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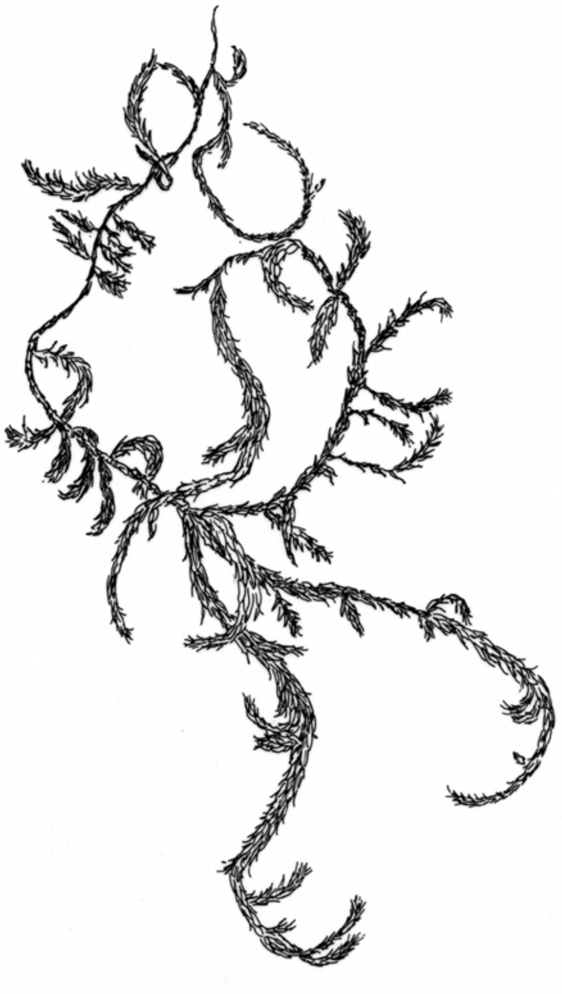
SANNA HUTTUNEN



Meteorium atrovariegatum

Helsinki 2004

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SANNA HUTTUNEN

Department of Biological and Environmental Sciences
University of Helsinki
Finland

Academic dissertation

To be presented with the permission of the Faculty of Biosciences
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on June 11th 2004 at 12 o'clock noon.

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This thesis is based on the following articles:

I Quandt, D., Huttunen, S., Streimann, H., Frahm, J.-P. and Frey, W. 2004: Molecular phylogenetics of the Meteoriaceae s. str.: focusing on the genera *Meteorium* and *Papillaria* — Molecular Phylogenetics and Evolution (in press).

II Huttunen, S. 2004: Bryophyte flora of the Hunan Province, China. 9. Meteoriaceae (Musci) I. *Chrysocladium, Duthiella, Meteorium, Pseudospiridentopsis, Sinskea, Toloxis,* and *Trachypodopsis* with identification key for Meteoriaceae in Hunan — Manuscript.

III Huttunen, S. and Ignatov, M. S. 2004: Phylogenetic analyses of Brachytheciaceae (Bryophyta) based on morphology, and sequence level data. — Cladistics 20: 151–183.

IV Quandt, D., Müller, K. and Huttunen, S. 2003: Characterisation of the chloroplast DNA *psb*T-H region and the influence of dyad symmetrical elements on phylogenetic structure. — Plant Biology 5: 400–410.

V Huttunen, S., Ignatov, M. S., Müller, K. and Quandt, D. 2004: Phylogeny and evolution of epiphytism in the three moss families Meteoriaceae, Brachytheciaceae and Lembophyllaceae. — Monographs in Systematic Botany (in press).

These are referred to in the text by their Roman numerals.

Contributions

The following table shows the major contributions of authors to the original articles or manuscripts.

	I	II	III	IV	V
Original idea	DQ, SH, HS	SH, TK	MI, SH	DQ	SH
Morphology	SH	SH	MI, SH	-	MI, SH
Molecular data	SH, DQ	-	SH	SH, DQ	SH, DQ
Manuscript preparation	DQ, SH	SH	SH, MI	DQ, KM, SH	SH, KM, DQ
Analyses:					
- Direct optimization and Nona analyses	SH	-	SH	-	SH
- ML and PAUP/PRAT analyses	DQ	-	-	DQ, KM	DQ
- Data exploration and/or analyses of phylogenetic structure	DQ	-	SH	DQ, KM	KM, SH

Initials refer to authors of the article in question, TK = Timo Koponen

Supervised by	Professor Timo Koponen Professor Jaakko Hyvönen University of Helsinki, Finland
Reviewed by	Dr. Lars Hedenäs Swedish Museum of Natural History, Stockholm, Sweden
	Dr. Jyrki Muona Finnish Natural History Museum, University of Helsinki, Finland
Examined by	Dr. Leena Myllys Department of Biology, University of Turku, Finland

Introduction

Pleurocarpous mosses are assumed to have radiated in forest biotopes dominated by angiosperms during the Tertiary, less than 65 My years ago (Krassilov & Schuster 1984, Buck 1991, Kürshner & Parolly 1999). The first moss with a growth habit resembling to that of pleurocarpous mosses is, however, a Permian *Rhizinigerites neuburgae* Meyen (Gomankov & Meyen 1987, Michael Ignatov, pers. comm.). Pleurocarpous species can be distinguished from other mosses by the position of their female reproductive structures. In pleurocarpy, the sporophytes develop on (reduced) lateral branches, while in most other mosses they are at the apices of shoots and hence terminate the apical growth (La Farge-England 1996). The forested habitats provided sheltered humid conditions, where the pleurocarpous mosses with continuous growth of the main shoot were able to form loose, weft-like moss colonies. This must have been an advantage in the competition for light and space on the floor layer of shady forests (Buck 1991). Today, this group with ca. 5000 species comprise half of all mosses (Buck & Goffinet 2000). Most pleurocarpous mosses belong to a monophyletic group forming the crown clade of all mosses, the division Bryophyta (De Luna *et al.* 2000, Newton *et al.* 2000, Goffinet *et al.* 2001, Shaw *et al.* 2003). Among Bryophyta the pleurocarpy is a derived, apomorphic character state which has evolved relatively recently, as the first ancestors of mosses date back to the lower Carbonifous era, ca. 350 mrd years ago (Thomas 1972). In addition to this crown clade, which includes the orders Hookeriales and Hypnales (Buck & Goffinet 2000), pleurocarpous mosses also exist in some other groups in Bryophyta. Thus this character is not a unique synapomorphy for this clade, but has evolved several times among mosses (Newton *et al.* 1999).

All the members of the Meteoriaceae and Brachytheciaceae, the families dealt with in this thesis, are pleurocarpous. In the traditional classifications, the pleurocarpous mosses are usually divided into three orders: Hookeriales, Hypnales, and Leucodontales (Fig. 1a; Brotherus 1925, Vitt 1984, Buck and Vitt 1986). These classifications rely mainly on the structure of the sporophyte generation and a few gametophytic characters. For example, in Buck and Vitt's (1986) classification, the Hypnales consisted mainly of terricolous species with a perfect "hypnalian" peristome (Fig. 2) and long leaf cells, while species in Leucodontales and Hookeriales were mostly epiphytes with erect capsules and reduced peristomes. The Leucodontales (= Isobryales by Brotherus 1925) were in Buck and Vitt's (1986) classification defined by a reduced "isobryalean" peristome (Fig. 2) and Hookeriales by a strong median furrow in the exostome, mitrate calyptrae, and ecostate leaves. In these classifications, the epiphytic Meteoriaceae with creeping shoots, erect capsules, and reduced isobryalean peristomes were placed in Leucodontales, and the mainly terricolous Brachytheciaceae with the perfect hypnalean peristome in Hypnales (Fig. 1a). The artificial nature of this kind of classifications was, however, already suspected before the first phylogenetic analyses of pleurocarpous mosses. Buck (1991) suggested that morphological character states delimiting the Leucodontales would be a result of convergent or parallel evolution due to similar habitats. Furthermore, Hedenäs (1989) pointed out the possible convergent origin of the reduced "isobryalean" peristomes. Thus, epiphytism, one of the character states defining Leucodontales, could have evolved in several independent lineages within pleurocarpous mosses, and this habitat shift would have led to the evolution of similar morphological character states in several epiphytic species.

The first phylogenetic analyses of family level relationships within pleurocarpous mosses were based on morphological data (Hedenäs 1995). Hedenäs (1995, 1996) noted the difficulties in defining the homologous characters between different groups of pleurocarpous mosses (for review see Hedenäs 1998). Probably due to the relatively simple structure of these plants, most of the character states appeared to have evolved repeatedly in different lineages (Hedenäs 1999a). This homoplastic evolution results in very complex patterns of character state combinations. This, in combination with the very low number of informative morphological characters, could hamper the phylogenetic analyses utilizing only morphological data (Hedenäs 1994, 1995, Buck *et al.* 2000b).

Despite difficulties, these first phylogenetic studies already showed that the traditional classification of pleurocarpous mosses into three orders: Hookeriales, Hypnales, and Leucodontales, did not describe the natural relationships (Hedenäs 1995). The Leucodontales were resolved as paraphyletic or polyphyletic grade, which could not be separated from Hypnales. This was later confirmed in

