Evolution of the Neckeraceae (Bryopsida)

Dissertation

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INTRODUCTION

Introduction

The group of pleurocarpous mosses comprises approximately 5000 species, which corresponds to about half of all mosses (Buck & Goffinet 2000). Bell et al. (2007) defined the pleurocarpous mosses, i.e. "the Core Pleurocarps", as a monophylum (Fig. 1), which consists of typically perennial mosses with creeping stems and abundant lateral branches. They are most diverse in tropical regions but several species exists even in arctic regions. In pleurocarpous mosses the archegonium and thus also sporophyte development is restricted to the apices of short, specialized lateral branches, in contrast to most other mosses, where archegonia and sporophytes develop terminally on the main axis (acrocarpous) or on major branches (cladocarpous).

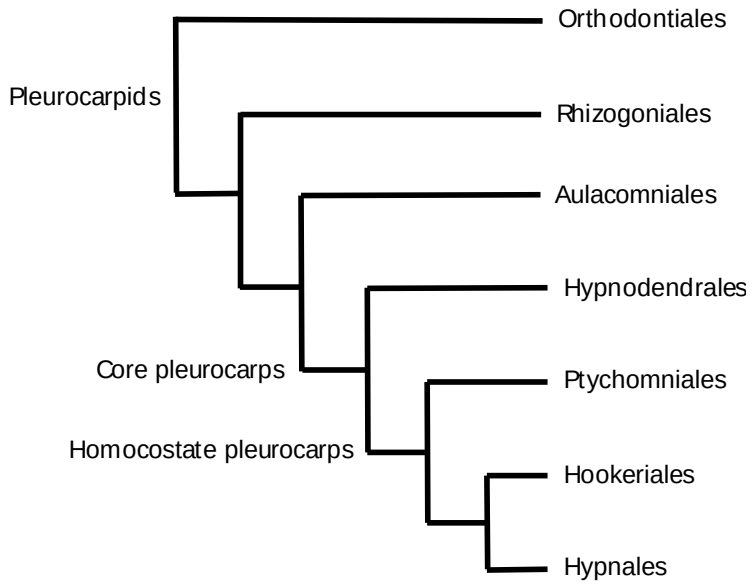
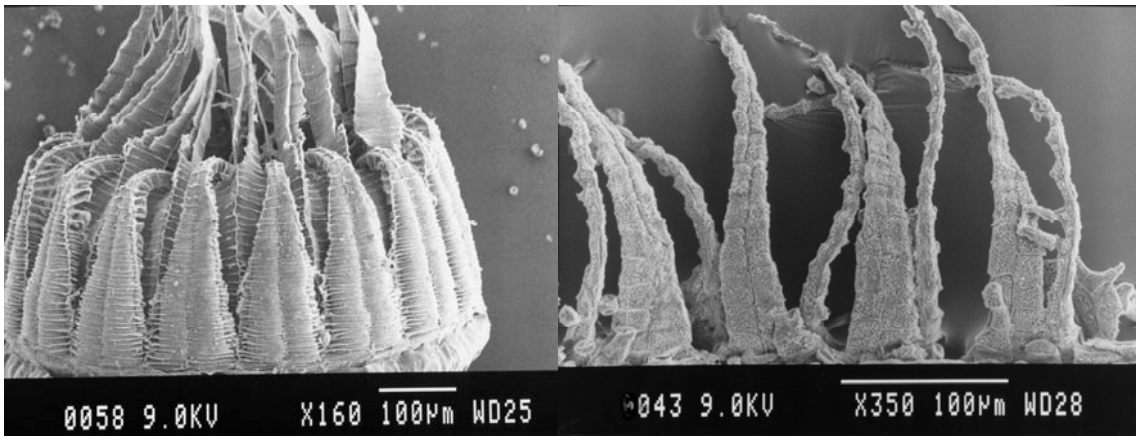


Figure 1. Phylogeny and classification of pleurocarpous mosses after Bell et al. (2007). The phylogeny is based on *nad5*, *rps4*, *rbcL* and *trnL* DNA sequences.

Traditionally, pleurocarpous mosses have been divided into three orders: the Hookeriales, Leucodontales (or Isobryales) and Hypnales, based mainly on their

sporophytic characters. Brotherus (1925) originally placed the Neckeraceae in the Leucodontales together with the Lembophyllaceae. The Neckeraceae has later been alternatively divided into two or three separate families: the Thamnobryaceae, the Neckeraceae and the Leptodontaceae. These families have been placed even in different orders (Neckeraceae and Leptodontaceae among the isobryalean mosses and Thamnobryaceae among hypnalean mosses) according to their peristome structure and the grade of peristome reduction. The perfect hypnoid peristome supposedly characterizing the "Thamnobryaceae" is compared with a reduced, so called neckeroid peristome in Figure 2.



a) b)

Figure 2. a) Perfect hypnoid peristome of *Porotrichum bigelovii* (Sull.) Kindb. (from *2189/5 Duell* 5.5.1981, H). b) reduced neckeroid peristome of *Neckeropsis liliana* (Renauld) Paris (from the holotype of *Neckera liliana* Renauld, PC). SEM-photos by Johannes Enroth.

A growing amount of evidence indicates that a grouping based on sporophytic characters is unnatural and due to convergent evolution (see chapter 3). According to the latest phylogenetic studies of pleurocarpous mosses, based on molecular data, the Neckeraceae belong to the order Hypnales (Buck & Goffinet 2000; Goffinet & Buck 2004) and the family shares a sister group relationship with the Lembophyllaceae (Quandt et al. in press; see also chapters 2 and 3).

In the most recent comprehensive classification (Goffinet et al. 2008) 28 genera are included in the Neckeraceae family. Of these, however, the monospecific *Crassiphyllum* is a synonym of *Thamnobryum* and the similarly monospecific *Metaneckera* is a

synonym of *Neckera* (the respective basionyms *Thamnobryum fernandesii* Sérgio and *Neckera menziesii* Drumm. should be used for these species), and *Dolichomitra* was shown by Quandt et al. (in press) to belong to the Lembophyllaceae s. lato. This classification was based on both morphological and molecular data, even if the molecular data used for these classifications were limited and did not cover all species of the family. Some previous studies based on molecular data have challenged the family concept of the Neckeraceae (Buck et al. 2000; Tsubota et al. 2002; Ignatov et al. 2007), indicating the need of a revision of the family.

The aim of this thesis is to clarify the family concept of the moss family Neckeraceae, to reveal its closest relatives and to show its position in a wider frame in relation to other pleurocarpous mosses as well as to provide new insights into the morphological evolution of the family. Phylogenetic reconstructions are based on extensive molecular data, using plastid, nuclear and mitochondrial sequences. The morphological features are studied and synapomorphies for each clade formed in the phylogenetic analyses are interpreted to see how sound the results are from a morphological point of view. A new delimitation of the family makes it necessary to reconsider the relevance of the morphological description and the morphological features characteristic to the family need to be reconsidered. Due to the new groupings, some changes in the morphological circumscriptions also on the genus level are necessary.

Chapter 1 gives a broad overview of the relationships of the pleurocarpous mosses and shows the need of changes in the definition of genera, families and the corresponding nomenclature in this group. Chapter 2 is a population genetic study of the genus *Thamnobryum*. The main aim of this chapter was to test the origin and species concept of some *Thamnobryum* species that are endemic to strictly restricted regions showing only minor differences in the morphological features in comparison to some more common species. In chapter 3 the monophyly of the Neckeraceae is tested. In addition, in this chapter the ancestral character states of some morphological characters within the Neckeraceae are reconstructed. Chapters 4 and 5 are resolving the genus composition and the relationships within the family more in detail.

Material, methods & related discussion

DNA sequencing

In pleurocarpous moss phylogenetics only few sequence markers have been commonly used. The most frequently used markers include the plastid marker *trnL-F* and the nuclear ITS1 & 2. Previous phylogenetic studies have shown that branch lengths among pleurocarpous mosses are usually extremely short (e.g. Buck et al. 2000a, Shaw et al. 2003a) and sometimes even the use of rapidly evolving DNA is not enough to solve the exact relationships within the chosen study group (e.g. Buchbender et al. 2006). Therefore, DNA sequence regions that are mainly non-coding and known to be fast evolving were chosen: ITS1 & 2 and the plastid region ranging from the end of the gene *rps*4 to the beginning of the gene *trnF* (Fig. 3). The latter includes the *trnT-L* and *rps4-trnT* spacers which have recently been successfully used by Hernandéz-Maqueda et al. (2008) in a phylogenetic study of the Grimmiaceae and seems to be a suitable marker for pleurocarpous moss systematics. Depending on the questions to be resolved, the rest of the sequence regions chosen varied slightly from study to study. The length, divergence and proportional contribution of the different sequence regions are compared (Table 4 in chapter 1) and their suitability for phylogenetic reconstruction of pleurocarpous mosses are discussed (especially in chapters 1 and 5).

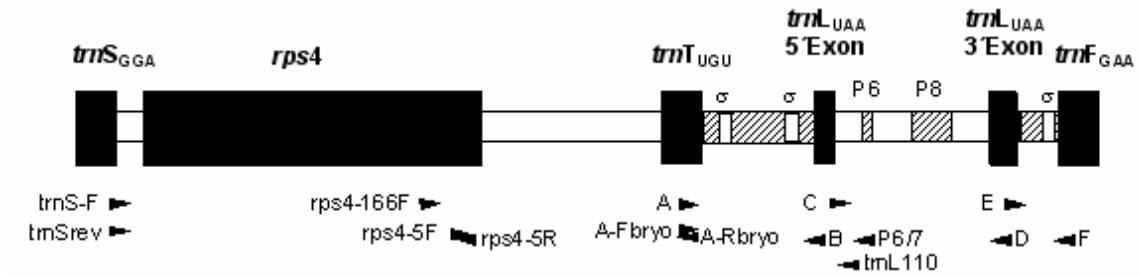


Figure 3. The plastid *trn*S-F region in land plants (Quandt et al. 2004). Location of amplification and sequencing primers are shown below.

To define the composition of the family in chapter 3, a well supported backbone structure was needed. A data set with a reduced taxon sampling and more than 6000 nucleotides (nt) from all genomes per taxon were sequenced. The compiled data set yielded a robust backbone topology that clarified the generic composition of the family.

In addition to ITS and *rps4-trnF* also the plastid *trnS-rps4* spacer as well as the *rpl16* group II intron and the mitochondrial *nad*5 gene including a group I intron were used. This was needed to guide the taxon sampling for the following more detailed analyses. The further analyses in chapters 4 & 5 concentrated on more detailed relationships within the family and genus composition. Therefore, such an extensive data set composed partly of conservative sequences that show very small amount of variation within the family was not a reasonable alternative. By reducing the sequence effort it was possible to include more taxa. Also the analyses in chapter 1 were dealing with more detailed relationships and the same regions were used here. *Rpl16* is not yet widely used in phylogenetic studies of pleurocarpous mosses Hedenäs (e.g. 2006) and Huttunen et al. (2008) being some of the few exceptions. A pilot study testing the phylogenetic utility of the group II intron in the Neckeraceae showed promising results and the region was thus included in most of the analyses (except in the analyses of chapter 2). However, the suitability of this region for phylogenetic analyses in pleurocarpous mosses is not yet reliably tested. Other unexplored sequence regions especially in non-coding chloroplast regions might offer a possibility to resolve the remaining questions in pleurocarpous moss systematics and would be worth to test.

Phylogenetic reconstruction

The programs MrBayes (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003) and PAUP*4.0b10/PRAP2 (Müller 2007) were used for phylogenetic reconstructions. PRAP2 enables the use of the parsimony ratchet method (Nixon 1999) with PAUP*4.0b10 (Swofford 2002) offering a quick and efficient way to perform analyses using parsimony as optimality criterion. No severe conflicts were noted in any of the analyses, but the phylogenetic trees retained by PAUP revealed considerably less resolution. Since indel coding is usually giving more information, the indels were coded employing a simple indel coding approach (Simmons & Ochoterena 2000) in all analyses. The indel coding rarely resulted in significant topological differences, but could affect the support values of different branches. Therefore, the results are shown in the form of a Bayesian tree complemented with the support values from the parsimony analyses, and both the values with and without indel coding are given along the branches. Indel coding seemed to provide additional phylogenetic information but also

to increase the homoplasy of the datasets (4 & 5). In the alignments including the *rpl16* intron the distribution of the indels were observed to differ from the phylogenetic grouping.

Ancestral state reconstruction

For the ancestral character state reconstruction (chapter 3) a data matrix containing 21 morphological characters were compiled for 45 species. To get a broad overview of the species including intraspecific variation, several specimens of each species were investigated. In some cases no material was available, and the morphological matrix was supplemented with information from the literature. The ancestral character states were reconstructed with BayesTraits (Pagel & Meade 2004).

Results & discussion

Phylogeny and classification of the Neckeraceae

The Neckeraceae is shown in this thesis to need adjustment in the family circumscription and generic delimitations. The Lembophyllaceae is confirmed to be the sistergroup of the Neckeraceae. The analyses in the current studies based on molecular data result in a genus composition of the Neckeraceae that is somewhat different from the traditional classification of the Neckeraceae (see Table 1 in chapter 3). The Neckeraceae are defined to include the following 26 genera: *Caduciella* Enroth , *Chileobryon* Enroth, *Circulifolium* S. Olsson, Enroth & D. Quandt, *Curvicladium* Enroth, *Echinodiopsis* S. Olsson, Enroth & D. Quandt, *Forsstroemia* Lindb., *Handeliobryum* Broth., *Himantocladium* (Mitt.) M. Fleisch., *Homalia* (Brid.) Bruch & Schimp., *Homaliodendron* M. Fleisch., *Hydrocryphaea* Dixon, *Leptodon* D. Mohr, *Neckera* Hedw., *Neckeropsis* Reichardt, *Neomacounia* Ireland, *Noguchiodendron* Ninh & Pócs, *Pendulothecium* Enroth & He, *Pinnatella* M. Fleisch., *Porotrichodendron* M. Fleisch., *Porotrichopsis* Herzog, *Porotrichum* (Brid.) Hampe, *Shevockia* Enroth, *Taiwanobryum* Nog, *Thamnobryum* Nieuwl., *Thamnomalia* S. Olsson, Enroth & D. Quandt and *Touwia* Ochyra. Of these genera, *Circulifolium*, *Echinodiopsis* and *Thamnomalia* are novel genera presented in this study for the first time. *Neomacounia*

and *Noguchiodendron* were not included in the analyses due to lacking material. *Cryptoleptodon* was eliminated after the synonymization with *Leptodon* and *Baldwiniella*, *Bissetia*, *Bryolawtonia*, *Dixonia*, *Homaliadelphus* and *Isodrepanium* were excluded from the family.

Morphological evolution within the Neckeraceae

This thesis gives a good overview of the evolution in the Neckeraceae. In addition to the reconstructed phylogeny, several morphological characters are analyzed together with the underlying selection pressures leading to the character evolution. From the ancestral state reconstructions made for both the habitat and some selected morphological characters, it seems that a similar pattern in the character state distribution and the habitat shift can be observed, peristome reduction being a good example (Fig. 4 in chapter 3). It is tempting to claim a correlation between habitat and morphology. In order to evaluate a likely correlation extensive correlation analyses in a Bayesian framework were tested. Some prominent characters (seta length, cilia, the stage of development of the basal membrane and the peristome in a dry stage) showed a clear correlation with the habitat. Some other characters that seemed to correlate with the habitat did not show a correlation on a statistically significant level (Table 1). The evolutionary patterns underlying the morphological characters are complex and a single event, like a shift in habitat can be the key event providing an explanation, but is not enough explain all the changes in character states (chapter 3).

Table 1. Correlation analyses for selected characters. Bayes Factor (BF) > 5 indicates strong support i.e based on three independent (I) and dependent (D) runs. Four characters show at least with some combinations of I and D runs strong evidence for correlated evolution. BF after 1,000,000,000 iterations, except * = BF after 2,500,000,000 iterations.

Character	Min Ln I	Max Ln I	Min Ln D	Max Ln D	Min BF	Max BF
Post fertilization growth of perichaetial leaves	-43,82	-43,70	-44,30	-43,65	0,35	-1,19
Operculum shape	-45,02	-43,97	-45,28	-44,61	0,81	-2,62
Dry peristome	-49,48	-49,31	-46,43	-46,43	**6,09**	**5,05**
Spore size	-52,10	-52,06	-51,96	-51,72	0,74	0,21
Basal membrane	-35,01	-34,63	-32,37	-32,00	**6,46**	*4,97*
Cilia	-42,84	-42,55	-40,24	-40,02	**6,15**	***5,74***
Peristome	-38,08	-37,80	-35,98	-35,81	4,54	3,64
Seta length	-41,69	-41,44	-38,57	-38,30	**6,78**	**5,74**

Conclusions

Many supposedly widely distributed genera of several species that seem to be morphologically coherent (*Echinodium*, *Homalia*, *Thamnobryum*, partly *Neckera*), were shown to be polyphyletic. They are replaced with smaller, geographically more restricted genera that at least in some cases (e.g. *Thamnomalia*, *Homalia* s.str., *Neckera* s.str.) seem to be morphologically heterogeneous. In other words, morphology can be misleading in the Neckeraceae even at the genus level and convergent evolution in both morphological and sequence level characters are common within the family (see especially chapter 3). Special habitat conditions have been shown to result in similar morphological structures also in several other moss groups (e.g. Buck 1991; Hedenäs 2001; Huttunen et al. 2004). This kind of convergent evolution is shown to occur in aquatic moss species by Vanderpoorten et al. (2002a), and seems to apply also in the case of *Thamnobryum alopecurum* and its endemic allies (chapter 1). However, similar morphological structures in similar aquatic habitat can naturally also be due to true phylogenetic relationships as is the case within Neckeraceae with *Handeliobryum sikkimense* and *Hydrocryphaea wardii*, or the three species of *Touwia* (*T. laticostata*, *T. negrosense* and *T. ellipticum*). The geographical patterns and grouping seem to have more phylogenetic significance than thought before.

CHAPTER 1

MIYABEACEAE, A NEW FAMILY OF PLEUROCARPOUS MOSSES

This study is submitted as: Olsson, S., Buchbender, V., Enroth, J., Hedenäs, L., Huttunen, S. & Quandt, D. Miyabeaceae, a new family of pleurocarpous mosses. The Bryologist.

1.1 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 treated either within the Leskeaceae (*Miyabea*) or Neckeraceae (*Homaliadelphus* and *Bissetia*) is persistently retrieved in various published phylogenies. 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 comprised the ITS1 & 2 (nuclear), the *trnS-rps4-trnT-trnL-trnF* cluster (plastid) as well as the *nad5* intron (mitochondrial). Although the phylogenetic reconstruction (MrBayes) 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 family 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 only one species occurring in North America.

1.2 Introduction

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. Cox & Hedderson 1999; Cox et al. 2000; Beckert et al. 2001; Bell et al. 2007; Quandt et al. 2007) we observe a considerable lack of resolution and support among the various pleurocarpous lineages (e.g. Buck et al. 2000a; Goffinet et al. 2001; Tsubota et al. 2002; Ignatov et al. 2007). This is especially evident in species rich and/or single marker analyses where phylogenies of homocostate pleurocarps (sensu Bell et al. 2007) 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 (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 (Huttunen et al. 2004; Hedenäs 2007; Quandt et al. in press).
Even if some families are reliably resolved through recent phylogenetic analyses (Quandt et al. 2003a; Huttunen et al. 2004; Huttunen et al. 2008; Quandt et al. in press), many inter- and intrafamiliar relationships remain unknown, especially considering the bryological "dust bins" such as e.g. 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 as proposed by the pleurocarps net (www.pleurocarps.eu).

However, among the few reported clades receiving considerable support an odd one was evident in the analysis based on the plastid *rbcL* gene by Tsubota et al. (2002) where *Miyabea fruticella*, *Homaliadelphus targionianus* and *Bissetia lingulata* (HMB-clade) that never have been considered as 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 (1992b) suggested a close relation to *Anomodon* and thus transferred it into the Anomodontaceae, what is not reflected in the most recent classification of mosses by Goffinet & Buck (2004). 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 that generic name turned out 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*** (R.S. Williams) Sharp. 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, also occurs in Japan.

Miyabea has three species that are narrowly distributed in Japan, Korea, and the eastern provinces of China (Watanabe 1972; Noguchi 1991; 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's (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 & Goffinet (2000) as well as Goffinet & Buck (2004) followed Brotherus's original concept and thought *Miyabea* is best placed in the Leskeaceae, even if the family's definition and circumscription differed considerably from Brotherus's 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. Therefore, we

combined sequence data of the ITS1-5.8S-ITS2 region (nuclear ribosomal DNA), the *nad5*-intron (mitochondrial DNA), and the *trnS-rps4-trnT-trnL-trnF* cluster (plastidal DNA). Finally, after showing the monophyly of the group, we will discuss the morphological synapomorphies distinguishing this clade.

1.3 Material and methods

Taxon sampling and molecular markers

60 taxa from 52 different genera representing 20 families of homocostate pleurocarps sensu Bell et al. (2007) (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 *rbcL* analysis of Tsubota et al. (2002). Family level treatment of the sampled taxa follows the most recent comprehensive classification of mosses by Goffinet & Buck (2004).

Sequencing was performed for three genomic regions: *i)* the internal transcribed spacer of nuclear ribosomal DNA (ITS1 & 2), including the 5.8S gene, *ii)* the group I intron residing in the mitochondrial *nad5* gene (and parts of the adjacent 5' and 3' exons of the gene) as well as *iii)* the plastidal *trnS-rps4-trnT-trnL-trnF* cluster, including 4 tRNAs (*trnS* (partial), *trnT*, *trnL*, *trnF* (partial)), a fast evolving gene (*rps4*), four spacers separating the coding regions, as well as one group I intron.Voucher details and EMBL accession numbers are listed in Table 1.

Table 1. Taxa used in the study with GenBank accession numbers for the sequenced or downloaded regions and voucher details if available. In some cases sequence data have been already submitted to GenBank from previous studies. Therefore accession numbers for *trnS-rps4-trnT-trnL-trnF* composed of up to three accession numbers.

Species name	Herbarium	Voucher ID	*trnS-rps4-trnT-trnL-trnF*	*nad5*	ITS
Anomodon giraldii Müll. Hal	H	H3194078	AM990342	FM161240	FM161075
Anomodon viticulosus (Hedw.) Hook. & Taylor	Buchbender	Buchbender 449	AM990343	FM161241	FM161076
Aulacomnium androgynum (Hedw.) Schwägr.	BM	Bell 1299	*rps4*: AF023811 *rps4-trnL*: AM990344; *trnL-F*: AY857795	AJ291564	FM161077
Bissetia lingulata (Mitt.) Broth.	H	H3194160	AM990346 *rps4*: AY908352	FM161243	FM161079
Boulaya mittenii (Broth.) Cardot	HIRO	Tanaka 7308	AM990347	FM161244	FM161080
Brachythecium rivulare Schimp.	H	Parnela s.n.	AM990348 *trnL-F*: AF397866	FM161245	FM161081
Callicostella cf. africana Mitt.	Enroth	Rikkinen et al. 21	AM990350	FM161247	FM161085
Cratoneuropsis relaxa (Hook. f. & Wilson) M. Fleisch.	MA	MA-Musci 15238	*rps4*: AY908244 *rps4-trnL*: AM990354 *trnL-F*: AY429494	FM161250	FM161089
Cryphaea amurensis Ignatov	Enroth	Ignatov 97-269	AM990355	FM161251	FM161090
Dichelodontium nitidum (Hook. f. & Wilson) Broth.	CHR	MacMillan, BH 99/14	*rps4*: AY449664 *rps4-trnL*: AM990359 *trnL-F*: AY449670	AY452347	-
Distichophyllum crispulum (Hook. f. & Wilson) Mitt.	H	H3207110	AM990360	FM161255	FM161096
Dolichomitriopsis diversiformis (Mitt.) Nog.	H, MHA	Nedoluzhko s.n.	*rps4*: AY908329 *rps4-trnL*: AM990362; *trnL-F*: AF397777	FM161257	FM161098
Entodon dregeanus (Hornsch.) Müll. Hal.	Quandt	Vanderpoorten FSA AM990363	FM161258	FM161100	
Forsstroemia trichomitria (Hedw.) Lindb.	Buchbender	Streimann 65120A	AM990365	FM161260	FM161103
Giraldiella levieri Müll. Hal.	Enroth	Enroth 70085	AM990366	FM161261	FM161104
Glossadelphus glossoides (Bosch & Sande Lac.) M. Fleisch.	S	B57848	AM990368	FM161263	FM161106
Glossadelphus ogatae Broth. & Yasuda	H	H3065706	AM990369	FM161264	FM161107
Gollania ruginosa (Mitt.) Broth.	H	Buck 23760	AM990370	FM161265	FM161108
Hampeella pallens (Sande Lac.) M. Fleisch.	H	H3205692	AM990371	FM161266	FM161109
Haplohymenium longinerve (Broth.) Broth.	H	H3069640	AM990372	FM161267	FM161111
Haplohymenium pseudotriste (Müll. Hal.) Broth.	H	H3069653	AM990373	FM161268	FM161112
Haplohymenium triste (Ces.) Kindb.	H	Enroth 63154	AM990374	FM161269	FM161113
Herpetineuron toccoae (Sull. & Lesq.) Cardot	Enroth	Enroth 70687	AM990375	FM161270	FM161114
Hildebrandtiella guyanensis (Mont.) W.R. Buck	Drehwald	Drehwald 4425	*rps4*: AY306927 *rps4-trnL*: AM990380 *trnL-F*: AF509559	FM161275	FM161119
Homaliadelphus targionianus (Mitt.) Dixon & P. de la Varde	H	Koponen et al. 55009	AM990388 *rps4*: AY908552	FM161283	FM161129
Homaliodendron exiguum (Bosch & Sande Lac.) M. Fleisch	B	B263509	AM990389	FM161284	FM161130

Hookeria acutifolia Hook. & Grev.	Enroth	Virtanen 61857	AM990393	FM161288	FM161137
Hylocomiastrum pyrenaicum (Spruce) M. Fleisch.	H, MHA	Ignatov & Bezgodov #773	AM990395	FM161290	FM161140
Hylocomiastrum umbratum (Ehrh. ex Hedw.) M. Fleisch.	H, MHA	Ignatov & Bezgodov #81	AM990396	FM161291	FM161141
Hypnodendron vitiense Mitt.	BM	Bell 480	*rps4*: AY524471 *rps4-trnL*: AM990397 *trnL-F*: AY524499	AY524526	FM161142
Hypnum cupressiforme Hedw.	Quandt	Quandt s.n.	AM990398	FM161292	FM161143
Lembophyllum divulsum (Hook. f. & Wilson) Lindb.	Frahm	Frahm 8-25	AM990402	FM161296	FM161146
Leptodon smithii (Hedw.) F. Weber & D. Mohr	B	B268385	AM990403 *rps4*: AY908261	FM161297	FM161147
Leptopterigynandrum Müll. Hal. sp.	Enroth	Koponen 46079	AM990404	FM161298	FM161148
Limbella tricostata (Sull.) Müll. Hal. ex E.B. Bartram	H	H3089826	AM990406 *rps4*: AY908572	FM161299	FM161150
Lindbergia brachyptera (Mitt.) Kindb.	H	H3194519	AM990407	FM161300	FM161151
Macrothamnium hylocomioides M. Fleisch.	H	Sloover 42870	AM990408	FM161301	FM161152
Meteorium polytrichum Dozy & Molk.	H	Streimann 57477	AM990410 *trnL-F*: AY044073	-	FM161153
Meteorium polytrichum Dozy & Molk.	Buchbender	Streimann 64800	AM990409	FM161302	-
Miyabea fruticella (Mitt.) Broth.	H	Koponen 45838	AM990411	FM161303	FM161154
Miyabea rotundifolia Cardot	H	Tan 93-771	AM990412	FM161304	FM161155
Neckera complanata (Hedw.) Huebener	Buchbender	Buchbender 204	AM990413	FM161305	FM161158
Papillaria crocea (Hampe) A. Jaeger	Buchbender	Streimann 47187	AM990420 *trnL-F:* AF509555	FM161313	FM161186
Phyllodon lingulatus (Cardot) W.R. Buck	H	H3065691	AM990367	FM161262	FM161105
Pinnatella minuta (Mitt.) Broth.	H	Rikkinen et al. 32	AM990424	FM161316	FM161194
Porotrichodendron robustum Broth.	B	B264620	AM990426	FM161318	FM161197
Pseudoleskeopsis zippelii (Dozy & Molk.) Broth.	Enroth	Enroth 71165	AM990433	FM161324	FM161206
Pseudotaxiphyllum fauriei (Cardot) Z. Iwats.	H	Enroth 70134	AM990434	FM161325	FM161207
Pterobryopsis hoehnelii (Müll. Hal.) Magill	Quandt	FSA 246	AM990435	FM161326	FM161208
Rigodium implexum Kunze ex Schwägr.	Quandt	Quandt A 10008	AM990436 *trnL-F*: AY429499	FM161327	FM161209
Scleropodium purum (Hedw.) Limpr.	Quandt	Quandt s.n.	AM990439	FM161329	FM161211
Straminergon stramineum (Dicks. ex Brid.) Hedenäs	DR	DR028753	AM990351	FM161330	FM161213
Taiwanobryum robustum Veloira	H	Taiwan 1544	AM990441	FM161331	FM161215
Taiwanobryum speciosum Nog.	H	Enroth 64877	AM990442 *rps4*: AY908272	FM161332	FM161216
Taxiphyllum aomoriense (Besch.) Z. Iwats.	H	Koponen 37279	AM990443	FM161333	FM161217
Thamnobryum alopecurum (Hedw.) Nieuwl. ex Gangulee	Buchbender	Buchbender s.n.	AM990444 *rps4*: AF023834	FM161334	FM161218
Thamnobryum subserratum (Hook. ex Harv.) Nog. & Z. Iwats.	H	Enroth 64595	AM990446	FM161336	FM161230
Trachyloma planifolium (Hedw.) Brid.	Bonn	Frahm No. 3-12	AM990449	FM161338	FM161234
Weymouthia cochlearifolia (Schwägr.) Dixon	CHR, Quandt	99-Mo1	AM990451	FM161340	FM161236

Weymouthia mollis (Hedw.) Broth.	CHR, Quandt	99-Mo2	AM990452 *rps4*: AY307014	-	FM161237
Zelometeorium patulum (Hdw.) Manuel	Quandt	Quandt A 10005	AM990453 *trnL-F*: AF397787	FM161342	FM161238

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 overnight in a 2 ml cap with round bottom. Afterwards 2 stainless steal 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 & 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 x 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 *trnL*110Rbryo a modification of *trnL*110 (Borsch et al. 2003). In addition two internal sequencing primers were newly designed (see Table 2) for sequencing of the *rps4-trnL* region. PCR settings were as follows: *trnS-rps4*: 3 min 94°C, 35 cycles (15 s 94°C, 30 s 50°C, 1 min 72°C), 7 min 72°C; *rps4-trnL*: 2 min 94°C, 30 cycles (1 min 94°C, 1 min 52°C, 1 min 30 s 68°C), 5 min 68°C; *trnL-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 *rps4-trnL* PCR-program with an increased number of cycles (up to 40 cycles) was frequently used for obtaining stronger products. Amplification of the *nad5* intron was performed using an (nested) approach described in Buchbender et al. (unpubl.) 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 sequences are deposited in GenBank, accession numbers are listed in Table 1.

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

Name	Sequenz	Direction	Autor	Region
trnS-F	TAC CGA GGG TTC GAA TC	F	Souza-Chies et al. (1997)	*trnS-rps4*
*rps5*rev	ATG TCC CGT TAT CGA GG	R	Nadot et al. (1994)	*trnS-rps4*
rps4-166F	CCA TAA TGA AAA CGT AAT TTT TG	F	Hernández–Maqueda et al. (2008)	*rps4-trnL*
*trnL*_P6/7Rbryo	CAT TGA GTC TCT GCA CCT	R	Quandt et al. (2004)	*rps4-trnL*
*trnL*110Rbryo	ATT TGG CTC AGG ATT **R**CT **Y**AT	R	modified from Borsch et al. (2003)	*rps4-trnL*
trnL-A-Rbryo	AGA GCA CCG CAC TTG TAA TG	R	Hernández–Maqueda et al. (2008)	*rps4-trnT* spacer
trnL-A-Fbryo	CAT TAC AAG TGC GGT GCT CT	F	Hernández–Maqueda et al. (2008)	*trnT-trnL* spacer
*trnT*_154R	AGT TTT AAG GCA ACA CTT TAT G	R	this study	*rps4-trnT* spacer & *trnT-trnL* spacer (partial)
*trnT*_154F	CAT AAA GTG TTG CCT TAA AAC T	F	this study	*trnT-trnL* spacer (partial) & *trnL* intron
*trnL-C*_mosses	CG**R** AAT **T**GG TAG ACG CTA CG	F	Quandt & Stech (2004)	*trnL-F*
trnL-F	ATT TGA ACT GGT GAC ACG AG	R	Taberlet et al. (1991)	*trnL-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. (submitted)	*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. (submitted)	*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 & Stech (2005). Simple sequence repeats were isolated based on strict motif recognition, hence overlapping motifs were considered non-homologous if the motifs could be derived independently from the adjacent region. Following the approach in Quandt et al. (2003a) and Quandt and Stech (Quandt & 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 reverse complemented. Regions of ambiguous alignment (hotspots) were exclued from phylogenetic analyses (compare 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 on 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 4 nt showing a length variation of more than 1 nt were excluded from the analyses. Location of hotspots are listed in Table 3). Alignments are provided on an appendix cd. Indels were incorporated in the analyses as binary data using a simple indel coding (SIC) strategy (Simmons & Ochoterena 2000) as implemented in the computer program SeqState (Müller 2005). SeqState generates a ready-to-use nexus 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% upweighting of the characters in the iterations. Heuristic bootstrap searches under parsimony were performed with 500 replicates and 10 random addition cycles per bootstrap replicate.

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 nt in Hypnodendron vitiense as well as 28 nt in Aulacomnium androgynum. § Location of the inversion is given with respect to the corrected and analysed matrix (i.e. the inversion is included as reverse complement).

No.	Position	Region (plastid)	No.	Position	Region (nuclear)
H1	701-703	*rps4-trnT* IGS	H16*	3925-3931	ITS1
H2	720-722	*rps4-trnT* IGS	H17	3980-3982	ITS1
H3	739-768	*rps4-trnT* IGS	H18	4044-4805	ITS1
H4	843-848	*rps4-trnT* IGS	H19	4833-4873	ITS1
H5	878-882	*rps4-trnT* IGS	H20	5013-5049	ITS1
H6	947-953	*rps4-trnT* IGS	H21	5054-5127	ITS1
H7	994-998	*rps4-trnT* IGS	H22	5231-5246	ITS1
H8	1059-1064	*rps4-trnT* IGS	H23	5416-5421	ITS1
H9	1221-1225	*rps4-trnT* IGS	H24	5659-5663	ITS1
H10	1549-1556	*trnT-trnL* IGS	H25	5829-5832	ITS2
H11	1698-1701	*trnT-trnL* IGS	H26	6126-6349	ITS2
H12	1832-1837	*trnT-trnL* IGS	H27	6410-6509	ITS2
H13	1864-1868	*trnT-trnL* IGS	H28	6664-7055	ITS2
H14	1902-1906	trnT-trnL IGS			
H15	2547-2550	*trnL-trnF* IGS			
I1 §	2496-2501	*trnL-trnF* IGS			

Bayesian analyses were performed with MrBayes v3.1.2 (Huelsenbeck & Ronquist 2001), applying the GTR+Γ+I model for the sequences data and the restriction site model for the binary indel partition. To allow for possible deviating substitution models for the different regions, the data set was divided into four partitions (partition 1: chloroplast DNA; partition 2: mitochondrial DNA; partition 3: nuclear DNA; partition 4: indels). The *a priori* probabilities supplied were those specified in 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; 2002b). Ten runs with four

chains (1.5×10^6 generations each) were run simultaneously, with the temperature of the single heated chain set to 0.2. Chains were sampled every 10 generations and the respective trees written to a tree file. Calculations of the consensus tree and of the posterior probability of clades were performed based upon the trees sampled after the chains converged (at generation 25,000). Consensus topologies and support values from the different methodological approaches were compiled and drawn using TreeGraph (Müller & Müller 2004). *Aulacomnium androgynum*. (Aulacomniales) and *Hypnodendron vitiense* (Hypnodendrales) were chosen as outgroup, representing the two nearest sister clades to homocostate pleurocarps (Bell et al. 2007).

