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Phylogenetic analyses reveal high levels of polyphyly among pleurocarpous lineages as well as novel clades

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Abstract. Phylogenetic analyses of the Hypnales usually show the same picture of poorly resolved trees with a large number of polyphyletic taxa and low support for the few reconstructed clades. One odd clade, however, consisting of three genera that are currently

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treated either within the Leskeaceae (*Miyabea*) or Neckeraceae (*Homaliadelphus* and *Bissetia*), was retrieved in a previously published phylogeny based on chloroplast *rbc*L. In order to elucidate the reliability of the observed *Homaliadelphus* - *Miyabea* - *Bissetia* -clade (HMB-clade) and to reveal its phylogenetic relationships a molecular study based on a representative set of hypnalean taxa was performed. Sequence data from all three genomes, namely the ITS1 and 2 (nuclear), the *trn*S-*rps*4-*trn*T-*trn*L-*trn*F cluster (plastid), the *nad*5 intron (mitochondrial), were analyzed. Although the phylogenetic reconstruction of the combined data set was not fully resolved regarding the backbone it clearly indicated the polyphyletic nature of various hypnalean families, such as the Leskeaceae, Hypnaceae, Hylocomiaceae, Neckeraceae, Leptodontaceae and Anomodontaceae with respect to the included taxa. In addition the results favor the inclusion of the Leptodontaceae and Thamnobryaceae in the Neckeraceae. The maximally supported HMB-clade consisting of the three genera *Homaliadelphus* (2–3 species), *Miyabea* (3 species) and *Bissetia* (1 species) is resolved sister to a so far unnamed clade comprising *Taxiphyllum aomoriense*, *Glossadelphus ogatae* and *Leptopterigynandrum*. The well-resolved and supported HMB-clade, here formally described as the Miyabeaceae, *fam. nov.* is additionally supported by morphological characters such as strongly incrassate, porose leaf cells, a relatively weak and diffuse costa and the presence of dwarf males. The latter are absent in the Neckeraceae and the Leskeaceae. It is essentially an East Asian family, with one species occurring in North America.

Keywords. *Glossadelphus*, *Taxiphyllum*, taxonomy, evolution, dwarf males, Miyabeaceae, Hypnales, phylogeny, *Homaliadelphus*, *Miyabea*, *Bissetia*.

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Although the monophyly of pleurocarpous mosses (homocostate pleurocarps sensu Bell et al. 2007) is beyond doubt and consistently resolved with moderate to high support in multigene analyses (e.g., Beckert et al. 2001; Bell et al. 2007; Cox et al. 2000; Cox & Hedderson 1999; Quandt et al. 2007) we observe a considerable lack of resolution and support among the various pleurocarpous lineages (e.g., Buck et al. 2000; Goffinet et al. 2001; Ignatov et al. 2007; Tsubota et al. 2002). This is especially evident in species-rich and/or single marker analyses where phylogenies of homocostate pleurocarps notoriously turn out as bushes instead of trees. However, the problem of identifying natural groups is not unique to molecular systematics as bryologists throughout the last century consistently faced this challenge while recognizing lineages solely based on the interpretation of morphological traits (e.g., Buck & Vitt 1986; Hedenäs 1995). The classification of pleurocarpous mosses even at the family level is in fact difficult, due to convergent evolution and homoplasy of morphological characters (Hedenäs 2007; Huttunen et al. 2004; Quandt et al. 2009).

Even if some families are reliably resolved through recent phylogenetic analyses (Huttunen et al. 2004; Quandt et al. 2003b, 2009; Vanderpoorten et al. 2002a), many inter- and intrafamilial relationships remain unknown, especially considering the bryological "dust bins," such as the Hypnaceae. Hence, although the new molecular tools boosted phylogenetic reconstructions, and therefore systematics, the prominent challenge in pleurocarpous moss systematics remains to identify and characterize natural higher order groups among the ca. 5000 pleurocarpous species and to relate these to each other (compare Shaw & Renzaglia 2004). This is complicated by the fact that sequence variation of the currently known markers among

hypnalean taxa is extremely low, even if non-coding regions are applied. Therefore, in order to obtain a reliable backbone of pleurocarpous mosses it seems that a high sequencing effort is required and/or new markers containing better phylogenetic signals need to be applied (see www.pleurocarps.eu).

Among the few reported pleurocarpous clades receiving considerable support, a curious one was evident in the analysis based on the plastid *rbc*L gene by Tsubota et al. (2002). In this analysis, *Miyabea fruticella*, *Homaliadelphus targionianus* and *Bissetia lingulata* (HMB-clade), taxa that have never been considered related and are currently placed in different families, unexpectedly formed a clade with high bootstrap-support. Preliminary examination of these taxa, however, revealed that they share a number of morphological features, some of which hint at affinities to the Anomodontaceae. This suggested that the clade could be natural and inspired us to perform the present molecular study.

Bissetia was placed in the Neckeraceae since its inception by Brotherus (1906), but Enroth (1992) suggested a close relation to *Anomodon* and thus transferred it into the Anomodontaceae, a treatment that was neither reflected in the classification of mosses by Goffinet and Buck (2004) nor in the most recent classification by Goffinet et al. (2009). The genus has only one species, *B. lingulata*, distributed in Japan and South Korea (Noguchi 1989).

The genus *Homaliopsis* was established by Dixon and Potier de la Varde (Dixon 1928), but the generic name was recognized to be a later homonym, and the taxon was renamed *Homaliadelphus* by Dixon and Potier de la Varde (Dixon 1931). It has been consistently placed in the Neckeraceae, mainly due to the wide and roundish, strongly complanate leaves and a very short or absent costa. Iwatsuki (1958) revised the genus and recognized three species, but Noguchi's (1989) treatment implies he thought there were only two, the generitype, *H. targionianus*, with three varieties, and *H. sharpii*. The former has a relatively wide distribution in SE Asia, ranging from Japan and Korea to India, while the latter is restricted to North America, or, if Iwatsuki's concept of *H. sharpii* var. *rotundatus* (= *H. targionianus* var. *rotundatus*) is accepted, then it also occurs in Japan.

Miyabea has three species that are narrowly distributed in Japan, Korea and the eastern provinces of China (Noguchi 1991; Watanabe 1972; Wu et al. 2002). Brotherus (1907) originally placed the genus in the Leskeaceae ''Gruppe'' Anomodonteae, which was later transferred to the Thuidiaceae as the subfamily Anomodontoideae (Brotherus 1925). That placement was accepted by Watanabe (1972), although in his treatment the generic contents of the subfamily differed somewhat from Brotherus' (1925). Some authors, such as Wu et al. (2002) have recognized that taxon as an independent family, the Anomodontaceae, and included *Miyabea* in it. However, Buck and Goffinet (2000) as well as Goffinet et al. (2009) followed Brotherus's original concept and thought *Miyabea* was best placed in the Leskeaceae, even if the family's definition and circumscription differed considerably from Brotherus' concept.

In order to elucidate the reliability of the *Homaliadelphus* - *Miyabea* - *Bissetia* - clade (in the following referred to as HMB-clade) and its phylogenetic position we used a molecular approach based on sequence data from all three genomes. We combined sequence data of the ITS1-5.8S-ITS2 region (nuclear ribosomal DNA), the *nad*5-intron (mitochondrial DNA) and the *trn*S-*rps*4-*trn*T-*trn*L-*trn*F cluster (plastidal DNA). Finally, after showing the monophyly of the group, we will discuss the morphological synapomorphies distinguishing this clade.

Materials and Methods

Taxon sampling and molecular markers. Fifty-eight taxa from 50 different genera representing 20 families of homocostate pleurocarps (Amblystegiaceae, Anomodontaceae, Brachytheciaceae, Entodontaceae, Calliergonaceae, Cryphaeaceae, Hookeriaceae, Hylocomiaceae, Hypnaceae, Lembophyllaceae, Leptodontaceae, Leskeaceae, Meteoriaceae, Neckeraceae, Plagiotheciaceae, Pterobryaceae, Ptychomniaceae, Rigodiaceae, Thuidiaceae, Trachylomataceae) were included in the analyses, plus two additional outgroup taxa from the Aulacomniaceae and Hypnodendraceae. Sampling was guided by previously suggested phylogenetic affinities of *Homaliadelphus*, *Bissetia* and

Miyabea, including the *rbc*L analysis of Tsubota et al. (2002). Family level treatment of the sampled taxa follows the most recent comprehensive classification of mosses by Goffinet et al. (2009).

