Europas. I. Grimmiaceae. Berlin, Germany.

———. 1930. Monographie der europäischen Grimmiaceen. Bibliotheca Botanica 101: 1–236.

Maddison, W. P. & D. R. Maddison. 1999. MacClade. 3.08a. Sinauer Associates.

Maier, E. 2002a. *Grimmia dissimulata* E.Maier *sp. nova*, and the taxonomic position of *Grimmia trichophylla* var. *meridionalis* Müll. Hal. (Musci, Grimmiaceae). Candollea 56: 281–300.

———. 2002b. The genus *Grimmia* (Musci, Grimmiaceae) in the Himalaya. Candollea 57: 143–238.

———. 2004. The formation of plicae in capsules of mosses of the order Bryales, with a focus on the genus *Grimmia* Hedw. Candollea 59: 51–63.

——— & P. Geissler. 1995. *Grimmia* in Mitteleuropa: ein Bestimmungsschlüssel. Herzogia 11: 1–80.

Muñoz, J. 1998a. A taxonomic revision of *Grimmia* subgenus *Orthogrimmia* (Musci, Grimmiaceae). Annals of the Missouri Botanical Garden 85: 367–403.

———. 1998b. Materials toward a revision of *Grimmia* (Musci: Grimmiaceae): nomenclature and taxonomy of *Grimmia longirostris*. Annals of the Missouri Botanical Garden 85: 352–363.

———. 1999. A revision of *Grimmia* (Musci, Grimmiaceae) in the Americas. 1: Latin America. Annals of the Missouri Botanical Garden 86: 118–191.

——— & F. Pando. 2000. A world synopsis of the genus *Grimmia* (Musci, Grimmiaceae). Monographs in Systematic Botany from the Missouri Botanical Garden 83: [i–viii] 1–133.

Neff, N. A. 1986. A rational basis for a priori character weighting. Systematic Zoology 35: 110–123.

Nyholm, E. 1998. Grimmiales. Illustrated Flora of Nordic Mosses 4: 287, 331–352. Nordic Bryological Society, Lund, Sweden.

Philippe, H., G. Lecointre, H. L. V. Lê & H. Le Guyader. 1996. A critical study of homoplasy in molecular data with the use of a morphologically based cladogram, and its consequences for character weighting. Molecular Biology and Evolution 13: 1174–1186.

Posada, D. & K. A. Crandall. 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14: 817–818.

Rambault, A. 2001. Se-Al. 2.0a8. University of Oxford.

Rodriguez, F., J. L. Oliver, A. Marin & J. R. Medina. 1990. The general stochastic model of nucleotide substitution. Journal of Theoretical Biology 142: 485–501.

Simmons, M. P. & H. Ochoterena. 2000. Gaps as characters in sequence-based phylogenetic analyses. Systematic Biology 49: 369–381.

Souza-Chies, T. T., G. Bittar, S. Nadot, L. Carter, E. Besin & B. Lejeune. 1997. Phylogenetic analysis of Iridaceae with parsimony and distance method using the plastid gene *rps*4. Plant Systematics and Evolution 204: 109–123.

Streiff, A. 2005. Morphological study of the genus *Grimmia* Hedw. (Grimmiaceae, Bryopsida). Journal of the Hattori Botanical Laboratory 97: 317–338.

Swofford, D. L. 2002. Paup. 4.0b10. Sinauer Associates.

Taberlet, P., L. Gielly, G. Pautou & J. Bouvet. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. Plant Molecular Biology 17: 1105–1109.

Tsubota, H., Y. Ageno, B. Estébanez, T. Yamaguchi & H. Deguchi. 2003. Molecular phylogeny of the Grimmiales (Musci) based on chloroplast *rbc*L sequences. Hikobia 14: 55–70.

Vanderpoorten, A., A. J. Shaw & B. Goffinet. 2001. Testing controversial alignments in *Amblystegium* and related genera (Amblystegiaceae: Bryopsida). Evidence from rDNA ITS sequences. Systematic Botany 26: 470–479.

Yang, Z. 1994. Maximum likelihood phylogenetic estimation from DNA sequences with variable rates over sites: approximate methods. Molecular Evolution 39: 306–314.

ms. received March 14, 2005; accepted January 27, 2006.