1.4 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 almost equally distributed between the plastid region (H1-15) and the nrDNA (H16-28), with no hotspots in *nad5*. As most of the hotspots in the plastid data were composed of poly-mononucleotide stretches that occasionally reached the critical amount of >10 nt, in some case 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 motives of varying length and uncertain homology assessment. This is reflected by more than double the amount of indels compared to the 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 nt insertion in the ITS1 of *Hypnodendron vitiense*. Length mutations in the *nad5* intron were rather limited and therefore alignment of *nad5* was straightforward. After exclusion of the hotspots and reverse complementing the hairpin-associated inversion in front of the *trnF* gene as described by Quandt et al. (2003a; 2004b) and Quandt & 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) provided only three quarters and *nad5* only half (1290) the amount of characters compared to the cp DNA *rps4* contained levels of variation and p.i.-sites as high as the non-coding regions and even outperformed the ITS region. This is almost turned upside down 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 %); *nad5*: 34 indels containing 25 p.i.-sites (74 %)), although the *nad5* 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.

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
trnS-F	2437	1671-1787	1710.90	22.868	4.345	2.667	27.235	14.602	227
nad5	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
trnS-rps4 IGS	60	16-46	32.383	3.755	9.187	1.821	41.667	23.333	9
rps4	609	609	609	-	3.092	6.41	30.328	16.066	0
rps4-trnT IGS	480	265-335	303.517	11.342	5.536	2.339	29.792	15	62
trnT	72	72	72	-	0.366	-	8.333	1.389	0
trnT-trnL IGS	582	252-336	276.717	12.897	7.5	1.848	26.976	14.433	98
trnL	85	85-85	85	-	0.23	-	3.529	2.353	0
trnL intron	463	254-345	270.667	16.452	4.071	2.294	24.19	13.391	47
trnL-trnF IGS	86	47-68	60.6	3.094	8.157	2.15	38.372	26.744	11
nad5 exon1	285	276-285	284.55	1.962	1.274	2.841	12.281	7.018	0
nad5 intron	899	821-842	830.933	3.27	1.528	5.243	22.024	8.899	34
nad5 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

Phylogenetic analyses

The parsimony analysis retained 1 most parsimonious tree (MPT, length 4848, CI= 0.557, RI= 0.515) with a considerable lack of supported resolution. The MPT showed no conflict with the results from the Bayesian inference. Therefore, only the MrBayes tree is illustrated in Fig. 1, complemented with bootstrap values of the parsimony analysis when applicable. Among homocostate pleurocarps species of 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 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* Broth. & Yasuda and *Taxiphyllum aomoriense* (Besch.) Z. Iwats. with affinities to the Anomodontaceae. Besides several expected clades, unexpected but well supported ones were found. These will be described in the following.

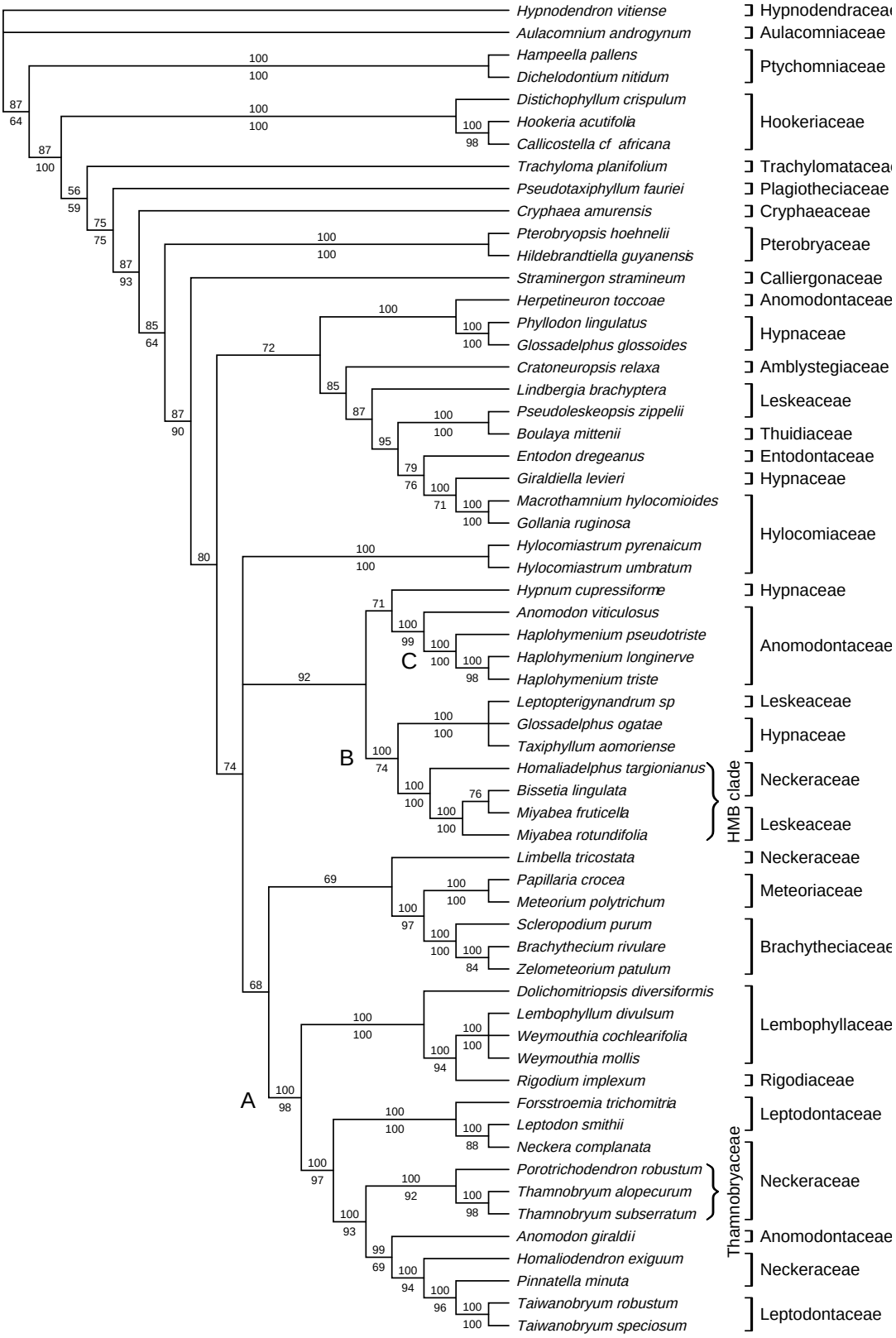


Figure 1. Majority consensus of trees sampled after stationarity in the Bayesian analysis of the matrix including indels, with posterior probabilities for individual clades above the branches. Values below the branches refer to bootstrap support values.

Three main clades where 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* (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. Together with the aforementioned species pairs *Entodon dregeanus* 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* & *Haplohymenium*). However, *Anomodon* itself is resolved as polyphyletic, with *A. giraldii* being deeply nested among the neckereacous taxa. On the other hand, the maximally supported *Homaliadelphus* - *Miyabea* - *Bissetia* - clade is sister to a small and morphologically heterogenous group consisting of *Taxiphyllum aomoriense*, *Leptopterigynandrum* sp. and *Glossadelphus ogatae*.

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

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

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

1.5 Discussion

Sequence variation of molecular markers

Although *rps4* as well as *trnL-F* are classic markers in molecular phylogenetics of bryophytes, the two spacers separating *rps4* from *trnT* and *trnT* from *trnL* have been largely ignored. Only the *trnT-L* IGS has been occasionally used with varying success exclusively on generic or population level (e.g. Frey et al. 1999; Pfeiffer et al. 2004;

Stech 2004). On deeper levels, however, Hernández–Maqueda et al. (2008) have been the first to successfully use both spacers combined with *rps4* and *trnL-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 *trnS-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 *trnL* intron, only the common inversion in front of *trnF* 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 *trnL* intron were quite similar, with the variability of the intron being relatively slightly smaller (see Table 4). The second included group I intron (*nad5*-intron), however, was more than double the size of the *trnL* 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 *rps4*, illustrating the fast evolving nature of this gene. In terms of sequence divergence ITS clearly outnumbered 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 nt 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 *nad5*-intron is the most efficient, as 74 % of the indels were p.i.-sites, although only 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 *nad5* 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 *rps4* and from 8.6 % (19.7 %) to 4.1 % (13.4 %) in the *trnL*-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. 2003) 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 *nad5* 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 *nad5* 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 4 nt (ranging from 2-8 nt). Despite its great use among early diverging diplolepideous-alternate mosses or hypnodendroid pleurocarps (Bell et al. 2007; Quandt et al. 2007) *nad5* seems to perform worse than plastid or nuclear regions in the Hypnales. This is nicely illustrated by the fact that *nad5* 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, *rps4* performed better compared to all other regions, even within the Hypnales (21.6 % variable sites; 11.8 % p.i-sites). To conclude, the observed little inter- and intrafamilial sequence divergence as well as the low content of p.i-sites among hypnalean *nad5* sequences rejects *nad5* 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 or dropping towards 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 as 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. Vanderpoorten et al. 2002a; Vanderpoorten et al. 2002b; Quandt & Huttunen 2004; Ignatov et al. 2007; Quandt et al. in press). However, among the Hypnales only few families, such as the Amblystegiaceae, Brachytheciaceae, Lembophyllaceae, Meteoriaceae and Leskeaceae have been revised recently with the aid of molecular data (e.g. Vanderpoorten et al. 2002a; Vanderpoorten et al. 2002b; Quandt et al. 2003b; Huttunen & Ignatov 2004; Huttunen & Quandt 2007; Ignatov et al. 2007; Quandt et al. in press). 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. 2000a; Tsubota et al. 2002; Ignatov et al. 2007).

***Lembophyllaceae/Rigodiaceae/Neckeraceae/"Thamnobryaceae"/Leptodontaceae-clade* (clade A)**

Following the classification of Goffinet & 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. in press, Stech et al. in press). The polyphyletic nature of the Neckeraceae and Leptodontaceae that was already 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 & 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 to 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 has 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 very similar *L. fryei* (R.S. Williams) Ochyra 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* (H.A. Crum & Steere) W.R. Buck, which differs clearly from the two above mentioned ones and was treated as *Vittia bartlettii* (H.A.Crum & Steere) Hedenäs & J.Muñoz, within the Amblystegiaceae (Hedenäs 2003) where it was also placed by, e.g., Buck (Buck 1998) and Goffinet & Buck (2004). The correct use of the generic name *Limbella* needs further study but we will not address the nomenclatural problem in the present paper, since it has no consequence in 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 & Higuchi (1999) found that *L. tricostata* (as *Sciaromium tricostatum* (Sull.) Mitt.) formed a clade with *Pleuroziopsis ruthenica* (Weinm.) Kindb. ex E. Britton, 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 comments in the discussion part 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 a new family: the Miyabeaceae. As mentioned above, the genus *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* Broth. was synonymized with *Phyllodon lingulatus* by Kis (2002), a concept which is adopted here. *Phyllodon* was placed in the Hypnaceae by Buck & 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* Müll. Hal.

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 the genus *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 *rbcL*.

Morphologically the Anomodontaceae *sensu* Goffinet & 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* 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 the Miyabeaceae. The main differences between the Anomodontaceae and the 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 ca midleaf at most, but usually ending well below midleaf. 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 its sister group does whereas 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

One of the most striking characters defining the Miyabeaceae within the context suggested by our results is the presence of dwarf males (Fig. 2), or phylloautoicy, in all genera (although not confirmed for every species). Dwarf males were reported for *Homaliadelphus laevidentatus* (S. Okamura) Z. Iwats. 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 of 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 range from 8 to 16 μm and the larger from 25-40 μm, while in *M. rotundifolia* the respective ranges are 12-22 and 29-38 μm. The sporophytes of the third species, *M. thuidioides* Broth., are unknown. Based on measurements of 50 spores from both of the specimens H3011293 (H) and H-BR0317006 (H-BR) we observed, a basically similar, but slightly less pronounced, anisospory in *Bissetia lingulata*. The spores largely fall in two size-classes, from 15 to 22 and from 25 to 31 μm, most of the spores being 20-22 or 25-27 μm. In

Homaliadelphus targionianus (specimen H3071598) 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 obligatorily phylloautoicous. The fact that *Homaliadelphus* holds a basal position in the clade suggests that the latter condition evolved in the *Bissetia-Miyabea*–lineage from a facultative one.

1.6 Formal description of the new pleurocarpous moss family, Miyabeaceae

Miyabeaceae Enroth, S. Olsson, Hedenäs, Huttunen, Buchbender & 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.

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 capsule and a reduced peristome with endostome absent or rudimentary.

The main morphological features of the *Miyabea-Bissetia-Homaliadelphus* -clade are as follows.

Plants small to medium sized. **Main stems** creeping, without a central strand, producing irregularly to subpinnately branched aerial stems with larger leaves and lacking a central strand. **Leaves** appressed-imbricate to complanate and more or less 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 they are also distinctly porose; marginal cells not differentiated, but in *Bissetia* towards base rather transverse in several rows; alar cells indistinct. Paraphyllia absent.

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. **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*). **Operculum** conical, with a long, oblique beak. **Calyptra** cucullate, naked or with few hairs. **Spores** 11-13 μm (*Homaliadelphus*) or anisosporous and ca 15-22 and 25-31 μm diameter (*Bissetia*, *Miyabea*).

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CHAPTER 2

THE ORIGIN OF THE BRITISH AND MACARONESIAN ENDEMIC *THAMNOBRYUM* SPECIES (NECKERACEAE)

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2.1 Abstract

The status and relationships of two British narrow endemic *Thamnobryum* species (*T. angustifolium* and *T. cataractarum*) as well as two Macaronesian endemics (*T. fernandesii* and *T. rudolphianum*) were investigated using nuclear (ITS1 & 2) and plastid (the *rps4-trnT-trnL-trnF* cluster) markers. Geographic structure present within a monophyletic *T. alopecurum* containing these narrow endemic taxa indicates that these submerged multistratose leaved forms in Britain and Madeira have been independently derived from the surrounding *T. alopecurum* populations and show convergent evolution in response to the extreme rheophilous habitat.

2.2 Introduction

Among the worldwide distributed species of *Thamnobryum* an unusually high proportion of endemics are reported from isolated island localities in Europe and Macaronesia. Four of the seven currently recognized species in this area (Hill et al. 2006) are narrow endemics. One species previously included in this genus, *T. cossyrense* (Bott.) A.J.E. Sm., endemic to the Mediterranean island of Pantelleria, is now considered synonymous with *Scorpiurium sendtneri* (Schimp.) M. Fleisch (Mastracci 2001). *Thamnobryum maderense* (Kindb.) Hedenäs is originally known from Madeira, the Azores and the Canary Islands. In recent times, however, new records from Portugal, southern Spain and Morocco (Jiménez et al. 2000) and the British Isles (Godfrey & Hodgetts 2006) have been reported. The species status is not unanimously accepted and some researchers treat *T. maderense* as a variety of the common *T. alopecurum* (Hedw.) Nieuwl. However, Frahm & Sabovljevic (2006) have argued that sub-complanate forms of *T. alopecurum* have been confounded with true *T. maderense,* which differs in its phyllotaxy, and that the specimens upon which the Stech et al., (2001) study based its conclusions are examples of the former. The recently described *T. rudolphianum* Mastracci (Mastracci 2004) is currently known only from four islands in the central and western Azores; the remaining endemics are even more geographically restricted. Two are restricted to single sites in the British Isles: *T.*

angustifolium (Holt.) Nieuwl. (Furness & Gilbert 1980) and *T. cataractarum* N. Hodgetts & Blockeel, (Hodgetts & Blockeel 1992), while *T. fernandesii* Sérgio is found in a limited area in the mountains of northern Madeira (Sérgio 1981; Hedenäs 1992). None of the very narrow endemic taxa (*T. angustifolium*, *T. cataractarum* or *T. fernandesii*) are known to produce sporophytes, or have any specialised means of propagation. Both *T. maderense* and *T. rudolphianum* produce capsules; the latter may also spread vegetatively by caducous branchlets (Mastracci 2004).

As a result of their extremely restricted range, these taxa have been accorded high conservation priority; all the single island endemics are listed in the Red Data Book of European Bryophytes (ECCB 1995). *T. fernandesii* is included on Appendix 1 of the Bern Convention and on Annex 2 of the EC Habitats and Species Directive. *T. angustifolium* was included on a list of the world's most threatened bryophytes (Hallingbäck & Hodgetts 2000) and is regarded as Critically Endangered in the UK Red Data Book (Church et al. 2001), protected under Schedule 8 of the Wildlife and Countryside Act, and since 1995 has been the subject of a UK Biodiversity Action Plan (Anon. 1995). *T. cataractarum* is regarded as vulnerable by Church et al. (2001) and it too was made the subject of a UK Biodiversity Action Plan in the second tranche of species (Anon. 1999).

The three insular endemic taxa show greater morphological similarities to one another than to the remaining European *Thamnobryum* species, indeed the characters differentiating *T. cataractarum* from *T. fernandesii* are largely related to stature, as acknowledged by the authors in their description (Hodgetts & Blockeel 1992); accordingly the status of the former species has been subject to question. All three taxa possess the features (reduced, partially bi- to multistratose laminas) that led Ochyra (1991) to recognise the novel genus *Crassiphyllum,* based on *C. fernandesii* (Sérgio) Ochyra. Significantly all of these plants are from similar habitats; all are submerged aquatics from at times fast-flowing water in deeply-shaded environments. Molecular studies (Stech & Frahm 1999; Stech et al. 1999; Stech & Frahm 2000; Stech & Frahm 2001), have demonstrated that many recently described narrow-endemic taxa from aquatic situations, e.g. *Gradsteinia andicola* Ochyra (formerly Donrichardsiaceae now Amblystegiaceae), *G. torrenticola* Ochyra, C. Schmidt & Bültman (formerly Donrichardsiaceae now *Platyhypnidium torrenticola* (Ochyra, C. Schmidt & Bültmann)

Ochyra & Bednarek-Ochyra; Brachytheciaceae), *Hypnobartlettia fontana* Ochyra (formerly Hypnobartlettiaceae now Amblystegiaceae), *Ochyraea tatrensis* Váňa (formerly Hypnobartlettiaceae now Amblystegiaceae), and *Platyhypnidium mutatum* Ochyra & Vanderp. (Brachytheciaceae) are genetically virtually indistinguishable from closely related and widespread species in the Amblystegiaceae and Brachytheciaceae, although many have been described as novel genera and in novel families. Stech & Frahm (2001) concluded that the transformation to a multistratose condition was therefore not a valuable diagnostic character at the family level and speculated further that it may occur widely in rheophilous situations. Conversely there are examples where taxa showing the multistratose condition have proven to be genetically distinct from similar unistratose-leaved taxa, for instance in the genera *Donrichardsia* H.A. Crum & L.E. Anderson (Vanderpoorten et al. 2002a) and *Vittia* Ochyra (Vanderpoorten et al. 2003).

How reliable then is the taxonomic recognition of these point or narrow endemic aquatic *Thamnobryum* species? Are they indeed more closely related one to another than to other *Thamnobryum* species, in which case is there evidence to support the recognition of the genus *Crassiphyllum*? Or are they merely local somatic mutants of the widespread and protean *T. alopecurum* (or other *Thamnobryum* species)? The answers to these questions are interesting biogeographically as well as having major implications for conservation effort and resource prioritisation.

Initial studies treating *T. maderense* and *T. fernandesii* unfortunately reach different conclusions. Whereas Stech et al. (2001), in a study of the taxonomic status of *T. maderense*, argue that *T. maderense* barely qualifies as a variety of *T. alopecurum*, Frahm & Sabovljevic (2006) reported a clear genetic distinction among the three taxa. In addition, Frahm & Sabovljevic (2006) observed a higher degree of similarity between internal transcribed spacer (ITS) sequences of nuclear ribosomal DNA (nrDNA) in *T. fernandesii* and *T. alopecurum* than between *T. alopecurum* and *T. maderense*. Frahm & Sabovljevic (2006) concluded that the high sequence divergence (9.1%) they report clearly indicates that "*T. fernandesii* could not be interpreted as a direct mutant of *T. alopecurum*". The possible relationships of the Madeiran endemic remained unresolved as the study did not include material of either British endemic. Furthermore, the levels of sequence divergence they reported would seem to be somewhat at odds with earlier

reports, based on a greater similarity in the more widely geographically separated *Thamnobryum* species, *T. alopecurum* and *T. pandum* (Hook. f. & Wilson) Jaeger from New Zealand (Stech et al. 2001). However, it has to be kept in mind that the study by Stech et al. (2001) did not include material from the Macaronesian islands. In the present study we therefore seek to address these issues using a broader array of molecular markers and a wider taxon representation.

Table 1. List of investigated specimens, with EMBL accession numbers for the regions sequenced and voucher details including voucher number, country of origin and the herbaria where the specimens are curated. In three cases sequence data were submitted to the database in previous studies and thus the entries for *rps4-trnT-trnL-trnF* are composed of two different accession numbers.

DNA no	Species name	Herbarium	Voucher ID	Locality	EMBL accession		
					rps4 - trnT - trnL - trnF	*rpl16*	ITS
	Lembophyllaceae						
SH103	*Lembophyllum clandestinum* (H. f & W.) Lindb. in Par.	H	Vitt 29644	New Zealand	AM990401; *trnLF* AF397823	FM160996	FM161145
SH146	*Dolichomitriopsis diversiformis* (Mitt.) Nog.	H, MHA	Nedoluzhko s.n.	Russia	AM990362; *trnLF* AF397777	FM160963	FM161098
B559	*Rigodium pseudothuidium* Dusén	NYBG	892248	Chile	-	-	FM161210
Rp47	*Rigodium pseudothuidium* Dusén	H	3134254	Chile	AM990438; *trnLF* AF543547	FM161051	-
	Neckeraceae						
B226	*Forsstroemia producta* (Hornsch.) Paris	H	Koponen 46545	China	FM201504	FM160967	FM161102
B193	*Neckera complanata* (Hedw.) Huebener	Buchbender	Buchbender 204	France	AM990413	FM161005	FM161158
B313	*Neckeropsis nitidula* (Mitt.) M. Fleisch.	S	B105713	Japan	AM990419	FM161030	FM161183
B476	*Pendulothecium punctatum* (Hook. f. & Wilson) Enroth & S. He	S	Sreimann 53845	New Zealand	AM990421	FM161033	FM161187
B472	*Pinnatella kuehliana* (Bosch & Sande Lac.) M. Fleisch.	Enroth	Müller S116	Indonesia	FM20150	FM161038	FM161192
B099	*Porotrichodendron robustum* Broth.	B	264620	Colombia	AM990426	FM200845	FM161197
SH372	*Porotrichopsis flacca* Herzog	S	Churchill et al. 10. Jan. 1991	Colombia	FM201506	FM161044	FM161199
B244	*Porotrichum bigelovii* (Sull.) Kindb.	H	Shevock & Kellman 27467	California	AM990428	FM161045	FM161200
B149	*Taiwanobryum speciosum* Nog.	H	Enroth 64877	China	AM990442	FM161055	FM161216
	Thamnobryum						
TB002	*Thamnobryum alopecurum* (Hedw.) Nieuwl. ex Gangulee	BM	Rumsey s.n.	England	-	-	FM201499

TB003	*Thamnobryum alopecurum* (Hedw.) Nieuwl. ex Gangulee	BM	Rumsey & Lansdown, s.n.	England	-	-	FM201500
TB004	*Thamnobryum alopecurum* fo protensum (Turner) Düll	BM	Coleridge 206	Madeira	FM201513	FM200846	FM201501
B238	*Thamnobryum alopecurum* (Hedw.) Nieuwl. ex Gangulee	Buchbender	Brohbachdal s.n. 11.7.2003	Germany	AM990444	FM161056	FM161218
TB005	*Thamnobryum angustifolium* (Holt) Nieuwl.	BM	Rumsey & Lansdown, s.n.	England	-	FM200847	FM201494
TB006	*Thamnobryum angustifolium* (Holt) Nieuwl.	BM	Rumsey & Lansdown, s.n.	England	FM201512	-	FM201495
TB008	*Thamnobryum cataractarum* N. Hodgetts & Blockeel	BM	Rumsey & Lansdown, s.n.	England	-	-	FM201497
TB009	*Thamnobryum cataractarum* N. Hodgetts & Blockeel	BM	Rumsey & Lansdown, s.n.	England	-	-	FM20149
B539	*Thamnobryum cataractarum* N. Hodgetts & Blockeel	S	B3725	England	FM201507	FM161057	FM161219
TB011	*Thamnobryum fernandesii* Sérgio	BM	Townsend s.n.	Madeira	-	-	FM201503
B549	*Thamnobryum fernandesii* Sérgio	S	B9965	Madeira	FM201508	FM161060	FM161222
SH300	*Thamnobryum maderense* (Kindb.) Hedenäs	S	B44108	Azores	AM990445	FM161061	FM161223
TB013	*Thamnobryum alopecurum* var..*maderense* (Kindb.) M. Stech, Ros & O. Werner (= *Thamnobryum maderense* sensu Jimenez et al. (2000))	MUB	14006	Spain	-	-	FM201502
B165	*Thamnobryum neckeroides* (Hook.) E. Lawton	NYBG	Buck 37648	USA, Oregon	FM201509	FM161062	FM161224
TB015	*Thamnobryum rudolphianum* Mastracci	BM	Mastracci s.n. (isotype)	Azores	-	-	FM201496
B574	*Thamnobryum rudolphianum* Mastracci	BM	919859	Azores	FM201510	FM161065	FM161228
B233	*Thamnobryum speciosum* (Broth.) Hoe	H	3141827	Hawaii	FM201511	FM161066	FM161229
B148	*Thamnobryum subserratum* (Hook. ex Harv.) Nog. & Z. Iwats.	H	Enroth 64595	China	AM990446	FM161067	FM161230

Table 2. Summary of the observed substitutions within the *Thamnobryum alopecurum* complex in the combined data set with reference to the location.

	rps4		*rps4-trnL* IGS			*trnL-F* IGS	*rpl16* intron				ITS1	ITS2	
T. alopecurum D	C	T	A	T	A	T	C	C	T	A	C	C	A
T. angustifolium	C	T	A	T	A	T	C	C	T	A	C	C	G
T. cataractarum	C	T	A	T	A	T	C	C	T	A	C	C	G
T. alopecurum Madeira	C	C	G	C	A	G	A	T	C	A	T	T	A
T. fernandesii	C	C	G	C	A	G	A	T	C	A	T	T	A
T. maderense	C	C	G	T	A	G	A	T	C	A	T	T	A
T. rudolphianum	T	C	G	T	G	G	A	T	C	T	T	T	A

2.3 Material and methods

Taxon sampling and molecular markers

Two data sets with different taxon sampling and molecular markers were compiled. In the first data set each recognized species of the *Thamnobryum alopecurum* complex was included only once, except *T. alopecurum* for which we included two specimens, one voucher originating from Madeira and one from mainland Europe. For this data set we sequenced three genomic regions: *i)* the internal transcribed spacer of nuclear ribosomal DNA (ITS1 & 2), including the 5.8S gene, *ii)* the plastid *rps4-trnT-trnL-trnF* cluster, including 2 tRNAs (*trnT*, *trnL*), the 5' 139 nt of *rps4*, three spacers separating the coding regions, as well as one group I intron in *trnL*, and *iii)* the group II intron in *rpl16* (plastid). This data set will be referred to as the combined data set in the following discussion. Sampling included a representative set of related Neckeraceae species according to the latest phylogenetic analyses by Olsson et al. (chapter 1 and 3) plus three representatives of the Lembophyllaceae that represent the sistergroup to Neckeraceae (compare Quandt et al. in press; chapter 1 and 3). The second data set solely consisted of ITS1 & 2 sequences for an increased number of exemplars from the *Thamnobryum alopecurum* complex, referred to as the ITS data set in the following. This data set also included one voucher of *Thamnobryum maderense sensu* Jimenez et al. (2000), from the Iberian Peninsula. While this has the sub-complanate growth form and some of the leaf shape characters of *T. maderense*, it is sterile and does not show the quadrifarious leaf arrangement suggested as typical of *T. maderense* by Frahm & Sabovlejic (2006). Voucher details and EMBL accession numbers for the sequenced regions are listed in Table 1.

DNA isolation, PCR amplification and sequencing

DNA was extracted using the DNeasy® Plant Mini Kit from Qiagen (Qiagen GmbH, Germany) following the manufacturer's protocol. Cleaning and grinding of plants prior to extraction followed Olsson et al. (chapter 1). Amplification of the ITS1-5.8S-ITS2 as well as the *rps4-trnF* region followed Olsson et al. (chapter 1), whereas the protocols for *rpl16* were obtained from Olsson et al. (unpubl.). Gel cleaned PCR products were sequenced by Macrogen Inc., South Korea (www.macrogen.com). DNA methods for

work conducted at the NHM in London followed Grundmann et al. (2006). Sequences were edited manually with PhyDE® v0.995 (Müller et al. 2005) and primer sequences eliminated. All sequences are deposited in EMBL.

Sequence analyses and phylogenetic analyses

Alignment of the sequence data was performed manually in PhyDE® v0.995 (Müller et al. 2005), based on the criteria laid out in Kelchner (2000), Borsch et al. (2003) and Quandt & Stech (2005). As length variation of the sequence data was very low, alignment was straightforward. Length variation in the plastid data was generally associated with simple sequence repeats (SSR) or poly-mononucleotide stretches. SSRs were positionally isolated based on strict motif recognition, hence overlapping motifs were considered non-homologous if the motifs could be derived independently from the adjacent region. The reported hairpin-associated inversion (Fig. 1) in the *trnL-F* intergenic spacer (IGS) (Quandt & Stech 2004; Quandt et al. 2004) was positionally isolated in the alignment and included in the analysis in reverse complement form in order to gain information from substitutions within the detected inversion, as discussed in Quandt et al. (2003) (compare Fig. 1 & 2). The type of the inversion was not coded in the phylogenetic analyses, as it is known to fluctuate at a population level (Quandt & Stech 2005). Secondary structures of the hairpin were calculated with RNAstructure 4.2 (Mathews et al. 2004). Alignments are provided on an appendix cd. In both data sets indels were incorporated 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 file containing the sequence alignment with an automatically generated indel matrix appended. Command files for using the parsimony ratchet (Nixon 1999) were generated using 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% upweighting of the characters in the iterations. Heuristic bootstrap searches under parsimony were performed with 1000 replicates and 10 random addition cycles per bootstrap replicate.

Bayesian analyses were performed with MrBayes v3.1.2 (Huelsenbeck & Ronquist 2001), applying the GTR+Γ+I model for the sequence data and the restriction site model for the binary indel partition. To allow for possible deviating substitution

models for the different regions, the data set was divided into four partitions (partition 1: *rps4-trnF*; partition 2: *rpl16*; partition 3: nuclear DNA; partition 4: indels). The *a priori* probabilities supplied were those specified in 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; Huelsenbeck et al. 2002b). Ten runs with four chains (10^6 generations each) were run simultaneously, with the temperature of the single heated chain set to 0.2. Chains were sampled every 10 generations and the respective trees written to a tree file. Calculations of the consensus tree and of the posterior probability of clades were performed based upon the trees sampled after the chains converged (< 25,000 generations). Consensus topologies and support values from the different methodological approaches were compiled and drawn using TreeGraph (Müller & Müller 2004).

A haplotype network for the ITS data set was calculated with TCS (Clement et al. 2000; Clement et al. 2002) using the default settings, with the exception that gaps were treated as missing data. Apart from the theoretical aspect that the 5th character state is not applicable, the specified gap treatment is necessary as otherwise the network is likely to break into two parts due to an observed 13 nt SSR in the ITS1 of *Thamnobryum subserratum* and *T. speciosum*. As TCS cannot run on a mixed matrix containing DNA and binary data the two binary coded indels were manually exchanged to A (1) and T (0), respectively.

2.4 Results & Discussion

The structural representations of the observed hairpin associated inversion upstream of *trnF* distinguish four types (a-d, compare Fig. 1) that can be transformed into each other via an inversion and/or substitution. For example, type a can be derived from type b through inversion of the terminal loop of the hairpin (Fig. 1). Type b is a derivate of c as a result of a transition in the terminal loop. This transition is, in fact, one of the many synapomorphic substitutions characterising *Thamnobryum*, but its information is lost if the inversion is positionally isolated or excluded from the analyses. As discussed in

Quandt, Müller & Huttunen (2003), including the reverse complement motif of the inversion retrieves the information of the substitution event, as indicated in Fig. 2, for the phylogenetic reconstructions, an approach followed here.

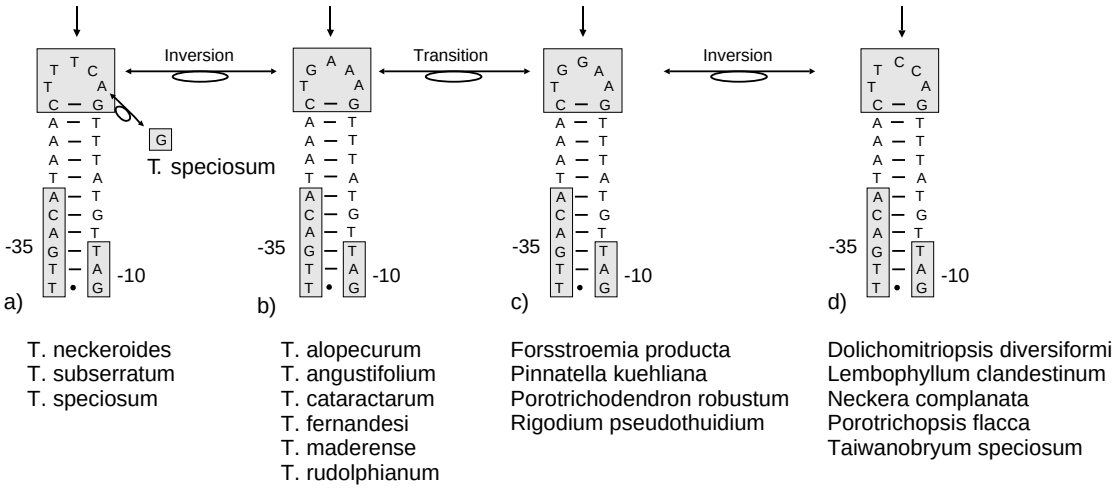

Figure 1. Structural representation of a hairpin associated inversion upstream of *trnF*. Four types (a-d) can be distinguished in the sequenced *trnL-F* IGS. For each type the species in which we observed the type is given below the structure. The -35 and -10 promotor elements are annotated in the helices and highlighted by a grey box. The terminal loop that harbours the inversion is also boxed in grey. Type a can be derived from type b and type d from type c respectively, via an inversion, whereas types b and c as well as d and a differ by a substitution in the inverted loop as indicated by the arrows. An observed transition in the terminal loop of the hairpin in *Thamnobryum speciosum* is also indicated.

```
motif a  TTGACATAAA-------CTTTCAGTTTATGTTAG        TTGACATAAACTGAAAGTTTATGTTAG
motif b  TTGACATAAACTGAAAG-------TTTATGTTAG   →    TTGACATAAACTGAAAGTTTATGTTAG
motif c  TTGACATAAACTGGAAG-------TTTATGTTAG        TTGACATAAACTGGAAGTTTATGTTAG
motif d  TTGACATAAA-------CTTCCAGTTTATGTTAG        TTGACATAAACTGGAAGTTTATGTTAG
```

Figure 2. Alignment of the four observed types of the hairpin associated inversion in the *trnL-F* IGS. The detected inversion has been positionally isolated in the alignment, but was included in the analyses as reverse complement in order to gain information from substitutions predating the inversion. Type a and b are characteristic for *Thamnobryum* and harbour a synapomorphic substitution in the terminal loop (indicated by an arrow in Fig. 1) that is illustrated in bold in the matrix. Reverse complemented nucleotides are shown in italics.