Sequencing was performed for three genomic regions: i) the internal transcribed spacers of nuclear ribosomal DNA (ITS1 & 2), including the 5.8S gene, ii) the group I intron residing in the mitochondrial *nad*5 gene (and parts of the adjacent 5′ and 3′ exons of the gene), and, iii) the plastidal *trn*S-*rps*4-*trn*T-*trn*L-*trn*F cluster, including four tRNAs (*trn*S (partial), *trn*T, *trn*L, *trn*F (partial)), a fast evolving gene (*rps*4), four spacers separating the coding regions, as well as one group I intron. Voucher details together with EMBL and GenBank accession numbers are listed in **Table 1**.

In addition to the material used for molecular work, several specimens were thoroughly screened for the presence of dwarf males, because they had previously been reported for two species of *Homaliadelphus* (Iwatsuki 1958; Sharp et al. 1994) but were unknown for *Bissetia* and *Miyabea*.

DNA isolation, PCR amplification and sequencing. Prior to DNA extraction, the dried specimens were cleaned with distilled water under a dissection microscope. Remaining contaminations were removed mechanically. Cleaned plant material was dried in an incubator at 70–80°C over night in a 2 ml cap with round bottom. Afterwards two stainless steel beads (5 mm) were added to each sample and crushed at 30 Hz for two times 1 min using a Mixer Mill (Retsch TissueLyser, Qiagen). From the resulting plant powder DNA was extracted using the DNeasy® Plant Mini Kit from Qiagen (Qiagen) following the manufacturer's protocol. Alternatively the CTAB-method described in Doyle and Doyle (1990) was employed. PCR amplifications (T3 Thermocycler and TGradient96, Biometra) were performed in 50 µl-reactions containing 1 U Taq DNA polymerase (peqGOLD Taq-Polymerase, peqlab Biotechnologie or Eppendorf), 1 mM dNTP mix of each 0.25 mM, 1 × buffer, 1.25–2.5 mM $MgCl_2$ and 20 pmol of each amplification primer. Amplification of the plastid region was generally performed in three sets following the approach described in Hernández-Maqueda et al. (2008). However, primer P6/7 was generally substituted with a new primer trnL110Rbryo, a modification of trnL110 (Borsch et al. 2003), and a new C-primer (modified from Taberlet et al. 1991) was designed. In addition two internal sequencing primers were newly designed (see **Table 2**) for sequencing of the *rps*4-*trn*L region. PCR settings were as follows: *trn*S-*rps*4: 3 min 94°C, 35 cycles (15 s 94°C, 30 s 50°C, 1 min 72°C), 7 min 72°C; *rps*4-*trn*L: 2 min 94°C, 30 cycles (1 min 94°C, 1 min 52°C, 1 min 30 s 68°C), 5 min 68°C; *trn*L-F: 2 min 94°C, 35 cycles (1 min 94°C, 1 min 55°C, 1 min 68°C), 5 min 68°C. A modification of the *rps*4-*trn*L PCR-program with an increased number of cycles (to 40) was frequently used for obtaining stronger products. Amplification of the *nad*5 intron was performed using a (nested) approach described in Buchbender (2009) with the following PCR profile: 1 min 30 s 96°C, 35 cycles (45 s 96°C, 1 min 55°C, 1 min 68°C), 7 min 68°C. The internal transcribed spacer of nuclear ribosomal DNA were amplified using the primers ITS5OW (Spagnuolo et al. 1999) and ITS4bryo (Stech et al. 2003) with an amplification profile of: 5 min 94°C, 40 cycles (1 min 94°C, 1 min 48°C, 45 s 68°C) with a time-increment of +4°C/cycle in the extension step, 7 min 68°C. In rare cases nested approaches were chosen using the internal primers SeqITS1 and SeqITS2. All primer sequences and references are given in **Table 2**. Generally multiple PCR products were pooled, concentrated and subsequently cleaned by running on 1.2% agarose gels. The excised PCR products were afterwards recovered by using the NucleoSpin Extract II kit (Macherey-Nagel) following the manufacturer's instructions. Sequencing reactions were performed using the DTCS QuickStart Reaction Kit (Beckman Coulter), applying the standard protocol supplied by the manufacturer for all reactions, using the PCR or internal primers. Extension products were run on a Beckman Coulter CEQ 8000. Alternatively, cleaned PCR products were sequenced by Macrogen Inc., South Korea (www.macrogen.com). Most sequences were generated by the authors, with some complementary sequences obtained from GenBank. Sequences were edited manually with PhyDE® v0.995 (Müller et al. 2005) and primer sequences eliminated. All generated sequences are deposited in EMBL, accession numbers are listed in **Table 1**.

Table 1. Taxa used in the study with EMBL and GenBank accession numbers for the sequenced or downloaded regions and voucher details if available. In some cases sequence data have been submitted to GenBank from previous studies. Therefore accession numbers for *trn*S-*rps*4-*trn*T-*trn*L-*trn*F may be composed of as many as three accession numbers.

Species	Herbarium	Voucher ID	*trn*S-*rps*4-*trn*T-*trn*L-*trn*F	*nad*5	ITS
Anomodon giraldii	H	*H3194078*	AM990342	FM161240	FM161075
Anomodon viticulosus	BUCHBENDER	*Buchbender 449*	AM990343	FM161241	FM161076
Aulacomnium androgynum	BM	*Bell 1299*	*rps*4: AF023811 *rps*4-*trn*L: AM990344 *trn*L-F: AY857795	AJ291564	FM161077
Bissetia lingulata	H	*H3194160*	AM990346 *rps*4: AY908352	FM161243	FM161079
Boulaya mittenii	HIRO	*Tanaka 7308*	AM990347	FM161244	FM161080
Brachythecium rivulare	H	*Parnela s.n.*	AM990348 *trn*L-F: AF397866	FM161245	FM161081
Callicostella cf. *africana*	ENROTH	*Rikkinen et al. 21*	AM990350	FM161247	FM161085
Cratoneuropsis relaxa	MA	*Musci 15238*	*rps*4: AY908244 *rps*4-*trn*L: AM990354 *trn*L-F: AY429494	FM161250	FM161089
Cryphaea amurensis	ENROTH	*Ignatov 97–269*	AM990355	FM161251	FM161090
Dichelodontium nitidum	CHR	*MacMillan, BH 99/14*	*rps*4: AY449664 *rps*4-*trn*L: AM990359 *trn*L-F: AY449670	AY452347	-
Distichophyllum crispulum	H	*H3207110*	AM990360	FM161255	FM161096
Dolichomitriopsis diversiformis	H, MHA	*Nedoluzhko s.n.*	*rps*4: AY908329 *rps*4-trnL: AM990362; *trn*L-F: AF397777	FM161257	FM161098
Entodon dregeanus	QUANDT	*Vanderpoorten FSA AM990363*	FM161258	FM161100	
Forsstroemia trichomitria	BUCHBENDER	*Streimann 65120A*	AM990365	FM161260	FM161103
Giraldiella levieri = *Pylasia levieri*	H	*Enroth 70085*	AM990366	FM161261	FM161104
Glossadelphus glossoides	S	*B57848*	AM990368	FM161263	FM161106
Glossadelphus ogatae	H	*H3065706*	AM990369	FM161264	FM161107
Gollania ruginosa	H	*Buck 23760*	AM990370	FM161265	FM161108
Hampeella pallens	H	*H3205692*	AM990371	FM161266	FM161109
Haplohymenium longinerve	H	*H3069640*	AM990372	FM161267	FM161111
Haplohymenium pseudotriste	H	*H3069653*	AM990373	FM161268	FM161112

Table 1. Continued.