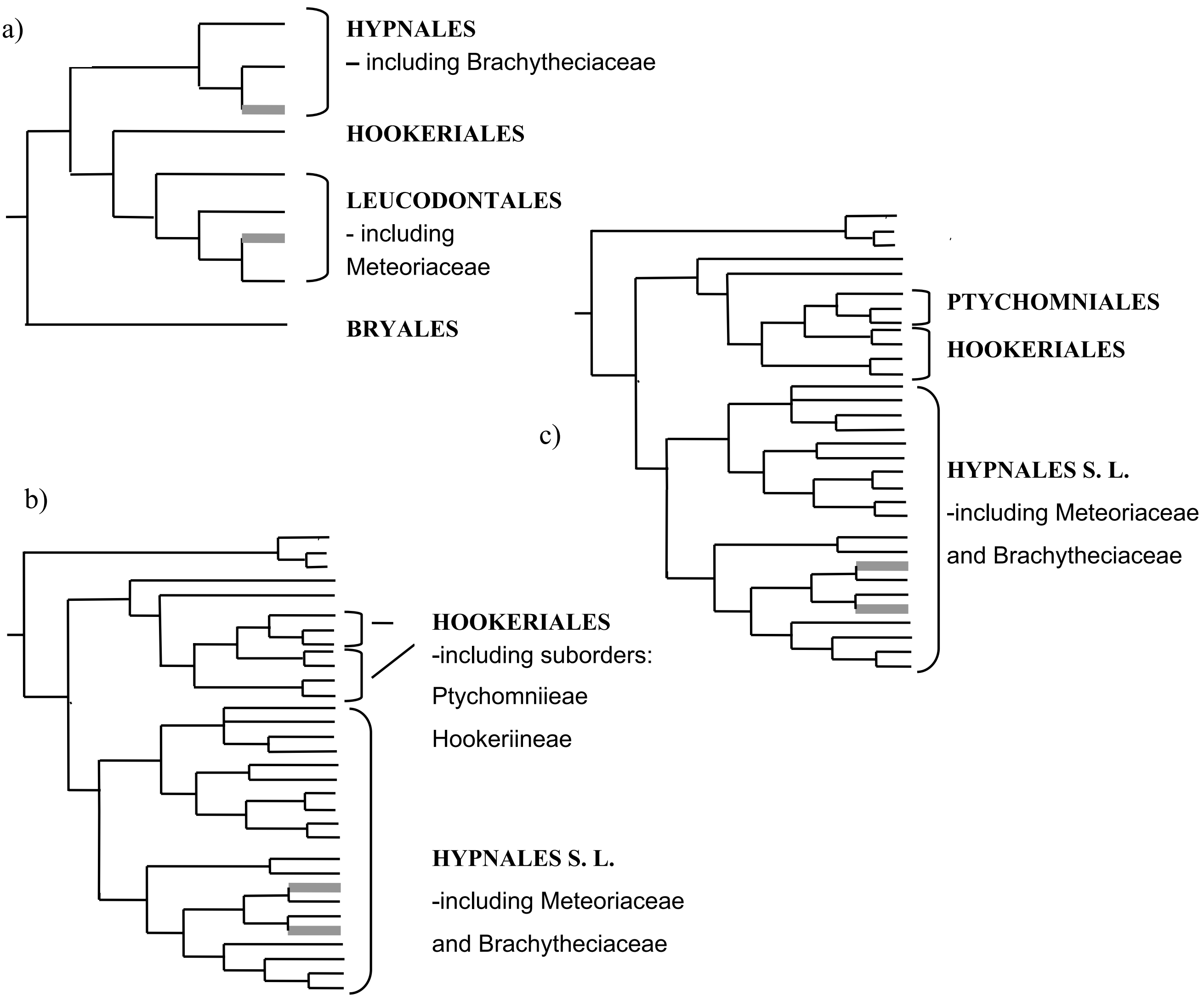


Figure 1. Phylogeny and classification of pleurocarpous mosses a) by Buck and Vitt (1986), b) Buck *et al.* (2000a) and Buck and Goffinet (2000), and c) Shaw *et al.* (2003). Although Buck and Vitt's classification is presented in form of phylogenetic tree, this classification is not based on phylogenetic analyses. Two latter phylogenies are based on analyses of DNA sequence data. The branches indicated with bolding present the position of the Meteoriaceae and Brachytheciaceae.

analyses based on molecular data (De Luna *et al.* 2000, Buck *et al.* 2000a), and the results were subsequently applied in a new classification by Buck and Goffinet (2000; Fig. 1b).

The most recent phylogenetic analyses of pleurocarpous mosses have revealed three major lineages: Ptychomniales, Hookeriales, and Hypnales (Fig. 1c; Shaw *et al.* 2003). The Hypnales, where both Meteoriaceae and Brachytheciaceae belong, is the largest order of mosses (Crosby *et al.* 1999, Buck & Goffinet 2000). Short branch lengths at the base of Hypnales (Buck *et al.* 2000a) and low DNA sequence variation (Shaw *et al.* 2002, Vanderpoorten *et al.* 2002) suggest that the Hypnales might have recently experienced a rapid radiation. Recently, Shaw *et al.* (2003) compared the phylogenetic diversity and molecular diversification rates between Hypnales and Hookeriales. Their results showed that despite the lower number of species in Hookeriales, molecular diversity is much higher within this order than is that of the species-rich Hypnales. Their study also supports the hypothesis that the Hypnales has experienced a relatively rapid diversification stage early in their history. The young age and the rapid evolution has also been suggested in studies dealing with Brachytheciaceae (McAdams 1982), and it is often suspected based on observations of the morphological variation within some hypnalean families. For example, taxonomy of the Brachytheciaceae, like some other families in Hypnales, is confounded with the high number of phenotypically plastic species, and this pattern might be due to recent and rapid speciation.

Research aim

Due to rapid radiation and the short branch lengths of cladograms, the phylogenetic relationships between families in Hypnales have mostly remained unresolved (Buck *et al.* 2000a, Shaw *et al.* 2003). In this thesis, both morphology and DNA sequence data from fairly rapidly evolving, non-coding genomic regions (Fig. 3) including several complex insertion and deletion (indel) events were used to resolve phylogenetic relationships within and between Meteoriaceae and Brachytheciaceae. The phylogeny was further used to study the morphological evolution evolution of epiphytism within these groups. To overcome the problems of ambiguous alignment of sequences, the direct optimization method as implemented in the program POY (Wheeler 1996, Wheeler *et al.* 2002) was used for phylogenetic analyses with parsimony as an optimality criterion. This method is computationally demanding compared to other methods, and thus analyses were run with parallel version of POY in computer clusters of the CSC, Center for Scientific Computing Ltd., Espoo, Finland.

In **I** and **II,** we aimed to test the monophyly of the Meteoriaceae and to evaluate morphological circumscription of the family and genera within it. In **III,** DNA sequence data and morphology provided the information for studies on the morphological evolution and relationships within Brachytheciaceae. This information is also crucial for improving the nomenclature of the family (Ignatov & Huttunen 2002). The *psb*T-H gene complex (Fig. 3b) is not yet widely used in phylogenetic analyses, and therefore its structure and phylogenetic utility in pleurocarpous mosses required further studies (**IV**). Comparisons of *psb*T-H sequences revealed a 9-bp inversion on the *psb*T-H spacer region, and assessment of positional homology was difficult for this region. The effect of different treatment of this region in phylogenetic analyses was tested in **III**. The last paper (**V**) concentrates on resolving the phylogenetic relationships between Meteoriaceae and Brachytheciaceae and detecting the evolution of epiphytism within these families. We test whether the hypothesis of phylogeny is altered by the convergent or parallel evolution of morphological characters that correlate with epiphytism. The direct optimization method was applied in all

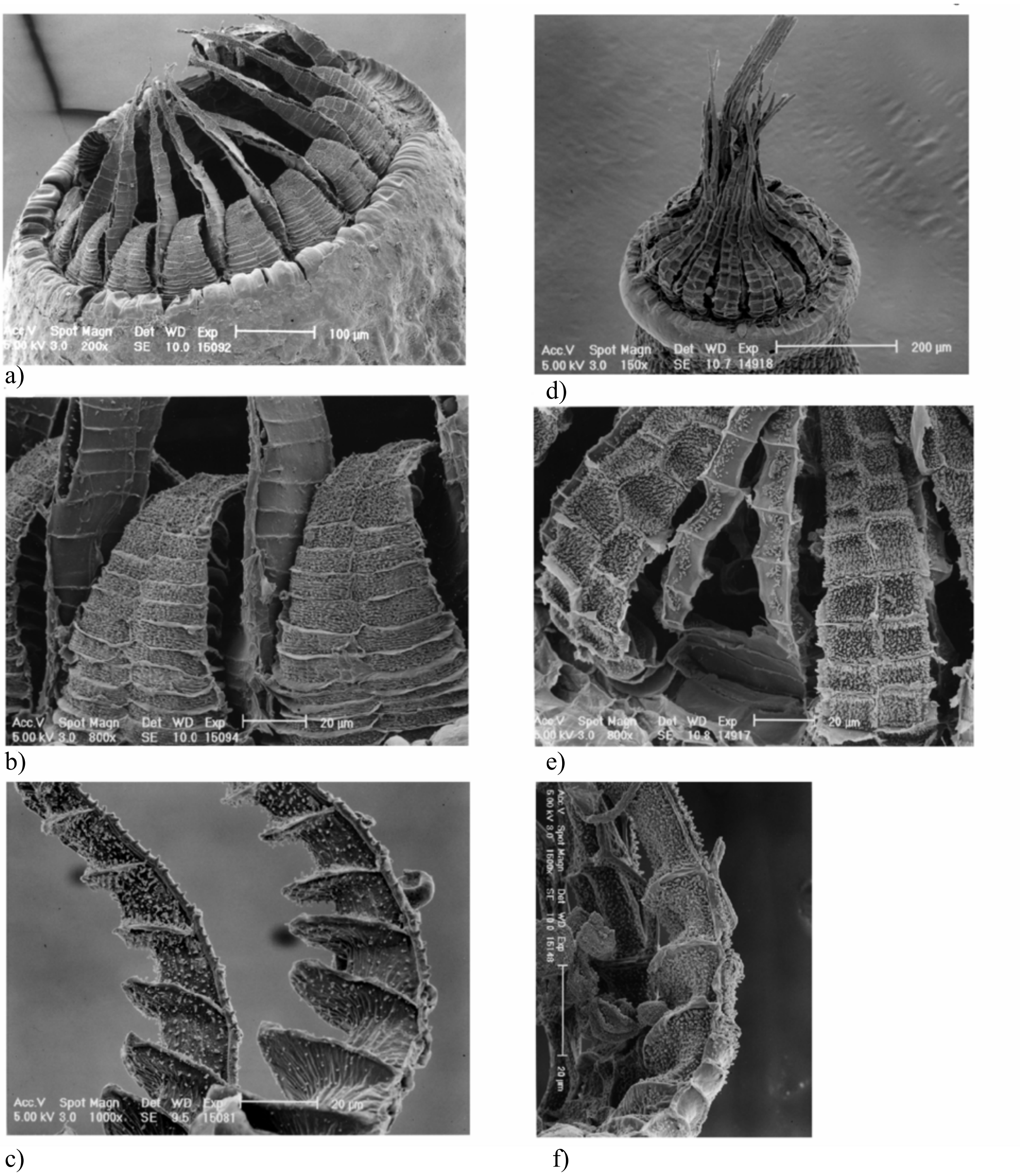
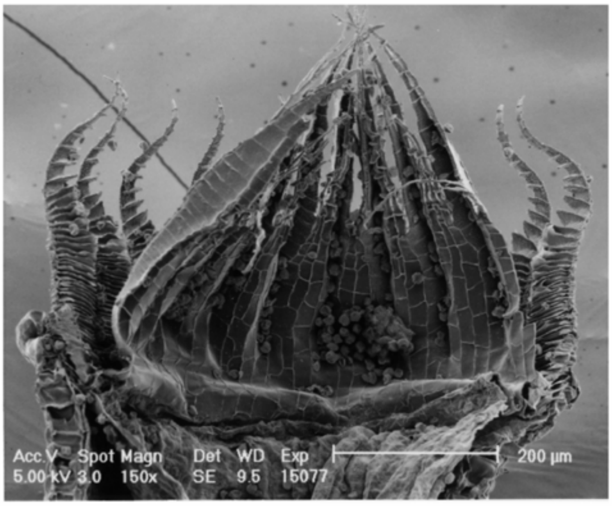


Figure 2. Perfect hypnoid peristomes (a-c and g-h) and specialized, isobryoid peristomes (d-f and i-k). a) Peristome and b) cross-striolate exostome outer surface in *Weymouthia mollis* (Hedw.) Broth. (Lembophyllacaeae) and c) elaborated trabecula on exostome inner surface in *Camptochaete arbuscula* (Sm.) Reichardt (Lembophyllaceae). d) Peristome and e) papillose exostome outer surface in *Trachypodopsis serrulata* (Palisot de Beauvois) M. Fleisch (Meteoriaceae), f) trabecula in *Neodicladiella pendula* (Sull.) W. R. Buck (Meteoriaceae).