Phylogenetic analyses of the combined data set clearly resolve the *Thamnobryum alopecurum* complex as monophyletic with maximal support regardless of the analytical method (Fig. 3). The remaining included *Thamnobryum* species are resolved sister to

the *Thamnobryum alopecurum* complex, which is attributed to the limited sampling size on the generic level. Among the *Thamnobryum alopecurum* complex two clades are resolved with high support, one comprising the specimens from the Azores and Madeira (PP 93; BS 85) and the second including the British and Central European specimens (PP 100; BS 100). Surprisingly, each clade contains the geographically corresponding *Thamnobryum alopecurum* specimen. This is highly interesting as it points towards the independent origin of the submerged aquatics from the surrounding *Thamnobryum alopecurum* populations, although conclusions should be drawn with caution as the sampling size is limited. However, a similar picture emerges considering the results from the ITS haplotype network analyses, although ITS variation in the *Thamnobryum alopecurum* complex did not exceed 0.26 % (p-distance). ITS variation among all included *Thamnobryum* specimens was a little higher (0.63 %) and reached 2.3 % among the Neckeraceae, and 2.9 % with outgroup taxa included. These results are in strong contrast to the extremely high sequence divergence values between *T. alopecurum*, *T. fernandesii*, and *T. maderense* reported by Frahm & Sabovljevic (2006), but resemble the results of Stech et al. (2001). Values reported by Frahm & Sabovljevic (2006) ranged between 9 % (*T. alopecurum* and *T. fernandesii*) to almost 50 % (*T. maderense* compared to *T. alopecurum* and *T. fernandesii*). These values are unusually high and even exceed reported ITS variation among all Neckeraceae (p-distance: 5.72 %) by 2 to 9 times (chapters 1 and 3). Therefore we express either serious doubt that the reported sequences correspond to the vouchers or question the quality of the sequence data. This correlates with the fact that BLAST searches of the submitted sequences (*Thamnobryum alopecurum* AM233514; *Thamnobryum fernandesii* AM233515) provide high similarity scores with various hypnalean taxa other than *Thamnobryum*, such as *Isothecium*. Only the submitted *T. maderense* (AM233516) sequence matches the ITS sequences reported here. However, especially towards the end of ITS2 this sequence has a high proportion of single nucleotide indels indicating that the original data (pherograms) were not cross-checked. Thus the molecular results reported by Frahm & Sabovljevic (2006) seem to be artificial and the reported sequences were not included in our phylogenetic analyses. In the network analysis reported here, the British populations cluster together, separated from the Macaronesian samples by the German populations. The *T. maderense sensu* Jimenez et al. (2000) sample from the Iberian

Peninsula shares the same haplotype as a specimen from Great Britain which superficially complicates the picture but gives support to the view of Frahm & Sabovlejic (2006) that these continental sub-complanate plants are distinct from true *T. maderense* and represent growth forms of *T. alopecurum*. However, the Macaronesian specimens all share the same ITS haplotype, distinct from the Central and South European as well as British specimens. ITS population level variation among the South to North European specimens seems to be higher compared to the Macaronesian samples (Fig. 4).

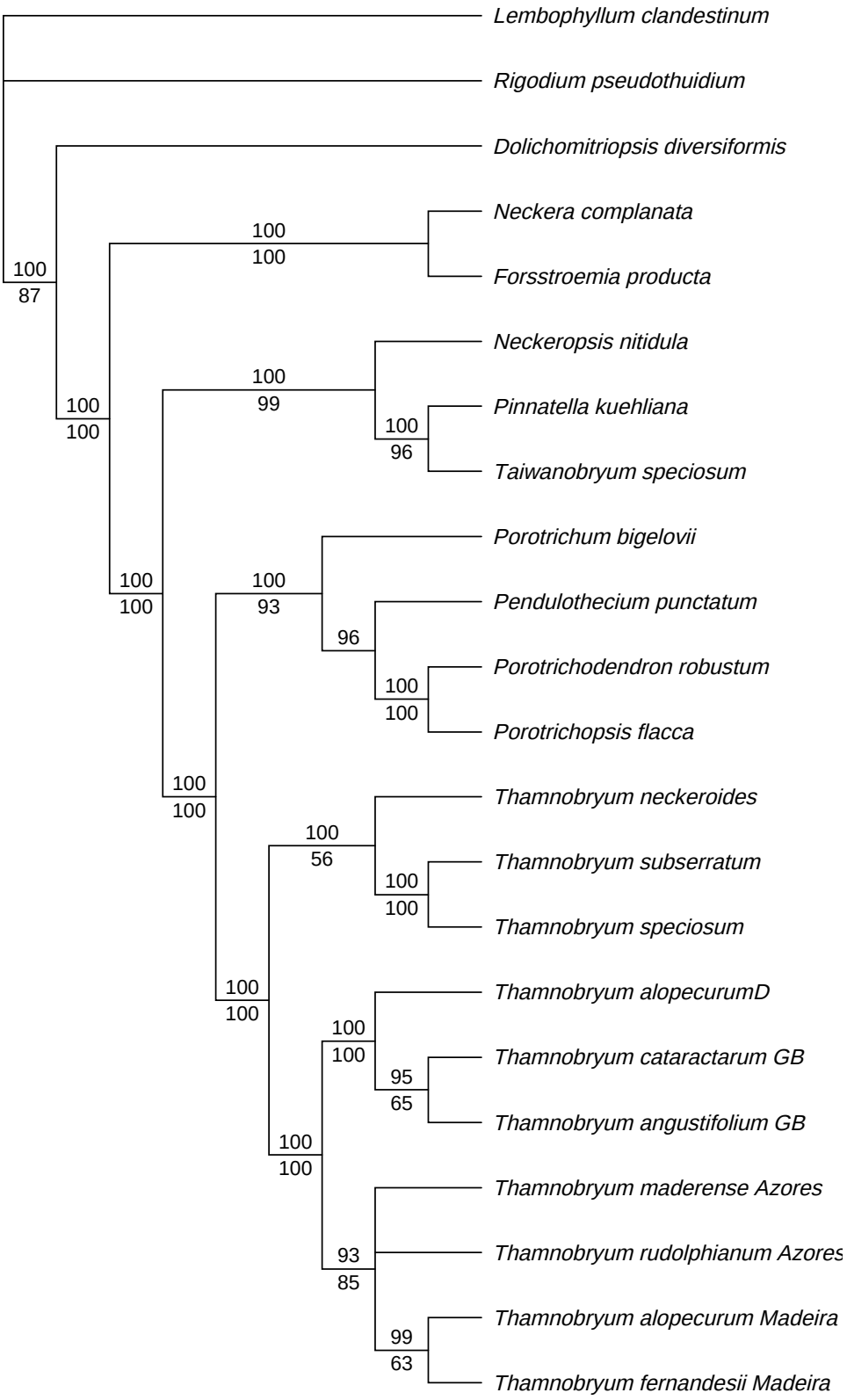

Figure 3. Bayesian phylogram based on the combined matrix, including the binary coded indels. Posterior probabilities are shown above the branches whereas bootstrap support from the parsimony analyses is indicated below.

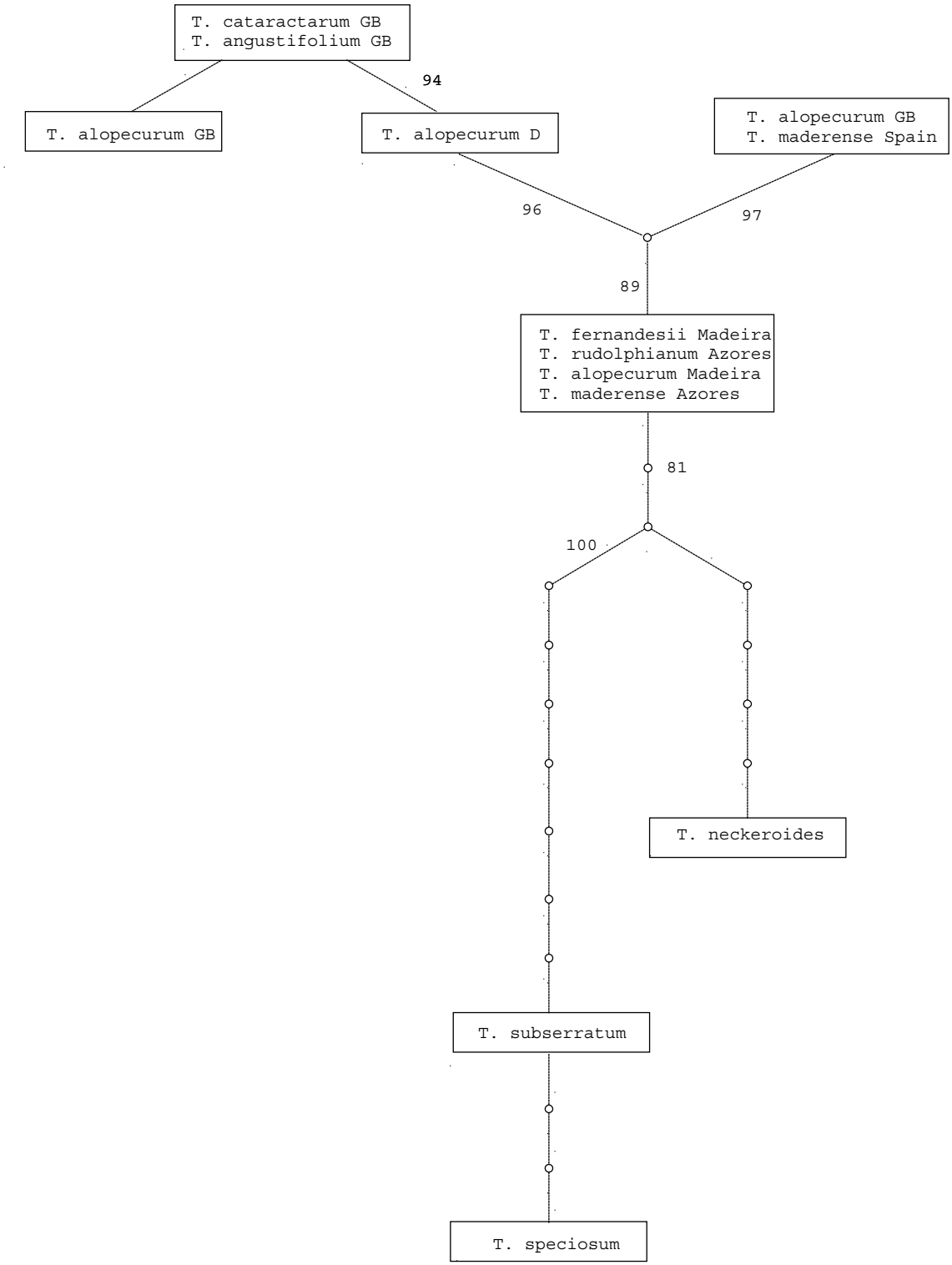

Figure 4. Haplotype network inferred from the ITS data for *Thamnobryum alopecurum* complex plus outgroup taxa. *Thamnobryum maderense sensu* Jimenez et al. (2000) was abreviated as *T. maderense* (Spain) in the haplotype network. Mutational steps separating the haplotypes are indicated by open circles. Confidence in the branches is indicated by posterior probabilities from an independent Bayesian analysis.

The above results are somewhat contradicted by the plastid data, as the Central European - British clade shares identical plastid sequences separating it from the Macaronesian clade by 6 substitutions (compare Table 2), with a high degree of variability inside the Macaronesian clade. For example, *T. rudolphianum* has a unique plastid sequence with two autapomorphic substitutions, while the Madeiran specimens of *T. alopecurum* and *T. fernandesii* share the same plastid haplotype differing from the remainder by one synapomorphic substitution (compare Table 2). Interestingly, the *rps4-trnL* IGS as well as the *rpl16* intron each provided as much information as the ITS data in terms of synapomorphic mutations, although they showed no variation inside the Central European – British clade.

The lack of ITS variation compared to the plastid variation among the Macaronesian morphospecies is somewhat surprising, as in biogeographically isolated populations one might expect a higher degree of ITS variation, as was observed in the plastid data. Therefore, one might speculate that the large variation in the plastid data inside the Macaronesian clade is a result of isolation leading to different copies of the plastid genome, whereas the identity of ITS copies is maintained via concerted evolution. Another option would be that chloroplast capture has been involved, i.e. hybridisation with subsequent introgression. Due to the limited sampling we refrain from providing a final conclusion at this point, and await future more detailed analyses.
In contrast to a similar study on *Isothecium* (Draper et al. 2007), our results on the *T. alopecurum* complex, although not as sophisticated as the *Isothecium* study, point towards convergence instead of a complicated network with a high degree of genetic exchange and cryptic speciation. On the other hand, the low molecular differentiation among as well as between the two *T. alopecurum* clades (Figs. 3 & 4), in combination with the high morphological variation among the taxa, could indicate possible morphological plasticity as reported for other moss taxa (e.g. Shaw & Allen 2000; Vanderpoorten et al. 2001; Vanderpoorten 2004). However, the fact that the reported extreme morphological aberrations generally occur in unusual habitats in different geographically isolated areas questions if these morphological observations can be reduced to mere phenotype plasticity. In contrast, if the plants had the genetic potential to develop morphologically different expressions in response to particular habitat conditions, one would assume that reports of such expressions, especially in Great

Britain, would be more frequent, because *Thamnobryum alopecurum* and the rheophilous habitats are very common there. However, more studies including culturing and transplantation experiments are needed to settle this issue.

To conclude, with regard to the submerged aquatics inside the *Thamnobryum alopecurum* complex we favour the convergent evolution hypothesis and postulate that the submerged aquatic morphospecies are derived from surrounding *T. alopecurum* populations. However, as the sampling is very limited more detailed population genetic analyses are urgently required to support or reject our findings. As is evident from the molecular analyses, the *T. alopecurum* complex provides an excellent study group for molecular as well as morphological evolution in mosses. Most importantly, the genetic basis of the morphological aberrations needs to be investigated. If we only examine morphological extremes we cannot know if they are due simply to up- and down-regulation of genes or true speciation via genetic differentiation. Therefore, because we now have the means to study the genetic basis of morphological differentiation, it is all the more important to ensure that these peculiar plants are preserved.

2.5 Acknowledgements

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CHAPTER 3

EVOLUTION OF THE NECKERACEAE: RESOLVING THE BACKBONE PHYLOGENY

This study is submitted as: Olsson, S., Buchbender, V., Enroth, J., Huttunen, S., Hedenäs, L. & Quandt, D. Evolution of the Neckeraceae: resolving the Backbone phylogeny. Systematics and Biodiversity.

3.1 Abstract

Earlier phylogenetic studies including neckeraceous species have indicated that the pleurocarpous moss family Neckeraceae shares a strongly supported sister group relationship with the Lembophyllaceae, but that the family delimitation of the former needs adjustment. To test the monophyly of the Neckeraceae as well as to redefine the family circumscription and to pinpoint its phylogenetic position in a larger context, a phylogenetic study based on molecular data was carried out. Sequence data was compiled, combining data from all three genomes: nuclear ITS1 & 2, plastid *trnS-rps4-trnT-trnL-trnF* and *rpl16*, and mitochondrial *nad5* intron. The Neckeraceae have sometimes been divided into the two families Neckeraceae and Thamnobryaceae, a division rejected here. Both parsimony and Bayesian analyses of molecular data reveal that the family concept of the Neckeraceae needs several further adjustments, such as the exclusion of some individual species and smaller genera as well as the inclusion of the Leptodontaceae. Within the family three well-supported clades (A, B, and C) can be destinguished. Members of clade A are mainly non-Asiatic and non-tropical. Most species have a weak costa and immersed capsules with reduced peristomes (mainly *Neckera* spp.) and the teeth at the leaf margins are usually unicellular. Clade B members are also mainly non-Asiatic. They are typically fairly robust, distinctly stipitate, having a single, at least relatively strong costa, long setae (capsules exserted), and the peristomes are well developed or only somewhat reduced. Members of clade C are essentially Asiatic and tropical. The species of this clade usually have a strong costa and a long seta, the seta often being mammillose in its upper part. Several neckeraceous genera that were recognized on a morphological basis are polyphyletic (e.g. *Neckera, Homalia, Thamnobryum, Porotrichum*). Ancestral state reconstructions revealed that currently used diagnostic traits, such as the leaf asymmetry and costa strength are highly homoplastic. Similarly, the reconstructions revealed that the "reduced" sporophyte features have evolved independently in each of the three clades.

3.2 Introduction

The pleurocarpous mosses, i.e. "the Core Pleurocarps" as defined by Bell et al. (2007) form a monophylum, which consists of typically perennial mosses with creeping stems and abundant lateral branches. In pleurocarpous mosses the archegonium and thus also sporophyte development is restricted to the apices of short, specialized lateral branches, in contrast to most other mosses, where archegonia and sporophytes develop terminally on the main axis (acrocarpous) or on major branches (cladocarpous).

The group of pleurocarpous mosses comprises approximately 5000 species, which corresponds to about half of all mosses (Buck & Goffinet 2000). Traditionally, pleurocarpous mosses have been divided into the orders Hookeriales, Leucodontales (or Isobryales) and Hypnales, with the Neckeraceae belonging to the Leucodontales (Brotherus 1925). Buck and Vitt (1986) defined the Hypnales as mainly terricolous species with an unreduced peristome (i.e. "perfect" or "well-developed"), and the Leucodontales were defined by a reduced peristome. Most likely this grouping does, however, not correspond to natural relationships, but is due to convergent peristome evolution in several lineages (e.g. Buck & Crum 1990; Buck 1991). Supported by molecular analyses the separation of the Leucodontales was therefore rejected (Buck et al. 2000b; Tsubota et al. 2002), thus the Neckeraceae are currently treated within the Hypnales (Goffinet & Buck 2004). The Hypnales have probably radiated relatively recently and rapidly, as indicated by the short branch lengths in the backbone of the Hypnales (Buck et al. 2000b) and low DNA sequence variation (Shaw 2000; Vanderpoorten et al. 2002a). Due to these problems, the phylogenetic relationships among the Hypnalean families are extremely difficult to reconstruct and remain largely unresolved (Buck et al. 2000b; Shaw et al. 2003). More analyses are needed to provide reliable answers addressing the evolution of this group. However, previous analyses highly support a close relationship between the Neckeraceae and the Lembophyllaceae (Quandt et al. in press; chapter 1), even if the current circumscription of the Neckeraceae is challenged (Buck et al. 2000b; Tsubota et al. 2002; Ignatov et al. 2007; Olsson et al. chapter 1).

The Neckeraceae as treated by Brotherus (1925) contained 16 genera grouped into three subfamilies: Leptodontoideae, Neckeroideae, and Thamnioideae (see Table 1). Walther (1983) accepted the division of the Neckeraceae into Leptodontoideae and Neckeroideae and recognized the Thamniaceae (later renamed Thamnobryaceae) as a separate family. The Leptodontaceae was erected by Schimper (1856), but it was generally not recognized until resurrected by Buck (1980) and employed by Buck and Vitt (1986). According to the current classification by Goffinet and Buck (2004), there are 28 genera in the Neckeraceae. However, no comprehensive genus-level revision of the family has been made. Recent studies based on a wider taxon sampling have already reduced the number of genera included. For example, *Homaliadelphus* and *Bissetia* belong to a newly erected family, the Miyabeaceae, and *Limbella tricostata* belongs near the Meteoriaceae and Brachytheciaceae (Olsson et al. submitted-a). On the other hand, Tsubota et al. (2002) provided evidence that members of the four genera (*Alsia, Forsstroemia, Leptodon, Taiwanobryum*) treated in Leptodontaceae by Goffinet and Buck (2004) belong to the Neckeraceae.

According to Enroth (1994b) and the chapters 1, 4 and 5 of this thesis we estimate the species number in the Neckeraceae to be around 200. The family has a wide geographic distribution, comprising largely tropical (*Neckeropsis, Pinnatella, Himantocladium, Porotrichodendron*) as well as predominantly temperate (*Neckera, Thamnobryum*) genera. The species are mainly epiphytic or epilithic, although some aquatic (rheophytic, i.e. growing in flowing water) species belong here as well. Members of the family are generally recognised by their usually large, glossy plants that have creeping stolons bearing very small leaves and tufts of rhizoids (Enroth 1989a), and more or less frondose (rarely dendroid) stems with or without distinct stipes. The leaf cells are almost always smooth, relatively short and firm-walled, and the marginal cells are typically quadrate to short-rectangular in few to several rows (Enroth 1994b). The sporophyte features are variable but usually fairly consistent within genera.

The suggested sistergroup of the Neckeraceae, the Lembophyllaceae, has undergone even more drastic changes in the generic composition. Originally with just four genera: *Lembophyllum*, *Camptochaete*, *Dolichomitra*, *Isothecium* (Brotherus 1909), the family later on expanded to contain 12 genera in Fleischer's (1906-1908; 1915-1923) and Brotherus' (1925) treatments. Due to changes in the interpretation of morphological characters this concept was later on considered to be unnatural and the

family was subsequently redefined to contain only two genera (*Lembophyllum* and *Camptochaete*) (e.g. Andrews 1952; Walther 1983; Buck & Vitt 1986; Crum 1991). Recently, however, the situation has reversed and the previously excluded taxa were again placed in the Lembophyllaceae (e.g. Tangney 1997; Crosby et al. 1999; Buck & Goffinet 2000; Quandt et al. 2000) up to the point that the latest revision based on molecular data nearly returned to the 1925 concept of Brotherus. Although the molecular analyses agree with the generic composition of Brotherus (1925) a clear morphological circumscription of the family is still lacking (Buchbender 2009; Quandt et al. in press). The Lembophyllaceae sensu Quandt et al. (in press) comprises a morphologically highly heterogeneous group of mainly epilithic or epiphytic plants with creeping stolons and often frondose stems bearing usually concave leaves. However, characters currently used to define the Lembophyllaceae as well as its sistergroup, the Neckeraceae, are not exclusive or discontinuous, hindering a clear morphological circumscription of both families. As a rule of thumb both families differ in their arrangement of leaves on the shoots. In the Neckeraceae the shoots are mostly complanate, whereas in the Lembophyllaceae they are usually terete, with the leaves being most often loosely appressed. In addition, both families differ in their habitat preferences, while the Neckeraceae are most diverse in tropical areas, whereas the Lembophyllaceae are essentially temperate.

There are several problems involved with morphology-based phylogenetic analyses of pleurocarpous moss relationships. Numerous characters can, in principle, be used if they are correctly understood and interpreted, but the often reduced morphology and abundant convergence implies homology problems. Thus, in many cases only a limited number of characters are informative. The simple structures observed in pleurocarpous mosses limit the number of potentially useful morphological characters for phylogenetic analyses. Therefore, at family level only about 50 to 100 morphological characters can be used (e.g.Hedenäs 1995, 1997; Pedersen & Hedenäs 2002; Vanderpoorten et al. 2002b; Huttunen & Ignatov 2004). Several previous studies have shown that morphological characters can be misleading with a high degree of convergent evolution even at the genus and species levels (Hedenäs 2001; Vanderpoorten et al. 2002a; Vanderpoorten et al. 2002b; Huttunen & Ignatov 2004). Also, morphological reduction has occurred several times in different moss lineages (e.g.Frey 1981). Therefore, the identification of relevant characters to be used in

pleurocarpous moss classification is crucial, and a failure to do this would result in an incorrect phylogenetic placement based on morphology (Hedenäs 1995). Sporophytes and the characters related to them have traditionally been considered the most important criteria in moss classification at all taxonomic levels. Sporophytes are, however, as subject to environmental pressures as the gametophytes (Hedenäs 2001, 2002), and they can be as homoplastic and therefore misleading in moss classifications as gametophyte characters at and above the family level (Buck 1991). A good example of parallel evolution of sporophytic characters was shown in a study by Huttunen et al. (2004), who concluded that structural reduction has independently taken place in the Brachytheciaceae in several lineages representing all four subfamilies.

This study, where our main focus is the family circumscription, is the first modern comprehensive and rigorous family-level study on the Neckeraceae. We test the monophyly of the Neckeraceae and evaluate its position in the pleurocarpous moss phylogeny with a representative set of taxa from the Neckeraceae and Lembophyllaceae, as well as from potentially closely related taxa. In addition to resolving the main patterns of relationships among the Neckeraceae and their relatives, we explore the morphological character evolution using Bayesian ancestral state reconstruction methods. We also shed light on some distinctive phytogeographic patterns among the Neckeraceae.

3.3 Material and methods

Taxon sampling and molecular markers

Seventy-three taxa from 47 different genera were included in the analysis. Thirty-eight members representing the Neckeraceae, Thamnobryaceae and Leptodontaceae, as well as supposedly neckeraceous species (according to Buck & Goffinet 2000) were included in the sampling. In addition, nine representatives of the Lembophyllaceae (according to Quandt et al. in press), and 24 outgroup species from several Hypnalean families as well as the Hookeriaceae were sampled. The selection of species was based on earlier treatments of the Neckeraceae (compare Table 1), as well as previous analyses by Olsson et al. (chapter 1) and Quandt et al (in press). Samples were sequenced for four genomic regions: the nuclear ribosomal ITS1 & 2, a mitochondrial group I intron

residing in *nad5* (and part of the gene) as well as two plastid regions: *rpl16* and *trnS-trnF*. The extensive *trnS-trnF* area includes the fast evolving protein coding gene *rps4*, four intergenic spacers (*trnS-rps4* IGS, *rps4-trnT* IGS, *trnT-trnL* IGS and *trnL-trnF* IGS), the *trnL* intron as well as four tRNAs genes (*trnS*, *trnT*, *trnL* and *trnF*). Species sampled, together with voucher information and EMBL accession numbers, are listed in Table 2.

Table 1. Overview of the different treatments of the Neckeraceae, including the Leptodontaceae and Thamnobryaceae (Thamniaceae). The treatment of the Neckeraceae by Goffinet and Buck (2004) is identical to Buck and Goffinet (2000), apart from the exclusion of *Porothamnium*. Buck and Vitt (1986) formally describe the Thamnobryaceae containing the dendroid Neckeraceae sensu Brotherus (1925) with cross-striolate exostomes (i.e. roughly the former subfamily Thamnoioideae Broth.).

Brotherus (1925)	**Vitt (Vitt 1984)**	**Walther (1983)**	**Buck & Goffinet (2000)**
Neckeraceae	**Neckeraceae**	**Neckeraceae**	**Leptodontaceae**
Leptodontoideae	*Baldwiniella*	**Leptodontoideae**	*Alsia*
Cryphidium	*Bissetia*	*Cryptoleptodon*	*Forsstroemia*
Leptodon	*Cryptoleptodon*	*Leptodon*	*Leptodon*
Cryptoleptodon	*Dolichomitra*	**Neckeroideae**	*Taiwanobryum*
Neckeroideae	*Handeliobryon*	*Homalia*	
Calyptothecium	*Himantocladium*	*Neckera*	**Neckeraceae**
Neckera	*Homalia*	*Metaneckera*	*Baldwiniella*
Neckeropsis	*Homaliadelphus*	*Neomacounia*	*Bissetia*
Bissetia	*Homaliodendron*	*Bissetia*	*Bryolawtonia*
Himantocladium	*Hydrocryphaea*	*Baldwiniella*	*Caduciella*
Baldwiniella	*Isodrepanium*	*Himantocladium*	*Crassiphyllum*
Homaliodendron	*Leptodon*	*Homaliadelphus*	*Cryptoleptodon*
Homalia	*Metaneckera*	*Homaliodendron*	*Curvicladium*
Thamnioideae	*Neckera*	*Neckeropsis*	*Dixonia*
Pinnatella	*Neckeropsis*		*Dolichomitra*
Handeliobryum	*Neomacounia*	**Thamniaceae**	*Handeliobryum*
Porotrichum	*Pinnatella*	*Porotrichum*	*Himantocladium*
Thamnium	*Porothamnium*	*Porothamnium*	*Homalia*
Porothamnium	*Porotrichodendron*	*Pinnatella*	*Homaliadelphus*
	Porotrichopsis	*Thamnobryum*	*Homaliodendron*
	Porotrichum	*Bestia*	*Hydrocryphaea*
	Thamnobryum	*Handeliobryum*	*Isodrepanium*
		Hydrocryphaea	*Metaneckera*
			Neckera
			Neckeropsis
			Neomacounia
			Noguchiodendron
			Pendulothecium
			Pinnatella
			Porothamnium
			Porotrichodendron
			Porotrichopsis
			Porotrichum
			Thamnobryum
			Touwia

Table2. Taxa used in the study with EMBL and GenBank accession numbers for the sequenced or downloaded (* = specimen that differs for the voucher details) regions and voucher details. In some cases sequence data have been already submitted to GenBank from previous studies. Therefore accession numbers for *trnS-rps4-trnT-trnL-trnF* are composed of up to three accession numbers. Herb. = Herbarium; ASV = Alfons Schäfer-Verwimp; VB = Volker Buchbender,

DNA no	Species name	Herb.	Voucher ID	GenBank accession			
				trnS-rps4-trnT-trnL-trnF	*rpl16*	*nad5*	ITS
SH424	*Andoa berthelotiana* (Mont.) Ochyra	S	B8333	AM990341	FM160947	FM161239	FM161074
B304	*Baldwiniella kealeensis* (Reichardt) E.B. Bartram	H	H3008991	AM990345; (*rps4* = AY908590*)	FM160948	FM161242	FM161078
B237	*Bissetia lingulata* (Mitt.) Broth.	H	H3194160	AM990346; (*rps4* = AY908352*)	FM160949	FM161243	FM161079
SH131	*Brachythecium rivulare* Schimp.	H	Parnela s.n. 19. May 1996	AM990348; (*trnLF* = AF397866)	FM160950	FM161245	FM161081
B222	*Bryolawtonia vancouveriensis* (Kindb.) D.H. Norris & Enroth	NYBG	J.R. Shevock 19202	AM990349	FM160951	FM161246	FM161082
B206	*Calyptothecium recurvulum* (Broth.) Broth.	NYBG	Withey 561	AM990352	FM160954	FM161248	FM161086
SH10	*Camptochaete arbuscula* (Sm.) Reichardt.	H	Streimann 51408	AM990353; (*rps4* = AY908330*)	FM160955	FM161249	FM161087
RJB10	*Cratoneuropsis relaxa* (Hook. f. & Wilson) M. Fleisch.	MA	MA-Musci 15238	AM990354; (*rps4* = AY908244*; *trnLF* = AY429494)	FM160956	FM161250	FM161089
B423	*Cryptoleptodon longisetus* (Mont.) Enroth	H	H3038483	AM990356; (*rps4* = AY908260*)	FM160957	FM161252	FM161091
B229	*Dacryophyllum falcifolium* Ireland	H	Shevock 27466	AM990357	FM160960	FM161253	FM161094
B115	*Dendroalsia abietina* (Hook.) E. Britton	B	B 230948	AM990358; (*rps4* = AY908185*)	FM160961	FM161254	FM161095
B224	*Dixonia thamnioides* (Broth. & Dixon) Horik. & Ando	NYBG	Akiyama Th-12	AM990361; (*rps4* = AY907956*)	FM160962	FM161256	FM161097
SH146	*Dolichomitriopsis diversiformis* (Mitt.) Nog.	H, MHA	Nedoluzhko s.n.	AM990362; (*rps4* = AY908329*; *trnLF* = AF397777)	FM160963	FM161257	FM161098
B258	*Echinodium umbrosum* Mitt A.Jaeger	ASV	Streimann 49634	EU434010; (*rps4* = AY908269*)	FM160965	AY908680*	EU477602
SH34	*Eurhynchiastrum pulchellum* (Hedw.) Ignatov & Huttunen	H	Koponen & Huttunen 1321	AM990364; (*trnLF* = AY044069)	FM160966	FM161259	FM161101
B196	*Forsstroemia trichomitria* (Hedw.) Lindb.	VBr	Streimann & Pocs 65120A	AM990365	FM160968	FM161260	FM161103
B349	*Heterocladium dimorphum* (Brid.) Schimp.	H	H3212307	AM990376	FM160970	FM161271	FM161115
B350	*Heterocladium heteropterum* (Brid.) Schimp.	H	H3070903	AM990377	FM160971	FM161272	FM161116
B351	*Heterocladium macounii* Best	H	H3212418	AM990378	FM160972	FM161273	FM161117
B352	*Heterocladium procurrens* (Mitt.) A. Jaeger	H	H3212289	AM990379	FM160973	FM161274	FM161118
B310	*Himantocladium plumula* (Nees) M. Fleisch.	H	Tan et al. 92-232	AM990381	FM160976	FM161276	FM161122
B422	*Homalia glabella* (Hedw.) Schimp.	H	Townsend 93/291	AM990382	FM160977	FM161277	FM161123
B111	*Homalia lusitanica* Schimp.	B	B 275202	AM990383	FM160978	FM161278	FM161124
B419	*Homalia pennatula* (Mitt. ex Dixon) S. He & Enroth	Enroth	ASV & Verwimp 16230	AM990384	FM160979	FM161279	FM161125
B218	*Homalia trichomanoides* (Hedw.) Schimp.	Olsson	Olsson 105	AM990385; (*rps4* = AY908276*)	FM160980	FM161280	FM161126
B305	*Homalia webbiana* (Mont.) Schimp.	S	S B42737	AM990386	FM160981	FM161281	FM161128
B474	*Homalia webbiana* (Mont.) Schimp.	H	Müller S116	AM990387	FM160982	FM161282	FM161127
B146	*Homaliadelphus targionianus* (Mitt.) Dixon & P. de la Varde	H	Koponen et al. 55009	AM990388; (*rps4* = AY908552*)	FM160983	FM161283	FM161129
B110	*Homaliodendron exiguum* (Bosch & Sande Lac.)	B	B 263509	AM990389	FM160984	FM161284	FM161130

	M. Fleisch						
SH249	*Homaliodendron microdendron* (Mont.) M. Fleisch.		Redfearn, Jr. 35901	AM990390	FM160987	FM161285	FM161133
B425	*Homaliodendron piniforme* (Brid.) Enroth	H	H3071962	AM990391	FM160988	FM161286	FM161134
SH35	*Homalothecium sericeum* (Hedw.) Schimp.	H	Koponen & Huttunen 1322	AM990392; (*rps4* = DQ294319*; *trnLF*= AF397805)	FM160990	FM161287	FM161136
B396	*Hookeria lucens* (Hedw.) Sm.	VB	Buchbender 466	AM990394; (*rps4* = AY306930*)	FM160991	FM161289	FM161138
B299	*Hypnum cupressiforme* Hedw.	Quandt	Quandt FSA 301	AM990398	FM160993	FM161292	FM161143
B205	*Isodrepanium lentulum* (Wilson) E. Britton	NYBG	Allen 8859	AM990399; (*rps4* = AY907964*)	FM160994	FM161293	FM161144
T51	*Isothecium myosuroides* Brid.	S	Sérgio 060604	AM990400; (*rps4* = AY306933*)	FM160995	FM161294	DQ294922
SH103	*Lembophyllum clandestinum* (H. f & W.) Lindb. in Par.	H	Vitt 29644	AM990401; (*trnLF* AF397823)	FM160996	FM161295	FM161145
B131	*Leptodon smithii* (Hedw.) F. Weber & D. Mohr	B	De Sloover 44851	AM990403; (*rps4* = AY908261*)	FM160997	FM161297	FM161147
B456	*Leucodon sciuroides* (Hedw.) Schwägr.	VB	Buchbender 293	AM990405; (*rps4* = AY908186*)	FM160998	AY908716*	FM161149
B341	*Limbella tricostata* (Sull.) Müll. Hal. ex E.B. Bartram	H	H3089826	AM990406; (*rps4* = AY908572*)	FM160999	FM161299	FM161150
SH22	*Meteorium polytrichum* Dozy & Molk.	H	Streimann 57477	(*rps4* = AM990410; *trnLF* = AY044073)	FM161001	-	FM161153
Mp6	*Meteorium polytrichum* Dozy & Molk.	VB	Streimann 64800	(*trnT* = AM990409)	FM161000	FM161302	-
B236	*Miyabea fruticella* (Mitt.) Broth.	H	Koponen 45838	AM990411	-	FM161303	FM161154
B413	*Miyabea rotundifolia* Cardot	H	Tan 93-771	AM990412	FM161002	FM161304	FM161155
B193	*Neckera complanata* (Hedw.) Huebener	VB	Buchbender 204	AM990413	FM161005	FM161305	FM161158
B308	*Neckera pennata* Hedw.	H	H3097380	-	-	FM161306	-
B347	*Neckera pennata* Hedw.	H	H3203794	AM990414; (*rps4* = AY908265*)	FM161016	-	FM161169
B307	*Neckera remota* Bruch & Schimp. ex Müll. Hal.	S	S B29895	AM990415	FM161018	FM161307	FM161171
SH301	*Neckera urnigera* Müll. Hal.	S	B15194	AM990416	FM161021	FM161308	FM161174
B247	*Neckeropsis calcicola* Nog.	H	Enroth 64632	AM990417	FM161025	FM161309	FM161178
B138	*Neckeropsis calcutensis* (M. Fleisch.) Enroth	H	H 3212832	AM990418	FM161026	FM161310	FM161179
B313	*Neckeropsis nitidula* (Mitt.) M. Fleisch.	S	S B105713	AM990419	FM161030	FM161311	FM161183
B152	*Orthostichella rigida* (Müll. Hal.) B.H.Allen & Magill	Quandt	Quandt A10001	AM990422; (*trnLF* = AF508315)	FM161032	FM161312	FM161185
B476	*Pendulothecium punctatum* (Hook. f. & Wilson) Enroth & S. He	S	Streimann 53845	AM990421	FM161033	FM161314	FM161187
B242	*Pinnatella alopecuroides* (Mitt.) M. Fleisch.	Enroth	ASV 16824	AM990423	FM161034	FM161315	FM161188
B150	*Pinnatella minuta* (Mitt.) Broth.	H	Rikkinen et al. 32	AM990424	FM161040	FM161316	FM161194
B309	*Pinnatella mucronata* (Bosch & Sande Lac.) M. Fleisch.	S	Hedenäs MY92-22	AM990425	FM161041	FM161317	FM161195
B294	*Porotrichodendron superbum* (Taylor) Broth.	H	H3121100	AM990427	FM161043	FM161319	FM161198
B098	*Porotrichum bigelovii* (Sull.) Kindb.	B	B230549		-	FM161320	-
B244	*Porotrichum bigelovii* (Sull.) Kindb.	H	Shevock & Kellman 27467	AM990428	FM161045		FM161200
B117	*Porotrichum frahmii* (Enroth) Enroth	B	B 255332	AM990429	FM161046	FM161321	FM161201
B369	*Porotrichum fruticosum* (Mitt.) A. Jaeger	H	Shevock 28269	AM990430	FM161047	FM161322	FM161202
B164	*Porotrichum substriatum* (Hampe) Mitt.	NYBG	Buck 32970	AM990431	FM161048	FM161323	FM161204
B114	*Prionodon densus* (Sw. ex Hedw.) Müll. Hal.	B	B 282645	AM990432; (*rps4* = AF143076*)	FM161049	AY908718*	FM161205
Ri29	*Rigodium implexum* Kunze ex Schwägr.	Quandt	Quandt A 10008	AM990436; (*trnLF* AY429499)	FM161050	FM161327	FM161209
B559	*Rigodium pseudothuidium* Dusén	NYBG	NYBG 00892248	(*rps4* = AM990437)	-	FM161328	FM161210
Rp47	*Rigodium pseudothuidium* Dusén	H3134254	Barrandegury 345	AM990438; (*trnLF* AF543547)	FM161051	-	-

B254	*Straminergon stramineum* (Dicks. ex Brid.) Hedenäs	DRD	DR028753	AM990351	FM161053	FM161330	FM161213
B573	*Symphyodon imbricatifolius* (Mitt.) S.P. Churchill	H	H3202267	AM990440; (*rps4* = AY306999*)	FM161054	AY452387*	FM161214
B149	*Taiwanobryum speciosum* Nog.	H	Enroth 64877	AM990442; (*rps4* = AY908272*)	FM161055	FM161332	FM161216
B238	*Thamnobryum alopecurum* (Hedw.) Nieuwl. ex Gangulee	VB	Buchbender s.n.	AM990444; (*rps4* = AF023834*)	FM161056	FM161334	FM161218
SH300	*Thamnobryum maderense* (Kindb.) Hedenäs	S	B44108	AM990445	FM161061	FM161335	FM161223
B148	*Thamnobryum subserratum* (Hook. ex Harv.) Nog. & Z. Iwats.	H	Enroth 64595	AM990446	FM161067	FM161336	FM161230
B429	*Thamnobryum tumidicaule* (K.A. Wagner) F.D. Bowers	H	H3141850	AM990447	FM161068	FM161337	FM161231
SH25	*Toloxis imponderosa* (Tayl) Buck	H	Norris 90418	AM990448; (*rps4* = AY908289*; *trnLF* = AY044067)	FM161069	AY908732*	FM161232
SH431	*Tripterocladium leucocladulum* (Müll. Hal.) A. Jaeger	H	H3150195	AM990450; (*rps4* = AY908334*; *trnLF* = AF509864)	FM161071	FM161339	FM161235
DQ	*Weymouthia mollis* (Hedw.) Broth.	CHR, Quandt	99-Mo2	AM990452; (*rps4* = AY307014*)	FM161072	-	FM161237

DNA isolation, PCR amplification and sequencing

DNA was extracted using the DNeasy® Plant Mini Kit from Qiagen (Qiagen GmbH, Germany) following the manufacturer's protocol. Cleaning and grinding of plants prior to extraction followed Olsson et al. (chapter 1). Amplification of the ITS1-5.8S-ITS2 as well as the *trnS-trnF* region followed Olsson et al. (chapter 1) and Hernández–Maqueda et al. (2008), respectively. Whereas the protocols for *rpl16* were obtained from Olsson et al. (unpubl.), *nad5* was amplified using the strategy and primers of Buchbender et al. (unpubl.). Gel cleaned PCR products were sequenced by Macrogen Inc., South Korea (www.macrogen.com). Sequences were edited manually with PhyDE® v0.995 (Müller et al. 2005) and primer sequences eliminated. All sequences are deposited in EMBL, accession numbers are listed in Table 1.