Species	Herbarium	Voucher ID	*trn*S-*rps*4-*trn*T-*trn*L-*trn*F	*nad*5	ITS
Haplohymenium triste	H	*Enroth 63154*	AM990374	FM161269	FM161113
Herpetineuron toccoae	H	*Enroth 70687*	AM990375	FM161270	FM161114
Hildebrandtiella guyanensis	DREHWALD	*Drehwald 4425*	*rps*4: AY306927 *rps*4-*trn*L: AM990380 *trn*L-F: AF509559	FM161275	FM161119
Homaliadelphus targionianus	H	*Koponen et al. 55009*	AM990388 *rps*4: AY908552	FM161283	FM161129
Homaliodendron exiguum	B	*B263509*	AM990389	FM161284	FM161130
Hookeria acutifolia	H	*Virtanen 61857*	AM990393	FM161288	FM161137
Hylocomiastrum pyrenaicum	H, MHA	*Ignatov & Bezgodov 773*	AM990395	FM161290	FM161140
Hylocomiastrum umbratum	H, MHA	*Ignatov & Bezgodov 81*	AM990396	FM161291	FM161141
Hypnodendron vitiense	BM	*Bell 480*	*rps*4: AY524471 *rps*4-*trn*L: AM990397 *trn*L-F: AY524499	AY524526	FM161142
Hypnum cupressiforme	QUANDT	*Quandt s.n.*	AM990398	FM161292	FM161143
Lembophyllum divulsum	FRAHM	*Frahm 8–25*	AM990402	FM161296	FM161146
Leptodon smithii	B	*B268385*	AM990403 *rps*4: AY908261	FM161297	FM161147
Leptopterigynandrum sp.	H	*Koponen 46079*	AM990404	FM161298	FM161148
Limbella tricostata	H	*H3089826*	AM990406 *rps*4: AY908572	FM161299	FM161150
Lindbergia brachyptera	H	*H3194519*	AM990407	FM161300	FM161151
Macrothamnium hylocomioides	H	*Sloover 42870*	AM990408	FM161301	FM161152
Meteorium polytrichum	H	*Streimann 57477*	AM990410 *trn*L-F: AY044073	-	FM161153
Meteorium polytrichum	BUCHBENDER	*Streimann 64800*	AM990409	FM161302	-
Miyabea fruticella	H	*Koponen 45838*	AM990411	FM161303	FM161154
Miyabea rotundifolia	H	*Tan 93–771*	AM990412	FM161304	FM161155
Neckera complanata	BUCHBENDER	*Buchbender 204*	AM990413	FM161305	FM161158
Papillaria crocea	BUCHBENDER	*Streimann 47187*	AM990420 *trn*L-F: AF509555	FM161313	FM161186
Phyllodon lingulatus	H	*H3065691*	AM990367	FM161262	FM161105

Table 1. Continued.

Species	Herbarium	Voucher ID	*trn*S-*rps*4-*trn*T-*trn*L-*trn*F	*nad*5	ITS
Pinnatella minuta	H	*Rikkinen et al. 32*	AM990424	FM161316	FM161194
Porotrichodendron robustum	H	*B264620*	AM990426	FM161318	FM161197
Pseudoleskeopsis zippelii	H	*Enroth 71165*	AM990433	FM161324	FM161206
Pseudotaxiphyllum fauriei	H	*Enroth 70134*	AM990434	FM161325	FM161207
Pterobryopsis hoehnelii	QUANDT	*FSA 246*	AM990435	FM161326	FM161208
Rigodium implexum	QUANDT	*Quandt A 10008*	AM990436	FM161327	FM161209
			*trn*L-F: AY429499		
Scleropodium purum	QUANDT	*Quandt s.n.*	AM990439	FM161329	FM161211
Straminergon stramineum	DR	*DR028753*	AM990351	FM161330	FM161213
Taiwanobryum robustum	H	*Taiwan 1544*	AM990441	FM161331	FM161215
Taiwanobryum speciosum	H	*Enroth 64877*	AM990442	FM161332	FM161216
			*rps*4: AY908272		
Taxiphyllum aomoriense	H	*Koponen 37279*	AM990443	FM161333	FM161217
Thamnobryum alopecurum	BUCHBENDER	*Buchbender s.n.*	AM990444	FM161334	FM161218
			*rps*4: AF023834		
Thamnobryum subserratum	H	*Enroth 64595*	AM990446	FM161336	FM161230
Trachyloma planifolium	BONN	*Frahm No. 3-12*	AM990449	FM161338	FM161234
Weymouthia cochlearifolia	CHR, QUANDT	*99-Mo1*	AM990451	FM161340	FM161236
Weymouthia mollis	CHR, QUANDT	*99-Mo2*	AM990452	-	FM161237
			*rps*4: AY307014		
Zelometeorium patulum	QUANDT	*Quandt A 10005*	AM990453	FM161342	FM161238
			*trn*L-F: AF397787		

Table 2. Primers used in the study. Modified nucleotides are printed in bold.

Name	Sequenz	Direction	Author	Region
trnS-F	TAC CGA GGG TTC GAA TC	F	Souza-Chies et al. (1997)	*trn*S-*rps4*
rps5rev	ATG TCC CGT TAT CGA GG	R	Nadot et al. (1994)	*trn*S-*rps4*
rps4-166F	CCA TAA TGA AAA CGT AAT TTT TG	F	Hernández-Maqueda et al. (2008)	*rps4-trn*L
trnL_P6/7Rbryo	CAT TGA GTC TCT GCA CCT	R	Quandt et al. (2004)	*rps4-trn*L
trnL110Rbryo	ATT TGG CTC AGG ATT **R**CT **Y**AT	R	modified from Borsch et al. (2003)	*rps4-trn*L
trnL-A-Rbryo	AGA GCA CCG CAC TTG TAA TG	R	Hernández-Maqueda et al. (2008)	*rps4-trn*T spacer
trnL-A-Fbryo	CAT TAC AAG TGC GGT GCT CT	F	Hernández-Maqueda et al. (2008)	*trn*T-t*rn*L spacer
trnT_154R	AGT TTT AAG GCA ACA CTT TAT G	R	this study	*rps4-trn*T spacer *&* *trn*T-t*rn*L spacer (partial)
trnT_154F	CAT AAA GTG TTG CCT TAA AAC T	F	this study	*trn*T-t*rn*L spacer (partial) *&* *trnL* intron
trnL-C_diplo	CG**R** AAT **T**GG TAG ACG CTA CG	F	This study modified from Taberlet et al. (1991)	*trn*L-F
trnL-F	ATT TGA ACT GGT GAC ACG AG	R	Taberlet et al. (1991)	*trn*L-F
ITS5OW	GGA GAA GTC GTA ACA AGG TTT CCG	F	Spagnuolo et al. (1999)	ITS1&2
ITS4_bryo	TCC TCC GCT TAG TGA TAT GC	R	Stech et al. (2003)	ITS1&2
SeqITS1	TTG CGT TCA AAG ACT CGA TGA	R	this study	ITS1
SeqITS2	AAC AAC TCT CAG CAA CGG	F	this study	ITS2
nad5_4F	GAA GGA GTA GGT CTC GCT TCA	F	Shaw et al. (2003a)	*nad5* intron
nad5_2220R	ATA TTC CAG TGG TTG CCG CG	R	Buchbender et al. (2009)	*nad5* intron
nad5_3R	AAA ACG CCT GCT GTT ACC AT	R	Shaw et al. (2003a)	*nad5* intron
nad5_IF2	CTT TTG TCG TGA AGA TTC G	F	Buchbender et al. (2009)	*nad5* intron

Sequence analyses and phylogenetic analyses. Alignment of the sequence data was done manually with PhyDE® v0.995, based on the criteria laid out in Kelchner (2000), Borsch et al. (2003) and Quandt and Stech (2005). Simple sequence repeats were isolated based on strict motif recognition (compare Kelchner 2000). Overlapping motifs that superficially contained identical motifs but deviated in length were considered non-homologous if the motifs could be derived independently from the adjacent region (compare tab. 4 in Quandt & Stech 2005). Following the approach in Quandt et al. (2003a) and Quandt and Stech (2004, 2005), the data matrix was screened for inversions using secondary structure models calculated with RNAstructure 4.2 (Mathews et al. 2004). Detected inversions were positionally separated in the alignment. As discussed in Quandt et al. (2003a) and Quandt and Stech (2004), presence or absence of detected inversions was not coded for the phylogenetic analyses. However, in order to gain information from substitutions within detected inversions, a second alignment file for the phylogenetic analyses was generated with the inversions included as reversed and complemented sequences. Regions of ambiguous alignment (hotspots) were exclued from phylogenetic analyses (**Table 3**). Hotspots in the data matrix were defined as positions with a high degree of length mutations where homology of sequence motifs could not be assessed. This is also true for poly-mononucleotide stretches as well as other microsatellite-like areas (e.g., $(AAT)_n$) that are prone to a high variation even at the population level (Provan et al. 2001 and references therein). As indel coding approaches on these areas are likely to result in a scoring of non-homologous events, poly-mononucleotide stretches longer than four nucleotides (nts) showing a length variation of > 1 nt were excluded from the analyses. Locations of hotspots are listed in **Table 3**. Alignments are available from the authors on request.