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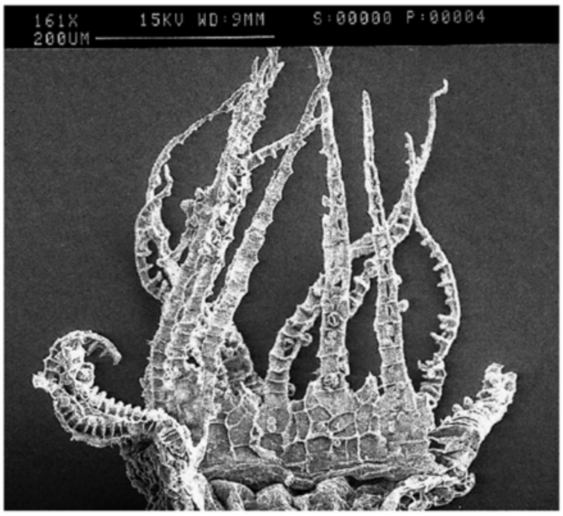

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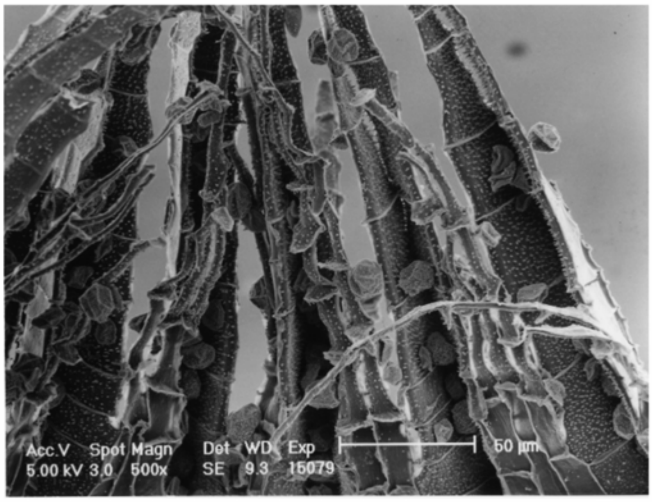

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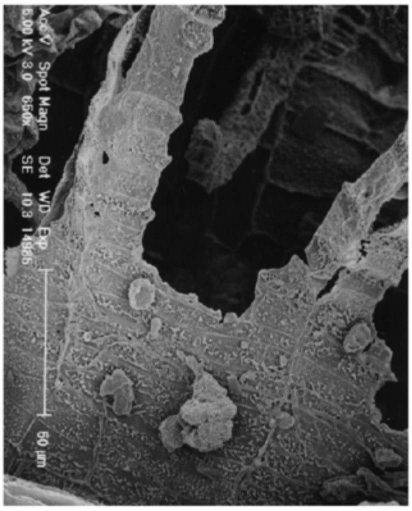
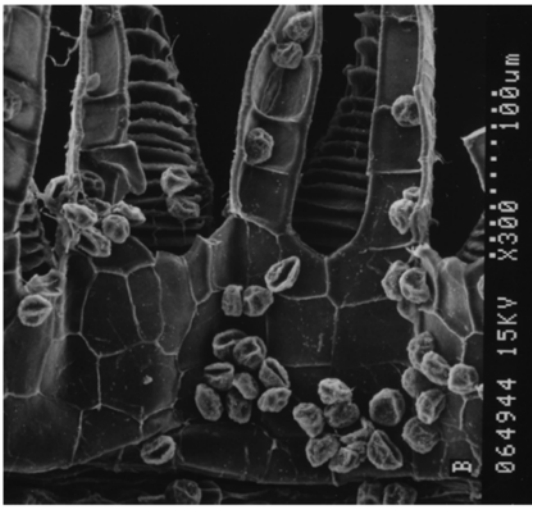


j)

Figure 2. continues. g) Endostome with high basal membrane, h) long cilia, and segments with narrow perforations in *Champtochaete arbuscula* (Sm.) Reichardt (Lembophyllaceae). i) Endostome with low basal membrane, reduced cilia which are absent at places, and segments with wide perforations in *Pseudobarbella levieri* (Renauldt & Cardot) Nog. (Meteoriaceae), j) cilia are lacking in *Aerobryopsis longissima* (Dozy & Molk.) M. Fleisch. (Meteoriaceae) and in *Meteoridium remotifolium* (Müll. Hal.) Manuel (Brachytheciaceae).



k)

phylogenetic analyses (**I**, **III**, **V**). This method is still fairly novel, and special attention is paid to its performance and results as compared to those obtained with more traditional methods (**I**, **V**).

Study groups

Meteoriaceae

Meteoriaceae consist of mainly epiphytic mosses occurring in humid tropical and warm temperate forests. Most of the Meteoriaceae are easy to recognize in the field as members of this family by their pendent habit, a character earlier regarded as important for delimitation of this family (Brotherus 1925, Noguchi 1976). Other gametophytic characters shared by most of the Meteoriaceae species include papillose leaf cells, monopodial growth, and a specific pattern of leaf-like pseudoparaphyllia that surround young branch primordia (Fig. 4). Otherwise, the morphological variation is fairly wide. Shoots may be either terete or complanate, and even in one plant, foliage and appearance of the shoots is plastic depending on the position of the shoot relative to the substrate. Leaf laminal cell shape varies from linear to rhomboidal and the leaf cell papillosity from uni- to pluripapillose. In the latter case, papillae may be either scattered over the cell lumen or arranged in rows over the lumen or cell walls. In sporophytes, seta length ranges from 1 mm to 2 to 3 cm. Capsules are most often erect and cylindrical to oblong, but in some cases inclined and almost globose. Peristomes in Meteoriaceae are usually described as “isobryoid” (Fig. 2d), but character states such as ornamentation of the outer surface of the exostome (Fig. 2e), height of the trabecula in the inner surface of the exostome (Fig. 2f), height of the endostomial cilia and basal membrane (Fig. 2i-j), structure of endostome segments (Fig. 2i), and the hygroscopic movements of the peristome still show a considerable variation within the family. The calyptra, which covers the developing sporophyte and later the capsule lid, is either hairy or naked.

The Meteoriaceae was originally established by Kindberg (1897) including only species occurring in North America: *Papillaria nigrescens* Swartz. (= *P. nigrescens* (Hedw.) A. Jaeger), *P. donnelii* Kindb. (= *P. nigrescens* (Hedw.) A. Jaeger) and *Meteorium pendulum* Sull. (= *Neodicladiella pendula* (Sull.) W. R. Buck). After this, Fleischer (1907) added several tropical genera and gave a detailed diagnosis for the family. In the first world-wide revision of the Meteoriaceae by Brotherus (1909), the family consisted of 13 genera, most of which are still in Meteoriaceae even in recent classifications (Table 1). By the second edition of *Natürlichen Pflanzenfamilien* (Brotherus 1925), active studies on tropical mosses had raised the number of genera to 18. Brotherus (1925) followed Fleischer’s (1907) division of Meteoriaceae and placed the genera in two subfamilies: Pilotrichelloideae and Meteorioideae. Pilotrichelloideae included species with smooth leaf cells and occasionally with double or without costa, while most of the species Meteorioideae had papillose leaf cells and a single costa which reaches up to mid-leaf. The family level classification in Brotherus (1925) was strongly affected by Fleischer’s work on tropical mosses (Fleischer 1907), and especially at higher taxonomical levels it mainly relied on sporophyte characters. After this “Brotherus–Fleischer” classification, the family concept remained fairly stable for decades (Table 1). The wide morphological variation within Meteoriaceae and pendent habit as the most important delimiting character, however, led to doubts about the naturalness of the family (Noguchi 1976, Norris & Koponen 1985, Buck 1994).

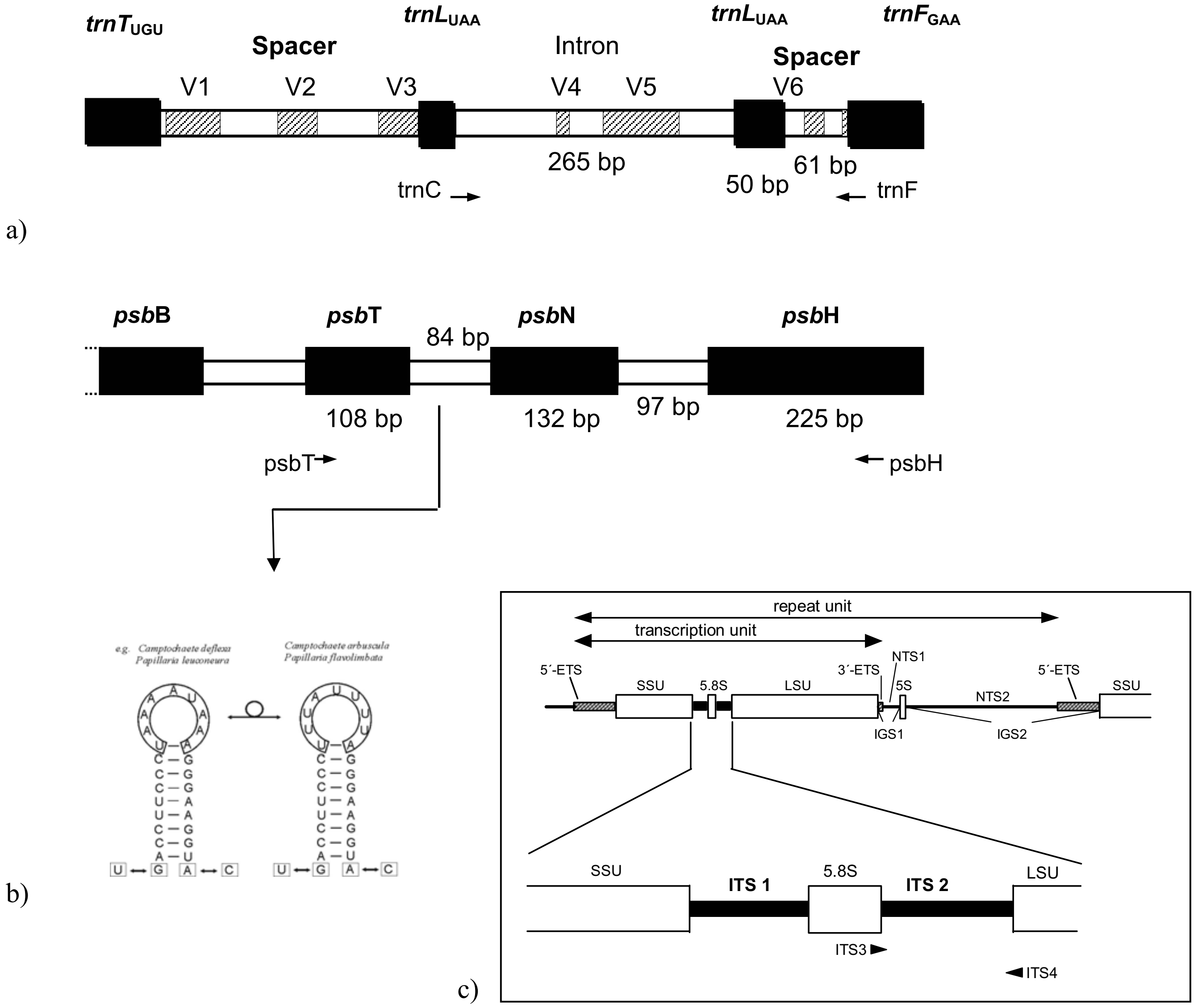
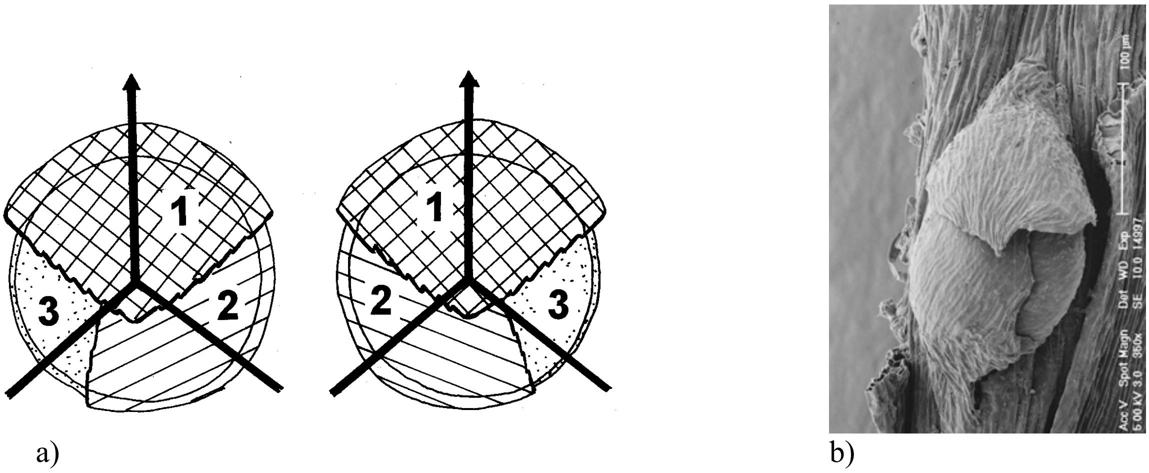


Figure 3. Overview of a) the chloroplast *trn*L–F region (Quandt & Stech 2004), b) the *psb*T-H gene complex; including a structural representation of the observed hairpin illustrating the 9 bp inversion found within the *psb*T-N spacer, and c) the nuclear ITS2 region (Quandt & Stech 2003) which were sequenced for phylogenetic analyses. Coding regions are represented by black boxes in a) and b) and with open boxes in c), and highly length-variable regions in *trn*L-F (V1-V7) by hatched boxes. Sizes with respect to *Meteorium polytrichum* Dozy & Molk. Primers and their orientation are indicated with small arrows.