Alignment, sequence analyses and phylogenetic reconstructions

Alignment of sequence data was done manually with PhyDE® v0.995 using the alignments of chapter 1 as scaffold and applying the alignment and hotspot definition approach described in Olsson et al. (chapter 1). The known inversion in front of *trnF* was positionally separated in the alignment (Quandt & Stech 2004), and included in the phylogenetic analyses as reverse complement in order to gain information from substitutions as discussed in Quandt et al. (2003a). Alignments are provided on an appendix cd. A ready-to-use nexus file containing the sequence alignment with an automatically generated binary indel matrix appended based on the simple indel coding approach of Simmons and Ochoterena (2000) was generated using the computer programme SeqState (Müller 2005). Command files for using the parsimony ratchet (Nixon 1999) were generated using the programme PRAP2 (Müller 2007) applying the default settings, and executed in PAUP 4.0b10 (Swofford 2002). Heuristic bootstrap searches under parsimony were performed with 1000 replicates.

Bayesian analyses were performed with MrBayes v3.1.2, applying the GTR+Γ+I model for the sequence data and the restriction site model for the binary indel partition. To allow for possibly deviating substitution models for the different regions, the data set was divided into five partitions (partition 1: *trnS-trnF* (plastid); partition 2: *rpl16* (plastid)*;* partition3: ITS1 & 2 (nuclear); partition 4: *nad5* (mitochondrial); partition 5: indels). The a priori probabilities supplied were those specified in the default settings of the programme. 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. (Huelsenbeck et al. 2001; 2002a). Ten runs with four chains (1×10^6 generations each) were run simultaneously, with the temperature of the single heated chain set to 0.5. Chains were sampled every 10 generations and the respective trees written to a tree file. Calculations of the consensus tree and of the posterior probability of clades were performed based upon the trees sampled after the chains converged (at generation 25,000). Consensus topologies and support values from the different methodological approaches were compiled and drawn using TreeGraph (Müller & Müller 2004).

Morphological data and ancestral state reconstruction

The morphological information for characters that are often discussed in connection with taxonomical delimitation of the Neckeraceae was compiled by the authors. The scored data are based both on the specimens used for molecular sampling and on additional material, since the specimens in the molecular study did not always include all characters (e.g., sporophytes). Moreover, morphological scoring based on several vouchers better reflects the infraspecific variation. When no herbarium material was available (in S or H), literature sources were used. Specimen information for taxa not included in the molecular study is presented in Appendix 2. Appendix 3 lists the characters that were scored as well as the resulting data matrix. Capsule orientation is described in relation to the axis of the seta, according to the terminology recently introduced by Hedenäs (2006). The longitudinal axis of an *orthotropous* capsule is parallel with the seta and its mouth points in the distal direction, *homotropous* is between orthotropous and orthogonal, the axis of an *orthogonal* capsule is perpendicular to that of the seta, *reclinate* is between orthogonal and antitropous, and the axis of an *antitropous* capsule is parallel with the seta and its mouth points towards the seta base.

The evolutionary history of each morphological character was reconstructed by determining the posterior probability with which each character state occurred in the ancestral species. We used the Markov chain model implemented in BayesTraits to estimate the posterior probability distributions of ancestral states at every node of the tree (Pagel & Meade 2004). The method takes into account the effect of phylogenetic uncertainty by using a Bayesian posterior tree sample in estimating the ancestral states.

With a perl script (written by Kai Müller, available from www.bioinf.web) 500 trees were randomly sampled among 1,000,010 trees from MrBayes analyses. Outgroups were excluded from ancestral state reconstructions. Trees were pruned with PAUP 4.0b10 (Swofford 2002) leaving the 45 ingroup taxa for the analyses (see Fig. 2-4). In BayesTraits the rate at which parameters get changed ('ratedev'), was set at the beginning of each run so that the acceptance rate of the proposed changes globally ranges between 20 and 40 %. A uniform distribution with a range of 0-100 was used as prior. Rate coefficients and ancestral character states were sampled every 500 generations to ensure independence from successive samplings. The chain was run for 5,050,000 generations. In order to circumvent issues associated with the fact that not all of the trees necessarily contain the internal nodes of interest, reconstructions were performed using a 'most recent common ancestor' approach that identifies, for each tree, the most recent common ancestor to a group of species and reconstructs the state at the node, then combines this information across trees (Pagel & Meade 2004).

3.4 Results

Phylogenetic analyses

The original alignment contained 6847 characters (3384 plastid, 2248 nuclear and 1215 mitochondrial). 27 hotspots with poly-mononucleotid repeats were recognized following chapter 1 and excluded from the analyses (Table 3). The resulting data matrix (with the inversion included as reverse complement) used for the phylogenetic analyses contained 6417 nucleotide characters, of which 5138 (80 %) were constant, 1279 (20 %) were variable and 664 (10 %) parsimony informative. After coding and including the 796 indels (209 plastid, 569 nuclear and 18 mitochondrial) the resulting matrix contained 7213 characters (5141 constant (71%), 2072 (29%) variable, 944 (13 %) parsimony informative). The parsimony analysis including indel coding retained 4 most parsimonious trees (MPT, length 4355, CI= 0.549, RI= 0.638), while the analysis excluding indels retained 35 MPTs (length 3091, CI=0.517, RI=0.637). The strict consensus trees of the parsimony analyses were well but not totally resolved.

The MrBayes trees from both analyses (with and without indel coding) are well resolved and highly supported with no incongruence. No supported topological conflicts between the strict consensus trees from the parsimony analyses and the majority rule trees from Bayesian analyses were observed. Therefore, only the MrBayes tree based on the analyses including indel coding is illustrated in Figure 1, complemented with information from the other analyses. Throughout the text posterior probabilities (PP) are listed first followed by the bootstrap support (BS) values. Values resulting from analyses with the SIC-matrix included precede the values from analyses without an indel coding approach. Thus support values from the different analyses will be referred to in the text following this scheme (PPsic /PP, BSsic / BS).

Table 3. Location, i.e. absolute position in the combined data set and corresponding region of mutational hotspots (H), including the observed inversion (I). § Location of the inversion is given with respect to the corrected and analysed matrix (i.e. the inversion is included as reverse complement).

No.	Position	Region	No.	Position	Region
H1	743-745	*rps4-trnT* IGS	H14	2920-2923	*rpl16* intron
H2	870-877	*rps4-trnT* IGS	H15	2941-2945	*rpl16* intron
H3	914-915	*rps4-trnT* IGS	H16	3311-3116	*rpl16* intron
H4	933-939	*rps4-trnT* IGS	H17	3325-3329	*rpl16* intron
H5	985-1001	*rps4-trnT* IGS	H18	3397-3400	*rpl16* intron
H6	1029-1031	*rps4-trnT* IGS	H19	3501-3506	ITS1
H7	1096-1098	*rps4-trnT* IGS	H20	3569-3573	ITS1
H8	1176-1179	*rps4-trnT* IGS	H21	4025-4031	ITS1
H9	1469-1481	*trnT-trnL* IGS	H22	4299-4307	ITS1
H10	1507-1510	*trnT-trnL* IGS	H23	4436-4439	ITS1
H11	1651-1656	*trnT-trnL* IGS	H24	4460-4462	ITS1
H12	1692-1710	*trnT-trnL* IGS	H25	4483-4487	ITS1
I1 §	2255-2261	*trnL-trnF IGS*	H26	4659-4664	ITS2
H13	2534-2541	*rpl16* intron	H27	4871-5131	ITS2

The Neckeraceae in its current circumscription is resolved as polyphyletic. Some taxa are actually resolved among outgroup taxa, such as *Baldwiniella kealeensis* and *Homalia pennatula*. The latter retains a close relation to *Symphyodon imbricatifolius*, with maximal support. The ingroup contains the Lembophyllaceae, the polyphyletically resolved *Heterocladium*, the Miyabeaceae, the polyphyletically resolved Neckeraceae as well as two representatives of the Hypnaceae. Among the ingroup taxa *Isodrepanium lentulum* branches off first, and does not belong to the Neckeraceae. The position of *Hypnum cupressiforme*, grouping together with the Miyabeaceae has only weak

support, while the Miyabeaceae receives full support regardless of the analysis method used. Some of the species currently placed in the Neckeraceae are forming a separate well supported cluster outside the Lembophyllaceae – Neckeraceae clade. This clade known as the OPP-clade (Quandt et al. in press) contains members of *Orthostichella*, *Porotrichum* plus *Dixonia thamnioides*, and *Homaliodendron piniforme*. The position of *Dixonia* has only moderate support (PP 90 / 93, BS 55 / -) but the rest of the clade gets maximal support. *Homalia webbiana*, like *Dacryophyllum falcifolium*, are closely related to this clade but branching off separately. The genus *Heterocladium* is resolved as polyphyletic, forming two pairs of species: *H. dimorphum* and *H. procurrens* cluster tightly together with maximal support, as well as *H. heteropterum* and *H. macounii*. The latter clade seems to be more closely related to the Lembophyllaceae than the first one, with good support for its position from the analysis including indel coding (PP 100 / 54, BS 98 / 75). The monophyly of the Lembophyllaceae is fully supported with all analysis methods, and the Lembophyllaceae being the sister group of the Neckeraceae reaches high statistical support, albeit only regarding Bayesian statistics. The Neckeraceae s. str. are divided into three distinct clades: clade A with *Neckera* as the main genus, clade B including *Thamnobryum* and its allies, and clade C with *Pinnatella* and *Neckeropsis* as the prominent genera. Some genera, e.g. *Neckera*, *Porotrichum,* and *Homalia* are clearly polyphyletic while others, such as *Pinnatella* and *Thamnobryum* form well supported clades including only a part of the species, thus not being monophyletic.

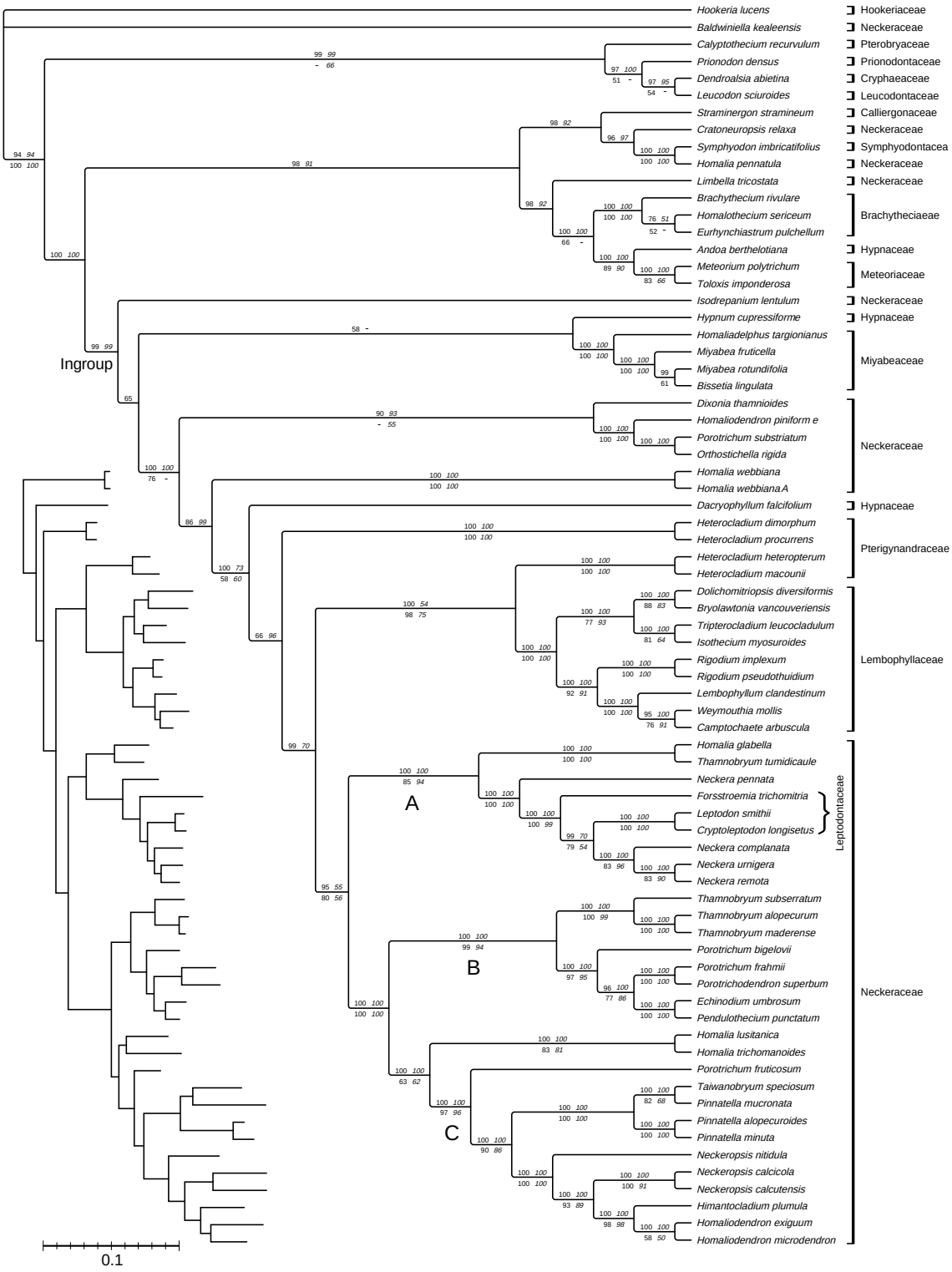

Figure 1. Majority consensus of trees sampled after stationarity in the Bayesian analysis of the matrix including indels. Values along the branches indicate posterior probabilities (above the branches) and bootstrap support values (below). The first value corresponds to the analyses with the indel coding matrix included in the analyses.

Ancestral state reconstructions of morphological characters

Ancestral state reconstructions revealed that the ancestor of the Lembophyllaceae – Neckeraceae clade (node I) had symmetric leaves (with posterior probability of 0.41 ± 0.16; Fig. 2), costa absent to weak (0.56 ± 0.18; Fig. 3), perfect peristome (0.83 ± 0.12; Fig. 4), a seta that was more that 9 mm long (0.69 ± 0.18) and orthogonal or widely homotropous (0.44 ± 0.16). The ancestor of all Neckeraceae species (at node II) differed from it by having clearly asymmetric leaves (with posterior probability of 0.56 ± 0.14), orthotropous to homotropous capsule (0.59 ± 0.16) and, with almost the same posterior probability, strong (0.43 ±0.11) or absent to weak (0.35 ± 0.13) costa. Within Neckeraceae the asymmetric leaves, strong costa, and perfect peristome are lost four times in different lineages. A short seta has evolved twice (in the clades A and C), and an orthotropous to homotropous capsule has been lost twice (in clade B as well as in the *Homalia lusitanica* - *H. trichomanoides* clade).

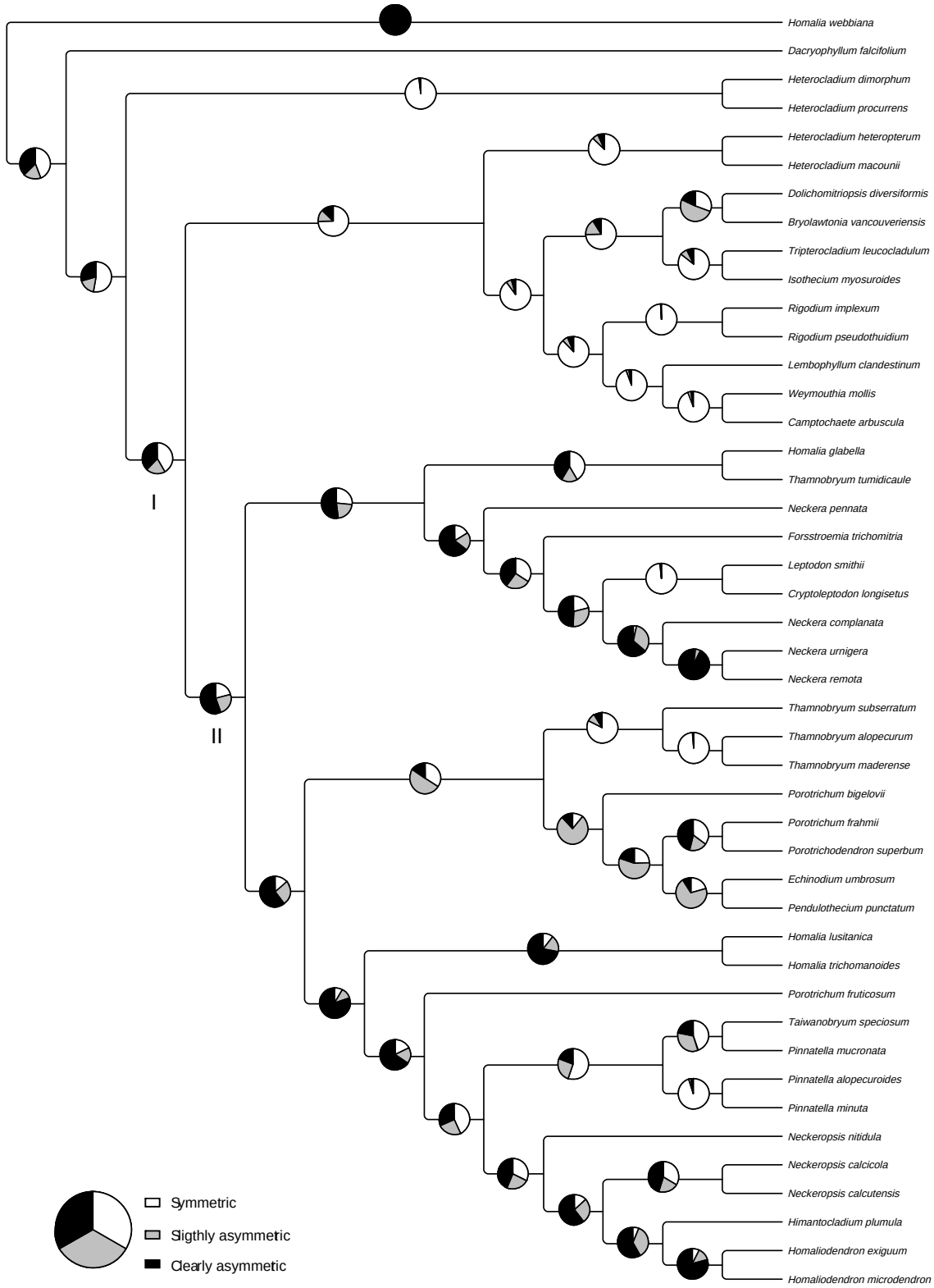

Figure 2. Ancestral character state reconstruction for leaf asymmetry among the ingroup. The circles plotted on the inferred Bayesian topology represent three states of leaf asymmetry (symmetric (white), slightly asymmetric (grey), clearly asymmetric (black)).

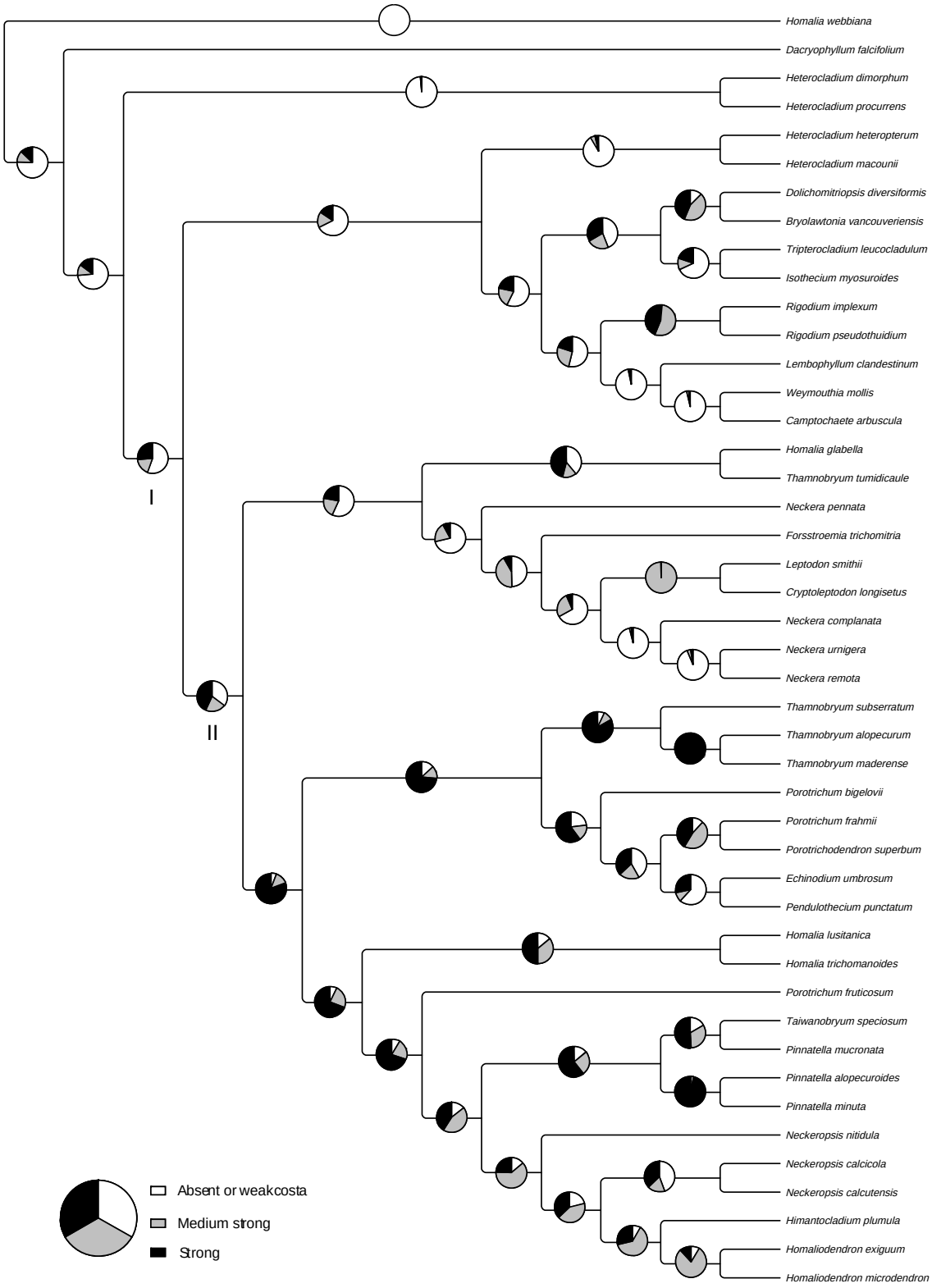

Figure 3. Ancestral character state reconstruction for strength of the leaf costa among the ingroup. The circles plotted on the inferred Bayesian topology represent three states of leaf asymmetry (absent or weak costa (white), medium strong costa (grey), strong costa (black)).

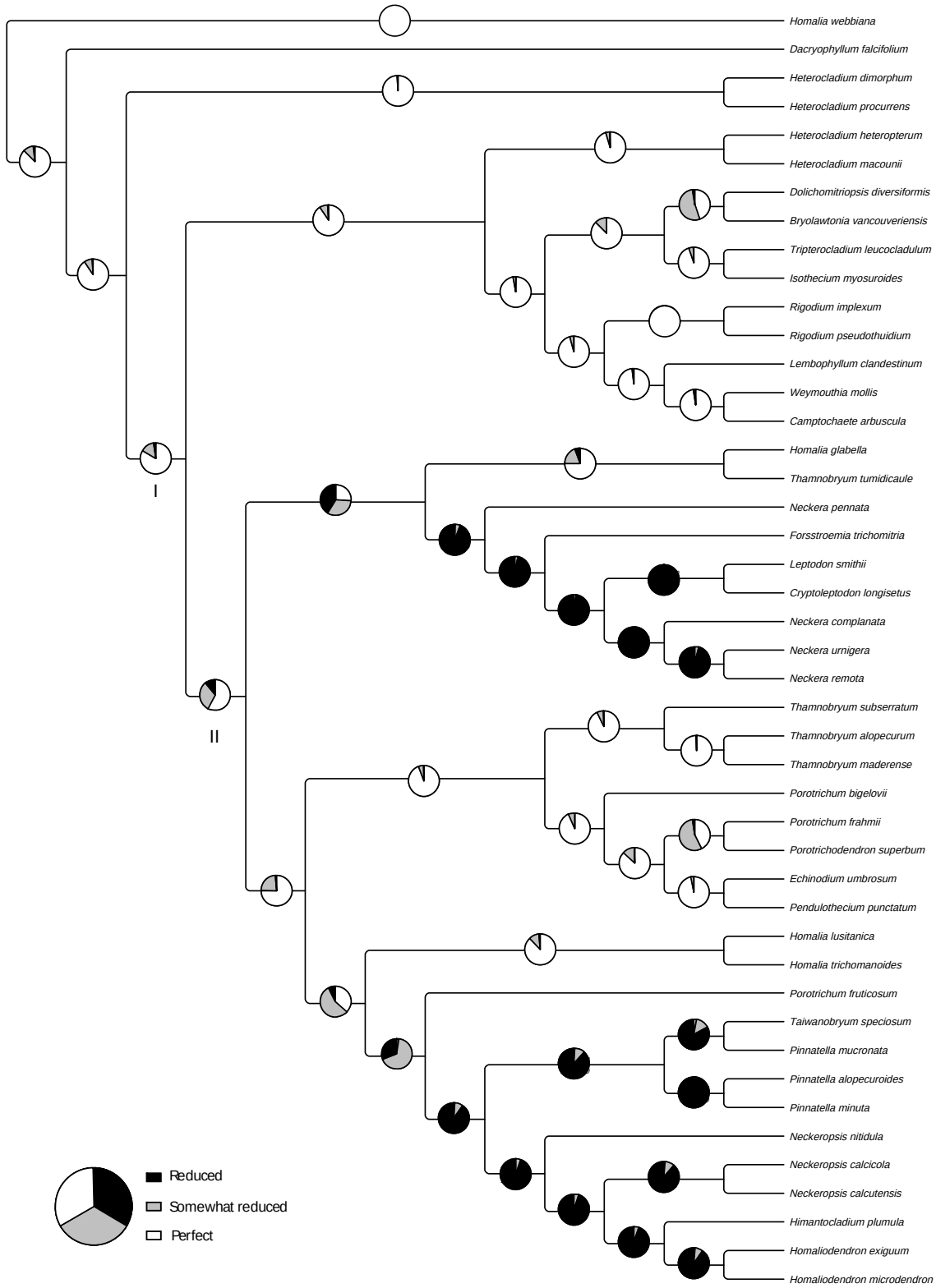

Figure 4. Ancestral character state reconstruction for peristome reduction among the ingroup. The circles plotted on the inferred Bayesian topology represent three states of leaf asymmetry aracter states (reduced (black), somewhat perfect (grey), perfect (white)).

3.5 Discussion

Phylogenetic position of the Neckeraceae

Our study supports a close relationship between the Neckeraceae and the Lembophyllaceae, as suggested by, e.g., Quandt et al. (2000; in press) and Stech et al. (2008). Already Brotherus (1925) placed the Neckeraceae close to the Lembophyllaceae in the order Isobryales (= Leucodontales) (see also Robinson 1975). Recent molecular analyses have not challenged this view, even if the monophyly of the Neckeraceae has been shown to be doubtful in its current circumscription (Buck et al. 2000b; Tsubota et al. 2002; Olsson et al. chapter 1). According to our results in Fig. 1 (compare chapter 1), the Neckeraceae include the species that have by some authors been previously placed in the Thamnobryaceae (Buck & Vitt 1986) and in the Leptodontaceae (Schimper 1856; Goffinet & Buck 2004). Thus the division of the Neckeraceae is rejected. The exact position of the Neckeraceae/ Lembophyllaceae clade among the pleurocarpous mosses still remains to be established, but the merging of the data into a broad study that is in preparation (cf. Buchbender et al. 2006) and includes representatives covering all pleurocarpous mosses will give further insight into this question.

The three clades that are resolved in the current analyses do not correspond to the subfamilies that Brotherus (1925) proposed. His subfamilies Leptodontoideae, Neckeroideae and Thamnioideae are shown to be polyphyletic since the clades in our analyses are composed of taxa belonging into at least two different subfamilies in the system of Brotherus (1925).

Trends in morphological evolution and phytogeographic patterns

Enroth (1994b) presented some hypotheses of primitive vs. advanced character states within the Neckeraceae. He postulated that reduction was the "key word" in the evolution, and that asymmetric leaves with a weak costa and fine dentation, irregular branching pattern, as well as a short seta with reduced peristome, would be advanced character states. Our results show that asymmetric leaves are ancestral in the Neckeraceae, but like Enroth (1994b) expected they support the hypothesis that the ancestor of the Neckeraceae had a strong costa, long seta, and perfect peristome. A notable observation is that for all these characters reduced states have evolved

independently several times within the family. In each of the three main clades the same trends towards more specialized structures can be observed in the sporophyte evolution: from antitropous, orthogonal or homotropous capsules to orthotropous; from long setae to short; and from perfect peristomes to variably reduced. These trends are strongest in clade A and weakest in clade B. One plausible reason for such morphological character changes may be a shift to epiphytic habitats that were repeatedly and independently conquered in the three different clades within the Neckeraceae. In each clade the basal taxa favour rocks or soil as substrates, while the more advanced ones are mainly epiphytic. The clades are also geographically differentiated. Clade A includes mainly non-Asiatic members, like clade B, where the truly tropical taxa are restricted to South-America, while clade C includes Asiatic and tropical members (except the basal *Homalia* and *Pinnatella minuta*, which occurs in Africa and S America).

In many other pleurocarpous moss families epiphytism is correlated with similar combinations of morphological character states (Hedenäs 2001; Huttunen et al. 2004). Especially structures of the sporophyte generation appear prone to evolve adaptations to new environmental conditions (Hedenäs 2001, 2002; Vanderpoorten et al. 2002b; Huttunen et al. 2004). It is clear that several morphological character states were independently acquired in the different Neckeraceae lineages, but further investigation is needed to unravel the evolutionary processes behind this. Factors that need to be studied further include both the genetic regulation of morphological characters and the evolutionary processes affecting morphology, including the role of habitat shifts in furthering character state changes. Although the primary factors promoting sporophytic reductions found in epiphytes are likely to affect spore dispersal, e.g., wind and humidity (Hedenäs 2001), reduced reproductive costs involved in producing reduced sporophytes also need to be considered in this context. It was only recently shown experimentally that sporophyte production incurs a cost in terms of reduced future gametophytic growth also in bryophytes (Ehrlén et al. 2000), and one may thus speculate that small and simple sporophytes "cost less" than large and elaborate ones to produce. If small sporophytes incur smaller reproductive costs than large ones they could potentially be advantageous in habitats where resources are limited, for example in epiphytic ones where low nutrient input or leaching may be problematic (cf. Smith 1982; Nadkarni 1984).

Morphological delimitation of the Neckeraceae

Our morphological studies revealed some new morphological characters that aid in family level delimitation especially between the Neckeraceae and the Lembophyllaceae. Even when the leaf cells are generally elongate, all members of the Neckeraceae have at least 1-2 marginal cell rows (Fig. 5) that are at least partly composed of quadrate to rectangular cells shorter than the corresponding inner laminal cells. In the Lembophyllaceae such a clearly differentiated leaf margin is not commonly present. *Baldwiniella kealeensis* and *Isodrepanium lentulum*, which according to our analyses do not belong in the Neckeraceae, lack such marginal cells. Furthermore, these two species share a bipolarity of character states in the two generations (cf. Enroth 1994): both have a distinctly advanced, "*Neckera*-like" gametophyte combined with a primitive type of sporophyte (long seta, homotropous capsules, cross-striolate lower exostome outsides and high basal membranes). Clearly, they have been placed in the Neckeraceae due to a superficial gametophytic resemblance to that family – fairly large, glossy plants with undulate and asymmetric leaves and a short, weak costa.