Table 3. Location (i.e., absolute position in the combined data set) and corresponding region of mutational hotspots (H), including the observed inversion (I). * autapomorphic insertion of 709 nts in *Hypnodendron vitiense* as well as 28 nts in *Aulacomnium androgynum*. § Location of the inversion is given with respect to the corrected and analyzed matrix (i.e., the inversion is included as reverse complement).

No.	Position	Region (plastid)	No.	Position	Region (nuclear)
H1	701–703	*rps*4-*trn*T IGS	H16*	3925–3931	ITS 1
H2	720–722	*rps*4-*trn*T IGS	H17	3980–3982	ITS 1
H3	739–768	*rps*4-*trn*T IGS	H18	4044–4805	ITS 1
H4	843–848	*rps*4-*trn*T IGS	H19	4833–4873	ITS 1
H5	878–882	*rps*4-*trn*T IGS	H20	5013–5049	ITS 1
H6	947–953	*rps*4-*trn*T IGS	H21	5054–5127	ITS 1
H7	994–998	*rps*4-*trn*T IGS	H22	5231–5246	ITS 1
H8	1059–1064	*rps*4-*trn*T IGS	H23	5416–5421	ITS 1
H9	1221–1225	*rps*4-*trn*T IGS	H24	5659–5663	ITS 1
H10	1549–1556	*trn*T-*trn*L IGS	H25	5829–5832	ITS 2
H11	1698–1701	*trn*T-*trn*L IGS	H26	6126–6349	ITS 2
H12	1832–1837	*trn*T-*trn*L IGS	H27	6410–6509	ITS 2
H13	1864–1868	*trn*T-*trn*L IGS	H28	6664–7055	ITS 2
H14	1902–1906	*trn*T-*trn*L IGS			
H15	2547–2550	*trn*L-*trn*F IGS			
I1§	2496–2501	*trn*L-*trn*F IGS			

Both parsimony and Bayesian analyses were performed using the information provided from indels and without indel coding. When indel coding was used, indels were incorporated in the analyses as binary data using a simple indel coding (SIC) strategy (Simmons & Ochoterena 2000) as implemented in SeqState (Müller 2005). SeqState generates a ready-to-use Nexus formatted data file containing the sequence alignment with an automatically generated indel matrix appended. Command files for using the parsimony ratchet (Nixon 1999) were generated using the program PRAP2 (Müller 2007) and executed in PAUP 4.0b10 (Swofford 2002). Ratchet settings were as follows: 10 random addition cycles of 200 iterations each, with 25% up-weighting of the characters in the iterations. Heuristic bootstrap searches under parsimony were performed with 500 replicates and 10 random addition cycles per bootstrap replicate.

Bayesian analyses were performed with MrBayes v3.1.2 (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003), applying the GTR+Γ+I model for the sequence data (as proposed by AIC in Modeltest 3.7) and the restriction site model for the binary indel partition (partition 4), with the ascertainment (coding) bias set to variable (lset coding = variable). To allow for possible deviating substitution models for the different regions, the sequence alignment was divided into three partitions (partition 1: chloroplast DNA; partition 2: mitochondrial DNA; partition 3: nuclear DNA). The specified prior probabilities supplied were those supplied by the default settings of the program. Posterior probability (PP) distributions of trees were created using the Metropolis-coupled Markov chain Monte Carlo (MCMCMC) method and the following search strategies suggested by Huelsenbeck et al. (2001, 2002). Ten runs with four chains (1.5×10^6 generations each) were run simultaneously (mcmc nruns = 10 nchains = 4 ngens = 1,500,000). Chains were sampled every ten generations and the respective trees written to a tree file. The ten runs mixed properly and the acceptances were within appropriate bounds. The program Tracer v1.4 (Rambaut & Drummond 2007) was used to calculate the burnin point and to examine the log likelihoods, ensuring that the runs were in the stationary phase. Since the first run was reaching its stationary phase later than the rest of the runs (at generation 650,000), this was set as the burnin point. The log likelihood values (lnL) were between 103 (run 8) and 333 (run 7), and the standard deviation varied from 0.521 to

0.962. Calculations of the consensus tree and of the posterior probability of clades were performed based upon the trees sampled after the chains converged. Consensus topologies and support values from the different methodological approaches were compiled and drawn using TreeGraph (Müller & Müller 2004).

Results

Alignment and sequence analyses. The original combined and aligned sequence matrix contained 7054 positions of which 2550 positions belong to the plastid partition, 1290 positions to the mitochondrial partition and 3214 positions to the nuclear ribosomal partition. In total 28 hotspots were assigned that were almost equally distributed between the plastid region (H1-15) and the nrDNA (H16-28), with no hotspots in *nad*5. As most of the hotspots in the plastid data were composed of poly-mononucleotide stretches that occasionally reached the critical amount of >10 nts, in some taxa sequencing problems were encountered. However, additional sequencing with internal primers generally solved this problem. Whereas hotspots in the plastid region exclusively consisted of poly-mononucleotide stretches or microsatellite-like repetitive elements, hotspots in the ITS region often consisted of complex motifs of varying length and uncertain homology assessment. This is reflected by more than double the amount of indels compared to the chloroplast (cp) data, although the nrDNA amplicon is only half the size. In addition large autapomorphic sequence stretches were observed in the ITS region such as a putative 709 nts insertion in the ITS1 of *Hypnodendron vitiense*. Length mutations in the *nad*5 intron were rather limited and therefore alignment of *nad*5 was straightforward. After exclusion of the hotspots and reverse complementing of the hairpin-associated inversion in front of the *trn*F gene as described by Quandt et al. (2003a, 2004) and Quandt and Stech (2004), 5575 nucleotide positions could be used in the phylogenetic analyses. Of these positions 21% were variable and 11% parsimony-informative. The plastid region provided slightly more variation (27%; 14.5% parsimony-informative (p.i.) sites) compared to the nuclear region (24%; 13.5% p.i. sites), whereas the mitochondrial data showed considerably lower variation (19%, 8% p.i. sites). Since the ITS region (1874 positions) provided only three quarters and *nad*5 only half (1290 positions) the number of positions compared to the cp data (2437 positions), the plastid region contributed the most to the analyses. Among the plastid partitions *rps*4 contained as high levels of variation and p.i. sites as the non-coding regions that were even higher than in the ITS data. This is almost reversed once indels are taken into account. 851 indels of which 268 were parsimony-informative were coded and used in the analyses. Here the nuclear indels (589 with 207 p.i. sites (35%)) vastly outnumbered the other regions (cpDNA: 227 indels containing 46 p.i. sites (20%); *nad*5: 34 indels containing 25 p.i. sites (74%)), although the *nad*5 indels provided a higher degree of p.i. sites. Detailed statistics considering the alignment, with the contribution of each region included, are listed in **Table 4**.