The first steps to redefine the delimitation of Meteoriaceae were made by Buck (1994), and since then the family concept has gone through some major changes. Buck (1994) synonymized Meteoriaceae with Trachypodaceae M. Fleisch. including six genera, *Diaphanodon* Renauld & Cardot, *Duthiella* Müll. Hal., *Pseudospiridentopsis* (Broth.) M. Fleisch., *Pseudotrachypus* Thériot, *Trachypus* Reinw. & Hornsch. and *Trachypodopsis* M. Fleisch., which still increased the diversity of morphology in the family. The circumscription of Trachypodaceae was already suspected earlier, and Norris and Koponen (1985) suggested that at least *Diaphanodon*, *Trachypus,* and *Trachypodopsis* could be closely related to Meteoriaceae. Along with these family-level rearrangements, Mentzel (1992), Buck (1994), and Mentzel and Schultze-Motel (1994) suggested some new generic groupings within Meteoriaceae. *Papillaria* Lorentz, the largest genus of the family, was split into three: *Toloxis* W. R. Buck, *Cryptopapillaria* M. Mentzel, and *Papillaria.* Similarly, species in *Floribundaria* M. Fleisch.*,* *Barbella* M. Fleisch., and *Chrysocladium* M. Fleisch. were also divided into several genera following the sectional divisions. Buck (1994) also excluded genera such as *Dolichomitriopsis* Okam., *Meteoriella* Okam., *Pilotrichella* (Müll. Hal.) Besch.*,* *Pseudopilotrichum* (Müll. Hal.) W. R. Buck & Allen *,* *Squamidium* (Müll. Hal.) Broth., and *Weymouthia* Broth. from the family. Brotherus (1925) placed most of these in the subfamily Pilotrichelloideae and Fleischer (1907) in the tribe Pilotrichellaleae. Due to the fact that Buck (1994) suspected that the Meteoriaceae might be a lineage deriving from the Brachytheciaceae, he still retained some doubtful genera such as *Aerobryum* Dozy & Molk., *Meteoridium* (Müll. Hal.) Manuel, and *Zelometeorium* Manuel within Meteoriaceae.

Despite these rearrangements, the morphological delimitation of Meteoriaceae remained ambiguous, and monophyly of the newly circumscribed family remained untested. The first phylogenetic analyses, however, challenged the monophyly of the Meteoriaceae. Studies by both Hedenäs (1995) and Buck *et al.* (2000a) revealed the polyphyly of the family as traditionally circumscribed. At the same time as the first molecular phylogenies of pleurocarpous mosses (see Buck *et al.* 2000a), some pendent genera were transferred from Meteoriaceae to other families such as Brachytheciaceae (e.g., *Aerobryum*, *Zelometeorium*, *Meteoridium*) or Lembophyllaceae (*Weymouthia, Neobarbella* Nog.) (Crosby *et al.* 1999, Buck & Goffinet 2000, Table 1). The



a) b)

Figure 4. a) Arrangement of pseudoparaphyllia around young branch primordia in the moss families Meteoriaceae and Brachytheciaceae, b) pseudoparaphyllia in *Squamidium brasiliense* (Hornsch.) Broth., Brachytheciaceae. Schematic drawing (a) by M. Ignatov (1999).

number of Meteoriaceae species in these studies was, however, very low and hence it was impossible to evaluate the phylogenetic relationships within the Meteoriaceae or to confirm the monophyly of the family. The position of some poorly understood monotypic genera such as *Ancistrodes* Hampe, *Lepyrodontopsis* Broth., *Aerolindigia* M. Menzel, *Lindigia* Hampe, and *Cryphaeophilum* M. Fleisch. also remained uncertain.

Brachytheciaceae

Brachytheciaceae are in many respects almost the opposite of Meteoriaceae. Diversification of this family has probably taken place in temperate and boreal regions of the northern hemisphere where the majority of species occur. Brachytheciaceae is one of the largest families among pleurocarpous mosses, probably including 250 to 350 species and 41 genera (Ignatov & Huttunen 2002), although the latest checklist of mosses contains a total of 570 accepted species (Crosby *et al.* 1999). Most of the species grow in forested habitats on cliffs, rocks, decaying wood, and soil, but some epiphytic and aquatic species exist. Plants most often form relatively loose wefts, and the primary shoots may be creeping and attached to the substrate. Morphological delimitation of the Brachytheciaceae has been problematic, due to the homoplasy of almost all morphological characters (see **III**). Those character states which in the bryological literature are commonly connected to Brachytheciaceae include smooth, long, and narrow leaf cells, presence of single costa in leaves, long seta, horizontal “brachythecioid”-type asymmetrical capsules, a perfect hypnoid peristome with elaborated hygroscopic movements, and an exostome with a reddish colour in lower half. Most of these characters, however, are not present in all species, and it has been difficult to find any stable characters diagnostic for the family. In this situation it is slightly surprising that the family status has not been questioned, and the Brachytheciaceae has even been regarded as one of the best defined among larger pleurocarpous moss families (Hedenäs 1989, 1992).

The first classification in which groupings resembling Brachytheciaceae as circumscribed today is the one by Bridel (1827). Six of his 28 sections, namely *Praelonga*, *Illeceebra*, *Muralia*, *Velutina*, *Plumosa,* and *Rutabula*, include most of the species of Brachytheciaceae known at that time except those of *Homalothecium* Schimp. This genus was, due to its erect capsules and modified peristome structure, not regarded as closely related to other Brachytheciaceae in the early classifications (see, for example, Bruch *et al.* 1851–1855). Although Bridel’s classification was based on a mere intuitive view of similarity between species, it appeared to be very close to current knowledge of phylogenetic relationships (**III**). Due to the intuitive nature of this classification, however, later attempts to find diagnostic characters to delimit these groups were unsuccessful. Despite this, characters such as operculum shape, used by Bruch *et al.* (1851–1855) for generic delimitation, are still widely used as diagnostic characters for the genera in Brachytheciaceae (see Robinson 1962, Nyholm 1965, Buck 1998, Ignatov *et al.* 1999). After Bridel, a similar tendency towards intuitive classifications can be found in the treatments by Schimper (1876), who formally established the family Brachytheciaceae, and by Lindberg (1879), who was the first to include *Homalothecium* and *Palamocladium* in the Brachytheciaceae.

During the 20th century, several attempts were made to improve generic classification within Brachytheciaceae. The largest genera, *Brachythecium* Schimp., *Rhynchostegium* Bruch & Schimp., *Rhynchostegiella* (Schimp.) Limpr., and *Eurhynchium* Bruch & Schimp. were considered too heterogeneous, and they were either divided into sections (Kindberg 1897) or split into smaller entities (for example, Warnstorf 1905, Fleischer 1923, Grout 1928, Robinson 1962, 1967, 1987).

Table 1.
Circumscriptions of the Brachytheciaceae and the Meteoriaceae in some classifications of mosses and new delimitations for these families (Ignatov & Huttunen 2002, Huttunen & Quandt 2004) based on phylogenetic analyses presented in **I**, **III**, and **V**. Species which, according to current knowledge (Quandt *et al.* 2004), belong to Lembophyllaceae marked with L.

Brotherus 1909	**Brotherus 1925**	**Vitt 1981**	**Crosby *et al.* 1999**	**Buck & Goffinet 2000**	**Huttunen & Quandt 2004**
Meteoriaceae	**Meteoriaceae**	**Meteoriaceae**	**Meteoriaceae**	**Meteoriaceae**	**Meteoriaceae**
Aerobryidium	Subfam. Pilotrichelloideae	*Aerobryidium*	*Aerobryidium*	*Aerobryidium*	*Aerobryidium*
Aerobryopsis	*Cryphaeophilum*	*Aerobryopsis*	*Aerobryopsis*	*Aerobryopsis*	*Aerobryopsis*
Aerobryum	*Duseniella* (= *Ancistrodes*)	*Aerobryum*	*Ancistrodes*	*Ancistrodes*	*Barbella*
Barbella	*Pilotrichella* L	*Ancistrodes*	*Barbella*	*Barbella*	*Barbellopsis*
Duseniella (= *Ancistrodes*)	*Squamidium*	*Barbella*	*Barbellopsis*	*Barbellopsis*	*Chrysocladium*
Floribundaria	*Weymouthia* L	*Barbellopsis*	*Bryowijkia*	*Chrysocladium*	*Cryptopapillaria*
Lindigia	Subfam. Meteorioideae	*Chrysocladium*	*Chrysocladium*	*Cryphaeophilum*	*Diaphanodon*
Meteoriopsis	*Aerobryidium*	*Cryphaeophilum*	*Cryphaeophilum*	*Cryptopapillaria*	*Duthiella*
Meteorium	*Aerobryopsis*	*Dolichomitriopsis* L	*Cryptopapillaria*	*Diaphanodon*	*Floribundaria*
Papillaria	*Aerobryum*	*Floribundaria*	*Diaphanodon*	*Duthiella*	*Meteoriopsis*
Pilotrichella L	*Barbella*	*Isotheciopsis*	*Dolichomitriopsis* L	*Floribundaria*	*Meteorium*
Squamidium	*Chrysocladium*	(=*Neobarbella*) L	*Duthiella*	*Lepyrodontopsis*	*Neodicladiella*
Weymouthia L	*Floribundaria*	*Lindigia*	*Floribundaria*	*Meteoriopsis*	*Neonoguchia*
	Lindigia	*Meteoriopsis*	*Lepyrodontopsis*	*Meteorium*	*“Papillaria”*
	Meteoridium	*Meteorium*	*Meteoriopsis*	*Neodicladiella*	*Pseudospiridentopsis*
	Meteoriella	*Papillaria*	*Meteorium*	*Neonoguchia*	*Pseudotrachypus*
	Meteoriopsis	*Pilotrichella* L	*Neodicladiella*	*Pseudospiridentopsis*	*Sinskea*
	Meteorium	*Pseudobarbella*	*Neonoguchia*	*Pseudotrachypus*	*Toloxis*
	Papillaria	*Squamidium*	*Papillaria*	*Sinskea*	*Trachycladiella*
		Weymouthia L	*Pseudobarbella*	*Toloxis*	*Trachypus*
			Pseudospiridentopsis	*Trachycladiella*	
			Pseudotrachypus	*Trachypus*	
			Sinskea		
			Toloxis		
			Trachycladiella		
			Trachypodopsis		
			Trachypus		