Another character state typical for the Neckeraceae seems to be a consistent lack of dwarf males. Such males have been found in most of the Lembophyllaceae genera (Tangney 2006; Buchbender 2009) and they have also been found in *Homaliadelphus* and *Bissetia*, which have been placed in the Neckeraceae before but which actually form a distinct family also including the genus *Miyabea* (chapter 1).

The genera included in the Neckeraceae in this analysis based on molecular data are somewhat different from those in the more traditional classifications of the Neckeraceae (Brotherus 1925; Enroth 1994b). In addition, there need to be some changes in the delimitation and contents of some genera. Below is a commentary on the genera that were earlier included in the Neckeraceae by some authors, but which are excluded from it in the present study.

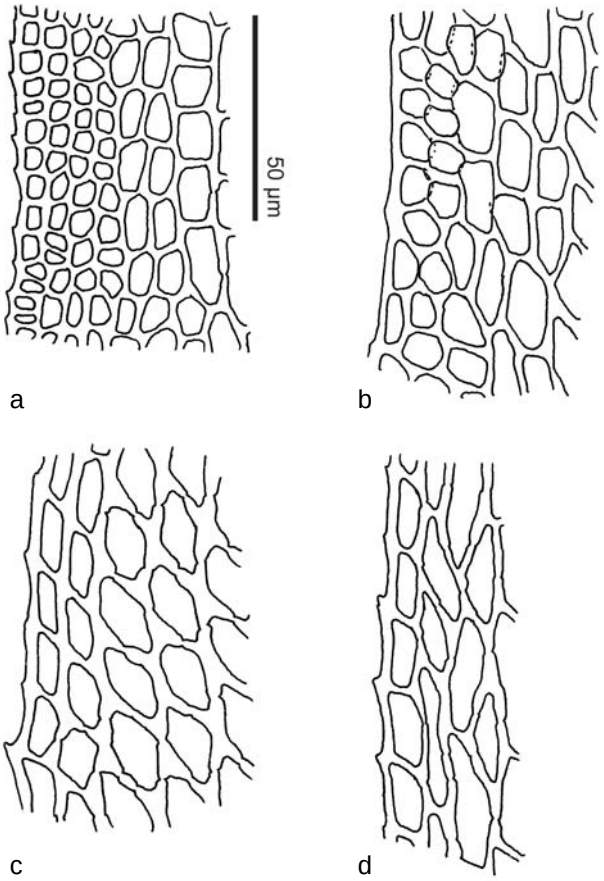

Figure 5. Variation in marginal leaf cells in the Neckeraceae. a. *Pinnatella alopecuroides* (redrawn from Enroth 1994c, fig 8g). b. *Curvicladium kurzii* (redrawn from Enroth 1993c, fig. 1d). c. *Neckera neckeroides*(redrawn from Enroth & Tan 1993, fig. 1d). d. *Neckera serrulatifolia* (redrawn from Enroth & Ji 2007, fig 2f)

3.6 Taxonomic changes

The families Thamnobryaceae and Leptodontaceae become synonyms of the Neckeraceae. Furthermore, several taxa are excluded from the Neckeraceae.

Baldwiniella kealeensis is an endemic of the Hawaii Islands. The exact relationships of the monospecific *Baldwiniella* need further elaboration, but it is clearly not at all closely related to the Neckeraceae.

Bryolawtonia vancouveriensis is another monospecific genus, from the California-Oregon district, and previously known as *Porotrichum vancouveriensis* and *Bestia vancouveriensis* (see Norris & Enroth 1990). It belongs in the Lembophyllaceae where it fits well together with e.g. *Isothecium*.

Homalia pennatula was previously placed in the genus *Symphyodon*, but He and Enroth (1995) and He (1997) treated it in *Homalia*. Their decision was based on overall gametophyte similarity to other *Homalia* species (leaf shape, irregularly serrulate upper leaf margins). However, the sporophytes are unknown and the sequence information as well as several morphological characters (variable costae and linear, projecting median leaf cells), support a placement in *Symphyodon*.

Homalia webbiana (see He 1997) and ***Dacryophyllum falcifolium*** (see Ireland 2004) as well as the genus *Heterocladium* do not belong in the Neckeraceae. Their accurate position among the pleurocarpous mosses remains to be solved in further studies. The genus *Heterocladium* is polyphyletic, since two of the species (*H. heteropterum* and *H. macounii*) nest within or as a sistergroup to the Lembophyllaceae while the other two (*H. dimorphum* and *H. procurrens*) do not.

Homaliadelphus and ***Bissetia*** appear together with *Miyabea* in a clade having strong support from both morphological and sequence data, supporting the results from Olsson et al. (chapter 1).

The clade known as **the OPP clade** (Quandt et al. in press), where *Homaliodendron piniforme* belongs to, together with *Dixonia thamnioides*, *Porotrichum substriatum* and *Orthostichella* (see also Allen & Magill 2007) is supported by this study but will not be discussed further, since it will be treated in the study by Buchbender (2009).

The monospecific genus ***Isodrepanium*** from Central and South America is apparently not belonging to the Neckeraceae and with the present taxon sampling it seems to represent a separate evolutionary lineage.

Limbella includes two species: *L. tricostata* from Hawaii and *L. fryei* from Oregon, excluding *Limbella bartlettii* (H.A. Crum & Steere) W.R. Buck (cf. chapter 1 for a more detailed discussion). They are big, stipitate, and morphologically rather similar to *Thamnobryum* s. str. species and *Handeliobryum*, growing on shady, often even wet places (sometimes in running water), on ground, stones and tree bases. The peristome is a perfect hypnoid one. *Limbella* was placed in the Thamnobryaceae by Ochyra (1987), who emphasized a close relationship with *Thamnobryum*. However, in our current analyses as well as in previous studies (chapter 1) it is located outside the Neckeraceae and close to the Brachytheciaceae and Meteoriaceae, where it seems to fit well according to morphology.

3.7 Acknowledgements

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3.8 Appendixes

Appendix 1. Voucher information of additional samples included in the morphological work.

Species	Voucher information
Bryolawtonia vancouveriensis	B117663 (S), Shevock 19202 (NY), Whittemore & Whittemore 4128 (NY)
Camptochaete arbuscula	B117121 (S), B108461 (S)
Cryptoleptodon longisetus	R.Düll s.n. (S), Hedenäs MA90-160 (S), Hedenäs MA91-285 (MADJ) (etc. in total 22 samples)
Dacryophyllum falcifolium	H3212880 (H); Ireland 2004
Dolichomitriopsis diversiformis	B118766 (S), B118767 (S), B118764 (S), B118765 (S)
Echinodium umbrosum	B121922 (S), B121923 (S), B121921 (S)
Forsstroemia trichomitria	B84146 (S), B84145 (S), B84147 (S)
Heterocladium heteropterum	B121947 (S), B121948 (S), B121949 (S), B96420 (S)
Heterocladium dimorphum	B121947 (S), B121948 (S), B121949 (S), B96420 (S)
Heterocladium macounii	B116655 (S), B116656 (S), B47516 (S), B116657 (S
Heterocladium procurens	B121953 (S), B121952 (S), B121950 (S), B121951 (S)
Himantocladium plumula	B21184 (S), Hedenäs MY92-346 (S), Hedenäs MY92-348 (S), Larsen et al. 3145 (S), F. Müller 52 (herb. Enroth); Enroth 1989
Homalia glabella	Schäfer-Verwimp & Verwimp 11102 (herb. Enroth), H3071327 (H); He 1997 (esp. Sporophyte)
Homalia lusitanica	H3071359 (H, with sporophyte), Baumgartner s.n. (S), Hedenäs MA90-173 (S) (etc. in total 41 samples); He 1997
Homalia trichomanoides	H3071483 (H), H3071487 (H, with sporophytes), Wallace s.n. Dec. 1924 (H, with sporophytes); He 1997
Homalia webbiana	Nóbrega et al. 5377 (MADJ), MA91-405 (S), Nóbrega 1612 (MADS), (etc. In total 21 samples)
Homaliodendron exiguum	H3071632 (H), H3071618 (H), Schäfer-Verwimp & Verwimp 16251 (herb. Enroth); Ninh 1984 (sporophytes)
Homaliodendron microdendron	Hedenäs MY92-432 (S), Chuang 6116; Schwarz 3919 (herb. Enroth); sporophyte Ninh 1984
Isothecium myosuroides	B81113 (S), 2 x Persson (S), Plantae exs. Canariens. 215 (S), Hedenäs MA91-382 (MADJ, S), Medelius (S), Thedenius (S), Löfvander (S), Hedenäs (S), B113596 (S), B113597 (S), B121995 (S)
Lembophyllum clandestinum	29388 (S), B116956 (S), Frahm 26-7(BONN)
Leptodon smithii	Hedenäs MA91-397 (S), Nóbrega 6031 (MADJ), Crundwell (S) (etc. In total 20 samples)
Neckera complanata	H-BR2896002 (H-BR, with spoprophytes), Pócs et al. 88300/H (herb. JE), Hedenäs MA91-182 (S) (etc. in total 13 specimen)
Neckera pennata	B16953 (S), B16954 (S), B16955 (S)
Neckera remota	Pócs, Mjatta & Linden 90021/AT (herb. JE), Pócs & Chuwa 88280/P (herb. JE), Pócs 6196 (herb. Enroth); De Sloover 1977
Neckera urnigera	H-BR2891006 (H-BR), H-BR2891003 (H-BR); Buck 1998
Neckeropsis calcicola	H- Enroth 64632; (1962); Touw 1962
Neckeropsis calcutensis	H3212832 (H), H3107863 (H); Enroth 1994
Neckeropsis nitidula	H3098207 (H), H3098202 (H); Touw 1962
Pendulothecium punctatum	(S) B108455, H53845, H-BR1992006; Enroth & He 1991
Pinnatella alopecuroides	H3107842 (H), H3107837 (H), Touw 8135 (L, with sporophytes); Enroth 1994
Pinnatella minuta	H3107998 (H), H3107985 (H), H3108009 (H, with sporophytes), H-BR3198009 (H-BR, with sporophytes); Enroth 1994
Pinnatella mucronata	Schumm & Schwarz 6396 (herb. Enroth), H3108017 (H), de Wilde & de Wilde Duyfjes 14492A (L, with sporophytes); Enroth 1994
Porotrichodendron superbum	Crosby & Crosby 5713 (S); Buck 1998
Porotrichum bigelovii	Specimen Fels 2. (S), Schofield 64050 (S); Lawton 1971
Porotrichum frahmii	Enroth 1996 (holotype cited there: Frahm et al. 671 (H), paratype: Frahm 92242 (H, DUIS)
Porotrichum fruticosum	Kanai, Murata & Togashi 236738 (NY, with sporophytes), Hooker s. n. (NY, with sporophytes), BM919198 (BM, with sporophytes), BM919205 (BM, with sporophytes)
Rigodium implexum	Dusén 798 (S), B116957 (S), Hollermayer 136 (S)
Rigodium pseudothuidium	Dusén 618 (S), B117484 (S), B117485 (S)
Taiwanobryum speciosum	H. Inoue: Bryophyta selecta exsiccata, no. 550 (S); Noguchi 1987-94.
Thamnobryum alopecurum	H3141550 (H, with sporophytes), H3141540 (H, with sporophytes), B42906 (S) (etc in total 45 specimen)
Thamnobryum maderense	Hedenäs MA91-114 (MADJ, S), Barros 2448 (MADS), 1880, R.Fritze", in herb. Kindberg (HOLO- and ISOTYPE), (S) (in total 42 specimen)
Thamnobryum subserratum	H3141840 (H, with sporophytes), H3141837 (H)
Thamnobryum tumidicaule	H3141847 (H); Buck 1998
Tripterocladium leucocladulum	B118062 (S), B118060 (S), B118061 (S)
Weymouthia mollis	B118277 (S), B118266 (S), B118268 (S), B118265 (S)

Appendix 2. Coding of morphological characters. – = non-applicable data; ? = missing data;
1. Costa absent to weak (indistinct or short and double) (0) medium (1) or strong (2).
2. Leaves symmetric (0) slightly asymmetric (1) clearly asymmetric (2).
3. Seta length (median values) <3.5 mm (0) 3.5-9 mm (1) or more than 9 mm (2).
4. Capsule orientation orthotropous to homotropous ("erect to inclined") (0) orthogonal ("horizontal") or widely homotropous (1) or reclinate to antitropous ("cernuous to pendulous") (2). Very variable form are treated as non-applicable as well as the variable orientation in *Heterocladium heteropterum*.
5. Peristome perfect (0) somewhat reduced (1) or reduced (2).

Character no.	1	2	3	4	5
Bryolawtonia vancouveriensis	2	1	1	1	0
Camptochaete arbuscula	0	0	1	1	0
Cryptoleptodon longisetus	2	0	1	0	0
Dacryophyllum falcifolium	0	2	?	?	?
Dolichomitriopsis diversiformis	1	0	1	0	1
Echinodium umbrosum	2	0	2	0	0
Forsstroemia trichomitria	1	0	0	0	2
Heterocladium heteropterum	0	0	1	-	0
Heterocladium dimorphum	0	0	2	1	0
Heterocladium macounii	0	0	2	1	0
Heterocladium procurens	0	0	2	1	0
Himantocladium plumula	2	1	0	0	2
Homalia glabella	0	2	2	0	0
Homalia lusitanica	2	2	2	1	0
Homalia trichomanoides	1	2	2	0	0
Homalia webbiana	0	2	2	0	0
Homaliodendron exiguum	1	2	0	0	2
Homaliodendron microdendron	1	2	0	0	2
Isothecium myosuroides	2	0	2	1	0
Lembophyllum clandestinum	0	0	2	1	0
Leptodon smithii	2	0	0	0	2
Neckera complanata	0	1	1	0	2
Neckera pennata	0	2	0	0	2
Neckera remota	0	2	0	0	2
Neckera urnigera	0	2	0	0	2
Neckeropsis calcicola	0	2	0	0	2
Neckeropsis calcutensis	2	0	0	0	2
Neckeropsis nitidula	1	0	0	0	2
Pendulothecium punctatum	0	1	2	2	0
Pinnatella alopecuroides	2	0	0	0	2
Pinnatella minuta	2	0	0	0	2
Pinnatella mucronata	1	1	1	0	2
Porotrichodendron robustum	1	0	-	-	-
Porotrichodendron superbum	1	0	2	0	1
Porotrichum bigelovii	2	1	2	-	0
Porotrichum frahmii	2	2	2	0	0
Porotrichum fruticosum	2	2	2	0	1
Rigodium implexum	2	0	2	2	0
Rigodium pseudothuidium	1	0	2	2	0
Taiwanobryum speciosum	2	0	2	0	2
Thamnobryum alopecurum	2	0	2	-	0
Thamnobryum maderense	2	0	2	1	0
Thamnobryum subserratum	2	0	2	-	0
Tripterocladium leucocladulum	0	0	2	0	0
Weymouthia mollis	0	0	0	0	0

CHAPTER 4

ON THE PARAPHYLY OF *NECKERA* AND *THAMNOBRYUM* (NECKERACEAE, BRYOPSIDA)

This study is submitted as: Olsson, S., Buchbender, V., Enroth, J., Hedenäs, L., Huttunen, S. & Quandt, D. On the paraphyly of *Neckera* and *Thamnobryum* (Neckeraceae, Bryopsida). Taxon.

4.1 Abstract

In chapter 3 the backbone structure of the pleurocarpous moss family Neckeraceae was resolved. The members of the family were shown to be distributed among three clades. This division is retained here in the analyses based on sequence data from the plastid *trnS-rps4-trnT-trnL-trnF* cluster and *rpl16* as well as nuclear ITS1 & 2. The detailed composition and phylogenetic relationships of two of the clades (the *Neckera* clade and the *Thamnobryum* clade) are discussed in detail. Consequently, the circumscriptions of *Homalia*, *Leptodon*, *Thamnobryum* and *Touwia* are amended, the new genera *Echinodiopsis* and *Thamnomalia,* both with two species, are formally described and several implied nomenclatural changes are proposed, including synonymisation of *Alsia* with *Neckera* and *Cryptoleptodon* with *Leptodon*.

4.2 Introduction

The pleurocarpous moss family Neckeraceae has a wide geographic distribution, comprising tropical and temperate genera. The members are mainly epiphytic or epilithic but there are some aquatic species as well. Most typically the Neckeraceae are large, glossy plants that have a creeping stolon bearing very small leaves and tufts of rhizoids, and more or less frondose (rarely dendroid) stems with or without distinct stipes. The leaf cells are almost always smooth, relatively short, and the marginal cells are typically quadrate to short-rectangular in few to several rows. The sporophyte features are variable but usually fairly consistent within genera. According to the current classification by Goffinet & Buck (2004) the family comprises 28 genera, although our previous and current analyses based on a wider taxon sampling suggest that several of these genera belong elsewhere (chapter 1, chapter 3).

The genera *Neckera* and *Thamnobryum* are two of the larger neckeraceous genera. In our previous study we resolved the backbone phylogeny and broad relationships of the Neckeraceae (chapter 3) and showed that after amendments the family becomes a monophyletic group consisting of three distinct clades. In this paper we will discuss in detail the composition, phylogenetic relationships and nomenclature of two of the clades: the *Neckera* clade and the *Thamnobryum* clade. Due to the

numerous taxonomic and nomenclatural changes needed, the rest of the Neckeraceae species are going to be treated in a separate forthcoming paper.

Previous major treatments of the genera in the Neckera and Thamnobryum clades

Alsia. *Alsia californica* (Hook. & Arn.) Sull. is a North American endemic, distributed along the west coast from Mexico to British Columbia. The monospecific *Alsia* was segregated from *Neckera* by Sullivant (1855). The family placements have varied; Lawton (1971) placed it in the Cryphaeaceae, Manuel (1975), Sharp et al. (1994) in the Leucodontaceae, and Buck & Goffinet (2000) in the Leptodontaceae. The stems are irregularly pinnately branched, bearing appressed, symmetric, somewhat concave leaves with an acute apex and a short, often forked costa. The leaf cells are smooth and incrassate with porose walls; the cells in the basal angles are smaller, often transverse and extending up the margins. There are abundant, leaf-like and often dissected paraphyllia on the stems and branches. The sexual condition is dioicous and the mostly erect, nearly symmetric capsules are exserted from large, differentiated and sheathing perichaetial leaves. These sporophyte characters as well as the reduced "neckeroid" double peristome do not markedly differ from many species of *Neckera.* The single character of *Alsia* "seriously" discordant in the Neckeraceae is the large groups of small, transverse cells in the leaf basal angles.

Anomodon. The genus *Anomodon* was originally segregated from *Neckera* (Hooker & Taylor 1818), including the species now known as *Anomodon viticulosus* (Hedw.) Hook & Taylor and *Antitrichia curtipendula* (Hedw.) Brid. An overview of the history of *Anomodon* was provided by Granzow-de la Cerda (1997), who placed the genus in the Anomodontaceae and also provided the latest revision and morphology-based phylogenetic analysis of the genus (Granzow-de la Cerda 1992, 1997). In the latter, *Haplohymenium* appeared as nested within *Anomodon*, and was therefore synonymised with the latter. Tsubota et al. (2002) got similar results based on plastid *rbc*L sequence data and furthermore, suggested *Anomodon giraldii* to have close affinities to the Neckeraceae. The genus, including *Haplohymenium*, has most species in eastern Asia. Its members are otherwise widespread in the Northern Hemisphere temperate to subtropical regions with scattered occurrences in the tropics and in the Southern Hemisphere.

Chileobryon *callicostelloides* (Thér.) Enroth, the sole species of the genus, was placed in the Anomodontaceae by Enroth (1992b). Previously it was included in the Neckeraceae as *Pinnatella callicostelloides* (Thér.) Broth., but the distinctly papillose laminal cells were thought to be a character justifying exclusion from the Neckeraceae. However, the general pattern of leaf areolation is not anomalous in the Neckeraceae. *Chileobryon callicostelloides* occurs only in the Juan Fernández Islands and mainland Chile (Enroth 1992b).

Cryptoleptodon was originally described by Renauld & Cardot (1900), but without any commentary on its relationships. The generitype of the genus is *Cryptoleptodon pluvinii* (Brid.) Broth., of which the previously used name *C. flexuosus* (Harv.) Renauld & Cardot is a synonym (Enroth 1992a). Several characters, however, suggest that it is close to *Leptodon*: the obtuse to rounded leaf apices, almost entire leaf margins, general leaf areolation, presence of paraphyllia (although more abundant in *Leptodon*), and the generally similar sporophytes, but with a spiculose peristome in *Leptodon* and a papillose one in *Cryptoleptodon* (cf. Buck 1980). *Cryptoleptodon* has been placed in the Neckeraceae subfam. Leptodontoideae (Brotherus 1925), in the Neckeraceae without any subfamilial division (Buck & Goffinet 2000), in the Leptodontaceae (e.g., Enroth 1992a) and also in the Pterobryaceae (e.g., Buck 1980). Four species have been placed in *Cryptoleptodon*: *C. pluvinii*, *C. rigidulus* (Mitt.) Broth, *C. acuminatus* M. Fleisch. and *C. longisetus* (Mont.) Enroth (Enroth 1992a). The two former species occur in the Himalayan region (Noguchi 1959; Gangulee 1976) and *C. pluvinii* disjunct in East Africa, while *C. longisetus* is known from the Canary Islands, Madeira and Cape Verde Islands (Düll 1980; Enroth 1992a; Hedenäs 1992). *Cryptoleptodon acuminatus* is an obscure taxon from "Ost-Indien" (for discussion, see below).

Curvicladium *kurzii* (Kindb.) Enroth was segregated from *Pinnatella* by Enroth (1993c). It was thought to resemble *Pinnatella* but it differs in the arcuate stems and branches, consistent absence of pseudoparaphyllia, multicellular apical teeth in the leaves, presence of post-fertilization growth of the perichaetial leaves, an 8-11 mm long, reddish brown and somewhat twisted seta, and frequent presence of reduced cilia in the endostome. The single species in its genus, it is known from the Himalayan region, Yunnan in China, and N Thailand.

Echinodium was revised by Churchill (1986), who recognized six extant species and an extinct one, the generitype being *E. madeirense* Jur. (=*E. spinosum* (Mitt.) Jur.). The distribution pattern was thought to be unique among mosses and probably relict: of the six extant species treated by Churchill (1986), four occur in Macaronesia and two in Australasia. The extinct (Pliocene) species is known from Europe. Churchill (1986), however, concluded that the monophyly of *Echinodium* was questionable. The chequered taxonomical history of *Echinodium* was reviewed by him, and he stated that in his view, "the only features that might be considered grouping characters" within *Echinodium* are the long-subulate leaves (with long-excurrent costae) that are plicate and have at least partially bistratose margins. The sporophyte is generally plesiomorphic, with long setae and inclined to pendulous capsules with an unreduced peristome. Most authors have until recently (e.g., Buck & Goffinet 2000) placed *Echinodium* in its own family, the Echinodiaceae, erected by Brotherus (1909). Also Churchill (1986) retained the family and suggested a relationship with the Thuidiaceae. Hedenäs (1992) suggested a relationship with *Isothecium* and *Pterigynandrum*. In the analysis by De Luna et al. (2000), however, *Echinodium umbrosum* (Mitt.) A. Jaeger was placed close to the Neckeraceae (*Neckera-Forsstroemia* clade), and in Tsubota et al. (2002) it was nested within the Neckeraceae. In a recent analysis Stech et al. (2008) show that *Echinodium* is indeed polyphyletic, and transfer *E. prolixum* (Mitt.) Broth. to the genus *Isothecium* in the Lembophyllaceae while *E. hispidum* (Hook. f. & Wilson) Reichardt and *E. umbrosum* belong to the Neckeraceae.

Forsstroemia was segregated from *Leptodon* by Lindberg (1863) to accommodate just one species, *F. trichomitria* (Hedw.) Lindb. In his monograph of *Forsstroemia*, Stark (1987) recognized ten species. Manuel (1974) placed the genus in the Leucodontaceae, but it was transferred to the Leptodontaceae by Buck (1980), a placement accepted by Stark (1987) and still unchanged (Goffinet & Buck 2004). Stark (1987) thought that *Leptodon* was the sister group of *Forsstroemia* within the Leptodontaceae subfam. Leptodontoideae. Stark (1987) characterized *Forsstroemia* by a suite of characters: 1) the branching pattern, in which a series of inflorescences alternates with a series of lateral branches, 2) costate leaves, 3) filamentous to foliose pseudoparaphyllia, 4) absence of a central strand in the stem, 5) uniseriate paraphyses that elongate after fertilization, 6) cucullate and hairy calyptrae, 7) erect capsules lacking annuli and

stomata, 8) relatively short setae, 9) hydrocastique exostomes and rudimentary endostomes, and 10) the sporophyte phenology in which the embryo overwinters. Although those characters render *Forsstroemia* a fairly morphologically coherent group, its possible monophyly has not been rigorously analysed. In the analysis by Tsubota *et al*. (2002) based on chloroplast *rbc*L, *Neckera urnigera* Müll. Hal. was nested in a clade in which the other species were *Forsstroemia trichomitria*, *F. japonica* (Besch.) Paris and *F. neckeroides* Broth. *Forsstroemia* has a wide general distribution in the tropical to warm-temperate regions, but the diversity centre is clearly in Asia, where several narrowly distributed endemic species occur.

Homalia was revised by He (1997), who recognized five species in it, with *H. trichomanoides* (Hedw.) Schimp. as the generitype. *Homalia trichomanoides* is widely distributed in the Northern Hemisphere, while the other species have much narrower distributions mainly in Europe and subtropical-tropical America. Morphologically *Homalia* seems to form a relatively homogeneous group. The plants are irregularly branched, glossy and have a strongly complanate leaf arrangement. The leaves are asymmetric, oblong-ovate to oblong-spathulate and have rounded or obtuse apices. The setae are elongate and the capsules have a well-differentiated annulus. The peristome is of the unreduced type.

Leptodon has currently two accepted species (Enroth 1992a). *Leptodon smithii* (Hedw.) F. Weber & D. Mohr, the generitype, has a temperate, disjunct and probably relict distribution, being known from N and S America, South and eastern Africa, the Mediterranean region, and eastern Australia and New Zealand (e.g., Pócs 1960; Nelson 1973), while *L. fuciformis* (Brid.) Enroth is endemic to the Réunion Island east of Madagascar (Enroth 1992a). The molecular diversity of *L. smithii* was recently studied by Mwafongo (2002) and Spagnuolo et al. (2007) for South Africa and SW Italy, respectively. *Leptodon* is characterized by pinnately to bipinnately branched fronds that are strongly inrolled when dry, the mostly rounded leaf apices, entire leaf margins, a single costa reaching typically up to ca half or two-thirds of the leaf length, the presence of leaf-like paraphyllia in abundance, the sheathing perichaetial leaves that enclose the ca 2 mm long seta, the emergent, erect capsule, and the reduced "neckeroid" peristome (cf. Nelson 1973).

Neckera is the largest genus in the Neckeraceae, with an estimated 50 species worldwide (Enroth 1994b), *N. pennata* Hedw. being the generitype. They are distributed mainly in the temperate and warm-temperate regions of the Northern Hemisphere, but have several Southern Hemisphere endemics as well. In the tropics species of *Neckera* do not occur in the lowlands but are restricted to mountain forests. There are endemic species in all continents except Antarctica, but the centre of species diversity is clearly in Asia. *Neckera* displays considerable morphological variability. The species may be distinctly stipitate-frondose (especially in a peculiar and strictly Asian group, (cf. Enroth 1996a; Enroth & Ji 2007) or lack a stipe. Lack of a stem central strand is a consistent feature, as is a complanate mode of branching. The leaves are mostly asymmetric, ovate-ligulate and with rounded to acute apices; often but not always the leaves are distinctly undulate and glossy. A costa may be lacking altogether or short (then often bifurcate to double) or long and single. The plants may be dioicous or autoicous. The setae vary from very short (less than 1 mm) to several cm long, and the capsule can be immersed or clearly exserted. Post-fertilization growth of the perichaetial leaves appears to be a consistent character, but its degree varies among species. The capsule is always erect and symmetric and the peristome is reduced, of the neckeroid-type. There is, however, much variation among the species in the peristomial details such as length and ornamentation of the exostome teeth and endostome segments, as well as in the presence / absence and height of an endostome basal membrane.

Pendulothecium, previously recognized as *Homalia* subgenus *Spathularia*, was established by Enroth & He (1991) in the Neckeraceae with *Pendulothecium auriculatum* (Wilson) Enroth & S. He as the generitype. It contains the species *P. auriculatum*, *P. oblongifolium* (Hook. f. & Wilson) Enroth & S. He and *P. punctatum* (Hook. f. & Wilson) Enroth & S. He, of which the former two are strictly endemic to New Zealand, while the latter also occurs on Norfolk Island. *Pendulothecium* differs from *Homalia* in several characters, including the pinnate to bipinnate and fairly dense mode of branching; the auriculate, mostly not complanate or spreading leaves; the non-twisted, thick setae; and the cernuous to pendulous, in wet state broad-oblong to ellipsoid capsules. The genus is currently well accepted (e.g., Fife 1995; Buck & Goffinet 2000).

Porotrichodendron was established by Fleischer (1906-1908), and is probably distributed nearly exclusively in South America (see Buck, 1998 for a discussion of the provenance of the type species *Porotrichodendron mahahaicum* (Müll. Hal.) M. Fleisch). It has less than five species (cf. Churchill & Linares 1995; Buck 1998), whose morphological boundaries are as yet fairly poorly understood due to a high degree of intraspecific variability. The genus can be characterized by concave, auriculate or cordate leaves with distinctly differentiated, thick-walled alar cells; several cm long setae; symmetric, erect capsules; very long-rostrate opercula; and a slightly reduced peristome with widely bordered exostome teeth that are at most moderately trabeculate at back and a relatively low basal membrane with no cilia or strongly reduced ones. *Porotrichodendron* has traditionally (e.g., Brotherus 1925) been placed in the Lembophyllaceae, but currently in the Neckeraceae (e.g., Enroth 1994b; Goffinet & Buck 2004).

Porotrichopsis. The monospecific genus *Porotrichopsis* was erected by Herzog (1916) and revised by Enroth (1995). *Porotrichopsis flacca* Herzog is a South American endemic, known from few collections from Bolivia and Colombia. The generic characters include the caducous branch leaves, often rendering the branches naked, and especially the strongly differentiated, short and coloured basal leaf cells that seem to form an abscission zone. The peristome is slightly reduced, e.g., lacking cilia and having perforate rather than fenestrate endostome segments. The exostome teeth are distinctly widely bordered. Herzog (1916) originally placed *Porotrichopsis* in the Neckeraceae, and Enroth (1995) somewhat hesitatingly agreed, suggesting a possible close relationship between *Porotrichopsis* and *Porotrichodendron.* An alternative placement has been the Lembophyllaceae (e.g., Brotherus 1925). Churchill & Linares (1995) and Gradstein et al. (2001) had *Porotrichopsis* along with some other stipitate-frondose genera in the Thamnobryaceae, an assemblage of genera that has never been well-defined and should be included in the Neckeraceae (e.g., Enroth 1994b; Buck 1998).

Porotrichum includes ca 15 species, the generitype being *Porotrichum longirostre* (Hook.) Mitt., distributed mainly in the tropical America and Africa (De Sloover 1983; Sastre-De Jesús 1987; Allen 1994; Buck 1998). The current generic circumscription covers also *Porothamnium* (e.g., Sastre-De Jesús 1987; Buck 2003; Enroth 2004),

which is thus a taxonomic synonym. The generic character state combination includes a distinctly stipitate-frondose habit, presence of a central strand in the stem, more or less complanate leaves, often spreading to squarrose stipe leaves, generally fusiform leaf cells, elongate, smooth and reddish setae, erect, symmetric capsules and a somewhat reduced peristome with moderately cross-striolate exostome teeth and a medium-high basal membrane wanting cilia or with 1-3 of them. The setae are shorter than in *Porotrichodendron*, which also differs in the more widely bordered exostome teeth and concave, often auriculate leaves with distinct groups of thick-walled alar cells. In comparison with *Homaliodendron*, the latter lacks a stem central strand, has appressed, overlapping stipe leaves and shorter, yellow setae often distinctly mammillose above.

Thamnobryum. The widely distributed yet mainly non-tropical genus was estimated to contain ca 35 species by Enroth (1994, Crosby et al. 2000 listed 42 species). It is characterized by dark green, dull, stipitate-frondose (sometimes dendroid) plants with non-overlapping stipe leaves appressed to the stipe and with plane margins. The leaves are mostly ovate or ovate-lanceolate, symmetric to slightly asymmetric and more or less complanate. A typical character is the very strong costa that may have abaxial spines near the tip. The seta is smooth and long, and the asymmetrical capsules vary from homotropous to reclinate (terminology follows Hedenäs, (2006)) and have phaneropore stomates at the base. The peristome is unreduced. A worldwide revision of *Thamnobryum* is underway (Mastracci 2003).

Thamnobryum subg. *Parathamnium* was established by (Ochyra 1990) for the aquatic, mainly SE Asian species *Thamnobryum ellipticum* (Bosch & Sande Lac.) Nog. & Z. Iwats. and *T. negrosense* (Bartr.) Z. Iwats. & B.C. Tan. They differ from the other species of *Thamnobryum* in several gametophyte characters, such as complanate leaf orientation, bi- to multistratose leaf margins that are crenulate to minutely serrulate, and the lack of dimorphism between the stipe leaves and the upper stem leaves. The sporophyte of *T. negrosense* is unknown, but that of *T. ellipticum* has an elongate seta, inclined capsule and a perfect hypnoid peristome (Fleischer 1906-1908). The lectotype species is *Thamnobryum ellipticum*. Later Ochyra (1991) recognized the taxon as an independent genus, *Parathamnium*, rendering *Thamnobryum* somewhat less heterogeneous.

Touwia, thus far a monospecific genus, was established and placed in the Neckeraceae by Ochyra (1986a). *Touwia laticostata* Ochyra is known from Queensland, Australia, where it grows on rocks in streambeds, and is at least periodically submerged. Originally known only from one gathering, more material has been found recently in the same area (Andi Cairns and Ryszard Ochyra, pers. comm.).

4.2 Material and methods

Taxon sampling and molecular markers

The taxon sampling was intended to be as complete as possible in terms of covering the morphological variation within the Neckeraceae. The results from preliminary analyses and earlier studies (chapters 1 and 3) together with previous taxonomic classification (e.g., Buck & Goffinet 2000; Goffinet & Buck 2004) were used as guidelines when choosing the species to be included. *Homalia webbiana* (Mont.) Schimp., *Heterocladium dimorphum* (Brid.) B.S.G. and *Heterocladium procurrens* (Mitt.) A. Jaeger together with the Lembophyllaceae clade were used as outgroup since they seem to be the closest relatives of the Neckeraceae (Quandt et al. in press; chapters 1 and 3). For this selection of taxa we sequenced three genomic regions: the internal transcribed spacer of nuclear ribosomal DNA (ITS1 & 2), the plastid *rps4-trnT-trnL-trnF* cluster (including the 3´of the *rps4* gene), and the group II intron in *rpl16* (plastid).

There are two genera that could not be included in the analyses due to lack of material. *Neomacounia nitida* (Lindb.) Ireland is a monospecific genus based on the basionym *Forsstroemia nitida* Lindb. It is known only from two specimens from Ontario, Canada, collected in 1862 and 1864 . The type locality and its surroundings were searched in the early 1970s to rediscover the taxon, but it was not found. It seems *Neomacounia* is extinct. Based on the description by Ireland (1974) there is nothing in the morphology of *Neomacounia* that belies a placement in the Neckeraceae; it is probably closely related to some *Neckera* species. *Noguchiodendron sphaerocarpum* (Nog.) Ninh & Pócs is the single species of *Noguchiodendron*, distributed in the Himalayan region and Thailand. As discussed by Ninh & Pócs (1981), it is probably closely related to *Homaliodendron*, where it originally was placed, but it differs in certain morphological characters in the gametophyte (e.g., presence of a central strand in the stem) as well as in the sporophyte (e.g., capsule shape, presence of an annulus),

justifying the maintenance of it as a separate genus. There was no adequately fresh material available for molecular analyses.