Phylogenetic analyses. The parsimony analysis including indel coding retained one most parsimonious tree (MPT, length 4848, CI= 0.557, RI= 0.515), while the analysis not including indels retained two MPTs (length 1385, CI=0,608, RI=0.724). Both of the parsimony consensus trees remained with a considerable lack of supported resolution. The MPTs showed no conflict with the results from the Bayesian inference. The results from analyses where indels were coded did not show any incongruence with the results from analyses without indel-coding, but resulted in slightly better resolved and supported trees. Therefore, only the analyses including indel-coding are discussed and only the MrBayes tree including indel-coding is illustrated in **Fig. 1**, complemented with bootstrap values (BS) of the parsimony analysis including indel-coding when applicable. Among homocostate pleurocarp species, the Ptychomniaceae (Ptychomniales) were resolved as the first branching clade and the Hookeriaceae (Hookeriales) sister to the Hypnales. Among the Hypnales (core ingroup) branching order is as follows: Trachylomataceae, Plagiotheciaceae, Cryphaeaceae, Pterobryaceae and Calliergonaceae. The relationships among these have moderate to high support. Although the backbone of the core ingroup is not fully resolved and lacks support in various parts, two main results are evident: i) the tree clearly indicates the polyphyletic nature of several hypnalean

Table 4. Sequence length, divergence and proportional contribution of the different regions to the data matrix as well as ti/tv ratios, number and distribution of indels. Number of characters, p-distance (p-dist.), transition/transversion ratio (ti/tv), variable sites, parsimony informative sites (p.i.) and number of indels are presented based on the data set with the hotspots excluded, whereas the length range together with the mean and the standard deviation (S.D.) are provided from the original alignment.

character set	No. chars.	length range [nt]	Mean [nt]	S.D.	p-dist. [%]	ti/tv	variable sites [%]	p.i. sites [%]	No. indels
*trn*S-F	2437	1671–1787	1710.90	22.868	4.345	2.667	27.235	14.602	227
*nad*5	1290	1098–1233	1201.08	31.438	1.439	6.748	18.837	7.984	34
ITS	1847	0–1379	705.15	129.683	9.815	1.424	23.714	13.481	589
*trn*S-*rps*4 IGS	60	16–46	32.383	3.755	9.187	1.821	41.667	23.333	9
*rps*4	609	609	609	-	3.092	6.41	30.328	16.066	0
*rps*4-*trn*T IGS	480	265–335	303.517	11.342	5.536	2.339	29.792	15	62
*trn*T	72	72	72	-	0.366	-	8.333	1.389	0
*trn*T-*trn*L IGS	582	252–336	276.717	12.897	7.5	1.848	26.976	14.433	98
*trn*L	85	85–85	85	-	0.23	-	3.529	2.353	0
*trn*L intron	463	254–345	270.667	16.452	4.071	2.294	24.19	13.391	47
*trn*L-*trn*F IGS	86	47–68	60.6	3.094	8.157	2.15	38.372	26.744	11
*nad*5 exon1	285	276–285	284.55	1.962	1.274	2.841	12.281	7.018	0
*nad*5 intron	899	821–842	830.933	3.27	1.528	5.243	22.024	8.899	34
*nad*5 exon2	106	0–106	n.a.	n.a.	1.109	0.564	9.434	2.83	0
ITS1	863	0–979	268.95	100.278	13.82	1.487	23.523	14.137	303
5.8S	162	0–161	157.383	20.492	1.102	0.647	11.111	4.321	3
ITS2	814	0–376	271.433	41.786	12.724	1.526	26.658	14.742	283

families, such as the Leskeaceae, Hypnaceae, Hylocomiaceae, Neckeraceae, Leptodontaceae and Anomodontaceae and ii) the maximally supported HMB-clade is resolved sister to a clade consisting of *Leptopterigynandrum*, *Glossadelphus ogatae* and *Taxiphyllum aomoriense* with affinities to the Anomodontaceae. Besides several expected clades, unexpected but well-supported ones were found. These will be described below.

Three main clades were resolved, although support at their basal nodes is often lacking. The first clade comprises a heterogeneous group of almost as many species as traditional families with an unsupported sister group relation to the rest of the core ingroup and can be divided into two sister groups. The first group within this clade contains *Cratoneuropsis relaxa* (Amblystegiaceae), *Lindbergia brachyptera*, *Pseudoleskeopsis zippelii* (both Leskeaceae), *Boulaya mittenii* (Thuidiaceae), *Entodon dregeanus* (Entodontaceae), *Giraldiella levieri* (*Pylaisia levieri*, see Arikawa 2004, Hypnaceae), *Macrothamnium hylocomioides* and *Gollania ruginosa* (both Hylocomiaceae) sister to a clade with *Phyllodon lingulatus* (syn. *Glossadelphus baldwinii*), *Glossadelphus glossoides* (both Hypnaceae) and *Herpetineuron toccoae* (Anomodontaceae). The third *Glossadelphus* s.l. (incl. *Phyllodon*) species, *G. ogatae* is resolved as sister to *Taxiphyllum aomoriense* and *Leptopterigynandrum* turning *Glossadelphus* polyphyletic. However, within this clade a close relationship of *Pseudoleskeopsis zippelii* with *Boulaya mittenii* as well as *Giraldiella levieri* with *Macrothamnium hylocomioides* and *Gollania ruginosa* is suggested, whereas *Hylocomiastrum* (Hylocomiaceae) is resolved elsewhere rendering the Hylocomiaceae polyphyletic. *Entodon dregeanus* together with the aforementioned species pairs forms a significantly supported grouping.

The second main clade received a posterior probability (PP) of 92% and contains on the one hand *Hypnum cupressiforme* sister to the highly supported Anomodontaceae s. str. (*Anomodon* and *Haplohymenium*). However, *Anomodon* itself is resolved as polyphyletic, with *A. giraldii* being deeply nested among the neckeraceous taxa. On the other hand, the maximally supported *Homaliadelphus* -

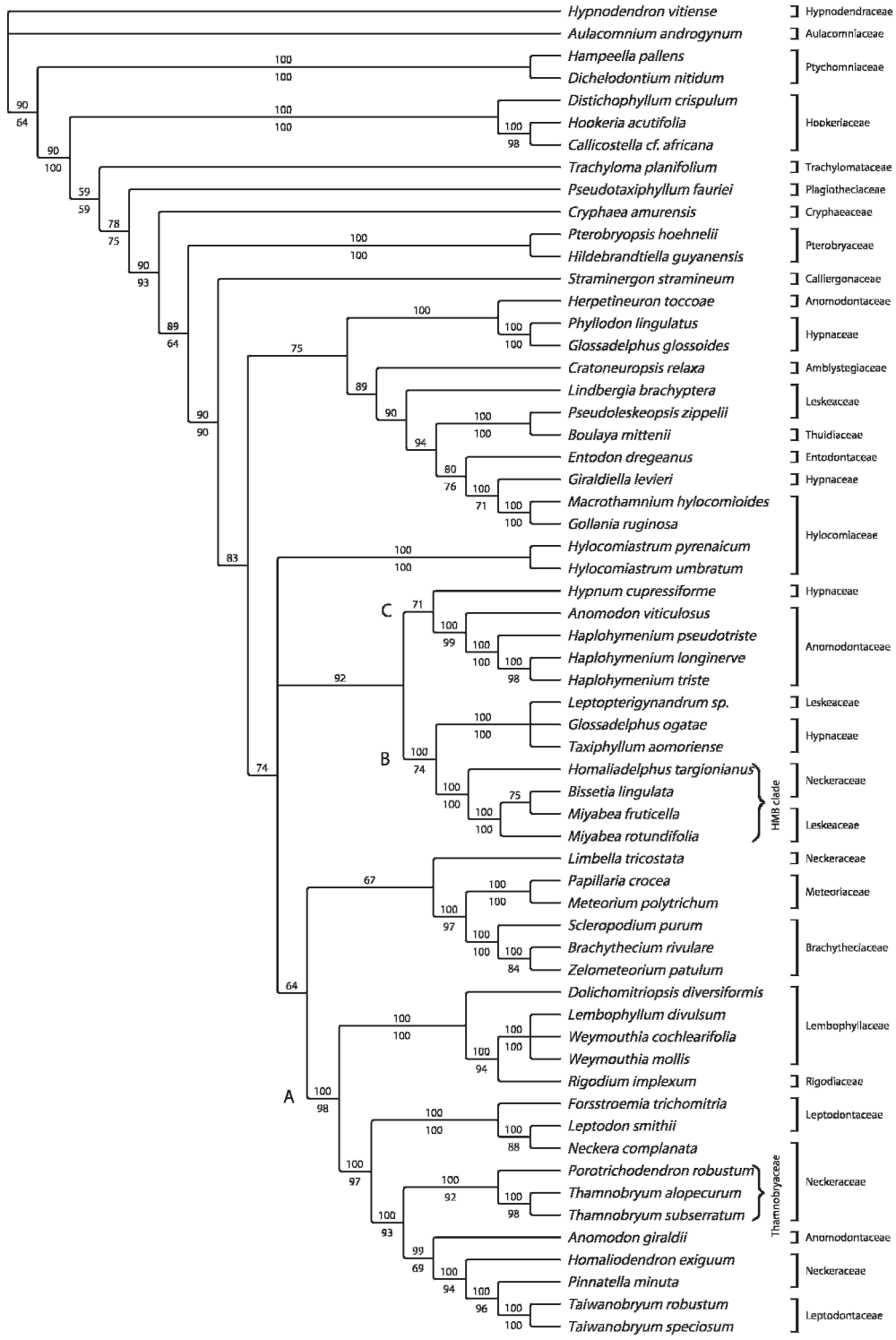

Figure 1. Majority consensus of trees sampled after stationarity in the Bayesian analysis of the matrix including indels, with posterior probabilities for individual clades above the branches. Values below the branches refer to bootstrap support values.