Brotherus 1909	**Brotherus 1925**	**Vitt 1981**	**Crosby et al. 1999**	**Buck & Goffinet 2000**	**Ignatov & Huttunen 2002**
Brachytheciaceae	**Brachytheciaceae**	**Brachytheciaceae**	**Brachytheciaceae**	**Brachyteciaceae**	**Brachytheciaceae**
Brachythecium	*Brachythecium*	*Brachythecium*	*Aerobryum*	*Aerobryum*	Subfam. Homalothecioideae
Bryhnia	*Bryhnia*	*Bryhnia*	*Aerolindigia*	*Aerolindigia*	*Brachytheciastrum*
Camptothecium	*Camptothecium*	*Bryoandersonia*	*Bestia* L	*Bestia* L	*Eurhynchiastrum*
Catagoniopsis	*Catagoniopsis*	*Camptothecium*	*Brachythecium*	*Brachythecium*	*Homalothecium*
Cirriphyllum	*Cirriphyllum*	*Catagoniopsis*	*Bryhnia*	*Bryhnia*	Subfam. Brachythecioideae
Eriodon	*Eriodon*	*Cirriphyllum*	*Bryoandersonia*	*Bryoandersonia*	*Brachytheciella*
Eurhynchium	*Eurhynchiella*	*Cratoneurella*	*Bryostreimannia*	*Bryostreimannia*	*Brachythecium*
Homalotheciella	*Eurhynchium*	*Eriodon*	*Camptothecium*	*Cirriphyllum*	*Bryhnia*
Homalothecium	*Flabellidium*	*Eurhynchiella*	*Cirriphyllum*	*Clasmatodon*	*Eurhynchiadelphus*
Muyroclada	*Homalotheciella*	*Eurhynchium*	*Clasmatodon*	*Cratoneurella*	*Kindbergia*
Okamuraea	*Homalothecium*	*Flabellidium*	*Cratoneurella*	*Eriodon*	*Myuroclada*
Oxyrrhynchium	*Ishibaea*	*Helicodontiadelphus*	*Eriodon*	*Eurhynchiella*	*Sciurohypnum*
Pleuropus	*Lepyrodontopsis*	*Homalotheciella*	*Eurhynchiella*	*Eurhynchium*	*Scleropodium*
Ptychodium	*Mandoniella*	*Homalothecium*	*Eurhynchium*	*Flabellidium*	*Unclejackia*
Rhynchostegiella	*Myuroclada*	*Isothecium*	*Flabellidium*	*Homalotheciella*	Subfam. Rhynchostegielloideae
Rhynchostegium	*Oxyrrhynchium*	*Kindbergia*	*Homalotheciella*	*Homalothecium*	*Aerolindigia*
Rigodium L	*Pleuropus*	*Kurohimehypnum*	*Homalothecium*	*Isothecium* L	*Cirriphyllum*
Scleropodium	*Rhynchostegiella*	*Lepyrodontopsis*	*Isothecium* L	*Juratzkaeella*	*Clasmatodon*
Scorpiurium	*Rhynchostegium*	*Mandoniella*	*Juratzkaeella*	*Lindigia*	*Donrichardsia*
Stenocarpidium	*Sciuroleskea*	*Myuroclada*	*Kindbergia*	*Mandoniella*	*Eurhynchiella*
	Scleropodium	*Oxyrrhynchium*	*Lindigia*	*Meteoridium*	*Flabellidium*
	Scorpiurium	*Palamocladium*	*Mandoniella*	*Muyroclada*	*Helicodontium*
	Tomenthypnum	*Plasteurhynchium*	*Meteoridium*	*Nobregaea*	*Homalotheciella*
		Pseudopleuropus	*Myuroclada*	*Palamocladium*	*Juratzkaeella*
		Pseudoscleropodium	*Nobregaea*	*Plasteurhynchium*	*Mandoniella*
		Rhynchostegiella	*Oxyrrhynchium*	*Platyhypnidium*	*Meteoridium*
		Rhynchostegium	*Palamocladium*	*Pseudopleuropus*	*Nobregaea*
		Rozea	*Platyhypnidium*	*Pseudoscleropodium*	*Okamuraea*
		Schimperella	*Pseudopleuropus*	*Puiggariopsis*	*Oxyrrhynchium*
		Sciuroleskea	*Pseudoscleropodium*	*Rhynchostegiella*	*Remyella*
		Scleropodium	*Puiggariopsis*	*Rhynchostegium*	*Rhynchostegiella*
		Scorpiurium	*Rhynchostegiella*	*Rozea*	*Schimperella*
		Tomenthypnum	*Rhynchostegium*	*Schimperella*	*Squamidium*
			Rozea	*Scleropodium*	*Zelometeorium*
			Schimperella	*Scorpiurium*	Subfam. Rhynchostegioideae
			Scleropodiopsis	*Squamidium*	*Platyhypnidium*
			Scleropodium	*Steerecleus*	*Rhynchostegium*
			Scorpiurium	*Stenocarpidiopsis*	*Eriodon*
			Squamidium	*Trachybryum*	*Aerobryum*
			Steerecleus	*Zelometeorium*	*Pseudoscleropodium*
			Stenoacarpidiopsis		*Bryoandersonia*
			Trachybryum		*Eurhynchium*
			Zelometeorium		*Plasteurrhynchium*
					Palamocladium
					Scorpiurium

Only a few of these changes were, however, accepted. At the same time, studies of tropical mosses also raised the number of described species and genera in Brachytheciaceae. Mainly due to the addition of these small or monotypic genera, the size of the family increased from Brotherus' (1925) 20 to the 34 genera recognized by Vitt in 1984 (Table 1).

As mentioned above, the first phylogenetic analyses and utilization of molecular data in systematics of pleurocarpous mosses coincide with the period of changing views of morphological evolution. The morphology of pleurocarpous mosses was actively studied to find new characters to use in classifications. These studies revealed some overlooked characters which seem to be typical for the Brachytheciaceae and some of its close relatives (Hedenäs 1989, Ignatov 1999). The presence of some very stable clades in the first phylogenetic analyses of pleurocarpous mosses also enabled some revision of the family-level classification (Buck *et al.* 2000b, Buck & Goffinet 2000). For example, Brachytheciaceae was found to be one of the most consistent clades in analyses by Buck *et al.* (2000a), and their results suggest that in order to maintain monophyly of the family, inclusion of some tropical taxa like *Aerobryum*, *Meteoridium*, and *Zelometeorium* was necessary (Table 1, Crosby *et al.* 1999, Buck & Goffinet 2000). These genera had morphological characters rather atypical for Brachytheciaceae in the traditional sense: most of them were epiphytes, often with pendent habit and variably modified sporophyte structure. Due to these differences, they were in earlier classifications placed in Meteoriaceae, and their position among Brachytheciaceae was difficult to define.

Relationships of Meteoriaceae and Brachytheciaceae

In early classifications, Meteoriaceae and Brachytheciaceae are placed in different orders: Meteoriaceae in the order Leucodontales (Isobryales) and Brachytheciaceae in the Hypnales (Fig. 1a). These two families were hence not suspected to be closely related. Even 20 years ago any study project concentrating on resolving relationships between them would probably have been regarded by most bryologists as a mere waste of time. This was mainly due to the prevailing view that the sporophyte generation in mosses represents an evolutionary conservative structure, which is important in higher level classifications. Due to differences in these characters, close relationships between the Meteoriaceae and Brachyteciaceae were regarded as unlikely.

Despite this, in some cases it was difficult to decide whether a species should be placed in the Meteoriaceae or Brachytheciaceae. In revisions of the genus *Lindigia* Hampe (Meteoriaceae), Visnadi and Allen (1991) and Menzel (1991) relied only on sporophyte characters and separated *Aerolindigia* (*Rhynchostegiella*) *capillacea* (Hornsch.) M. Menzel (= *Lindigia capillacea* (Hornsch.) Hampe) and *L. debilis* (Mitt.) A. Jaeger even into different families (Table 1), although without sporophytes these species are almost indistinguishable. Later on, Buck (1994) and Ignatov *et al.* (1999) also raised the question of delimitation of Meteoriaceae and Brachytheciaceae. Buck (1994) transferred Meteoriaceae from Leucodontales to Hypnales and regarded it as closely related to the Brachytheciaceae. He suspected monophyly of the Meteoriaceae and proposed to merge it with Brachytheciaceae. Ignatov (1999) found that Meteoriaceae and Bracytheciaceae share a unique pattern of foliose pseudoparaphyllia, which are small leaf-like structures surrounding young branch primordia (Fig. 4). In these two families, branch primordia are covered by three pseudoparaphyllia, and the uppermost of them is always pointing towards the base of the shoot, while two others are arranged at a 120-degree angle. This character seems to be the only potential synapomorphy connecting these two families.

Changes in ordinal level classification of pleurocarpous mosses (Fig. 1b–c; Buck *et al.* 2000b, Buck & Goffinet 2000) confirmed the potentially close relationships between the Meteoriaceae and Brachytheciaceae. It now seemed possible that the epiphytic Meteoriaceae could have been derived from some terricolous Brachytheciaceae-type ancestor. Following Buck's (1994) ideas, typical features of Meteoriaceae, such as papillose leaf cells, pendent habit, short seta, and modified, hygrocastique peristome structure, were easy to explain as adaptations to epiphytic habitats (for details see **V**; Norris & Koponen 1985). All of these characters are common in pleurocarpous epiphytes, and they were shown to have evolved several times in different lineages within Hypnales s. l. (Buck *et al.* 2000a, De Luna *et al.* 2000). A sister group relationship between the Meteoriaceae and Brachytheciaceae, the family status of the Meteoriaceae, and the evolution of epiphytism within these two families remained, however, unstudied.

Material and methods

DNA sequence data and morphology as a source of information in phylogeny reconstruction

Use of DNA sequence data for detecting phylogenetic relationships is based on between-specimen differences in nucleotide composition in selected genomic regions. Because of the conservative nature of most coding DNA, more variable non-coding regions have become widely used, especially at lower taxonomic level studies in plant systematics. Among pleurocarpous mosses, most of the widely used coding DNA regions do not contain enough phylogenetic information for resolving family-level relationships. Because of this problem, we used the information on three mainly non-coding DNA sequence regions for phylogeny reconstruction: chloroplast *psb*T-H and *trn*L-F, and nuclear ITS2 (Fig. 3; **I**, **III**, **V**). In pleurocarpous mosses, these non-coding genomic regions are often used in studies concentrating on genus or family level (Shaw & Allen 2000, Vanderpoorten *et al.* 2003, Quandt *et al.* 2004), although in higher plants and often also in acrocarpous mosses, they are used for resolving species or even population-level relationships. These regions were sequenced a) with primers trnC and trnF for the *trn*L-F region (Fig. 3a; Taberlet *et al.* 1991), b) psbT and psbH for the *psb*T-H gene complex (Fig. 3b; Hong *et al.* 1995), and c) ITS3 and ITS4 for ITS2 (Fig. 3c; White *et al.* 1990). A total of 185 specimens were sequenced, and the sequences showed some length variation in all three regions (Table 2).