DNA isolation, PCR-amplification and sequencing

DNA was extracted using the DNeasy® Plant Mini Kit from Qiagen (Qiagen GmbH, Germany) following the manufacturer's protocol. Methods of cleaning and grinding of plants prior to extraction and amplification of the ITS1-5.8S-ITS2 as well as the *rps4-trnT-trnF* region followed Olsson et al. (chapter 1), whereas the protocols for *rpl16* were obtained from Olsson et al. (unpubl.). Gel cleaned PCR products were sequenced by Macrogen Inc., South Korea (www.macrogen.com). Sequences were edited manually with PhyDE® v0.995 (Müller et al. 2005) and primer sequences were eliminated. All sequences are deposited in EMBL; accession numbers are listed together with voucher information in Table 1.

Table 1. List of specimens used in the study including EMBL or GenBank accession numbers for the sequenced or downloaded regions and voucher details. Order of accession numbers*: rps4- trnF, rpl16,* ITS. In three cases sequence data have been already submitted to GenBank from previous studies and thus the accession numbers for *rps4-trnT-trnL-trnF* are composed of two different accession numbers. * denotes taxa for which nomenclatural changes are suggested in this article.

DNA no	**Species name**	**Herbarium**	**Voucher ID**	**GenBank accession *(rps4) - trnT & trnL - trnF***	*rpl16*	**ITS**
B116	*Alsia californica* (Hook. & Arn.) Sull.	B	Bryo 234031	FM210280	FM160946	FM161073
B141	*Anomodon giraldii* Müll. Hal.*	H	H3194078	AM990342	FM210763	FM161075
SH10	*Camptochaete arbuscula* var. tumida (Sm.) Reichardt.	H	Streimann 51408	AM990353	FM160955	FM161087
B617	*Chileobryon callicostelloides* (Broth. ex Thér.) Enroth	H	H 3107865	FM210283	FM200841	FM161088
B423	*Cryptoleptodon longisetus* (Mont.) Enroth *	H	H3038483	AM990356	FM160957	FM161091
B421	*Cryptoleptodon pluvinii* (Brid.) Broth.*	Huttunen	Huttunen s.n., China, Hunan.	FM210284	FM160958	FM161092
B223	*Curvicladium kurzii* (Kindb.) Enroth	NYBG	Akiyama Th-85	FM210285	FM160959	FM161093
SH146	*Dolichomitriopsis diversiformis* (Mitt.) Nog.	H, MHA	Nedoluzhko s.n.	AM990362; *trnLF* AF397777	FM160963	FM161098
B195	*Echinodium hispidum* (Hook. f. & Wilson) Reichardt	Buchbender	Downing s.n., 29.10.2000	FM210286	FM160964	FM161099
B258	*Echinodium umbrosum* var. glaucoviride	Schäfer-Verwimp	Streimann 49634	EU434010	FM160965	EU477602
B349	*Heterocladium dimorphum* (Brid.) Schimp.	H	H3212307	AM990376	FM160970	FM161115
B352	*Heterocladium procurrens* (Mitt.) A. Jaeger	H	H3212289	AM990379	FM160973	FM161118
B422	*Homalia glabella* (Hedw.) Schimp.	H	Townsend 93/291	AM990382	FM160977	FM161123
B111	*Homalia lusitanica* Schimp.	B	B275202	AM990383	FM160978	FM161124
B218	*Homalia trichomanoides* (Hedw.) Schimp.	Quandt	Olsson 105	AM990385	FM160980	FM161126
B474	*Homalia webbiana* (Mont.) Schimp.	H	Müller K68	AM990387	FM160982	FM161127
B110	*Homaliodendron exiguum* (Bosch & Sande Lac.) M. Fleisch	B	B263509	AM990389	FM160984	FM161130
B230	*Homaliodendron flabellatum* (Sm.) M. Fleisch.	H	H3071675	FM210290	FM160985	FM161132
B424	*Homaliodendron neckeroides* Broth.	H	H3071953	FM210306	FM161015	FM161168
SH103	*Lembophyllum clandestinum* (H. f & W.) Lindb. in Par.	H	Vitt 29644	AM990401; *trnLF* AF397823	FM160996	FM161145
B131	*Leptodon smithii* (Hedw.) F. Weber & D. Mohr	B	B268385	AM990403	FM160997	FM161147
B226	*Forsstroemia producta* (Hornsch.) Paris *	H	Koponen 46545	FM201504	FM160967	FM161102
B196	*Forsstroemia trichomitria* (Hedw.) Lindb.*	Buchbender	Streimann & Pocs 65120A	AM990365	FM160968	FM161103
B253	*Neckera besseri* (Lobarzewski) Jur.	Quandt	Olsson 107	FM210294	FM161003	FM161156
B367	*Neckera brownii* Dixon	H	Tangney 2330	FM210295	FM161004	FM161157
B193	*Neckera complanata* (Hedw.) Huebener	Buchbender	Buchbender 204	AM990413	FM161005	FM161158
B248	*Neckera crenulata* Harv.	H	Long 33980	FM210297	FM161006	FM161159
B192	*Neckera crispa* Hedw.	Buchbender	Buchbender 385	FM210298	FM161007	FM161160
B127	*Neckera douglasii* Hook.	B	B253879	FM210299	FM161008	FM161161
B249	*Neckera goughiana* Mitt.	H	Koponen 46476	FM210300	FM161009	FM161162
B128	*Neckera himalayana* Mitt.	B	B253876	FM210301	FM161010	FM161163
B427	*Neckera hymenodonta* Müll. Hal.	H	H3206871	FM210302	FM161011	FM161164
B471	*Neckera intermedia* Brid.	H	Samaniego & Manso, 12.10.1999	FM210303	FM161012	FM161165
B106	*Neckera jamesonii* Taylor	B	B264587	FM210304	FM161013	FM161166
B161	*Neckera menziesii* Drumm.	NYBG	Halse 4878	FM210305	FM161014	FM161167
B347	*Neckera pennata* Hedw.	H	H3203794	AM990414	FM161016	FM161169
B250	*Neckera polyclada* Müll. Hal.	H	Koponen 45441	FM210307	FM161017	FM161170

B307	*Neckera remota* Bruch & Schimp. ex Müll. Hal.	S	B29895	AM990415	FM161018	FM161171
B105	*Neckera scabridens* Müll. Hal.	H	Kürschner et al. 95-498	FM210308	FM161019	FM161172
B470	*Neckera submacrocarpa* Dixon	Enroth	Pocs 90021/AL	FM210309	FM161020	FM161173
SH301	*Neckera urnigera* Müll. Hal.	S	B15194	AM990416	FM161021	FM161174
B544	*Neckera valentiniana* Besch.	Bolus Herb., Univ. Cape Town	Hedderson 16404	FM210310	FM161022	FM161175
B298	*Neckera warburgii* Broth.	B	Bryo 253855	FM210311	FM161023	FM161176
B251	*Neckera yezoana* Besch.	H	Enroth 70675	FM210312	FM161024	FM161177
B313	*Neckeropsis nitidula* (Mitt.) M. Fleisch.	S	B105713	AM990419	FM161030	FM161183
B476	*Pendulothecium punctatum* (Hook. f. & Wilson) Enroth & S. He	S	Streimann 53845	AM990421	FM161033	FM161187
B260	*Pinnatella anacamptolepis* (Müll. Hal.) Broth.	S	B104516	FM210318	FM161036	FM161190
B472	*Pinnatella kuehliana* (Bosch & Sande Lac.) M. Fleisch.	Enroth	Müller S116	FM20150	FM161038	FM161192
B099	*Porotrichodendron robustum* Broth.	B	B264620	AM990426	FM200845	FM161197
B294	*Porotrichodendron superbum* (Taylor) Broth.	H	H3121100	AM990427	FM161043	FM161198
SH372	*Porotrichopsis flacca* Herzog	S	Churchill et al. 10. Jan. 1991	FM201506	FM161044	FM161199
B244	*Porotrichum bigelovii* (Sull.) Kindb.	H	Shevock & Kellman 27467	AM990428	FM161045	FM161200
B117	*Porotrichum frahmii* (Enroth) Enroth	B	B255332	AM990429	FM161046	FM161201
SH252	*Porotrichum madagassum* Kiaer ex Besch.	Vanderpoorten, Quandt	Easton Cape, Flora South Africa 244	FM210322	FM210764	FM161203
B559	*Rigodium pseudothuidium* Dusén	NYBG	NYBG 00892248	-	-	FM161210
Rp47	*Rigodium pseudothuidium* Dusén	H	H3134254	AM990438; *trnLF* AF543547	FM161051	-
B149	*Taiwanobryum speciosum* Nog.	H	Enroth 64877	AM990442	FM161055	FM161216
B238	*Thamnobryum alopecurum* (Hedw.) Nieuwl. ex Gangulee	Buchbender	Buchbender s.n. 11.7.2003	AM990444	FM161056	FM161218
B539	*Thamnobryum cataractarum* N. Hodgetts & Blockeel	S	B3725	FM201507	FM161057	FM161219
B546	*Thamnobryum ellipticum* (Bosch & Sande Lac.) Nieuwl. *	Enroth	Müller S114	FM210325	FM161058	FM161220
B190	*Thamnobryum fasciculatum* (Sw. ex Hedw.) I. Sastre	NYBG	Buck 26902	FM210326	FM161059	FM161221
B549	*Thamnobryum fernandesii* Sérgio	S	B9965	FM201508	FM161060	FM161222
SH300	*Thamnobryum maderense* (Kindb.) Hedenäs	S	B44108	AM990445	FM161061	FM161223
B165	*Thamnobryum neckeroides* (Hook.) E. Lawton	NYBG	Buck 37648	FM201509	FM161062	FM161224
B420	*Thamnobryum negrosense* (E.B. Bartram) Z. Iwats. & B.C. Tan *	H	Schäfer-Verwimp & Verwimp 16852	FM210327	FM161063	FM161225
B311	*Thamnobryum pandum* (Hook. f. & Wilson) I.G. Stone & G.A.M. Scott	H	H3208440	FM210328	FM161064	FM161226
B120	*Thamnobryum pumilum* (Hook. & Wilson) B.C. Tan	B	B268163	FM210329	FM200843	FM161227
B574	*Thamnobryum rudolphianum* Mastracci	BM	BM000919859	FM201510	FM161065	FM161228
B233	*Thamnobryum speciosum* (Broth.) Hoe	H	H3141827	FM201511	FM161066	FM161229
B148	*Thamnobryum subserratum* (Hook. ex Harv.) Nog. & Z. Iwats.	H	Enroth 64595	AM990446	FM161067	FM161230
B429	*Thamnobryum tumidicaule* (K.A. Wagner) F.D. Bowers	H	H3141850	AM990447	FM161068	FM161231
B261	*Touwia laticostata* Ochyra	Quandt	Cairns 27.8. 2005	FM210330	FM161070	FM161233
DQ	*Weymouthia mollis* (Hedw.) Broth.	CHR, Quandt	99-Mo2	AM990452	FM161072	FM161237

Sequence analyses and phylogenetic analyses

Alignment of the sequence data was performed manually in PhyDE® v0.995 (Müller et al. 2005), based on the criteria laid out in Kelchner (2000), Borsch et al. (2003) and Quandt & Stech (2005) using the alignment of Olsson et al. (chapter1) as scaffold. As length variation of the sequence data was very low, alignment was straight forward. The reported hairpin associated inversion in the *trnL-F* intergenic spacer (IGS) (Quandt et al. 2004a; Quandt & Stech 2005) was positionally isolated in the alignment and included in the analysis as reverse complement in order to gain information from substitutions within the detected inversion, as discussed in Quandt et al. (2003a). Alignments are provided on an appendix cd. Indels were incorporated as binary data using a simple indel coding (SIC) strategy (Simmons & Ochoterena 2000) as implemented in SeqState (Müller 2005). Command files for using the parsimony ratchet (Nixon 1999) were generated using PRAP2 (Müller 2007) and executed in PAUP 4.0b10 (Swofford 2002). Ratchet settings were as followed: 10 random addition cycles of 200 iterations each, with 25% upweighting of the characters in the iterations. Heuristic bootstrap searches under parsimony were performed with 1000 replicates and 10 random addition cycles per bootstrap replicate.

Bayesian analyses were performed with MrBayes v3.1.2 (Huelsenbeck & Ronquist 2001), applying the GTR+Γ+I model for the sequences data and the restriction site model for the binary indel partition. To allow for possible deviating substitution models for the different regions, the data set was divided into four partitions (partition 1: *rps*4-*trn*F; partition 2: *rpl*16; partition 3: nuclear DNA; partition 4: indels). The *a priori* probabilities supplied were those specified in the default settings of the program. Posterior probability (PP) distributions of trees were calculated using the Metropolis-coupled Markov chain Monte Carlo (MCMCMC) method and the search strategies suggested by (Huelsenbeck et al. 2001; Huelsenbeck et al. 2002b). Ten runs with four chains ($2.5\ 10^7$ generations each) were run simultaneously, with the temperature of the single heated chain set to 0.1. Chains were sampled every 10 generations and the respective trees written to a tree file. Calculations of the consensus tree and of the posterior probability of clades were performed based upon the trees sampled after the chains converged (< generation 50 000). Consensus topologies and support values from the different methodological approaches were compiled and drawn using TreeGraph (Müller & Müller 2004)

4.4 Results

Alignment and sequence analyses

In total 12 hotspots with poly-homonucleotid repeats were recognized following Olsson et al. (chapter 1) and excluded from the analyses. Hotspots were more frequent in the plastid region (H1-9), while only three were found in the nrDNA (H10-12). The resulting combined and aligned sequence matrix contained 3507 positions of which 1499 positions belong to the *rps4-trnT-trnL-trnF* partition, 904 positions to the *rpl16* partition and 1104 positions to the nuclear ribosomal partition. 2806 of the characters were constant and 404 characters were parsimony-informative. Including the data matrix based on indel coding raised the number of parsimony-informative characters to 557 (a total of 3859 characters with 1021 being variable).

Phylogenetic analyses

The parsimony analysis without indel coding retained 20 most parsimonious trees (MPT, length 1477, CI= 0.558, RI=0.785). After inclusion of the indel matrix 711 MPTs were retained (length 2057, CI= 0.571, RI= 0.778). The strict consensus tree of both analyses showed no conflict with the results from the Bayesian inference, but had less resolution compared to the MrBayes tree. Therefore, only the MrBayes tree is illustrated in Fig. 1, with posterior probabilities (PP) indicated and complemented with bootstrap values (BS) of the parsimony analysis when applicable. When the indel matrix was included in the analyses, the only topological difference observed was the poorly resolved position of *Forsstroemia producta* (Hornsch.) Paris. However, differences in the magnitude of support values at some of the nodes were observed. Therefore, both of the values without and with the indel matrix included are illustrated and discussed. Values resulting from analyses without an indel coding approach precede the values from analyses with the SIC-matrix included. Thus support values from the different analyses will be referred to in the text following this scheme (PP / PPsic, BS / BSsic).

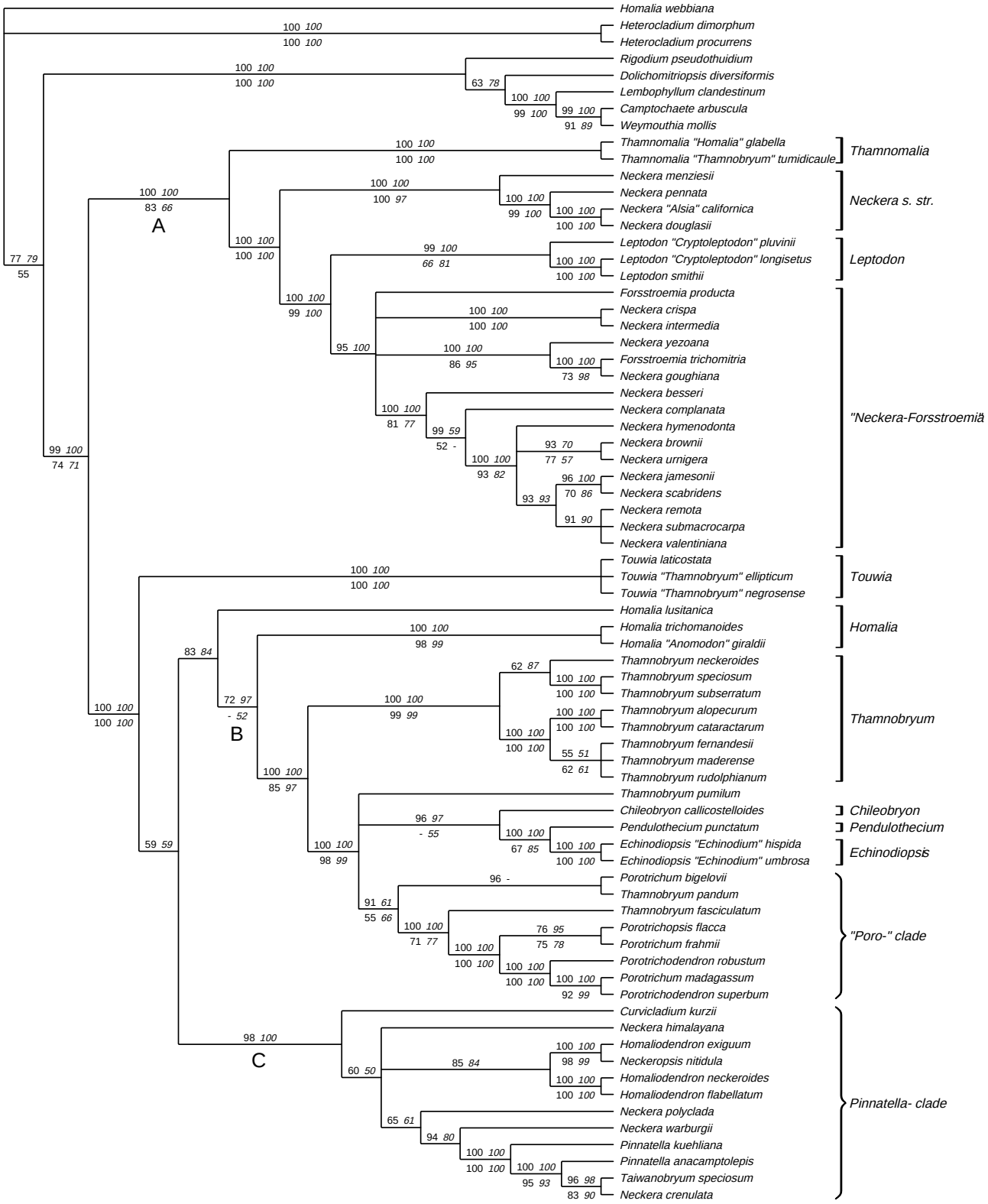

Figure 1. Phylogenetic relationships of selected Neckeraceae taxa based on *rps4-trnT-trnL-trnF, rpl16* and ITS1 & 2 sequences. The PP values from the MrBayes analyses (without indel coding first, then with indel coding) are indicated above, the bootstrap values of the parsimony analysis below when applicable (without indel coding first, then with indel coding).

The ingroup species belong to the Neckeraceae as defined by Olsson et al. (chapter 3). Three clades can be distinguished: clade A formed by *Neckera* and related taxa, clade B having *Thamnobryum* as the most prominent genus and clade C including *Pinnatella* and *Neckeropsis* among others. The positions of a clade including *Touwia laticostata* to *Thamnobryum negrosense*, as well as a clade formed of *Homalia lusitanica* Schimp., *H. trichomanoides* and *Anomodon giraldii* Müll. Hal. remain unresolved within the Neckeraceae.

In addition to most *Neckera* species, *Forsstroemia*, *Cryptoleptodon* and *Leptodon*, *Alsia californica, Homalia glabella* (Hedw.) Schimp. and *Thamnobryum tumidicaule* (K.A. Wagner) F.D. Bowers belong to clade A, which receives maximum Bayesian support (PP 100). The two last mentioned species render *Homalia* and *Thamnobryum* polyphyletic and form a clade (PP 100, BS 100) that is resolved as a sistergroup to all the remaining taxa in this clade. The remaining taxa can further be divided into two clades: one including *Neckera menziesii* Hook., *Neckera pennata*, *Alsia californica* and *Neckera douglasii* Hook. (PP 100, BS 97–100) and the other formed of the rest of the taxa (PP 100, BS 99–100). *Leptodon* and *Cryptoleptodon* are resolved in a clade separated from the others when the indel matrix is not included in the analyses, but when the indel matrix is included, this clade remains in an unresolved position.

Clade B is divided into two well defined clades: one includes only *Thamnobryum* species and the other has species of *Thamnobryum*, *Chileobryon*, *Pendulothecium*, *Echinodium*, *Porotrichum*, *Porotrichopsis* and *Porotrichodendron*, rendering the genera *Porotrichum* and *Porotrichodendron* polyphyletic. Both clades get high support values (PP 100, BS 98–99), but the relationships within the clades are not totally resolved.

Clade C is composed of diverse taxa: *Pinnatella*, *Neckeropsis*, *Homaliodendron*, *Taiwanobryum*, *Curvicladium* and some *Neckera* species. Even if the clade receives high support in the Bayesian analyses (PP 98 / 100), the internal nodes in this clade are neither totally resolved nor well supported, except for a clade containing *Pinnatella kuehliana* (Bosch & Sande Lac.) M. Fleisch., *P. anacamptolepis* (Müll. Hal.) Broth., *Taiwanobryum speciosum* Nog. and *Neckera crenulata* Harv. (PP 100, BS 100) and two small clades with *Homaliodendron exiguum* (Bosch & Sande Lac.) M. Fleisch. together with *Neckeropsis nitidula* (Mitt.) M. Fleisch. (PP 100, BS 98–99) and *Homaliodendron*

neckeroides Broth. together with *Homaliodendron flabellatum* (Sm.) M. Fleisch. (PP 100, BS 100), respectively.

4.5 Discussion

Phylogenetic analyses and taxonomic relationships

We show that *Leptodon* and *Cryptoleptodon* actually form only one genus and that the Leptodontaceae should be merged with the Neckeraceae (see also chapter 3). Furthermore, we clarify the position of *Anomodon giraldii*, *Touwia laticostata* and the genus *Chileobryon* and suggest a new circumscription for *Thamnobryum*. However, some phylogenetic relationships within the group still remain unsolved. The composition of the polyphyletic genera *Porotrichum* and *Porotrichodendron* are addressed in a separate study (Buchbender 2009), and so are the more detailed contents of clade C (chapter 5). A comprehensive revision of the large and heterogeneous genus *Neckera* requires further study.

Even if additional data is most often expected to increase resolution and group support seems inclusion of the simple indel coding (SIC) in the phylogenetic analyses to have negative effects for the phylogeny based on our data set. Especially in parsimony analyses inclusion of indel data lead to lower resolution, yielded a great increase in MPTs (711 instead of 20) and lower retention index (RI). Posterior probability values for some groups such as the clade consisting of *Neckera* species from *Neckera complanata* to *Neckera valentiana*, were also clearly higher without indel data. We assume this to be due to likely convergent evolution of the the coded indels that can give slightly misleading evolutionary information. For some groups, however, inclusion of the indel matrix lead to better support (for example *Pendulothecium – Echinodiopsis* clade, clade B excluding *Homalia lusitanica*, clade B excluding *Homalia lusitanica*, *H. trichomanoides* and *Anomodon giraldii* and the *Thamnobryum neckeroides – T. subserratum* clade). The support seems to be due to a combination of indels more than to significant single indel events, since only few indels supporting these groups were found. The *Pendulothecium – Echinodiopsis* clade is supported by an indel in the *rpl16* region (position 1778-1791 in the final alignment) and the B clade excluding *Homalia lusitanica*, *H. trichomanoides* and *Anomodon giraldii* is supported by three indels in the ITS region (positions 2685-2687, 2723-2725 and 3211-3213).

The polyphyly of the genus *Homalia* is intriguing, since it is a morphologically fairly coherent group (cf. He 1997). Especially unexpected was the grouping of *Anomodon giraldii* together with *Homalia trichomanoides*. In the current analyses *Homalia lusitanica* is not included in this clade, which contradicts our previous results based on a more extensive sequence data (chapter 3). Since it might be an artefact due to less sequence level information available, we want to refrain from nomenclatural changes considering *H. lusitanica* and wait for further studies to clarify the exact phylogenetic position of it. *Anomodon giraldii* is morphologically very different from *Homalia trichomanoides*, therefore its position in the phylogeny was verified by confirming carefully the identification, the origin of isolated DNA and DNA sequences. Thus we suggest *A. giraldii* to be renamed as *Homalia giraldii*, these two species (possibly together with *Homalia lusitanica*) forming the genus *Homalia* s. str.

One remarkable character state of *A. giraldii* that was previously thought not to occur in the Neckeraceae is the papillose leaf cells. However, also *Chileobryon callicostelloides*, removed by Enroth (1992b) from the Neckeraceae mainly because of this character state, has papillose leaf cells. In other pleurocarpous taxa leaf cell papillosity has been shown to be an important diagnostic character for separating the families Meteoriaceae and Brachytheciaceae (Huttunen & Ignatov 2004), but it is quite inconsistent for example in the Thuidiaceae – Leskeaceae –complex (cf. Buck & Crum 1990), although it can generally be used to define genera also there. *Anomodon giraldii* seems to be the only "*Anomodon* species" belonging to the Neckeraceae (Tsubota et al. 2002; Olsson et al. chapter 1). *Anomodon*, as currently understood, is most diverse in Asia (cf. Iwatsuki 1963; Granzow-de la Cerda 1992) and has only one species with smooth leaf cells. The rest of the genus *Homalia* is highly polyphyletic: *H. glabella* belongs to clade A while *H. webbiana* and *H. pennatula* (Dixon) S. He & Enroth have been excluded from the Neckeraceae (see chapter 3).

Clade A. This group includes mainly non-Asiatic species, some of which have a wide, often disjunct (possibly relict) distribution, e.g., *Neckera menziesii*, *Leptodon smithii*, *Forsstroemia trichomitria* and *F. producta.* Most of the species belonging to this group have a relatively weak costa and immersed capsules (mainly *Neckera* spp.) and the teeth at the leaf margins are usually unicellular. *Thamnobryum tumidicaule* and *Homalia glabella* form the first diverging branch in clade A. The high support for this clade

implies recognition at the genus level, and for this purpose we below describe and discuss the genus *Thamnomalia*.

The genus *Neckera* as currently understood has been shown to possibly not be monophyletic in earlier studies (Tsubota et al. 2004; Ignatov et al. 2007; Olsson et al. chapter 3), which is confirmed here with a more comprehensive taxon sampling. In the current analyses we include taxa that well cover the morphological variation and geographical extent of the genus. Since *Neckera pennata* is the type of the genus, the clade including that species, *N. menziesii*, *N. douglasii* and *N. californica* (syn. *Alsia californica*), forms *Neckera* s. str. The majority of the species currently belonging to the genus *Neckera* belong to a clade also including *Forsstroemia producta* and *F. trichomitria* (generitype). A clade formed by *Neckera* and *Forsstroemia* was also hint at by the results of Tsubota et al. (2002), but due to the sparse taxon sampling (*Forsstroemia trichomitria*, *F. japonica*, *F. neckeroides* and *Neckera urnigera*) the supporting evidence remained weak. Since the relationships between *N. besseri* (Lobarz.) Jur. to *N. valentiniana* Besch. and the remaining members of the "*Forsstroemia-Neckera*" clade are not satisfactorily resolved, and the taxon sampling is still relatively incomplete, we refrain from making nomenclatural changes in this group. It is evident, however, that at some point one or several genera accommodating these "*Neckera*" species will have to be raised. It might be mentioned that the Australasian *N. hymenodonta* Müll. Hal. has previously been treated as a taxonomic synonym of *N. pennata* (e.g., Fife 1995). As Ji & Enroth (2008) showed, *N. hymenodonta* is clearly different from *N. pennata* (e.g., the former has paraphyllia) and in the present analysis it becomes placed in the "*Forsstroemia-Neckera* –clade".

Also, the four "*Neckera*" species belonging to the clade C will be discussed in detail in chapter 5: *N. himalayana* Mitt., *N. polyclada* Müll. Hal., *N. warburgii* Broth. and *N. crenulata*. They are morphologically different from the other *Neckera* species and form a peculiar group of robust Asian species (Enroth 1996b). According to our results they are neither closely related to the "true" *Neckera*s or those in the clade containing also *Forsstroemia*, nor are they forming a clade together.

Leptodon smithii and the two *Cryptoleptodon* species form a clade, implying that *Cryptoleptodon* as a genus is not justifiable and it should be included in *Leptodon*, as it traditionally has been (e.g., Jaeger & Sauerbeck 1876-1879). It has been suggested in previous studies (Maeda et al. 2000; Goffinet et al. 2001; Tsubota et al. 2004) that *Forsstroemia, Echinodium, Leptodon*, and *Anomodon giraldii* have close affinities with

the *Neckera* species, although based on a limited dataset (see also chapter 1 and 3). The morphological similarity between *Forsstroemia* and *Leptodon* was pointed out by Stark (1987), and the affinities of *Forsstroemia* to the Neckeraceae (when the Leptodontaceae become included in it) receive morphological support from Buck (1980) and Enroth (1992a).

Clade A contains some phytogeographically distinct and evolutionarily informative groupings and structure. In clade A, the basal group formed of *Homalia glabella* and *Thamnobryum tumidicaule* is South American and tropical. The next basal group with four species of *Neckera* (s. str.) is essentially temperate and North American, with the exception of *N. pennata* which has a much wider distribution especially in the northern Hemisphere and which may in fact contain more than one species (cf. Appelgren & Cronberg 1999). It thus seems that this group originated and diversified in the "New World", since apart from *N. pennata*, none of the European (*N. complanata* (Hedw.) Huebener, *N. crispa* Hedw., *N. intermedia* Brid., *N. besseri*) Asian (*N. yezoana* Besch., *N. goughiana* Mitt.) or African (*N. remota* Bruch & Schimp. ex Müll. Hal., *N. submacrocarpa* Dixon, *N. valentiniana*) species belong in *Neckera* s. str. It should be noted that the South American species *N. urnigera*, *N. jamesonii* Taylor and *N. scabridens* Müll. Hal. as well as the species from New Zealand *N. brownii* Dixon and *N. hymenodonta* and the three African species just mentioned form a clade with a maximum MB support (Fig. 1), with the African species grouping together. It is thus clear that the "*Neckera*-characters" deeply undulate, complanate and asymmetric leaves and a weak costa were acquired independently in *Neckera* s. str. and in the "*Forsstroemia-Neckera* clade". These characters are notably absent in the *Leptodon* clade.

Clade B. The members of the clade B are mainly non-Asiatic; the truly tropical taxa in this clade are limited to South America. Nearly all taxa have a single, at least relatively strong costa, and most taxa are fairly robust, and distinctly stipitate. In addition, the setae are long (capsules exserted) and the peristomes are perfect or only somewhat reduced (in *Porotrichodendron*) but not to a „neckeroid" state as in clade A. As Enroth & Tan (1994) pointed out, the Thamnobryaceae, comprising "the dendroid Neckeraceae sensu Brotherus (1929) with cross-striolate exostomes" (Buck & Vitt 1986), cannot be kept separate from the Neckeraceae. Our current analyses (see also chapters 1 and 5) based on molecular data confirms this, all "Thamnobryaceae" species being included in

the Neckeraceae. The placement of *Chileobryon callicostelloides*, a monospecific genus from Chile (including the Juan Fernandez Islands), among the pleurocarpous mosses has been uncertain. Our analyses support the view of Brotherus (1925), who placed the species in the Neckeraceae as *Pinnatella callicostelloides*. It is in fact not close to *Pinnatella* but forms a group together with *Pendulothecium punctatum*, *Echinodium hispidum* and *E. umbrosum*. The clade is southern amphi-Pacific in distribution, covering Chile, New Zealand and Norfolk Island (cf. Churchill 1986; Enroth & He 1991; Enroth 1992b).

Porotrichodendron would be monophyletic when *Porotrichum madagassum* Kiaer ex Besch. is included in it, a grouping that gets morphological support in addition to the molecular evidence. In the actual paper, however, the taxon sampling is not complete, and the analyses from Buchbender (2009) suggest that these genera need a thorough revision. Therefore we refrain at the moment from new nomenclatural combinations in this group.

The genus *Thamnobryum* is polyphyletic. The species in the clade together with the generitype *T. alopecurum* (Hedw.) Nieuwl. ex Gangulee form *Thamnobryum* s. str. *Thamnobryum tumidicaule* is placed in the *Neckera* group and placed in a new genus. *Thamnobryum ellipticum* and *T. negrosense* form a clade together with *Touwia laticostata* (thus far the single species in its genus), and we suggest that they are included in the genus *Touwia*. This grouping is morphologically sound since the two *Thamnobryum* species have earlier been noted to be morphologically distinct (see introduction), and they share morphological similarities with *Touwia*. The three species of *Touwia* have a restricted distribution area in Australasia and SE Asia (Ochyra 1986b; Enroth 1989a; Ochyra 1990) and they are rheophytic (growing in running water) species only known from gametophytes. However, all the rheophytic taxa in the Neckeraceae (cf. Enroth 1999) do not form a monophyletic group despite some similar morphological adaptations. Thus, the rheophytic *Thamnobryum*s (*T. fernandesii* Sérgio, *T. cataractarum* N. Hodgetts & Blockeel and *T. angustifolium* (Holt.) Nieuwl.) are closely related to *T. alopecurum* but in several separate lineages (Chapter 5) and *T. pumilum* (Hook. f. & Wilson) Nieuwl. remains in an unresolved position. We hope that the upcoming study by Buchbender et al. (unpubl.) will resolve the remaining questions related to the position of *T. pumilum* and the "*Poro*-"clade, where several nomenclatural changes will be needed. It should be noted that after *Thamnobryum tumidicaule* and *T. fasciculatum* (Hedw.) Sastre (see Fig. 1) become removed from that genus, the single

species placed in *Thamnobryum* and occurring in the South American continent is the peculiar *T. liesneri* B.H. Allen & S.P. Churchill from Venezuela (Allen & Churchill 2002).

The Australasian *Echinodium hispidum* and *E. umbrosum* were shown by Stech et al. (2008) not to belong in *Echinodium* s. str., and were thus transferred to *Thamnobryum*. With a more extensive taxon sampling it is clear, however, that these species do not belong in *Thamnobryum*. The sporophytes of the two *Echinodiums* and *Pendulothecium* are almost identical, but the apohysal stomata in the former are immersed (vs. superficial in *Pendulothecium*) and the spores are smaller (12-14 µm in the *Echinodiums* and 16-20 µm in *Pendulothecium*; (cf. Churchill 1986; Enroth & He 1991)). However, there are clearer differences in the gametophytes, justifying erecting a new genus *Echinodiopsis* for *Echinodium hispidum* and *E. umbrosum*. Those two species have a stem central strand (lacking in *Pendulothecium*), foliose pseudoparaphyllia (lacking in *Pendulothecium*), long, very strong and excurrent costae with internal differentiation (ending in midleaf or reaching to 5/6 leaf length at most, of homogeneous cells), and a completely different leaf shape with bistratose parts. The clade formed of *Chileobryon*, *Pendulothecium* and *Echinodiopsis* is phytogeographically coherent and southern amphi-Pacific. *Chileobryon* is known from the Juan Fernández Islands and mainland Chile, while the two other genera are distributed in Australasia, especially in New Zealand and some of the adjacent islands. All species also grow in very similar, moist and shady habitats, with soil and rocks being the preferred substrates, but also on tree bases and logs (Churchill 1986; Enroth & He 1991; Enroth 1992b).

4.6 Taxonomic and nomenclatural novelties and changes

Neckera Hedw., Spec. Musc.: 200. 1801, *nom. cons.*

Generitype: *Neckera pennata* Hedw. (*typ. cons.*)

= ***Alsia*** Sull., Proc. Am. Ac. Arts Sci. 3: 184. 1855, *syn. nov*.

Generitype: *Alsia californica* (Hook. f. & Arn.) Sull. (≡ *Neckera californica* Hook. f. & Arn.)

This synonymization implies that the accepted name of *Alsia californica* is *Neckera californica*, the basionym.

Leptodon D. Mohr, Observ. Bot.: 27. 1803, ***nom. cons.***
Generitype: *Leptodon smithii* (Hedw.) Weber & D. Mohr (≡ *Hypnum smithii* Hedw.)
= ***Cryptoleptodon*** Renauld & Cardot, Bull. Soc. R. Bot. Belgique 38: 30. 1900, ***syn. nov***.
Generitype (see Enroth, 1992a): *Cryptoleptodon pluvinii* (Brid.) Broth.

This synonymisation implies that the accepted name of *Cryptoleptodon pluvinii* is *Leptodon pluvinii* (Brid.) A. Jaeger, the accepted name of *Cryptoleptodon longisetus* (Mont.) Enroth is *Leptodon longisetus* Mont., and the accepted name of *Cryptoleptodon rigidulus* (Mitt.) Broth. is *Leptodon rigidulus* (Mitt.) A. Jaeger.