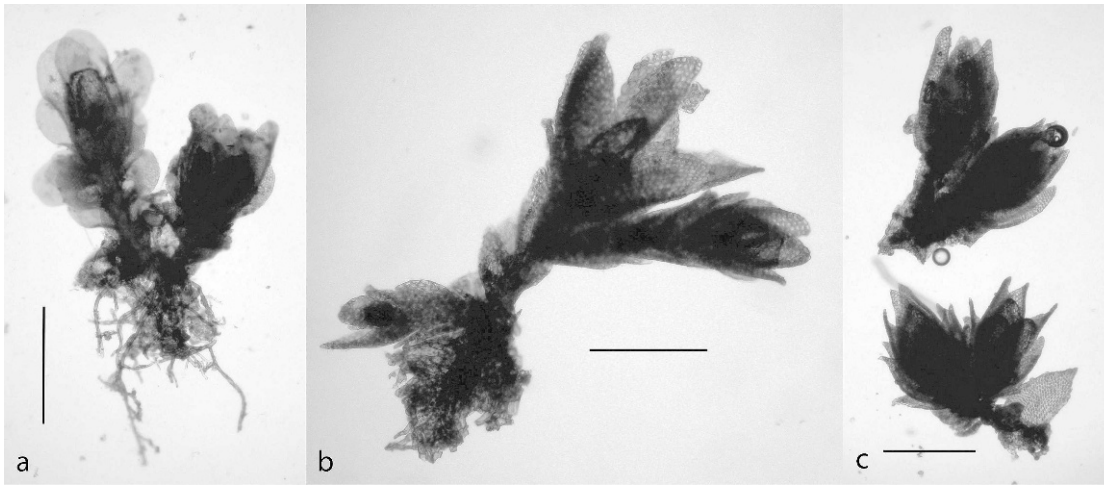

Figure 2. Dwarf males. **a.** *Homaliadelphus targionianus* (*Redfearn Jr. 35536*, s). Scale bar = 0.3 mm. **b.** *Bissetia lingulata* (*Mayebara s.n.*, s: *B121919*). Scale bar = 0.2 mm. **c.** *Miyabea fruticella* (*Ando s.n.*, s: *B121920*). Scale bar = 0.3 mm.

Miyabea - *Bissetia* - clade is sister to a small and morphologically ill-defined group consisting of *Taxiphyllum aomoriense*, *Leptopterigynandrum* sp. and *Glossadelphus ogatae*.

The third main clade consists of: i) a well-supported (PP 100, BS 97) Meteoriaceae-Brachytheciaceae sister group that clusters with *Limbella tricostata*, albeit with no support, and ii) a strongly supported (PP 100, BS 98), Lembophyllaceae/Rigodiaceae/Neckeraceae/Thamnobryaceae/Leptodontaceae-clade also including *Anomodon giraldii*. Among the latter the Rigodiaceae are resolved nested within the maximally supported (PP 100, BS 100) Lembophyllaceae sister to the highly supported (PP 100, BS 97) Neckeraceae/Thamnobryaceae/Leptodontaceae. The former Thamnobryaceae are nested among the representatives of the polyphyletic Neckeraceae and Leptodontaceae.

Dwarf males (Fig. 2a–c). Within the HMB-clade, specimens with dwarf males were found in *Homaliadelphus sharpii* [U.S.A. Tennessee, 15 Mar 1931, *Sharp*, *North American Musci Perfecti 232* (s)], *Homaliadelphus targionianus* var. *targionianus* [China. Sichuan, *Redfearn Jr. 35536* (s)], *Bissetia lingulata* [Japan. Kyushū: Kumamoto, *K. Mayebara* (s; reg. no. B121918); Kyushū: Kumamoto, *K. Mayebara* (s; reg. no. B121919)] and *Miyabea fruticella* [Japan. Hiroshima Pref.: Sandan-kyo, *H. Ando* (s; reg. no. B121920)].

Discussion

Sequence variation of molecular markers. Although *rps*4 as well as *trn*L-F are classic markers in molecular phylogenetics of bryophytes, the two spacers separating *rps*4 from *trn*T and *trn*T from *trn*L have been largely ignored. Only the *trn*T-L IGS has been occasionally used with varying success exclusively on generic or population levels (e.g., Frey et al. 1999; Pfeiffer et al. 2004; Stech 2004). On deeper levels, however, Hernández-Maqueda et al. (2008) were the first to successfully use both spacers combined with *rps*4 and *trn*L-F in a phylogenetic study on the Grimmiaceae, an approach that was followed here. Reported sequence variation by Hernández-Maqueda et al. (2008) of the *trn*S-F region was similar to the values observed in our analyses (25% variable sites, 16.4% p.i. sites versus 27.2% variable sites; 14.6 p.i. sites), although their study only dealt with intrafamily level relationships. In contrast to Hernández-Maqueda et al. (2008) who reported various inversions often combined with a complex structural evolution of the *trn*L intron, only the common inversion in front of *trn*F was observed in the data set. Sequence characteristics (length, number of characters, p.i. sites, etc.) of both non-coding plastid spacers as well as the *trn*L intron were quite similar, with the variability of the intron being relatively slightly smaller (see **Table 4**). The second included group I intron (*nad*5-intron), however, was more than double the size of the *trn*L intron, but

contained roughly 30% less indels and a lower relative amount of variable and parsimony-informative sites. As in Quandt et al. (2007) the highest relative amount of parsimony-informative sites was observed in *rps*4, illustrating the fast-evolving nature of this gene. In terms of sequence divergence, ITS clearly diverged more than the organellar regions (see **Table 4**) which is surprisingly not reflected in the relative amount of p.i. sites that are comparable to the non-coding plastid regions. Although the ITS region represents a relatively short amplicon the alignment resulted in a fairly high number of positions attributed to the high number of indels that additionally displayed a high length variation. The largest indel (autapomorphic) with 709 nts was found in *Hypnodendron vitiense*. The high amount of indels together with the fact that one third of the indels were parsimony-informative, in contrast to one fifth in the cp data, almost doubled the p.i. sites of the nrDNA partition. In terms of parsimony information obtained from indels the *nad*5 intron is the most efficient, as 74% of the indels were p.i. sites, although only a few indels were recorded (34). However, as considerable parts of the length mutations in the plastid as well as in the nuclear data were excluded from the analyses (excluded hotspots), the number of length mutations, i.e., indels, represents only a proportion of those actually present.