The sequenced *psb*T-H region consisted of partial *psb*T and *psb*H genes and the whole *psb*N gene between them, all of which code for small proteins of photosystem II. All length variation appeared to be in *psb*T-N and *psb*N-H spacers, which are situated between the coding regions. In *trn*L-F, length variation is concentrated in the *trn*L intron, which is situated within the anticodon loop of the tRNA$^{\text{Leu}}$. Some indel events have also occurred in the non-coding *trn*L-F spacer. Of all three regions, nuclear Internal Transcripted Spacer 2 (ITS2) between the ribosome coding 5,8S and 26S genes was the most variable.

In analyses of Brachytheciaceae (**III**) and of relationships of the Meteoriaceae and Brachytheciaceae (**V**) information from morphology was utilized together with molecular data for phylogeny reconstruction. In Brachytheciaceae, the morphological data matrix included 63, and in Meteoriaceae–Brachytheciaceae a total of 60 characters. Morphological information was in all species primarily based on the DNA voucher specimen. In most species, however, not all characters were available in these specimens, for example, due to lack of sporophytes. This was especially a

Table 2. Length variation (bp) in the analyzed ITS2 (nrDNA), and chloroplast *psb*T-H and *trn*L-F sequences; mean and standard deviation (SD) also shown.

	Length (bp)
ITS2	336 – 450 (372.2 SD 18.82)
*psb*T-H	505 – 533 (523.5 SD 3.22)
*trn*L-F	406 – 472 (422.5 SD 8.59)

problem in Meteoriaceae, where sporophytes are in many species rare or even unknown. In these cases, the information from voucher specimens was supplemented with information from other specimens which were morphologically as close as possible to the sequenced ones.

Phylogeny reconstruction and direct optimization

If the non-coding DNA strands include multiple indel events of different length, their alignment is a complex task. Usually several equally justified alignments exist even in fairly simple cases, and these different alignments may result in a different hypothesis of phylogenetic relationships (see Morrison & Ellis 1997). Despite this, most phylogenetic studies make no attempt to explore the effect of different alignments on phylogeny. In this thesis, unambiguous alignment of DNA sequences appeared to be impossible, mainly due to the extensive length variation of ITS2 sequences. To avoid subjectivity in alignment and to obtain the optimal hypothesis also of character transformations between DNA sequence data, direct optimization (Wheeler 1996) was utilized for phylogenetic analyses. This method requires no separate alignment step prior to analyses, but the search for optimal tree topology and character transformations are combined into one process. Traditionally, alignment of DNA sequences is an independent step preceding phylogenetic analyses, a step which attempts to identify homologous nucleotide positions between sequences from different taxa. The true homologies (i.e., synapomorphies originating from a unique transformation event in cladogram) in this alignment can be distinguished by running cladistic analysis (see de Pinna 1991), but this information is not used for improving the homology statements in the original alignment. In direct optimization, a separate alignment step is lacking, and “alignment”, meaning the matrix presenting optimal transformations between DNA sequences, is obtained only as a result of analyses. In this matrix, only shared synapomorphies are presented in each column, which distinguishes it from traditional alignments.

Parsimony served as an optimality criterion in all direct optimization analyses performed in this thesis (**I**, **III**, **V**). Thus, cladograms and character transformation series between DNA sequences that demanded the lowest number of evolutionary changes were regarded as the best hypothesis to explain the evolution of the groups studied. Direct optimization utilizes the character optimization in up and down passes, and using these procedures, the unaligned sequences are optimized on cladograms (Wheeler 1996, Wheeler *et al.* 2004). Transformations in sequence data can in this way be optimized on a tree by constructing hypothetical ancestral sequences in each node. During analyses, millions of topologies and character optimizations are tested to find the shortest tree(s) and the most parsimonious character transformations for DNA sequences. Unlike in traditional phylogenetic analyses of static alignments, homology between DNA sequences is dynamic, and single analyses may result in several hypotheses for character transformations in the data (“alignment”), each of which is connected to a different optimal topology. This property allows to study the effect of different homology statements on tree topology. Dynamic homology assessment also implies that adding taxa or characters also has the potential to affect putative homology statements. During the analyses, all data submitted will be co-optimized, and hence any change in available data may affect not only the topology obtained but also character transformations in all data partitions (except morphology). The most important fact is, however, that direct optimization for the first time enables search for the parsimonious hypothesis of character homology in connection with a search for the most parsimonious topology.

Currently, direct optimization is available only in one phylogenetic program, POY (Wheeler *et al.* 2002). This method is computationally extremely demanding compared to traditional parsimony methods, and to obtain reliable results even with moderately large data sets, parallel computing is necessary. In this thesis I applied parallel computing with eight processors in computer clusters at the Center for Scientific Computing Ltd. (CSC), Espoo, Finland in all direct optimization analyses (**I**, **III**, **V**). In order to save computation time, DNA sequences were first cut into shorter fragments within conserved regions by the program Winclada (Nixon 1999a). Then unaligned sequences were submitted to analyses together with a morphological data matrix, and analysed with direct optimization. Due to limitations of available computation time, each analysis included the maximum of 15 to 20 random addition sequences which each included 14 random addition builds (see Appendices in **I**, **III,** and **V**). Methods like branch swapping, (both tree connection bisection, TBR, and subtree pruning and rerafting, SPR), tree-drifting, and tree-fusing (Golobof 1999) were used to explore the tree space. To study the effect of different analysis parameters on the topology obtained, different gap costs were used (**I**, **III,** and **V**). Although this kind of sensitivity analyses (see Wheeler 1995) have achieved almost the status of a standard method for data exploration in direct optimization analyses, its value for pointing the most parsimonious results or for evaluating the stability of results of phylogenetic analyses has recently been criticized (Frost *et al.* 2001, Grant & Kluge 2003). I agree that the only weighting scheme resulting in a parsimonious result is the equal weighting of all characters. Exploration of different gap costs in this thesis (**I**, **III**) can thus now be deemed unnecessary.

Due to the novelty of the method, its results were also compared to those obtained with more traditional methods (**I** and **V**). In these studies, sequence data was aligned manually, using the DNA structure and mutational mechanisms as a background information for alignment (see Kelchner 2000, 2002, Borsch *et al.* 2003, Quandt 2002, **IV**). Data were analysed with parsimony (**I**, **V**) and maximum likelihood (**I**) as optimality criteria with the programs PAUP (Swofford 2002) and PRAP (Müller 2004) or NONA (Goloboff 1994) in conjunction with the Winclada (Nixon 1999a) shell. PRAP enables the use of the parsimony ratchet method (Nixon 1999b) in PAUP analyses, which has been shown to improve efficiency of the analyses, especially those of large data sets (Nixon 1999b, Goloboff 1999).

Tests of correlated evolution

The evolution of some morphological characters has been suspected to correlate with epiphytism (see **V**). This hypothesis was tested with the Concentrated Changes Test (CTT) by Maddison (1990), which utilizes results from phylogenetic analyses and aims to test whether character state changes in a dependent character (all morphological characters in the data matrix in **V**) are concentrated on branches of the cladogram where epiphytism (independent character) is present. For testing, character coding of all 60 morphological characters in **V** was transformed into binary state coding which increased the number of characters to 64. Correlation was tested for all of these characters with program MacClade 3.05 (Maddison & Maddison 1992).

Comparisons between results and robustness of the hypothesis on gap treatment and homoplastic characters

Due to differences in treatment and weighting of gaps (missing data or ”fifth” state with different weightings) and differing optimality criteria (parsimony vs. ML), not all tree lengths between analyses are comparable. To evaluate results in each paper (**I**, **III**, **V**), the Incongruence Length Difference metrix (ILD; Mickevich & Farris 1981, Farris *et al.* 1995) was applied. This is based on differences in tree lengths between combined analyses and analyses of individual data partitions, i.e. ILD = (tree length of combined data set - —tree lengths of individual data sets) / tree length of combined data set. ILD thus measures how well data from different sources (different genomic regions, morphological data) “fit” together, i.e. are congruent.

In phylogenetic analyses, the effect of ”noisy” or biased data on the phylogenetic hypothesis is a frequent concern for various reasons (see Wenzel & Siddall 1999). While noise, meaning here random homoplastic data, only rarely reduces the ability to recover the parsimonious topology, it still may affect the ”stability” of the hypothesis. Biased data due, for example, to adaptations to certain environmental conditions may instead have the potential to mislead the phylogenetic analyses. If the effect is less pronounced, it may also affect ”stability” or ”strength” of the clades. The analyses performed in this thesis, tested the contribution of the 9 bp inversion on the *psb*T-N spacer (Fig. 3b) on phylogeny reconstruction (**III**, **IV**). In addition, in article **V**, we confirmed that the parallel or convergent evolution of the characters correlated with epiphytism do not affect the hypothesis of relationships among Brachytheciaceae and Meteoriaceae (see also Hedenäs 1994, 1995, Buck *et al.* 2000b). Although exclusion of noisy or biased data is often recommended, discriminating between a ”correct” and a noisy or biased phylogenetic signal is usually impossible. The aim in the studies presented here (**III**, **IV**, **V**) was only to test whether this kind of data does affect the results of phylogenetic relationships, not to pinpoint characters to be excluded.

The contribution of the characters correlated with epiphytism (**V**) and the *psb*T-N loop region (**IV**) to phylogeny reconstruction was studied using the method developed by Kai Müller in **IV**. It is based on comparison of support for clades 1) in the analyses where doubtful characters are deleted and 2) in analyses where the same number of parsimony-informative characters are randomly deleted. Phylogenetic analyses were also performed in **III** and **V,** both including all and excluding doubtful characters to see how that affects the resulting phylogenetic hypotheses. Congruence between data partitions in both of these analyses was also detected with ILD.

Results and discussion

Evolutionary relationships of Meteoriaceae and Brachytheciaceae

Results presented in this thesis support the monophyly of both Meteoriaceae and Brachytheciaceae, with the exclusion of some smaller genera. These families also appeared in all analyses as sister clades, hence supporting the ideas presented by Buck (1994) and Ignatov (1999). This unique pattern of pseudoparaphyllia (Fig. 4) has evolved only once and is a synapomorphy for the Meteoriaceae – Brachytheciaceae clade. The Brachytheciaceae and Meteoriaceae can be distinguished from each other by one additional character, leaf cell papillosity (**V**). In the Brachytheciaceae, all species lack true papillae, although some prorate papillae formed by projecting leaf laminal cells are present in some species. Meteoriaceae have variously arranged papillae on leaf cells. In some rare cases, such as *Barbellopsis trichophora* (Montagne) W. R. Buck, however, they may be secondarily lost.

Phylogeny and classification of Meteoriaceae

Meteoriaceae appeared to be a monophyletic sister of Brachytheciaceae (**I**, **V**). Monophyly of the family, however, demands exclusion of genera such as *Ancistrodes* (Müll. Hal.) Crosby, *Cryphaeophilum* (Dusen) M. Fleisch., *Lepyrodontopsis* (Hedw.) Broth., and *Lindigia* (Mitt.) A. Jaeger, which all are monotypic, pendent genera from South America (**I**, Table 1). Based on the molecular data, it seems that *Ancistrodes* should be placed within Hookeriales and *Cryphaeophilum* in Cryphaeaceae (**I**), but the phylogenetic position of *Lindigia* and *Lepyrodontopsis* remains ambiguous. The species of Trachypodaceae are resolved as a polyphyletic group within Meteoriaceae, thus confirming the synonymization by Buck (1994). Synapomorphies for delimiting this newly defined Meteoriaceae include the Brachytheciaceae – Meteoriaceae pseudoparaphyllia pattern (Fig. 4) and papillose leaf cells. Quandt and Huttunen (2004) showed that pendent growth habit, which in early classifications served to delimit Meteoriaceae, has evolved several times in different lineages among pleurocarpous mosses, and thus cannot be used alone as a diagnostic character for the family. Although the morphological similarities between the Lembophyllaceae and Meteoriaceae are often emphasized (as in Allen & Magill 2003), these two families are not closely related (**V**, Quandt *et al.* 2004).