A fourth species placed in *Cryptoleptodon* is *C. acuminatus* M. Fleisch. (Fleischer 1917). It was based on a specimen in Carl Müller's herbarium from "Ost-Indien". No type material has been located (cf. Enroth, 1992a), but for nomenclatural reasons also that taxon is here transferred to *Leptodon*. It is unclear what "Ost-Indien" in the protologue means, but according to Gangulee (1976) the three species (*acuminatus, pluvinii, rigidulus*) "are localized in North-Western India, adjacent Western Tibet and Pakistan".

Leptodon acuminatus (M. Fleisch.) S. Olsson, Enroth & D. Quandt, ***comb. nov.***
≡ *Leptodon pluvinii* (Brid.) A. Jaeger var. *foliis acuminatulis* Müll. Hal. ex M. Fleisch., Hedwigia 59: 212. 1917; *Cryptoleptodon acuminatus* M. Fleisch., Hedwigia 59: 212. 1917.

Thamnomalia S. Olsson, Enroth & D. Quandt, ***gen. nov***.

Genus hoc cognoscitur caulibus frondosis, irregulatim ramosis, areolatione foliorum cellulis apicalibus parietibus satis crassis et cellulis medianis parietibus clare tenuioribus et cellulis alaribus infirme vel haud differentiatis. Species duo praecipue in America centrali et in archipelago Indiae occidentalis distributae sunt et plerumque ad rupes in silvis humidis habitant.

Generitype: ***Thamnomalia glabella*** (Hedw.) S. Olsson, Enroth & D. Quandt

Thamnomalia glabella (Hedw.) S. Olsson, Enroth & D. Quandt, *comb. nov.*
Basionym: *Leskea glabella* Hedw., Sp. Musc. Frond.: 235. 1801; *Neckera glabella* (Hedw.) F. Weber & D. Mohr, Index Mus. Pl. Crypt.: 3. 1803; *Hypnum glabellum* (Hedw.) Sw. ex P. Beauv., Prodr. Aethéogam.: 64. 1805; *Homalia glabella* (Hedw.) Bruch & Schimp., Bryol. Eur. 5, fasc. 44-45, Monogr. 1: 54. 1850.

Thamnomalia tumidicaulis (K.A. Wagner) S. Olsson, Enroth & D. Quandt, *comb. nov*.
Basionym: *Thamnium tumidicaule* K.A. Wagner, Bryologist 55: 145. 1952; *Thamnobryum tumidicaule* (K. A. Wagner) F.D. Bowers, Bryologist 77: 162. 1974.

The two species of *Thamnomalia* have very similar geographic ranges. Both species occur in Central America and the West Indies; *T. glabella* is also known from SE Brazil (cf. He, 1997; Buck, 1998). Both species grow mainly on rocks and rarely on tree trunks; *T. glabella* thrives at 400-2500 m and *T. tumidicaulis* at 600-1200 m (Buck 1998).

Sporophytes are known only for *T. glabella* and they were described by He (1997). The gametophytes of the two species are fairly different. The shared features include the frondose habit with rather irregularly branched stems, the complanate leaves (strongly so in *T. glabella*), and the leaf areolation pattern. The apical cells are relatively strongly incrassate and sometimes porose, while the median laminal and their subjacent cells have clearly thinner walls. The alar cells are scarcely if at all differentiated. Both species have foliose pseudoparahyllia, but in *T. glabella* they are intermingled with filamentous ones. Most of the other gametophyte characters distinguish rather than unite *T. glabella* and *T. tumidicaulis*. The leaves of *T. glabella* are clearly asymmetric, those of *T. tumidicaulis* are symmetric; *T. glabella* has a very weak and short, often double costa, while that of *T. tumidicaulis* is single and very strong, ending shortly below the leaf apex; and the apical teeth in the leaves of *T. glabella* are unicellular, while those of *T. tumidicaulis* are often composed of 2-3 cells. *Thamnomalia tumidicaulis* also has a distinct stem central strand, but *T. glabella* seems to show some variation in this character. He (1997) said in the verbal description of *Homalia glabella* that a central strand is absent, but the illustration (fig. 109) shows a small central strand. Since the

presence or absence of a central strand is one of the most consistent features at the species level in the Neckeraceae, it is probable that He's (1997) concept of *H. glabella* actually contains more than one species. Further studies are needed.

Echinodiopsis S. Olsson, Enroth & D. Quandt, *gen. nov*.

Genus hoc simile generis *Echinodii* in Macaronesia, se praecipue cellulis alaribus non differentiatis, cellulis foliorum plerumque leviter mamillosis et seta gradatim verus capsulam inspissata differt. In Australasia distributum est.

Generitype: *Echinodiopsis hispida* (Hook. f. & Wilson) S. Olsson, Enroth & D. Quandt

Echinodiopsis hispida (Hook. f. & Wilson) S. Olsson, Enroth & D. Quandt, *comb. nov.*
Basionym: *Hypnum hispidum* Hook. f. & Wilson, London J. Bot. 3: 552. 1844; *Leskea hispida* (Hook. f. & Wilson) Mitt., J. Linn. Soc. Bot. 4: 91. 1859; *Echinodium hispidum* (Hook. f. & Wilson) Reichardt, Reise Oestern. Freg. Novara Bot. 1(3): 127. 1870; *Thamnobryum hispidum* (Hook. f. & Wilson) Stech, Sim-Sim, Tangney & D. Quandt, Org. Div. Evol. XX: 9.

Echinodiopsis umbrosa (Mitt.) S. Olsson, Enroth & D. Quandt, *comb. nov.*
Basionym: *Leskea umbrosum* Mitt., J. Linn. Soc. Bot. 4: 92. 1859; *Echinodium umbrosum* (Mitt.) Jaeg., Ber. St. Gall. Naturw. Ges. 1876-77: 314. 1878; *Thamnobryum umbrosum* (Mitt.) Stech, Sim-Sim, Tangney & D. Quandt, Org. Div. Evol. XX: 9.

Echinodiopsis umbrosa* var. *glauco-viride (Mitt.) S. Olsson, Enroth & D. Quandt, *comb. nov.*
Basionym: *Hypnum glauco-viride* Mitt. in Hook. f., Handb. New Zealand Fl.: 473. 1867; *Sciaromium glauco-viride* (Mitt.) Mitt. in Seem., Fl. Vit.: 400. 1873; *Echinodium glauco-viride* (Mitt.) Jaeg., Ber. St. Gall. Naturw. Ges. 1876-77: 314. 1878; *Echinodium hispidum* var. *glauco-viride* (Mitt.) Dixon, New Zealand Inst. Bull. 3(5): 249. 1927; *Echinodium umbrosum* var. *glauco-viride* (Mitt.) S.P. Churchill, J. Bryol. 14: 129. 1986; *Thamnobryum umbrosum* var. *glauco-viride* (Mitt.) Stech, Sim-Sim, Tangney & D. Quandt, Org. Div. Evol. XX: 9.

Echinodiopsis is characterized by dark-green to blackish, dull, variably branched plants that thrive in shady, moist places and most often grow on rocks or soil, sometimes also on tree bases. The leaves are narrow and lanceolate or subulate from a triangular or an ovate base. The costa is very strong, long-excurrent in *E. hispida* and percurrent to short-excurrent in *E. umbrosa*. The leaf margins and apical parts of the lamina are at least partly bistratose. Alar cells are not differentiated. The pseudoparaphyllia are leaf-like. The plants are dioicous. The seta is red or reddish-orange, distinctly flares below the apophysis, which has immersed stomata, and the capsule orientation varies from reclinate to antitropous, sometimes homotropous. There is a well-differentiated annulus of 1-3 cell rows. The peristome is unreduced.

Stech et al. (2008) tabulated the morphological distinctions in the gametophytes of *Echinodium* s.str. and the two species placed here in *Echinodiopsis*. Most of the differences are rather relative, and the single clear-cut one is the well-differentiated alar cells in *Echinodium* vs. the non-differentiated alar cells in *Echinodiopsis*. There are also some differences in the sporophytes. In *Echinodiopsis* the capsules are mostly cernuous to pendulous, while in *Echinodium* they vary from nearly erect to horizontal (Hedenäs, 1992). The seta in *Echinodiopsis* distinctly flares below the apophysis. The stomata in *Echinodium* (at least in E. setigerum and E. renauldii, cf. Hedenäs 1992) are superficial, but in *Echinodiopsis* they are immersed (Churchill 1986; Bell et al.) . The differences between *Echinodiopsis* and its closest relative *Pendulothecium* were discussed above.

Touwia Ochyra, J. Bryol. 14: 103. 1986. Basionym: *Thamnium* Schimp. sect. *Parathamnium* M. Fleisch., Musci Fl. Buitenzorg 3: 930. 1908, *syn. nov*.; *Thamnobryum* Nieuwl. subg. *Parathamnium* (M. Fleisch.) Ochyra, J. Hattori Bot. Lab. 68: 301. 1990; *Parathamnium* (M. Fleisch.) Ochyra, Fragm. Flor. Geobot. 36(1): 77. 1991.

Touwia laticostata Ochyra, J. Bryol. 14: 103. 1986 (generitype).

Touwia elliptica (Bosch & Sande Lac.) S. Olsson, Enroth & Quandt, *comb. nov*. Basionym: *Porotrichum ellipticum* Bosch & Sande Lac., Bryol. Jav. 2: 70. 1863; *Thamnium ellipticum* (Bosch & Sande Lac.) Kindb., Hedwigia 41: 247. 1902; *Thamnobryum ellipticum* (Bosch & Sande Lac.) Nog. & Z. Iwats., J. Hattori Bot. Lab.

36: 470. 1972; *Parathamnium ellipticum* (Bosch & Sande Lac.) Ochyra, Fragm. Flor. Geobot. 36(1): 77. 1991.

Touwia negrosensis (E.B. Bartr.) S. Olsson, Enroth & Quandt, *comb. nov.* Basionym: *Thamnium negrosense* E.B. Bartr., Philipp. J. Sci. 68: 251. 1939; *Thamnobryum negrosense* (E.B. Bartr.) Z. Iwats. & B.C. Tan, Miscell. Bryol. Lichenol. 7(7): 152. 1977; *Parathamnium negrosense* (E.B. Bartr.) Ochyra, Fragm. Flor. Geobot. 36(1): 77.1991.

Homalia giraldii (Müll. Hal.) S. Olsson, Enroth & D. Quandt, *comb. nov.* Basionym: *Anomodon giraldii* Müll. Hal.,Nuov. Giorn. Bot. Ital. n. ser. 3: 117. 1896.

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CHAPTER 5

PHYLOGENETIC RELATIONSHIPS IN THE "*PINNATELLA*" CLADE OF THE MOSS FAMILY NECKERACEAE (BRYOPHYTA)

This study is submitted as: Olsson, S., Buchbender, V., Enroth, J., Hedenäs, L., Huttunen, S. & Quandt, D. Phylogenetic relationships in the "*Pinnatella*" clade of the moss family Neckeraceae (Bryophyta). Organisms Diversity & Evolution.

5.1 Abstract

The family Neckeraceae is composed of three distinct clades, of which two, i.e. the *Neckera* and *Thamnobryum* clades are well defined. The third clade consisting of species belonging to *Caduciella*, *Curvicladium*, *Handeliobryum*, *Himantocladium*, *Homaliodendron*, *Hydrocryphaea*, *Neckera*, *Neckeropsis*, *Pinnatella*, *Shevockia* and *Taiwanobryum*, is in the focus of this study. Based on sequence data from the plastid *trnS-rps4-trnT-trnL-trnF* cluster and the *rpl16* intron as well as nuclear ITS1 & 2 phylogenetic relationships of these genera are reconstructed. The nearest relatives of this clade are resolved shedding more light on the evolution of the family. The genera belonging to the clade and its generic composition are discussed, emphasizing the polyphyly and redefinition of *Pinnatella*, *Neckeropsis* and *Homaliodendron*. The positions of *Touwia* and *Homalia* within the family are addressed in an additional analysis based on extended sequence data. We suggest several taxonomical changes including the description of the new genus *Circulifolium* (comprising the former *Homaliodendron exiguum* and *H. microdendron*).

5.2 Introduction

The pleurocarpous mosses are typically represented by a creeping, branching habit and are with around 5000 species a land plant group of considerable size. In pleurocarpous mosses the sporophyte development is restricted to the apices of short, lateral branches in contrast to most other mosses. According to the latest studies, the pleurocarpous mosses as defined by Bell et al. (2005) form a monophylum that can be divided into four orders: Hypnodendrales, Ptychomniales, Hookeriales and Hypnales, the Neckeraceae belonging to the latter. The Neckeraceae are consisting of mainly temperate and tropical species, and the species number is estimated to be around 200 (Enroth 1994b; chapters 1, 3 and 4). The species are epiphytic or epilithic, but there are some aquatic (rheophytic) ones as well.

In our previous studies (chapter 3) we dealt with the relationships of the Neckeraceae, the Lembophyllaceae and related taxa. We showed that the circumscription of

the Neckeraceae needs some adjustment and that the family can be divided into three distinct clades that we named the *Neckera* clade, the *Thamnobryum* clade and the "*Pinnatella"* clade, according to the most species-rich genus in each clade except the last one. The purpose of this study is to analyse in more detail the composition of the "*Pinnatella*" clade, which is not clearly characterized by only a single dominant genus, instead three major genera are *Pinnatella*, *Homaliodendron* and *Neckeropsis* located in this group.

The "*Pinnatella*" clade is mainly tropical (except *Handeliobryum*) and Asiatic, only *Pinnatella minuta* occurring in Africa and South America. The members of this clade usually have a strong costa and a long seta; a weak costa and immersed capsules are found only in some species of *Neckeropsis*; *Hydrocryphaea* and one species of *Homaliodendron*. The seta is often mammillose in its upper part, a character state shared by all *Pinnatella* species for which the sporophytes are known, *Taiwanobryum*, *Neckeropsis calcutensis*, *Neckera crenulata*, *Neckera himalayana* and occasionally by *Homaliodendron flabellatum*. The seta is consistently smooth in *Himantocladium*.

In our earlier phylogenetic analyses of the Neckeraceae the position of the *Homalia* clade (*Homalia lusitanica*, *Homalia trichomanoides* and *Homalia giraldii*) remained controversial. It was at one time resolved as sister to the "*Pinnatella*" clade (chapter 3) and sometimes to the *Thamnobryum* clade, but with low support (chapter 4). Morphologically the *Homalia* clade is heterogenic, and is not clearly belonging together with any of the bigger clades. Therefore, in this study we attempted to address the relationship of the *Homalia* clade within the Neckeraceae with additional analyses including five sequence markers.

5.3 Material and methods

Taxon sampling and molecular markers

The material used was taken from herbarium specimens; voucher numbers of the specimens and herbaria are listed in Table 1 together with authors of Latin names. In the analyses 58 taxa from 25 different genera were included. The ingroup species were selected based on previous classifications (e.g. Goffinet & Buck 2004; chapter 1), our earlier molecular analyses of a wider taxon sampling (chapter 3), as well as morphological characters, to cover the morphological variation within the study group as completely as possible. The outgroup species were selected from the other Neckeraceae clades that were resolved sister in our previous analyses (see chapter 3), and from the Lembophyllaceae, which are sister to the whole Neckeraceae (Quandt et al. in press; chapter 3). *Homalia webbiana*, *Heterocladium dimorphum* and *Heterocladium procurrens* are the most distant outgroups in this analysis. Since the sequence variation within the family turned out to be low, we chose for the phylogenetic reconstructions markers that are known to evolve fast: intern transcribed spacers 1 & 2 of nuclear ribosomal DNA, the plastid *rpl16* intron, as well as the plastid *trnT-trnL* and *trnL-trnF* intergenic spacers (IGS) and the *trnL*-intron.

To resolve the broader relationships of the "*Pinnatella*" clade and to pinpoint the position of *Homalia* (*Homalia trichomanoides*, *Anomodon giraldii* and *Homalia lusitanica*) and *Touwia*, an analysis with a reduced taxon sampling was conducted. This reduced data set is based on data from our previous study resolving the backbone phylogeny of the Neckeraceae (Quandt el al. in press; chapter 1) and modified by adding taxa relevant to the present study. The material used including voucher details and EMBL numbers of the specimens are listed in Table 2. The reduced data set contains two additional markers (*rps4* and *nad5*, see Olsson et al. chapter 1 for details and amplification strategy). Since some of the material is used in both of the analyses, the tables are partly overlapping. For the second set of analyses we utilized the same methods and settings as for the first one.

Table 1. List of taxa used in the study with EMBL or GenBank accession numbers for the sequenced or downloaded regions and voucher details. In two cases sequences were submitted to GenBank in previous studies and thus the accession numbers for *rps4-trnT-trnL-trnF* are composed of two different accession numbers. * denotes taxa for which nomenclatural changes are suggested in this article.

Species name	Herbarium	Voucher ID	GenBank accession		
			rps4 - trnT & trnL - trnF	*rpl16*	ITS1&2
Anomodon giraldii Müll. Hal = *Homalia giraldii* (Müll. Hal.) S. Olsson, Enroth & D. Quandt	H	H3194078	AM990342	FM210763	FM161075
Caduciella guangdongensis Enroth *	Enroth	Koponen et al. 57241	FM210281	FM160952	FM161083
Caduciella mariei (Besch.) Enroth	Enroth	Koponen 28035	FM210282	FM160953	FM161084
Camptochaete arbuscula var. tumida (Sm.) Reichardt.	H	Streimann 51408	AM990353	FM160955	FM161087
Chileobryon callicostelloides (Broth. ex Thér.) Enroth	H	H 3107865	FM210283	FM200841	FM161088
Curvicladium kurzii (Kindb.)Enroth	NYBG	Akiyama Th-85	FM210285	FM160959	FM161093
Dolichomitriopsis diversiformis (Mitt.) Nog.	H, MHA	Nedoluzhko s.n.	AM990362; *trnLF* AF397777	FM160963	FM161098
Echinodium hispidum (Hook. f. & Wilson) Reichardt	Buchbender	Downing s.n., 29.10.2000	FM210286	FM160964	FM161099
Forsstroemia producta (Hornsch.) Paris	H	Koponen 46545	FM201504	FM160967	FM161102
Handeliobryum sikkimense (Paris) Ochyra	H	Redfearn et al. 33981	FM210287	FM160969	FM161110
Heterocladium dimorphum (Brid.) Schimp.	H	H3212307	AM990376	FM160970	FM161115
Heterocladium procurrens (Mitt.) A. Jaeger	H	H3212289	AM990379	FM160973	FM161118
Himantocladium cyclophyllum (Müll. Hal.) M. Fleisch. *	NYBG	Redfearn Jr. 36081	FM210288	FM160974	FM161120
Himantocladium implanum (Mitt.) M. Fleisch.	NYBG	De Sloover 21124	FM210289	FM160975	FM161121
Himantocladium plumula (Nees) M. Fleisch.	H	Tan et al. 92-232	AM990381	FM160976	FM161122
Homalia lusitanica Schimp.	B	B275202	AM990383	FM160978	FM161124
Homalia trichomanoides (Hedw.) Schimp.	Quandt	Olsson 105	AM990385	FM160980	FM161126
Homalia webbiana (Mont.) Schimp.	H	Müller K68	AM990387	FM160982	FM161127
Homaliodendron exiguum (Bosch & Sande Lac.) M. Fleisch *	B	B263509	AM990389	FM160984	FM161130
Homaliodendron flabellatum (Sm.) M. Fleisch.	H	H3071675	FM210290	FM160985	FM161132
Homaliodendron flabellatum (Sm.) M. Fleisch.	Enroth	Schwarz 3801	FM210291	FM160986	FM161131
Homaliodendron microdendron (Mont.) M. Fleisch. *	H	Redfearn, Jr. 35901	AM990390	FM160987	FM161133
Homaliodendron neckeroides Broth.	H	H3071953	FM210306	FM161015	FM161168
Homaliodendron scalpellifolium (Mitt.) M. Fleisch.	H	H3071976	FM210292	FM160989	FM161135
Hydrocryphaea wardii Dix.	H	Shevock 23460	FM210293	FM160992	FM161139
Lembophyllum clandestinum (H. f & W.) Lindb. in Par.	H	Vitt 29644	AM990401; *trnLF* AF397823	FM160996	FM161145
Neckera complanata (Hedw.) Huebener	Buchbender	Buchbender 204	AM990413	FM161005	FM161158
Neckera crenulata Harv. *	H	Long 33980	FM210297	FM161006	FM161159
Neckera crispa Hedw.	Buchbender	Buchbender 385	FM210298	FM161007	FM161160
Neckera himalayana Mitt.	B	B253876	FM210301	FM161010	FM161163
Neckera pennata Hedw.	H	H3203794	AM990414	FM161016	FM161169
Neckera polyclada Müll. Hal.	H	Koponen 45441	FM210307	FM161017	FM161170
Neckera warburgii Broth.	B	Bryo 253855	FM210311	FM161023	FM161176

Neckeropsis calcicola Nog.	H	Enroth 64632	AM990417	FM161025	FM161178
Neckeropsis calcutensis (M. Fleisch.) Enroth	H	H3212832	AM990418	FM161026	FM161179
Neckeropsis disticha (Hedw.) Kindb.	NYBG	Heras 901/93	FM210313	FM161027	FM161180
Neckeropsis fimbriata (Harv.) M. Fleisch.	Enroth	Schäfer-Verwimp 16212	FM210314	FM161028	FM161181
Neckeropsis gracilenta (Bosch & Sande Lac.) M. Fleisch.	S	B105716	FM210315	FM161029	FM161182
Neckeropsis nitidula (Mitt.) M. Fleisch.	S	B105713	AM990419	FM161030	FM161183
Neckeropsis undulata (Hedw.) Reichardt	B	B238406	FM210316	FM161031	FM161184
Pendulothecium punctatum (Hook. f. & Wilson) Enroth & S. He	S	Streimann 53845	AM990421	FM161033	FM161187
Pinnatella alopecuroides (Mitt.) M. Fleisch.	Enroth	Schäfer-Verwimp 16824	AM990423	FM161034	FM161188
Pinnatella ambigua (Bosch & Sande Lac.) M. Fleisch.	Enroth	Schäfer-Verwimp 16252	FM210317	FM161035	FM161189
Pinnatella anacamptolepis (Müll. Hal.) Broth. *	S	B104516	FM210318	FM161036	FM161190
Pinnatella foreauana Thér. & P. de la Varde	H	Linis 757-03	FM210319	FM161037	FM161191
Pinnatella kuehliana (Bosch & Sande Lac.) M. Fleisch.	Enroth	Müller S116	FM20150	FM161038	FM161192
Pinnatella makinoi (Broth.) Broth.	HIRO	Deguchi 36762	FM210320	FM161039	FM161193
Pinnatella minuta (Mitt.) Broth.	H	Rikkinen et al. 32	AM990424	FM161040	FM161194
Pinnatella mucronata (Bosch & Sande Lac.) M. Fleisch. *	S	Hedenäs MY92-22	AM990425	FM161041	FM161195
Pinnatella taiwanensis Nog.	Enroth	Koponen et al. 54169	FM210321	FM161042	FM161196
Porotrichodendron superbum (Taylor) Broth.	H	H3121100	AM990427	FM161043	FM161198
Porotrichum fruticosum (Mitt.) A. Jaeger *	H	Shevock 28269	AM990430	FM161047	FM161202
Rigodium pseudothuidium Dusén	H	H3134254	AM990438; *trnLF* AF543547	FM161051	-
Shevockia inunctocarpa Enroth & M.C.Ji	Enroth	Shevock 25325	FM210323	FM161052	FM161212
Taiwanobryum robustum Veloira	H	Taiwan 1544	AM990441	FM864218	FM161215
Taiwanobryum speciosum Nog.	H	Enroth 64877	AM990442	FM161055	FM161216
Thamnobryum alopecurum (Hedw.) Nieuwl. ex Gangulee	Buchbender	Buchbender s.n. 11.7.2003	AM990444	FM161056	FM161218
Thamnobryum ellipticum (Bosch & Sande Lac.) Nieuwl. = *Touwia elliptica* (Bosch & Sande Lac.) S. Olsson, Enroth & Quandt	Enroth	Müller S114	FM210325	FM161058	FM161220
Thamnobryum maderense (Kindb.) Hedenäs	S	B44108	AM990445	FM161061	FM161223
Thamnobryum negrosense (E.B. Bartram) Z. Iwats. & B.C. Tan = *Touwia negrosensis* (E.B. Bartr.) S. Olsson, Enroth & Quandt	H	Schäfer-Verwimp & Verwimp 16852	FM210327	FM161063	FM161225
Thamnobryum pumilum (Hook. & Wilson) B.C. Tan	B	B268163	FM210329	FM200843	FM161227
Touwia laticostata Ochyra	Quandt	Cairns 27.8. 2005	FM210330	FM161070	FM161233
Weymouthia mollis (Hedw.) Broth.	CHR, Quandt	99-Mo2	AM990452	FM161072	FM161237

Table 2. List of specimens used in the study including EMBL or GenBank accession numbers for the sequenced or downloaded regions and voucher details. In two cases sequences were submitted to GenBank in previous studies and thus the accession numbers for *rps4-trnT-trnL-trnF* are composed of two different accession numbers. * denotes sequences from differing specimens obtained from the GenBank.

DNA no	Species name	Herbarium	Voucher ID	GenBank accession				
				rps4 - trnF	***rps4***	***rpl16***	***nad5***	**ITS**
B141	*Anomodon giraldii = Homalia giraldii*	H	H3194078	AM990342		FM210763	FM161240	FM161075
SH10	*Camptochaete arbuscula* var. tumida (Sm.) Reichardt.	H	Streimann 51408	AM990353	AY908330*	FM160955	FM161249	FM161087
B617	*Chileobryon callicostelloides* (Broth. ex Thér.) Enroth	H	H 3107865	FM210283	FM882222	FM200841	FM882226	FM161088
B423	*Cryptoleptodon longisetus* (Mont.) Enroth	H	H3038483	AM990356	AY908260*	FM160957	FM161252	FM161091
B223	*Curvicladium kurzii* (Kindb.) Enroth	NYBG	Akiyama Th-85	FM210285	AY908266*	FM160959	AY908670*	FM161093
SH146	*Dolichomitriopsis diversiformis* (Mitt.) Nog.	H, MHA	Nedoluzhko s.n.	AM990362; *trnLF* AF397777	AY908329*	FM160963	FM161257	FM161098
B258	*Echinodium umbrosum* var. glaucoviride	Schäfer-Verwimp	Streimann 49634	EU434010	AY908269*	FM160965	AY908680*	EU477602
B196	*Forsstroemia trichomitria* (Hedw.) Lindb.	Buchbender	Streimann & Pocs 65120A	AM990365		FM160968	FM161260	FM161103
B349	*Heterocladium dimorphum* (Brid.) Schimp.	H	H3212307	AM990376		FM160970	FM161271	FM161115
B352	*Heterocladium procurrens* (Mitt.) A. Jaeger	H	H3212289	AM990379		FM160973	FM161274	FM161118
B310	*Himantocladium plumula* (Nees) M. Fleisch.	H	Tan et al. 92-232	AM990381		FM160976	FM161276	FM161122
B422	*Homalia glabella* (Hedw.) Schimp.	H	Townsend 93/291	AM990382		FM160977	FM161277	FM161123
B111	*Homalia lusitanica* Schimp.	B	B275202	AM990383		FM160978	FM161278	FM161124
B218	*Homalia trichomanoides* (Hedw.) Schimp.	Quandt	Olsson 105	AM990385	AY908276*	FM160980	FM161280	FM161126
B474	*Homalia webbiana* (Mont.) Schimp.	H	Müller K68	AM990387		FM160982	FM161282	FM161127
B110	*Homaliodendron exiguum* (Bosch & Sande Lac.) M. Fleisch	B	B263509	AM990389		FM160984	FM161284	FM161130
B230	*Homaliodendron flabellatum* (Sm.) M. Fleisch.	H	H3071675	FM210290	AY908271*	FM160985	AY908671*	FM161132
SH249	*Homaliodendron microdendron* (Mont.) M. Fleisch.	H	Redfearn, Jr. 35901	AM990390		FM160987	FM161285	FM161133
SH103	*Lembophyllum clandestinum* (H. f & W.) Lindb. in Par.	H	Vitt 29644	AM990401; *trnLF* AF397823		FM160996	FM161295	FM161145
B131	*Leptodon smithii* (Hedw.) F. Weber & D. Mohr	B	B268385	AM990403	AY908261*	FM160997	FM161297	FM161147
B193	*Neckera complanata* (Hedw.) Huebener	Buchbender	Buchbender 204	AM990413		FM161005	FM161305	FM161158
B128	*Neckera himalayana* Mitt.	B	B253876	FM210301	FM882219	FM161010	FM882223	FM161163
B347	*Neckera pennata* Hedw.	H	H3203794	AM990414	AY908265*	FM161016	-	FM161169
B250	*Neckera polyclada* Müll. Hal.	H	Koponen 45441	FM210307	FM882220	FM161017	FM882224	FM161170
B307	*Neckera remota* Bruch & Schimp. ex Müll. Hal.	S	B29895	AM990415		FM161018	FM161307	FM161171
SH301	*Neckera urnigera* Müll. Hal.	S	B15194	AM990416		FM161021	FM161308	FM161174
B247	*Neckeropsis calcicola* Nog.	H	Enroth 646	AM990417		FM161025	FM161309	FM161178
B138	*Neckeropsis calcutensis* (M. Fleisch.) Enroth	H	H3212832	AM990418		FM161026	FM161310	FM161179

B313	*Neckeropsis nitidula* (Mitt.) M. Fleisch.	S	B105713	AM990419		FM161030	FM161311	FM161183
B476	*Pendulothecium punctatum* (Hook. f. & Wilson) Enroth & S. He	S	Streimann 53845	AM990421		FM161033	FM161314	FM161187
B242	*Pinnatella alopecuroides* (Mitt.) M. Fleisch.	Enroth	Schäfer-Verwimp 16824	AM990423		FM161034	FM161315	FM161188
B150	*Pinnatella minuta* (Mitt.) Broth.	H	Rikkinen et al. 32	AM990424		FM161040	FM161316	FM161194
B309	*Pinnatella mucronata* (Bosch & Sande Lac.) M. Fleisch.	S	Hedenäs MY92-22	AM990425		FM161041	FM161317	FM161195
B294	*Porotrichodendron superbum* (Taylor) Broth.	H	H3121100	AM990427		FM161043	FM161319	FM161198
B098	*Porotrichum bigelovii* (Sull.) Kindb.	B	B230549	-		-	FM161320	-
B244	*Porotrichum bigelovii* (Sull.) Kindb.	H	Shevock & Kellman 27467	AM990428		FM161045	-	FM161200
B369	*Porotrichum fruticosum* (Mitt.) A. Jaeger	H	Shevock 28269	AM990430		FM161047	FM161322	FM161202
B559	*Rigodium pseudothuidium* Dusén	NYBG	NYBG 00892248	-	AM990437	-	FM161328	FM161210
Rp47	*Rigodium pseudothuidium* Dusén	H	H3134254	AM990438; *trnLF* AF543547	-	FM161051	-	-
B149	*Taiwanobryum speciosum* Nog.	H	Enroth 64877	AM990442	AY908272*	FM161055	FM161332	FM161216
B238	*Thamnobryum alopecurum* (Hedw.) Nieuwl. ex Gangulee	Buchbender	Buchbender s.n. 11.7.2003	AM990444	AF023834*	FM161056	FM161334	FM161218
B546	*Thamnobryum ellipticum = Touwia elliptica*	Enroth	Müller S114	FM210325	AY908270*	FM161058	AY908674*	FM161220
SH300	*Thamnobryum maderense* (Kindb.) Hedenäs	S	B44108	AM990445		FM161061	FM161335	FM161223
B148	*Thamnobryum subserratum* (Hook. ex Harv.) Nog. & Z. Iwats.	H	Enroth 64595	AM990446		FM161067	FM161336	FM161230
B429	*Thamnobryum tumidicaule* (K.A. Wagner) F.D. Bowers	H	H3141850	AM990447		FM161068	FM161337	FM161231
B261	*Touwia laticostata* Ochyra	Quandt	Cairns 27.8. 2005	FM210330	FM882221	FM161070	FM882225	FM161233
SH15	*Weymouthia mollis* (Hedw.) Broth.	H	Streimann 58249	-	-	-	FM161341	-
DQ	*Weymouthia mollis* (Hedw.) Broth.	CHR, Quandt	99-Mo2	AM990452	AY307014*	FM161072	-	FM161237

DNA isolation, PCR amplification and sequencing

DNA was extracted using the DNeasy® Plant Mini Kit from Qiagen (Qiagen GmbH, Germany) following the manufacturer's protocol. For details of the DNA extraction, PCR amplification of the ITS1-5.8S-ITS2 and the *rps4-trnT-trnL-trnF* cluster, purification protocols and sequencing strategies employed, see Olsson et al. (chapter 1). The amplification protocols for *rpl16* are described in Olsson et al. (unpubl.). The cleaned PCR products were sequenced by Macrogen Inc., South Korea (www.macrogen.com). Primer sequences were eliminated before depositing in EMBL, the corresponding accession numbers are listed in Table 1.

Sequence edition and phylogenetic analyses

Nucleotide sequences were edited manually and aligned using PhyDE® v0.995 (Kelchner 2000), based on the criteria laid out in Kelchner (2005) and Quandt and Stech (2005). The alignment process was straight forward due to low sequence length variation. The reported hairpin associated inversion in the *trnL-F* intergenic spacer (IGS) (Quandt et al. 2003a; Quandt et al. 2004a) was positionally isolated in the alignment and included in the analysis as reverse complement in order to gain information from substitutions within the detected inversion, as discussed in Quandt et al. (2000). Indels were incorporated as binary data using a simple indel coding (SIC) strategy (Müller 2005) as implemented in SeqState (Nixon 1999). Command files for using the parsimony ratchet (Müller 2007) were generated using PRAP2 (Swofford 2002) and executed in PAUP 4.0b10 (Huelsenbeck & Ronquist 2001). Ratchet settings were as followed: 10 random addition cycles of 200 iterations each, with 25% upweighting of the characters in the iterations. Heuristic bootstrap searches under parsimony were performed with 1000 replicates and 10 random addition cycles per bootstrap replicate.

Bayesian analyses were performed with MrBayes v3.1.2 (Olsson et al. submitted-b), applying the GTR+Γ+I model for the sequences data and the restriction site model for the binary indel partition. To allow for possible deviating substitution models for the different regions, the data set was divided into four partitions (partition 1: *rps4-trnF*; partition 2: nuclear DNA; partition 3: *rpl16*; partition 4: indels). The *a priori* probabilities supplied were those specified in the default settings of the program. Posterior probability (PP) distributions of trees were calculated using the Metropolis-coupled Markov chain Monte

Carlo (MCMCMC) method and the search strategies suggested by (Huelsenbeck et al. 2002b; Müller & Müller 2004). Ten runs with four chains (2.5 10^7 generations each) were run simultaneously, with the temperature of the single heated chain set to 0.1. Chains were sampled every 10 generations and the respective trees written to a tree file. Calculations of the consensus tree and of the posterior probability of clades were performed based upon the trees sampled after the chains converged (< generation 50 000). Consensus topologies and support values from the different methodological approaches were compiled and drawn using TreeGraph (chapter 1). The alignments and trees are provided on an appendix cd.

5.4 Results

Alignment and sequence analyses

Before analysing the matrix, 14 hotspots with poly-homonucleotide repeats were recognized and excluded from the analyses following Olsson et al. chapter 1. Hotspots were regularly distributed among the partitions: six hotspots were located in the plastid *rps4-trnF* region (H1-H6), while the rest of the hot spots were located in the nrDNA and the *rpl16* intron, with four in each region. The resulting alignment contained 3891 positions of which 1429 positions belong to the (*rps4)-trnT-trnL-trnF* partition, 1554 positions to the nuclear ribosomal partition and 908 positions to the *rpl16* partition. 3142 of the characters were constant and 434 characters were parsimony-informative. In the data matrix where the information based on indel coding was included, a total of 4416 positions were available. This additional data raised the parsimony-informative characters to 677 but the constant characters remained the same.

The second data set contained after the exclusion of the hotspots (in total 11 hotspots) 5222 positions of which 1916 positions belong to the *rps4-trnT-trnL-trnF* partition, 865 to the *rpl16* partition, 1281 to the nad5 region and 1160 to the ITS. 4477 of the characters were constant and 407 characters were parsimony-informative. When the information based on indel coding was included, the data matrix included 5568 positions (4485 constant and 549 parsimony informative).