In comparison with a recent phylogenetic study addressing the evolution of diplolepideous-alternate mosses and applying almost the same marker combinations (Quandt et al. 2007), we observe only half the sequence variability and p.i. sites in our data set. Whereas the *nad*5 intron displayed a p-distance of 4.4% with 32.5% of the characters being variable and 18.8% parsimony-informative, among a representative set of diplolepideous-alternate mosses, the same marker in our data set displays a p-distance of 1.4% with only 18.8% variable and 8% informative sites. In addition, the number of indels is only half as large (34) compared to a representative set of diplolepideous-alternate mosses (63). Similarly, the sequence variation (p-distance) and content of p.i. sites drops in the plastid markers from 6.7% (29.1%) to 3.1% (16.1%) in *rps*4 and from 8.6% (19.7%) to 4.1% (13.4%) in the *trn*L-intron. One reason for this phenomenon could be that the Hypnales represent the derived and rapidly radiated branch of diplolepideous-alternate mosses (cf. Shaw et al. 2003b) that has not allowed the accumulation/fixation of synapomorphic mutations. As mentioned above, the low sequence variation among the hypnalean taxa is pronounced in the mitochondrial *nad*5 where sequence variation merely reaches 1.5% and the percentage of parsimony-informative sites is only half of the values found in the plastid or nuclear markers. Whereas *nad*5 contained several large indels characteristic for the different groupings among hypnodendroid pleurocarps (Bell et al. 2007), indels in the present data set usually comprise small simple sequence repeats of only 2–8 nts. Despite its great use among early diverging diplolepideous-alternate mosses or hypnodendroid pleurocarps (Bell et al. 2007; Quandt et al. 2007) *nad*5 seems to perform worse than plastid or nuclear regions in the Hypnales. This is nicely illustrated by the fact that *nad*5 contains only 4.1% p.i. sites (overall variability = 9.8%) in the Hypnales, whereas the plastid as well as the nuclear data set contained 11.8–13.7% p.i. sites (overall variability = 21.6–22.8%). Again, *rps*4 performed better compared to all other regions, even within the Hypnales (21.6% variable sites; 11.8% p.i. sites). To conclude, the observed minimal inter- and intrafamilial sequence divergence as well as the low content of p.i. sites among hypnalean *nad*5 sequences rejects *nad*5 as a cost-efficient marker for inferring relationships among the Hypnales. Moreover, because overall sequence divergence as well as phylogenetic signal of the traditional markers is faint in the Hypnales the sequencing effort needs to be extended compared to previous studies among diplolepideous taxa and/or new markers are urgently needed in order to gain a well-resolved and supported tree of the Hypnales.

Phylogenetic analyses. It is not surprising that several families included in the analyses are resolved polyphyletic, since the discrepancy between molecular phylogenetic results and previous morphological concepts of pleurocarpous mosses, which is due to morphological convergence or plasticity, is evident from several recent phylogenetic analyses (e.g., Ignatov et al. 2007; Quandt et al. 2009; Quandt & Huttunen 2004; Vanderpoorten et al.

2002a, b). However, among the Hypnales only a few families, such as the Amblystegiaceae, Brachytheciaceae, Lembophyllaceae, Meteoriaceae and Leskeaceae have been revised recently with the aid of molecular data (e.g., Huttunen et al. 2004; Huttunen & Quandt 2007; Ignatov et al. 2007; Quandt et al. 2003b, 2009; Vanderpoorten et al. 2002a, b). In contrast to previous molecular studies on other pleurocarpous families the Leskeaceae have been reported scattered all over the trees suggesting that "the Leskeaceae in the traditional circumscription is rather a concept than a taxon" (Ignatov et al. 2007), which is also indicated in the present analysis. Few molecular-based attempts have been made to elucidate the relationships among hypnalean families, and with limited success due to the low phylogenetic signal of the traditional markers (Buck et al. 2000; Ignatov et al. 2007; Tsubota et al. 2002).

***Lembophyllaceae/Rigodiaceae/Neckeraceae/Thamnobryaceae/Leptodontaceae-clade* (clade A).** Following the classification of Goffinet and Buck (2004) we have maintained the Rigodiaceae so far, although recent studies have already transferred *Rigodium* and the Rigodiaceae to the Lembophyllaceae (Quandt et al. 2009; Stech et al. 2008). The polyphyletic nature of the Neckeraceae and Leptodontaceae previously indicated by the analyses of Ignatov et al. (2007) and Tsubota et al. (2002) is supported in our analyses based on a somewhat broader sampling of both families. Our results indicate that the Leptodontaceae should be merged with the Neckeraceae. The highly supported monophyletic Thamnobryaceae (cf. Buck & Vitt 1986) are nested among the traditional Neckeraceae and Leptodontaceae and should therefore also be included in the Neckeraceae as already suggested by Enroth and Tan (1994) and Buck (1998). The placement of *Anomodon giraldii* within the Neckeraceae was already suggested by Tsubota et al. (2002), but we refrain from transferring the species to a new or existing Neckeraceae genus as the sampling of the Neckeraceae is presently too small and the phylogenetic position therefore too uncertain. The generic concepts of the Neckeraceae and the phylogenetic position of *A. giraldii* will be discussed in detail in later papers. However, it is already clear that a more broadly defined Neckeraceae have a highly supported sister group relationship with the Lembophyllaceae.

In addition to the confusion within this clade, several members of the Neckeraceae are resolved outside of clade A, including *Homaliadelphus*, *Bissetia* and *Limbella tricostata*. Whereas, *Homaliadelphus* and *Bissetia* largely constitute the HMB-clade (see below), *Limbella tricostata* clusters with the Brachytheciaceae and Meteoriaceae. A detailed taxonomical and nomenclatural treatment of *Limbella* (consisting of the Hawaiian endemic *L. tricostata* and the closely similar *L. fryei* from Oregon) was provided by Ochyra (1987), who placed the genus in the Thamnobryaceae (= Neckeraceae in our concept). There is, however, a third species, currently called *Limbella bartlettii*, which differs clearly from the two above mentioned ones and was treated as *Vittia bartlettii*, within the Amblystegiaceae (Hedenäs 2003), the family where it was also placed by, e.g., Buck (1998: 211) and Goffinet and Buck (2004). The correct use of the generic name *Limbella* needs further clarification but we will not address the associated nomenclatural problems in the present paper, since it has no bearing on our study. In our analysis *L. tricostata* and, by implication, very probably also *L. fryei*, are related to the Brachytheciaceae-Meteoriaceae clade. It should be noted, however, that Arikawa and Higuchi (1999) found that *L. tricostata* (as *Sciaromium tricostatum*) formed a clade with *Pleuroziopsis ruthenica*, the single species in the family Pleuroziops(id)aceae (Goffinet & Buck 2004), although the support for the clade was quite low.

Taxiphyllum-Glossadelphus-Leptopterigynandrum-Miyabea-Bissetia-Homaliadelphus *clade* (clade B). Tsubota et al. (2002) reported an odd "*Taxiphyllum-Glossadelphus-Miyabea-Bissetia-Homaliadelphus*-clade," but with no further discussion, which basically set the stage for the present analyses. In the analyses by Tsubota et al. (2002), a clade formed by *Taxiphyllum aomoriense* and *Glossadelphus ogatae* (both illustrated in Noguchi 1994) was sister to the HMB-clade that is here formally recognized as the Miyabeaceae. As mentioned above, *Glossadelphus* is resolved as polyphyletic in the present analysis, something that

was not observed in previous studies due to limited sample size. A detailed screening of the literature revealed numerous systematic and taxonomic problems associated with this genus. When the type of *Glossadelphus* was transferred to *Phyllodon* by Buck (1987) the generic name *Glossadelphus* became redundant. However, only a limited set of *Glossadelphus* species were moved to other genera. The names *Glossadelphus ogatae* and *G. glossoides* are therefore still used here, whereas *G. baldwinii* was synonymized with *Phyllodon lingulatus* by Kis (2002), a concept which is adopted here. *Phyllodon* was placed in the Hypnaceae by Buck and Goffinet (2000). Regardless of whether the genus is named *Phyllodon* or *Glossadelphus*, it is polyphyletic according to our analysis. While *G. ogatae* groups with *Taxiphyllum aomoriense*, *Phyllodon lingulatus* and *G. glossoides* form a clade with *Herpetineuron toccoae*. This is highly interesting since based on our sampling the proposed affinity of *Phyllodon* with *Taxiphyllum* (Buck 1987) seems to be true only for *Glossadelphus ogatae*. Much additional work seems to be warranted to solve the systematic and taxonomic problems within this group.