Within Meteoriaceae two clades appear in most of analyses: the *Meteorium – Papillaria* clade containing *Meteorium* Dozy & Molk. sensu Buck (1994) and *Chrysocladium* M. Fleisch., and the clade with the rest of the family (**I**, **V**). Some variation, however, occurs in the content of these clades. The taxa with the most uncertain position include the *Toloxis* clade with *Toloxis* W. R. Buck, *Papillaria intricata* (Mitt.) Müll. Hal. & Broth., *Cryptopapillaria fuscescens* (Hook.) M. Menzel, and *Trachypus bicolor* Reinw. & Hornsch. (Figs. 3–5 in **I**, Fig. 2 in **V**).

Our findings for relationships within *Meteorium – Papillaria* clade support Buck's (1994) synonymization of *Meteorium* and *Papillaria* Lorentz. Based on his observations of leaf cell papillosity in South American *Papillaria* and *Meteorium*, he concluded that this character cannot be used for separating these genera, and even at species level its variation does not support separating *Papillaria deppei* (Müll. Hal.) A. Jaeger and *Meteorium illecebrum* (Hedw.) Broth. as distinct species. In our analyses, the type species of *Meteorium* (*Meteorium polytrichum* Dozy & Molk.) and *Papillaria* (*Papillaria nigrescens* (Hedw.) A. Jaeger) are resolved in the same clade with all other species of *Meteorium* (clade M in Figs. 1-5, **I**; *Meteorium* clade in Fig. 2, **V**). The rest of the *Papillaria* species, however, form their own, well-supported clade sister to *Meteorium* (clade P in Figs. 1-5, **I**; *Papillaria* clade in Fig. 2, **V**). These two groups also have some morphological characters which separate them from each other, but the final nomenclatural clarification still awaits more detailed phylogenetic analyses of the Meteoriaceae. The *Papillaria* clade should probably be segregated into a genus of its own, but due to the position of the type species of *Papillaria*, either a new name or conservation of the name *Papillaria* Lorentz with a new type is needed (**I**). The name *Meteorium* Dozy & Molk. has been misused, almost since the establishment of the genus, but these problems, including conflicting synonymization with *Aerobryidium* M. Fleisch., are now resolved (Huttunen *et al.* 2004). The position of *Chrysocladium* may affect the generic circumscription of

Meteorium and *Papillaria*. In most analyses, however, it remained outside the *Meteorium* clade and could thus be kept as its own monotypic genus (Figs. 4 and 5 in **I**, and Fig. 2 in **V**).

In analyses including morphological characters (Fig. 2 in **V**), synapomorphies for the *Meteorium* – *Papillaria* clade include a totally papillose exostome outer surface, relatively large spores (> 20 µm), black coloration at the base of shoots which is absent only in species of the *Toloxis* – *Cryptopapillaria* clade, and an only slightly serrulate or subentire leaf margin. Morphological studies on *Chrysocladium*, *Meteorium,* and *Papillaria* reveal some novel character combinations, which seem to be present only in these groups (**I**, **II**, **V**). All species in this clade have axillary hairs consisting of 1 to 4 short brown basal cells and roundish, hyaline apical cells (Fig. 6 in **I**). Shoots are terete, and due to straight leaf insertion, the basal part of the leaf is erect or appressed to the shoot. Peristomes have hygrocastique movements, a smooth or papillose exostome outer surface, and very low or almost non-existent trabeculae on the exostome inner surface. Similarity of peristome structure in this clade was also evident in SEM studies of species in the *Meteorium* – *Papillaria* clade. In SEM micrographs, the exostome in these species bends very strongly into the capsule (Fig. 5 in **II**), which I have never seen in any fresh or herbarium specimens. This might be due to extreme drying during preparation for SEM in combination with the low basal membrane and slender segments of the endostome, very low trabeculae at the inner surface of the exostome, and shortness of exostome teeth in relation to the width of the capsule mouth. Similarity of peristome structure in *Meteorium* and *Papillaria* was also noted by Noguchi (1976), who regarded them as closely related. Especially striking are densely branching and often biseriate axillary hairs, which are diagnostic for species in the *Papillaria* clade (Fig. 6a in **I**).

Division of the species into two major clades in the *Meteorium* – *Papillaria* clade also seems to reflect their geographical distribution (**I**). The *Meteorium* clade is most diverse in warm temperate and tropical South East Asia, especially southern China, the eastern Himalayas, and Indochina, although some South American (*M. illecebrum*) and almost pantropical species (*P. nigrescens*) also exist. In the *Papillaria* clade, distribution of species is concentrated in Australia, the southern Pacific and South America. *Diaphanodon blandus* (Harv.) Renauld & Cardot and most often also *Trachypus bicolor* Reinw. & Hornsch., which earlier were both placed in Trachypodaceae, were resolved as members of the *Meteorium* – *Papillaria* clade (**V**). Some of the cladograms (Fig. 1 in **V**) allow retention of *Toloxis* and *Cryptopapillaria* within *Papillaria,* although analyses of molecular data never revealed this sister group relationship (Figs. 1–5 in **I**; Figs. 1 and 3 in **V**). All species in the *Toloxis* clade have been rather recently separated from *Papillaria* (Mentzel 1992, Buck 1994), but several morphological differences distinguish them as independent genera. *Cryptopapillaria*, however, seems to be polyphyletic, because *C. penicillata* (Dozy & Molk.) M. Menzel was resolved within the *Papillaria* clade.

Clades in the second major lineage of Meteoriaceae, clade A/B (clade B in Figs. 1–5 in **I**; clade A in Figs. 1–3 in **V**), lend support to generic divisions suggested by Buck (1994) and Menzel & Schultze-Motel (1994). *Sinskea* W. R. Buck (= *Chrysocladium* sect. *Chrysosquarridium* M. Fleisch.) is not closely related to *Chrysocladium retrorsum* (Mitt.) M. Fleisch., the type species of *Chrysocladium*. *Floribundaria* M. Fleisch. is most often resolved as a basal member of the clade A/B (Figs. 4–5 in **I**, Fig. 2 in **V**), while *Trachycladiella* (= *Floribundaria* sect. *Trachycladiella* M. Fleisch.) is always a sister to *Barbellopsis* Broth. or to a clade including *Barbella* M. Fleisch., *Neodicladiella* W. R. Buck, and *Barbellopsis*. For clade A/B, no morphological synapomorphies appeared (**V**). Most species have at least some cross-striolation at the base of the exostome teeth. Complanate leaf arrangement, at least on branches, is also a character state which occurs in only the

A/B clade. It is present in *Floribundaria*, and in all other species in a clade including, for example, *Sinskea* and *Barbella* except *Trachypodopsis serrulata* (Fig. 2 in **V**).

Phylogenetic analyses revealed that most of the speculations on generic relationships in the early taxonomic publications are hampered by homoplasy of morphological characters and lack of information on some valuable characters. As suspected by Buck (1994), a special type of papillosity where numerous papillae are arranged on rows "over the cell walls" (an expression based on the impression obtained in light microscopy; see Fig 7b in **I**) has evolved at least three times within the Meteoriaceae, in *Trachycladiella*, *Trachypus* and *Cryptopapillaria*. Pluripapillose leaf cells were also revealed to be a plesiomorphic character state in Meteoriaceae, as suggested by Noguchi (1976), although his argument was based on a slightly different view of relationships within Meteoriaceae. A unipapillose state has been evolved from this state at least twice, in the *Meteorium* clade and in the A/B clade. The majority of the Trachypodaceae species are in phylogenetic analyses resolved as a basal grade within most major clades in Meteoriaceae (Fig. 2 in **V**) or within the A/B clade. Only two species of these, namely the genus *Pseudotrachypus* P. de la Varde & Thériot, formed a monophyletic clade. The usefulness of axillary hair characters in pleurocarpous mosses has been doubted (Buck 1980, Hedenäs 1990), but our studies of Meteoriaceae show that their structure seems to include ample information for generic classification. In some group, such as the *Papillaria* clade, they might serve as a good diagnostic character (**I**, **II**). Within the Meteoriaceae, axillary hair characters that could be informative on the genus level include: 1) presence/absence of brown basal cells, 2) tendency forward axillary hairs consisting of numerous cells, 3) shape of cells, and 4) shape of the most apical cell in each axillary hair, which is in some groups (e.g., *Aerobryopsis* M. Fleisch.), for example, distinctly bent.

Based only on molecular data, the position of some species, such as *Papillaria intricata* remained somewhat problematic. This species is very similar to *Toloxis* species, but all analyses retain it as a sister to the *Cryptopapillaria–Toloxis* clade. Solving nomenclatural issues within this clade would demand synonymization of these two genera, which, however, are morphologically rather distinct. Taxonomic sampling of Meteoriaceae in **I** and **V** is also insufficient for resolving some of the central questions in this family, such as delimitation and relationships of *Aerobryopsis* and *Aerobryidium* (see Noguchi 1976, Buck 1994) and circumscription of *Pseudotrachypus* and *Barbella* (see Buck 1994).

Phylogeny and classification of Brachytheciaceae

Based on results in **III,** a new generic classification is proposed for Brachytheciaceae (Ignatov & Huttunen 2002). It is divided into four subfamilies: Rhynchostegioideae, Rhynchostegielloideae, Homalothecioideae, and Brachythecioideae (**III**, Table 1). Phylogenetic analyses confirmed the polyphyly of some of the largest genera within Brachytheciaceae, namely *Brachythecium* Schimp., *Eurhynchium* Bruch & Schimp., *Cirriphyllum* Grout, and *Rhynchostegiella* (Schimp.) Limpr. On the other hand, *Rhynchostegium* Bruch & Schimp. and *Homalothecium* Schimp. were resolved as monophyletic entities. Results supported the placement of several mainly tropical epiphytic genera in Brachytheciaceae, including, for example, *Aerobryum* Dozy & Molk., *Aerolindigia* M. Menzel, *Homalotheciella* (Cardot) Broth., *Meteoridium* (Müll. Hal.) Manuel, *Okamuraea* Broth., *Squamidium* (Müll. Hal.) Broth., *Unclejackia* Ignatov, T. J. Kop. & D. Norris, and *Zelometeorium* Manuel.

Brachythecium s. l. was split into three genera: *Sciurohypnum* (Hampe) Hampe, including species earlier placed in *Brachythecium* sections *Cirriphyllopsis* Broth. and *Reflexa* Limpr.; *Brachytheciastrum* Ignatov & Huttunen, comprising the species of *Brachythecium* sect. *Velutina* Broth.; and *Brachythecium* with the majority of species in the former *Brachythecium* s. l. Of these three genera, *Sciurohypnum* and *Brachythecium* are closely related to each other and form the majority of the subfamily Brachythecioideae. While most of the tropical epiphytes were resolved within two clades in Rhynchostegielloideae and Rhynchostegioideae, *Unclejackia* was revealed to be closely related to *Brachythecium* sensu stricto. In addition, *Bryhnia* Kaur. and *Eurhynchium eustegium* (Besch.) Dixon were resolved as members of the Brachythecioideae. *E. eustegium* was hence placed in a new genus, *Eurhynchiadelphus* Ignatov, Huttunen & T. J. Kop. (Ignatov & Huttunen 2002).