Phylogenetic analyses

The parsimony analysis with indel coding retained 566 most parsimonious trees (length 2548, CI = 0.558, RI = 0.701), while analysis without indel coding retained 1440 most parsimonious trees (length 1595, CI = 0.562, RI = 0.720). The strict consensus tree of these showed no conflict with the results from the Bayesian inference, but had less resolution compared to the MrBayes tree. Therefore, only the MrBayes tree is illustrated in Fig. 1, with posterior probabilities (PP) indicated and complemented with bootstrap values (BS) of the parsimony analysis when applicable. Values resulting from analyses with an indel coding approach precede the values from analyses with the SIC-matrix excluded. Thus support values from the different analyses will be referred to in the text following this scheme (PPsic / PP / BSsic / BS).

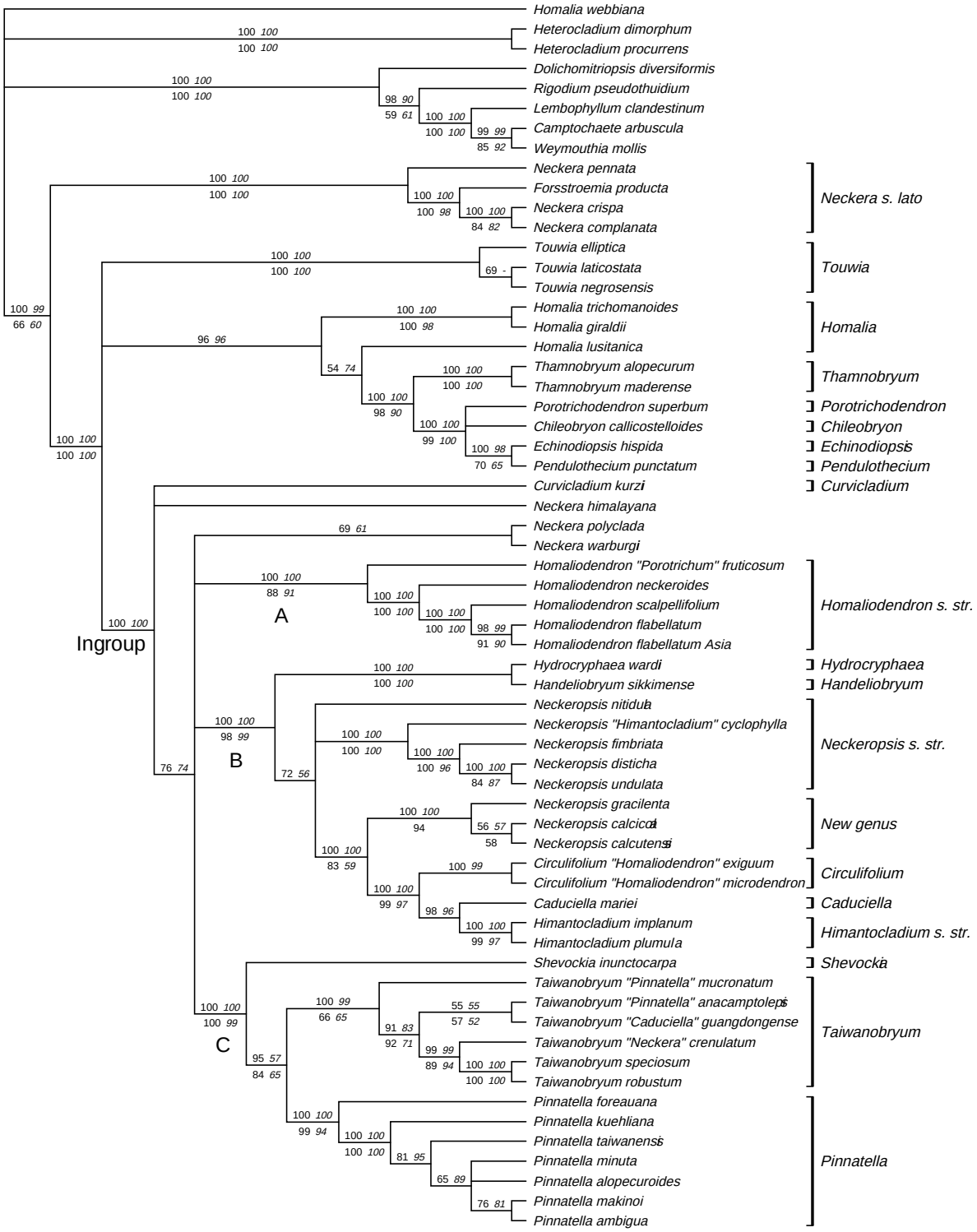

Figure 1. Phylogenetic relationships of selected Neckeraceae taxa based on *rps4-trnT-trnL-trnF*, *rpl16* and ITS1 & 2 sequences. The PP values from the MrBayes analyses (with indel coding first, then without indel coding) are indicated above, the bootstrap values of the parsimony analysis below when applicable (with indel coding first, then without indel coding).

The outgroup species belonging to the Neckeraceae form the following clades: a fully supported *Neckera*-clade (PP 100, BS 100), a clade containing *Thamnobryum* among other genera (PP 100 /100, BS 98 / 90) and the genus *Touwia* well supported (PP 100 / 100, BS 100 / 100) but in an unresolved position, these groups being congruent with earlier studies Olsson et al. (chapter 3). The ingroup is well supported in the Bayesian analyses (PP 100) including species from *Caduciella*, *Curvicladium*, *Handeliobryum*, *Himantocladium, Homaliodendron, Hydrocryphaea*, *Neckera*, *Neckeropsis*, *Pinnatella*, the recently described genus *Shevockia*, and *Taiwanobryum*. The ingroup is divided into three clades, whereas *Curvicladium kurzii* and three *Neckera* species (*N. himalayana, N. polyclada* and *N. warburgii*) are located outside of these clades. The first clade is well supported (PP 100 / 100, BS 88 / 91) and includes *Homaliodendron* species (*H. neckeroides*, *H. scalpellifolium* and *H. flabellatum*) together with *Porotrichum fruticosum*. This grouping shows *Homaliodendron* to be polyphyletic, since some "*Homaliodendron*" species are found in the next clade. The second clade gets high support in both the Bayesian and parsimony analyses (PP 100 / 100, BS 98 / 99) and includes the monospecific genera *Hydrocryphaea* and *Handeliobryum*, as well as *Neckeropsis*, *Circulifolium*, *Caduciella* and *Himantocladium*. Even if all *Himantocladium* and *Neckeropsis* species are situated within this clade, the relationships within the clade render these genera non-monophyletic. Also *Caduciella* with just two species turns out to be polyphyletic, because one of the species referred to this genus is found in the next clade. The third clade is highly supported in the analyses (PP 100 / 100, BS 100 / 99). It includes all *Pinnatella* species as well as *Caduciella guangdongensis*, *Neckera crenulata*, *Shevockia inunctocarpa*, *Taiwanobryum speciosum* and *T. robustum*. *Shevockia inunctocarpa* is resolved as the sistergroup to the remaining species in the clade. The *Pinnatella* species are divided into two groups in a non-monophyletic way, the bigger group (*Pinnatella s. str*.) receiving good statistical support (PP 100 / 100, BS 99 / 94).

The results of the second set of analyses that were performed to resolve the relationships within the Neckeraceae in a wider frame and focusing on the problematic placement of *Homalia* and *Touwia* are illustrated in Figure 2. The results from the Bayesian analyses without indel coding suggests that *Homalia lusitanica* do form a clade together with *Homalia trichomanoides* and *Homalia giraldii*, but the support remains low (PP 74).

Furthermore, this analysis suggests that both *Touwia* and *Homalia* s. str. belong to the "*Pinnatella*" clade (i.e. the ingroup species treated in the current study), even if the position does not receive statistical support. The exact position of these taxa is a particularly difficult problem to solve, since even five markers used do not provide enough information for resolving their position reliable. The Bayesian analysis without an indel coding approach was the one with the highest resolution. Therefore this is the one shown in the Figure 2 completed with the support values from the other analyses. The support values from the different analyses will be referred to in the text following this scheme (PP / PPsic / BS / BSsic).

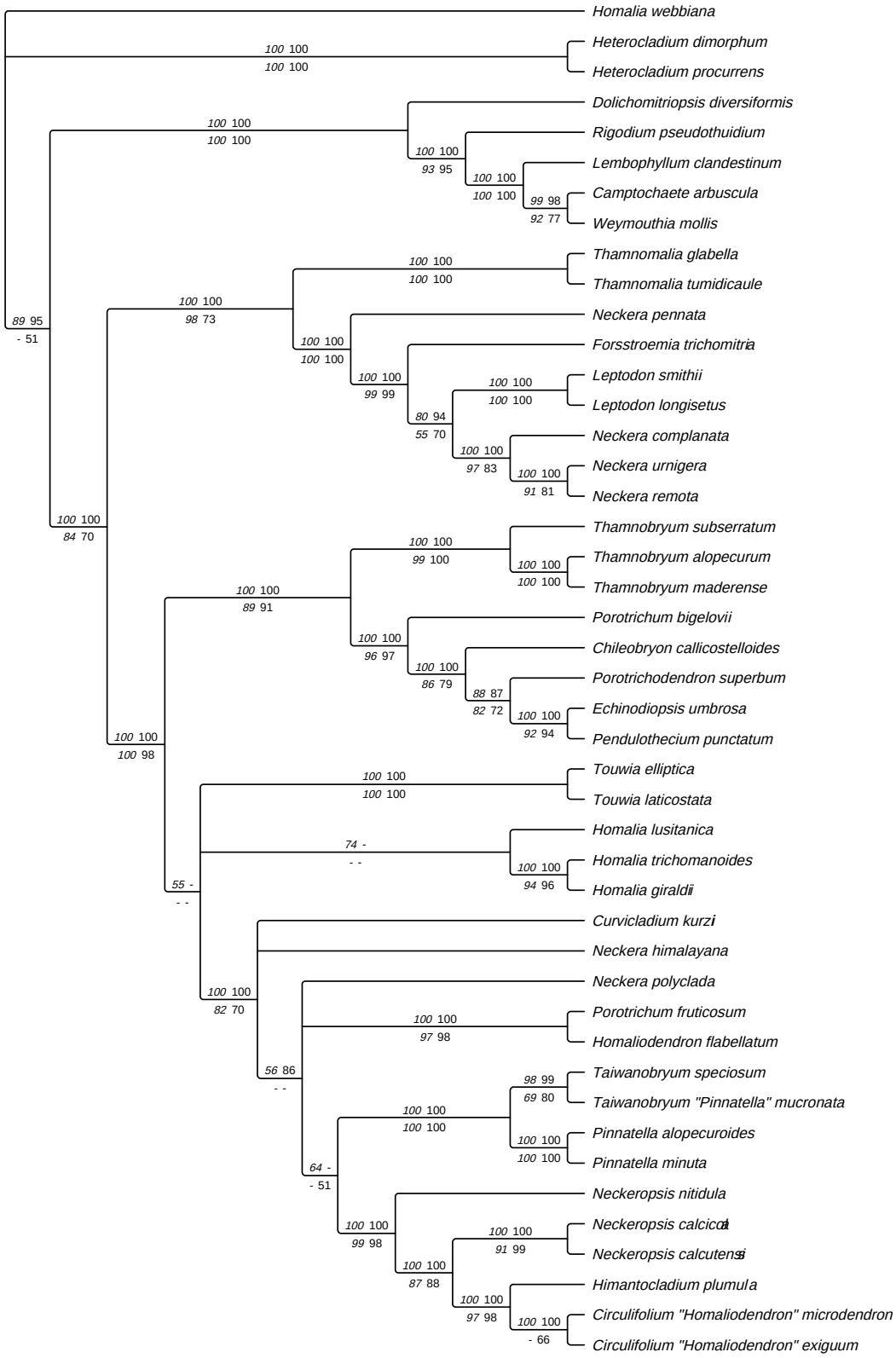

Figure 2. Analysis clarifying the relationships of *Homalia lusitanica* based on a combined data set based on *trnS-rps4-trnT-trnL-trnF*, *rpl16*, ITS1 & 2 and *nad5* intron sequences. The PP values from the MrBayes analyses (with indel coding first, then without indel coding) are indicated above, the bootstrap values of the parsimony analysis below when applicable (with indel coding first, then without indel coding).

5.5 Discussion

Phylogenetic analyses and taxonomic relationships

The analyses in chapter 3 resulted in a robust backbone structure for the Neckeraceae. This was used to guide the taxon sampling in further analyses and showed that more detailed analyses with additional molecular data were needed to resolve the circumscriptions of the genera belonging to the "*Pinnatella*" clade, since some genera (e.g. *Pinnatella* and *Neckeropsis*) appeared to be polyphyletic. The inclusion of *Anomodon giraldii* and the genus *Touwia* into the backbone data set clarified the branching order and the relationships of the sister groups of the "*Pinnatella*" clade. In the more detailed study, taxon sampling for the "*Pinnatella*" clade was increased and the phylogenetic relationships turned out to be more complicated than they appeared to be at the first glance, resulting in the loss of resolution in some branches. This is a natural consequence of adding more taxa and using fewer markers. However, no true conflicts exist between the results of our different analyses, since the apparently conflicting branches are not statistically supported, except for the position of the *Homalia* group. Our present results contradict our previous results (Ninh 1984) regarding the placement of *Homalia lusitanica*, since in the previous study this species formed a clade together with *H. trichomanoides* with maximum support. The analysis based on more extensive sequence data resolves this incongruence and supports a clade including the two *Homalia* species together with *Anomodon giraldii*. This demonstrates that even when a laborious sequencing effort was committed, resulting in an alignment including almost 4000 positions, additional sequence data are needed to resolve a few remaining questions regarding the phylogenetic relationships within the family.

Ingroup relationships and previous major treatments of the genera in the Pinnatella group

Based on the results of the present study, *Homaliodendron exiguum* and *H. microdendron* are not closely related to other *Homaliodendron* species. They should be placed in a separate genus; we therefore rise Fleischer´s (1905-1906) section *Circulifolia* to the generic level, and name it *Circulifolium.* Due to the polyphyly of *Caduciella*, *Himantocladium*, *Homaliodendron*, *Neckeropsis*, *Pinnatella*, *Shevockia* and *Taiwanobryum*, we also suggest some changes in their generic delimitations.

Clade A

Homaliodendron. *Homaliodendron* is a tropical genus, with the centre of diversity in Asia. Ninh (1989b) revised the Indonesian taxa and recognized ten species, but Enroth (1905-1906) found that some of them could not be distinguished from the highly variable and wide-ranging *H. flabellatum*. The genus was divided into the sections *Homaliodendron* and *Circulifolia* by Fleischer (1984), and Ninh (1989b) followed that notion. Given the apparent high variability of *H. flabellatum* (Enroth 1994b), the species number in *Homaliodendron* was estimated to be about 12 by Enroth (1984), with two species in section *Circulifolia* and the rest in section *Homaliodendron*. All species of *Homaliodendron* are stipitate-frondose (*H. exiguum* not distinctly so) and have appressed, usually overlapping stipe leaves. In the stems a central strand is not differentiated. The leaf dentation is very coarse in section *Homaliodendron*, in which there are large, multicellular teeth in the apical parts of the leaves, but in section *Circulifolia* the marginal teeth are small and unicellular. All species are dioicous. The seta is 1.5-4.5 mm long (Ninh 1984), smooth or in the upper part mammillose, and yellow. The capsules are exserted, orthotropous and symmetric and have 5 to 12 stomata in the apophysis (Olsson et al. submitted-b). The peristome is of the reduced neckeroid type. The lower dorsal plates of the exostome teeth are often somewhat cross-striolate, and their upper parts, as well as the endostome segments, are variably papillose. *Homaliodendron piniforme* (Olsson et al chapter 3), occurring in Africa and S America was shown not to belong within the Neckeraceae (Enroth 1989b).

Due to its polyphyletic nature *Homaliodendron* has to be divided into two groups. The generitype of the genus is *H. flabellatum*. *Homaliodendron scalpellifolium, H. neckeroides* and *Porotrichum fruticosum* group together with it forming the first group, *Homaliodendron* s. str. Our analyses included one *H. flabellatum* from the Philippines and one from Honduras. Previously the South American specimens have been named *H. decompositum* and the Asian ones *H. flabellatum,* but some researchers have claimed them to be one and same species (Brotherus 1929). Our data shows that they are closely related, but to determine the number of species more specimen should be included. *Homaliodendron neckeroides* was so named by Brotherus (1929; 1994), but recognized by Enroth and Tan (1929) as *Neckera neckeroides* based mainly on the sporophyte and

perichaetial leaf characters, especially the immersed capsule typical in *Neckera* but not encountered in any other species of *Homaliodendron*. According to our results, we suggest to use the original placement and name, *Homaliodendron neckeroides* (Ninh 1984).

Porotrichum fruticosum is resolved in a sister group relation to the *Homaliodendron* species, but differs from them mainly in the spreading rather than appressed stipe leaves and a much longer seta (over 1.5 cm when in the other species it does not exceed ca 4.5 mm). In addition, it has a higher (ca 130 μm) endostome basal membrane and reduced cilia between the segments, the latter lacking in the other *Homaliodendron* species. However, it differs much more from the rest of the *Porotrichum* species. It occurs only in the general Himalayan region while no other species of *Porotrichum* is known from Asia. Furthermore, the lack of a central strand in the stem (which is also lacking in *Homaliodendron*), the very thick-walled and porose laminal cells (also found in *Homaliodendron* s. str.) and the large composite marginal teeth in the leaves (present in some species of *Porotrichum* but much more pronounced in and typical of *Homaliodendron* s. str.) all suggest a close relationship with *Homaliodendron* s. str. Since it is clearly not justified to keep this species in *Porotrichum* or to establish a new genus for it, we suggest renaming it as *Homaliodendron fruticosum*.

Clade B

The members of the second group in *Homaliodendron*, *H. microdendron* and *H. exiguum,* belong to the clade B and a new genus is warranted to accommodate them. Fleischer (1905-1906) placed these two species in his *Homaliodendron* sect. *Circulifolia*, therefore we propose the name *Circulifolium*. They differ from *H. flabellatum* and its allies (cf. Ochyra 1986a; Enroth 1989b) in being typically smaller, having more strongly complanate leaves, in the minute, crenulate leaf dentation, in the filiform rather than leaf-like pseudoprapaphyllia, and in the relatively thin-walled, non-porose laminal cells. The sporophytes do not markedly differ.

Handeliobryum and Hydrocryphaea. In a detailed taxonomic analysis of *Handeliobryum*, Ochyra (1931) recognized only one species and placed it in the Thamnobryaceae. *Handeliobryum sikkimense* is a rheophytic moss growing in fast-flowing streams in the Himalayan region, including Yunnan in China. It is a very stout, rigid plant, with a

dendroid habit, well-differentiated stipe leaves, a very strong costa, and a bistratose leaf lamina with multistratose margins.

Hydrocryphaea was originally (Manuel 1975) placed in the Cryphaeaceae, as the generic name implies. Manuel (1999) thought it was related to the "thamnobryoid" Neckeraceae, a view agreed with by Enroth (Shevock et al. 2006). The single species, *H. wardii*, is known from North India, China (Yunnan), N Vietnam and N Laos, and recently several new locations have been spotted especially in Yunnan (Shevock et al. 2006). It grows at least periodically submerged in flowing water. It is a rigid plant with a strong, subpercurrent costa in the weakly limbate leaves. The seta is just up to 0.2 mm long, rendering the erect capsule deeply immersed among the perichaetial leaves. The peristome is reduced, basically of the "neckeroid" type, but there is no basal membrane in the endostome (Touw 1962).

Handeliobryum and *Hydrocryphaea* are both Asian taxa growing in flowing water and in the same general area. Even if some of the characters that the species share with each other may have been independently evolved due to the similar habitats, the molecular data support them being closely related. Yet their gametophytes differ (cf. Ochyra 1986; Shevock et al. 2006) so there is no justification for uniting the species in one genus, particularly since the sporophytes of *Handeliobryum* remain undescribed.

Neckeropsis. As currently defined, *Neckeropsis* is a pantropical genus with 27 species. The majority of the taxa are Asian (Touw 1972; Sastre-De Jesús 1987; Touw & Ochyra 1987; Ochyra & Enroth 1989), while there are four species in South America (Enroth & Magill 1994; Enroth 1995) and eight in Africa (Higuchi et al. 1989; Enroth 1993b). The section *Pseudo-Paraphysanthus* of *Neckeropsis* consists of rheophytic taxa with several morphological adaptations to the harsh environment (Touw 1962; Ochyra & Enroth 1989; Enroth 1999). In the papers cited above, the genus has been revised separately for South America, Africa and Asia-Oceania, but it has not been subject to rigorous phylogenetic analysis yet. *Neckeropsis* consists of non-stipitate (except *N. cyclophylla*), typically remotely and irregularly branched plants with a complanate, "pseudotetrastichous" (cf. Sastre-De Jesús 1987) foliation and lacking a central strand in the stem. The leaves may be undulate or not, and the leaf apex is mostly obtuse, rounded or truncate. The sexual condition varies according to species. Post-fertilization growth of the perichaetial leaves is

common and often considerable. In some species the perichaetial paraphyses become leaf-like and multiseriate; they have been called "ramenta" (e.g. Enroth 1990; Buck 1998). The seta is short, rendering the sporophytes immersed in most species. The capsules are erect and symmetric, and the peristome is of the reduced neckeroid type with spiculose-papillose exostome teeth and endostome segments, and wanting cilia. The generitype is *Neckeropsis undulata*.

According to our results, *Neckeropsis* is polyphyletic and divides into two groups. To the *Neckeropsis* s. str. belong *N. disticha*, *N. undulata,* and *N. fimbriata*. While *N. disticha* and *N. undulata* are synoicous, *H. cyclophyllum* and *N. fimbriata* are dioicous. All species in this group have a fairly strong costa, but the leaves may be distinctly undulate (*N. fimbriata, N. undulata*) or not. A synapomorphy shared by *N. disticha, N. undulata* and *N. fimbriata* is the presence of ramenta, or modified, leaf-like paraphyses. Such paraphyses are absent in *H. cyclophyllum* and in all species in the other "*Neckeropsis*" clade. There are, however, three more species in Asia that also have ramenta: *N. andamana*, *N. crinita* and *N. nano-disticha* (Touw 1962). It remains to be studied if those three also belong in *Neckeropsis* s. str. One feature that seems to be common to all species of *Neckeropsis* s. l. is the absence of apophysal stomata (Touw 1962), but that needs to be confirmed. The basal *Himantocladium cyclophyllum* is somewhat anomalous in this group, since it is stipitate, has non-auriculate leaves, has an exserted capsule with apophysal stomata, and lacks ramenta. However, the support for the group is maximal.

In the other group including *N. calcicola*, *N. gracilenta* and *N. calcutensis* all of these species are dioicous. The latter species was treated by Enroth (1994c) in *Neckeropsis*, but due to some morphological characters (especially the leaf areolation strongly reminiscent of *Pinnatella alopecuroides*) it was later treated in *Pinnatella* by Enroth (1906-1908). Although *Neckeropis* as currently circumscribed is clearly polyphyletic, we do not feel it justified to make any taxonomic rearrangements yet, mainly because our analysis contains only seven of the 27 species. Also, the group containing *N. calcicola*, *N. gracilenta* and *N. calcutensis* is morphologically very heterogeneous and more taxa must be sampled in it. Furthermore, *Neckeropsis nitidula* is closely related to the rest of the *Neckeropsis* species but remains in an unresolved position.

Himantocladium. The tropical genus *Himantocladium* was established by Fleischer (1992c) and revised by Enroth (1994a), who recognized eight species. The latter author subdivided the genus into the two sections *Himantocladium*, with five synoicous species, and *Cyclophyllum*, with three dioicous species. Later Enroth (1962) transferred one of the dioicous species (*H. warburgii*) back to its original genus *Neckera*, leaving *Himantocladium* with seven species. In our analysis *N. warburgii* forms a clade with *N. polyclada*, but the clade is in an unresolved position and the support for the clade is weak. *Himantocladium* is an Asian-Oceanian group, with just one species present in the Seychelles. A close relationship between *Himantocladium* and *Neckeropis* was emphasized by Touw (1989a) as well as Enroth (1991); they also discussed the generic distinctions. *Himantocladium* is characterized by the following combination of characters: stipitate-frondose plants, with the fronds usually branching sub-pinnately or pinnately; absence of a central strand in the stem; appressed, overlapping stipe leaves; fairly strong, single costa; absence of post-fertilization growth of the perichaetial leaves; a straw-yellow seta up to 2.0 (rarely 2.5) mm long; erect, symmetrical capsules that have 2-3 apophysal stomata; and a reduced, spiculose-papillose "neckeroid" peristome. The generitype is *Himantocladium implanum*. In the present paper we transfer *H. cyclophyllum* to *Neckeropsis*. This leaves *Himantocladium* with six species, just one of which (*H. formosicum*, endemic to Taiwan) is dioicous. Its relationships need further study.

Caduciella. *Caduciella* was described and placed in the Leptodontaceae by Enroth (1993a) to accommodate just one species, *Caduciella mariei*, previously known as *Pinnatella mariei*. A second species (*C. guangdongensis*) from SE China was described as new two years later (Fleischer 1905-1906). The total distribution area (of *C. mariei*) ranges from Tanzania to India and SE China, Thailand and Vietnam through Indonesia and New Guinea to Queensland in Australia; it is also known from Micronesia. The two species of *Caduciella* are small, stipitate-frondose plants, with overlapping and appressed stipe leaves. There is no central strand in the stem. The costa is single and reaches to midleaf or above and the leaf margins are entire or serrulate near the leaf apex. The leaf cells are in distinct rows and the pseudoparaphyllia are numerous and leaf-like. The species are also connected by the presence of caducous distal branch leaves, often leaving the branch tips naked. This

type of vegetative propagation is uncommon in the Neckeraceae as a whole. Sporophytes are unknown for both of the species.

According to the current analyses, *Caduciella mariei* is closely related to *Himantocladium implanum* and *H. plumula*. Due to the much smaller size, entire leaf margins, leaf areolation, numerous leaf-like paraphyllia, and caducous leaves we recognize *Caduciella* as a genus distinct from *Himantocladium* and encompassing only *C. mariei*.

Clade C

Pinnatella* and *Shevockia. The pantropical genus *Pinnatella* was established by Fleischer (1994c) and monographed by Enroth (1994). The latter author recognized 15 species, of which only *P. minuta* occurs in South America and continental Africa, the other species being mainly Asian-Oceanic. He subdivided the genus into the subgenera *Urocladium* with three species and *Pinnatella* with 12 species. The subdivision resulted from a cladistic analysis based on 44 morphological characters (see also Enroth 1989a). That analysis did not support an earlier subdivision by Enroth (1990), in which he established the section *Tenuinervia* for two species (*P. anacamptolepis* and *P. mucronata*) which, in contrast to the rest of *Pinnatella*, share a relatively weak costa and median laminal cells distinctly longer than the apical ones. The current number of species in *Pinnatella* is 13, since *P. calcutensis* actually belongs in *Neckeropsis*, a notion advocated by Enroth (2006) before the monographic study. *Pinnatella anacamptolepis* was transferred to the recently described genus *Shevockia* by Enroth and Ji (1981), but our current analysis does not support that. In general terms *Pinnatella* consists of stipitate-frondose plants, with usually pinnately to bi-pinnately branched fronds. The stipe leaves are distinctly differentiated, not overlapping and spreading. The laminal cells are short and the marginal cells quadrate to short-elongate in a few to several rows; the cell corners are often more or less elevated to form small papillae. The costa is single and strong, often reaching near the leaf apex. All species for which gametangia are known are dioicous and there is no post-fertilization growth of the perichaetial leaves. The seta is straw-yellow, 2.0-4.5 mm long, straight and mamillose in the upper part. The capsule is orthotropous and symmetric, with up to five phaneroporous stomata in the apophysis. The peristome is double, reduced (”neckeroid-type”), with densely spiculose papillose exostome teeth and endostome segments. There are no cilia in

the endostome. Vegetative propagation takes place through flagelliform, microphyllous branches produced in the leaf axils.

The genus *Pinnatella* becomes polyphyletic in the current study. Since *Pinnatella kuehliana* is the generitype, the clade including this species corresponds to *Pinnatella* s. str. *Shevockia inunctocarpa* is the only representative of its genus since *S. anacamptolepis* (synonym of *Pinnatella anacamptolepis)*, appears together with *Taiwanobryum*. Into this well-supported *Taiwanobryum* clade *Pinnatella mucronata*, *Pinnatella anacamptolepis*, *Neckera crenulata*, *Taiwanobryum speciosum*, *T. robustum* and *Caduciella guangdongensis* belong as well. Since *C. mariei* is the type species of the genus, *Caduciella guangdongensis* needs to be renamed. We suggest the transfer of all of these species into the genus *Taiwanobryum*.

Taiwanobryum. *Taiwanobryum* in its previous circumscription, with two species (*T. speciosum* being the generitype) occurs in East Asia, from Japan through Taiwan and SE China to the Philippines and Borneo. It has usually been placed in the Prionodontaceae (e.g. Buck & Goffinet 2000), but more recently in the Leptodontaceae by Buck and Goffinet (2002), who included only *Prionodon* in the Prionodontaceae. In the phylogenetic analysis by Tsubota et al. (1981), *Taiwanobryum speciosum* appeared in the Neckeraceae, close to *Pinnatella ambigua*. Lai and Koponen (2000) suggested a close relationship between *Taiwanobryum robustum* and *Neolindbergia* (*brassii*), based mainly on the peculiar gemmate-tipped, axillary rhizoids; however, *Neolindbergia* is currently placed in the heterogeneous Pterobryaceae (Enroth 1994c).

The gametophytic characters of the two species thus far constituting *Taiwanobryum* are very similar; the sporophyte of *T. robustum* remains unknown. The plants are relatively robust, sparsely branched plants with a poorly defined stipe, crowded, ovate-lanceolate leaves with coarsely toothed margins in the upper parts, a strong, single costa, strongly incrassate and, especially in *T. robustum*, porose walls of the laminal cells, an elongate seta that is mammillose in its upper part, an orthotropous, symmetrical capsule and a reduced peristome with papillose exostome teeth and no endostome.

Adding the four species *T. crenulatum, T. mucronatum, T. anacamptolepis* and *T. guangdongense* renders *Taiwanobryum* far more heterogeneous and difficult to define morphologically, especially relative to *Pinnatella*. The robust *T. crenulatum* fits relatively

well with *T. speciosum* and *T. robustum*, but the three other taxa pose problems in this grouping. Among themselves, they form a morphologically "acceptable" group, being relatively small, often densely branched, with a relatively weak costa mostly ending near midleaf, and slightly asymmetric leaves with mucronate apices. However, they also differ markedly from each other. For example, the stipe leaves of *T. mucronatum* are spreading and not overlapping, while in the two other species they are overlapping, squarrose in *T. anacamptolepis* and appressed in *T. guangdongense*. *Taiwanobryum mucronatum* has a stem central strand, while the two other species do not. The leaf cell walls of *T. anacamptolepis* are incrassate and porose, but thinner and non-porose in the other two. The pseudoparaphyllia of *T. anacamptolepis* and *T. guangdongense* are numerous, but *T. mucronatum* has much fewer of them. Of the three, the sporophyte is known only for *T. mucronatum*, and it much resembles that of *Pinnatella*, but has a clearly more strongly mammillose seta (Enroth 1994c).

5.6 Taxonomic and Nomenclatural changes

Circulifolium S. Olsson & Enroth, *gen. nov.*
Generitype: *Circulifolium microdendron* (Mont.) S. Olsson, Enroth & D. Quandt

Genus hoc ab *Homaliodendro* praecipue statura plantae minore, foliis valde complanatis, cellulis foliorum non porosis, dentibus unicellularis foliorum, apicibus foliorum rotundatis vel truncatis, apicibus obtusis foliorum perichaetialium et pseudoparaphylliis filiformibus differt.

Circulifolium exiguum (Bosch & Sande Lac.) S. Olsson, Enroth & D. Quandt, *comb. nov*. - Basionym: *Homalia exigua* Bosch & Sande Lac. in Dozy & Molk., Bryol. Jav. 2: 55. 1862; *Thamnium exiguum* (Bosch & Sande Lac.) Kindb., Hedwigia 41: 240. 1902; *Homaliodendron exiguum* (Bosch & Sande Lac.) M. Fleisch., Musci Fl. Buitenzorg 3: 897. 1908.

Circulifolium microdendron (Mont.) S. Olsson, Enroth & D. Quandt, *comb. nov.* - Basionym: *Hookeria microdendron* Mont., Ann. Sci. Nat. Bot. sér. 2(19): 240. 1843; *Hypnum microdendron* (Mont.) Müll. Hal., Syn. Musc. Frond. 2: 231. 1851; *Homaliodendron microdendron* (Mont.) M. Fleisch., Hedwigia 45: 78. 1906.

Neckeropsis cyclophylla (Müll. Hal.) S. Olsson, Enroth & Quandt, *comb. nov.* - Basionym: *Neckera cyclophylla* Müll. Hal., Syn. Musc. Frond. 2: 664. 1851; *Thamnium cyclophyllum* (Müll. Hal.) Kindb., Hedwigia 41: 224. 1902; *Himantocladium cyclophyllum* (Müll. Hal.) M. Fleisch., Musci Fl. Buitenzorg 3: 887. 1908.

Taiwanobryum mucronatum (Bosch & Sande Lac.) S. Olsson, Enroth & D. Quandt, *comb. nov.* -
Basionym: *Neckera mucronata* Bosch & Sande Lac. in Dozy & Molk., Bryol. Jav. 2: 68. 1863; *Porotrichum mucronatum* (Bosch & Sande Lac.) Broth., Monsunia 1: 49. 1899; *Thamnium mucronatum* (Bosch & Sande Lac.) Kindb., Hedwigia 41: 249. 1902; *Pinnatella mucronata* (Bosch & Sande Lac.) M. Fleisch., Hedwigia 45: 80. 1906.

Taiwanobryum anacamptolepis (Müll. Hal.) S. Olsson, Enroth & D. Quandt, *comb. nov.* -
Basionym: *Neckera anacamptolepis* Müll. Hal., Syn. Musc. Frond. 2: 663. 1851; *Thamnium anacamptolepis* (Müll. Hal.) Kindb., Hedwigia 41: 251. 1902; *Pinnatella anacamptolepis* (Müll. Hal.) Broth., Nat. Pflanzenfam. 1(3): 857. 1906; *Shevockia anacamptolepis* (Müll. Hal.) Enroth, J. Hattori Bot. Lab. 100: 74. 2006.

Taiwanobryum guangdongense (Enroth) S. Olsson, Enroth & D. Quandt, *comb. nov.* -
Basionym: *Caduciella guangdongensis* Enroth, Bryologist 96: 471. 1994.

Taiwanobryum crenulatum **(Harv.)** S. Olsson, Enroth & D. Quandt, *comb. nov* -
Basionym: *Neckera crenulata* Harv. *in* Hook., Icon. Pl. Rar. 1: 21. f. 6. 1836.

Homaliodendron fruticosum (Mitt.) S. Olsson, Enroth & D. Quandt, *comb. nov.* -
Basionym: *Porotrichum fruticosum* (Mitt.) A. Jaeger, Ber. Thätigk. St. Gallischen Naturwiss. Ges. 1875-76: 306, Sp. Musc. 2. 1877.

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University studies

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International experience

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PUBLICATION LIST

2008

OLSSON S, RUMSEY F, GRUNDMANN M, RUSSEL S, ENROTH J, QUANDT D. 2008. Origin of British and Macaronesian endemic *Thamnobryum* species (Neckeraceae). Journal of Bryology

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2005

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STENROOS S, MYLLYS L, HUHTINEN S., LAUKKA T, OLSSON S, MARSH T, LESONEN A, PUOLASMAA A, HYVÖNEN J & DÖBBELER P. Microfungi on mosses and lichens. Biodiversity framework MOSSE seminar, Espoo/ Finland.

ERKLÄRUNG

Ich versichere, dass ich die von mir vorgelegte Dissertation selbstständig angefertigt, die benutzten Quellen und Hilfsmittel vollständig angegeben und die Stellen der Arbeit, die anderen Werken im Wortlaut oder dem Sinn nach entnommen sind als Entlehnung kenntlich gemacht habe; dass diese Dissertation noch keiner anderen Fakultät oder Universität zur Prüfung vorgelegen hat. Die Bestimmungen der Promotionsordnung sind mir bekannt.