From a morphological point of view, a sister group relationship between the Miyabeaceae and the *Taxiphyllum-Glossadelphus* clade is difficult to sustain. Both the latter genera have homotropous to orthogonal or antitropous (terms adopted from Hedenäs 2007), more or less asymmetric capsules with an essentially unreduced peristome. The leaf cells are clearly elongate and not nearly as strongly incrassate as in the Miyabeaceae. In our analysis, an unidentified Chinese species of *Leptopterigynandrum* is nested in the *Taxiphyllum-Glossadelphus* clade, which makes this assemblage more difficult to circumscribe morphologically. However, already Ignatov et al. (2007) noticed that, e.g., *Leptopterigynandrum austro-alpinum* clusters with *Taxiphyllum* and *Glossadelphus ogatae*. *Leptopterigynandrum* is currently placed in the Leskeaceae (Buck & Goffinet 2000; Goffinet & Buck 2004) and it resembles members of the Miyabeaceae in the orthotropous capsules and reduced peristome. However, its leaf characters, including the only somewhat decurrent bases, lanceolate and acute to acuminate apices, distinctly bifurcate costa and only slightly incrassate, minutely multipapillose leaf cells (e.g., Crum & Buck 1994), bear no resemblance to the Miyabeaceae. As far as we know, dwarf males have not been reported for any species placed in *Taxiphyllum*, *Glossadelphus*/*Phyllodon* or *Leptopterigynandrum*. The sister group of the Miyabeaceae is thus morphologically heterogeneous and in need of further analyses.

Anomodontaceae (clade C). The polyphyly of *Anomodon* is consistent with the results of Tsubota et al. (2002). Both analyses show *A. giraldii* nested within the Neckeraceae. As the type species of *Herpetineuron* is forming a maximally supported branch with *Phyllodon* s. l. (see above) outside the Anomodontaceae, *Herpetineuron* should be excluded from the family, even if its family level relationship remains uncertain. This is in sharp contrast to the analyses by Tsubota et al. (2002) where *Herpetineuron toccoae* is clearly resolved within the Anomodontaceae based on *rbc*L.

Morphologically the Anomodontaceae *sensu* Goffinet and Buck (2004) represent the closest match for the HMB-clade which is to some extent supported by the molecular analyses (**Fig. 1**). Several species of *Anomodon* and *Haplohymenium* (the latter was included in *Anomodon* by Granzow-de la Cerda 1997) have orthotropous capsules with basically similarly reduced peristomes as in the Miyabeaceae, although the exostomes of *Miyabea* and *Bissetia* differ in their strongly lamellate dorsal plates, strongly trabeculate ventral plates and cristate tooth margins. *Haplohymenium* and species such as *Anomodon viticulosus* and *A. rugelii* have leaf shapes reminiscent of the Miyabeaceae, having decurrent bases and obtuse to rounded apices. A further similarity is the strongly incrassate leaf cells, at least partly porose, found in both the Anomodontaceae and Miyabeaceae. The main differences between the Anomodontaceae and Miyabeaceae are as follows. In the Anomodontaceae the leaf cells are strongly papillose to prorulose, but in the Miyabeaceae they are smooth. Those taxa of the Anomodontaceae that have character states resembling the Miyabeaceae mentioned above, have a strong and well-defined costa almost reaching the leaf apex or at least above mid-leaf; in the Miyabeaceae, the costa is absent (*Homaliadelphus*) or, when present, weak and diffuse

(not sharply defined from the adjacent laminal cells) and mostly reaching to about midleaf at most, but usually ending well below it. Also, to our knowledge, dwarf males have not been reported for any species in the Anomodontaceae. Considering the fact that the *Anomodon-Haplohymenium* clade shares more morphological characters with the Miyabeaceae than the sister group of the latter does, but molecular data suggest that it is more distantly related, the Miyabeaceae obviously represent a morphologically very well-defined clade sharply delimited from its nearest relatives.

Dwarf males (Fig. 2). One of the most striking characters defining the Miyabeaceae within the context suggested by our results is the presence of dwarf males, or phyllodioicy, in all genera (although not confirmed for every species). Dwarf males were reported for *Homaliadelphus laevidentatus* by Iwatsuki (1958) and for *H. sharpii* (var. *sharpii*) by Sharp et al. (1994), but they have so far gone unnoticed for *Bissetia* and *Miyabea*. Noguchi (1989) considered *B. lingulata* as dioicous and stated that all examined herbarium material of this species comprised female plants. In addition, he found no male plants despite thorough investigation. Watanabe (1972) stated that species of *Miyabea* are dioicous, but failed to describe male plants or perigonia, as did also Noguchi (1991) and Wu et al. (2002). Watanabe (1972), however, described the spores of *Miyabea fruticella* and *M. rotundifolia* as dimorphic, that is, falling in two distinct size-classes and thus exhibiting anisospory, which is often ''correlated with presence of dwarf males'' in mosses (Mogensen 1983; see also Ramsay 1979). In *M. fruticella* the smaller spores are 8–16 μm and the larger 25–40 μm, while in *M. rotundifolia* the respective ranges are 12–22 and 29–38 μm. Sporophytes of the third species, *M. thuidioides*, are unknown. Based on measurements of 50 spores from both of the specimens *3011293* (H) and *0317006* (H-BR), we observed a basically similar but slightly less pronounced anisospory in *Bissetia lingulata*. The spores largely fall in two size-classes, 15–22 and 25–31 μm, most of the spores being 20–22 or 25–27 μm. In *Homaliadelphus targionianus* (specimen H*3071598*) the spores are very similar, 11–13 μm in diameter. Based on our own observations and on the literature cited above, the genus *Homaliadelphus* is facultatively phylloautoicous, while *Bissetia* and *Miyabea* are obligately so. The fact that *Homaliadelphus* shares a sister group relation to *Bissetia* and *Miyabea* might indicate that the latter condition evolved from a falcultative one.

Description of the Miyabeaceae

Miyabeaceae Enroth, S. Olsson, Buchbender, Hedenäs, Huttunen & D. Quandt, *fam. nov. Plantae huius familiae foliis basi decurrentibus vel lobatis, apice late acutis, obtusis vel rotundatis, cellulis foliorum laevibus, parietibus cellularum praecipue ad basim mediumque folii valde incrassatis et porosis, costa nulla vel invalida, brevi et diffusa, plantis masculinis pumilibus praesentibus in generibus omnibus, seta longa, capsula erecta, peristomio reducto cum endostomio rudimentali vel nullo proprio.*

Type genus: *Miyabea* Broth., Nat. Pflanzenfam. 1(3): 984. 1907.

Other genera included: *Bissetia*, *Homaliadelphus*.

Description. Plants small to medium-sized. Main stems creeping, without a central strand, producing irregularly to subpinnately branched aerial stems with larger leaves. Paraphyllia absent. Leaves appressed-imbricate to complanate and±homomallous when dry, ovate to ligulate or nearly rounded, base distinctly decurrent or lobed; leaf apices broadly acute to obtuse or rounded; leaf margins entire below and crenulate to toothed near apex, or entire throughout; costa absent or diffuse and ill-defined, reaching to mid-leaf or rarely to ¾ of leaf length; laminal cells smooth, incrassate, especially so in central parts from midleaf to leaf base, where also distinctly porose; marginal cells not differentiated, but in *Bissetia* towards base rather transverse in several rows; alar cells indistinct. Dioicous and phyllodioicous. Setae elongate, 3–12 mm long, smooth, twisted or not; capsules orthotropous, symmetric, cylindric to obovoid; apophysal stomata few, phaneropore, round-pored; annulus absent or very poorly defined; operculum conical, obliquely long-rostrate; peristome reduced; exostome teeth smooth to papillose, not striate, in *Bissetia* and *Miyabea* lamellate at front, strongly trabeculate at back and with cristate margins;

endostome fragmentary (Noguchi 1991) or absent (*Miyabea*) to strongly reduced with fragile segments often adhering to exostome (*Homaliadelphus*, *Bissetia*). Calyptra cucullate, naked or with few hairs. Spores 11–13 μm (*Homaliadelphus*) or anisosporous and ca. 15–22 and 25–31 μm (*Bissetia*) or 8–22 and 25–40 μm in diam. (*Miyabea*).

Discussion. The family is characterized by decurrent to lobed leaf bases, smooth, thick-walled, often porose laminal cells, especially in the median parts of the leaves, broadly acute to obtuse or rounded leaf apices, absence of costa or presence of a weak, short and rather diffuse one, presence of dwarf males in all genera, elongate seta, orthotropous, symmetrical capsules and a reduced peristome with endostome absent or rudimentary.

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