Unlike *Sciurohypnum* and *Brachythecium* s. str., *Brachytheciastrum* is a close relative of *Homalothecium* s. l. belonging to the subfamily Homalothecioideae. Species in *Homalothecium* have sometimes been divided into three genera: *Camptothecium* Schimp., *Homalothecium* s. str., and *Trachybryum* (Broth.) W. B. Schofield, but this division was shown to lead to unnatural groupings. *Homalothecium* s. str., which includes species with more specialized peristome structure than do to those in *Camptothecium,* comprised the most derived members in two lineages within *Homalothecium* s. lat, while *Camptothecium* and *Trachybryum* were resolved at the base of this clade. In addition to *Homalothecium* and *Brachytheciastrum*, the Homalothecioideae included *Eurhynchium pulchellum* (Hedw.) Jenn., which was placed in a new genus *Eurhynchiastrum* Ignatov & Huttunen.

In addition to the monophyletic *Rhynchostegium*, the subfamily Rhynchostegioideae includes *Eurhynchium* s. str., consisting of *E. striatum* (Hedw.) Schimp. and *E. angustirete* (Broth.) T. J. Kop.; *Palamocladium* Müll. Hal.; *Pseudoscleropodium* (Limpr.) M. Fleisch.; and *Scorpiurium* Schimp. The Southeast Asiatic epiphytic genus *Aerobryum* was revealed to be sister to *Pseudoscleropodium*. *Eurhynchium striatulum* (Spruce) M. Fleisch. is separated as a genus of its own, *Plasteurhynchium* M. Fleisch., which, however, belongs to same subfamily with *Eurhynchium* sensu stricto. *Platyhypnidium riparioides,* a species very close to *P. muelleri* (A. Jaeger) Müll. Hal., the type species of genus, was resolved as closely related to *Rhynchostegium.* Although it was still retained as a genus of its own, it probably should be included in *Rhynchostegium* (see Ignatov & Huttunen 2002). The genus *Platyhypnidium* M. Fleisch. is strikingly polyphyletic; of the three members of the genus included in cladistic analyses (**III**), *P. riparioides* (Hedw.) Dixon was resolved in Rhynchostegioideae, *P. patulifolium* (Cardot & Thériot) Broth. was transferred to *Donrichardsia* Crum & Anderson in Rhychostegielloideae, and *Platyhypnidium austrinum* (Hook. & Wilson) M. Fleisch. was found in the same subfamily but not closely related to *P. patulifolium*.

The subfamily Rhynchostegielloideae included some novel and slightly surprising groupings. Tropical epiphyte genera in this subfamily were resolved mainly within two clades: *Squamidium*, *Meteoridium*, and *Zelometeorium* comprised one very stable clade, and another clade was formed by *Aerolindigia*, *Clasmatodon* Hook. & Wilson, *Helicodontium* Schwägr., *Homalotheciella*, and *Remyella* Müll. Hal. The aquatic *Donrichardsia macroneuron* (Grout) Crum & Anderson, which had earlier been placed in Amblystegiaceae (Crum & Anderson 1979), was revealed to be closely related to *Platyhypnidium patulifolium*, a rare and also aquatic southeast Asiatic moss species. It was subsequently transferred to the genus *Donrichardsia*. *Donrichardsia* is sister to *Oxyrrhynchium* (Schimp.) Warnst., which includes a group of species centered around *Oxyrrhynchium hians* (Hedw.) Loeske. *Oxyrrhynchium* was separated from *Eurhynchium* already by Warnstorf in 1905, but due to difficulties in understanding its morphological circumscription, it has most often been dealt with as a subgenus, or as a mere synonym of *Eurhynchium* (see Ignatov & Isoviita 2003). An Asiatic genus, *Okamuraea*, was also revealed to be a member of the Brachytheciaceae, although most of the recent classifications have placed it in Leskeaceae (e.g., Noguchi 1991, Crosby *et al.* 1999, Buck & Goffinet 2000). Our analyses resolved it as sister to *Cirriphyllum* Grout.

The results from phylogenetic analyses (**III**) proved to be very stable. The hypothesis of relationships between taxa remained almost the same despite some changes in morphological character matrix and addition of taxa (compare **III** and **V**). Placement of some species, however, is still in need of further study. The position of *Bryoandersonia illecebra* (Hedw.) H. Robinson was unstable in analyses of Brachytheciaceae (**III**), and unlike these first direct optimization analyses, in larger analyses including a wider selection of taxa (**V**) it was resolved as close to Rhynchostegioideae (see also Ignatov & Huttunen 2002). *Kindbergia* Ochyra switched its position from the Brachythecioideae to Homalothecioideae as result of adding taxa in analyses (**III** and **V**), and it also revealed itself to be problematic in analyses of Brachytheciaceae (**III**). On the subfamily level, the basal nodes within Brachytheciaceae remained poorly supported, and the hypothesis of relationships between them was altered by addition of taxa (**III**, **V**). However, it seems likely that the Homalothecioideae and Brachythecioideae represent the most derived groups in Brachytheciaceae (**V**). The Rhynchostegioideae and Rhynchostegielloideae, which together include the majority of the tropical species in the family, are either basal to the Homalothecioideae – Brachythecioideae clade (**V**, Fig. 4 in **III**), or sister to it (Fig. 2 in **III**).

Evolution of the 9-bp inversion in the *psb*T-N spacer was revealed to be homoplastic in a data set including a wide selection of pleurocarpous mosses. It also significantly reduced the robustness of phylogenetic hypotheses derived from that data (**IV**). Within the Brachytheciaceae, however, five distinct forms of this region were observed which are synapomorphies for different clades. Inversion has apparently occurred only three times in this family, once in Rhynchostegielloideae, where all species have an inverted A-rich form of the loop region (see **IV**, **III**) and twice in Rhynchostegioideae, in *Aerobryum* and *Rhynchostegium serrulatum* (Hedw.) A. Jaeger. In light of these results, this inversion is very informative within the family. Although its inclusion in phylogenetic analyses leads to difficulties in homology statements, I see no reason to exclude this information (**III**). Our observations on inversion in the *psb*T-N loop region confirm once again the fact that increased homoplasy in analyses based on different data sets cannot serve as a universal rule for all other data sets, but globally homoplasious characters may still be locally informative (see Wenzel & Siddall 1999).

Results from phylogenetic analyses support the homoplastic nature of most of the morphological characters in Brachytheciaceae (**III**). Those characters which were earlier used to delimit genera — such as a rostrate operculum and a costa ending in a spine — have evolved several times in most of the subfamilies. Within each subfamily exist some lineages leading to a specialised sporophyte structure (**V**). Although a combination of only two characters: pattern of pseudoparaphyllia and smooth leaf cells, can serve in delimiting the Brachytheciaceae from all other pleurocarpous moss families, the morphological character combinations defining genera within the family remain in most cases ambiguous. Despite this, some generic morphological characters apparently exist, although it seems hard to formulate them into a generic diagnosis. This is evident from the consistency between some early ”intuitive” classifications and the results of phylogenetic analyses.

Morphological evolution and evolution of epiphytism within the Meteoriaceae – Brachytheciaceae clade

Evolution of morphological characters in Meteoriaceae and Brachytheciaceae was shown to be prone to homoplasy. In most cases only very few synapomorphies exist for major clades in both families, or they were totally lacking. Even characters that traditionally have been regarded as reliable diagnostic characters at genus level are hampered by homoplasy (see Fig. 2 in **III**). The most striking phenomenon in Meteoriaceae and Brachytheciaceae was the morphological evolution of epiphytic species. According to the results shown in **V**, epiphytic habitats have been conquered several times within the Meteoriaceae–Brachytheciaceae clade (Fig. 4 in **V**). This ecological shift correlates with the evolution of several characters, especially sporophytic ones (**V**). Although sporophytic characters were earlier thought to be conservative, these results, together with those of some earlier studies (Hedenäs 1999b & 2001, Buck *et al.* 2000a, Vanderpoorten *et al.* 2002) support the view that they are instead responding to environmental conditions even more easily than do gametophytic characters. For example, seta length, spore size, hygroscopic movements of the peristome, endostome basal membrane and cilia height, and the ornamentation of lower part of the exostome were found to correlate with epiphytism (Fig. 2). Very similar sporophyte structures appear in distantly related species. For example, the peristome in *Squamidium brasiliense* (Hornsch.) Broth. (Brachytheciaceae, Rhynchostegielloideae) with its exostome outer surface and even endostome basal membrane covered by large branching papillae, resembles very closely the peristome of *Trachypus bicolor* Reinw. & Hornsch. (Meteoriaceae). In the gametophyte generation, characters such as a long flexuous leaf acumen, absence of a central strand in the stem, blackish coloration on bases of shoots and pendent growth habit correlate with epiphytism. Correlation of these characters with epiphytic habitats has been suspected earlier, and in the bryological publications they are often regarded as adaptations to epiphytism (Grout 1908, Patterson 1953, Vitt 1981, Buck & Vitt 1986, Buck 1991, Hedenäs 1998 & 2001). Despite the connection of these characters with epiphytism and striking similarities between distantly related epiphytic species, sporophyte structure within, for instance, the epiphytic family Meteoriaceae still shows rather wide variation. For example, several epiphytic Meteoriaceae species have xerocastique peristomes with variable cross-striolated exostomes and a high endostomial basal membrane, and the species with these characteristics often appear to be unrelated in phylogeny (**V**).

Direct optimization and phylogenetic analyses of Meteoriaceae and Brachytheciaceae

Despite considerable sequence length variation, the hypothesis of relationships and character transformations in direct optimization analyses remained fairly stable (**I**, **III**, **V**). Even variation in gap costs, or treatment of the 9-bp inversion on the *psb*T-N spacer affected the position of only a few taxa (**I**, **III**). Direct optimization is sometimes said to perform poorly if data include sequences with long indel events (Belshaw & Quicke 2002). Although some species included had indel events spanning up to 55 bp (**I**, **V**), in analyses performed for this thesis they showed no negative effects. The effect of increasing sequence length variation on direct optimization analyses was tested in **III**. According to these result, direct optimization is able to find the same topology despite an increase in genetic distance between specimens, (Table 3, Figs. 2 and 3 in **III**). Addition of distantly related outgroup species to analyses actually improved the congruece among data partitions (Table 5 in **III**) and increased the resolution within the ingroup (Figs. 2 and 3 in **III**). Results of phylogenetic relationships were also robust for addition of terminals or data (compare Fig. 4 in **I**, Fig. 2 in **III**, and Fig. 2 in **V**).

Direct optimization and parsimony analyses of static alignments revealed very similar topology, although some variation was observed in arrangement of some major clades (**I**, **V**). The position of these clades was dependent on homology statements, and they also had lower branch support values in all analyses. In direct optimization, information from indel events was used in phylogeny reconstruction, while in analyses of static manual alignments, gaps were treated as missing data. Hence some of the differences between topologies (e.g., Figs. 3 and 4 in **I**, Figs. 1 and 3 in **V**) is explained by the amount of data used. Some of the differences between topologies are still also due to the method used in analyses, i.e., direct optimization vs. static alignment (Figs. 3 and 5 in **I**). Due to inclusion of gap information, clades obtained generally higher support in direct optimization analyses (Figs. 4 and 5 in **I**). In this case, too, some of the differences in clade support values are, however, due to differing homology statements (Figs. 3 and 4 in **I**, Figs. 1 and 3 in **V**). Results in this thesis show that inclusion of gap information in analyses affected only some minor details of topology (**I**, **III**), although the number of parsimony-informative characters increased significantly if POY character transformation matrices were studied as ordinary alignments (Table 4 in **I**).

In direct opimization, the common practise is to explore the effect of different gap costs on phylogenetic relationships (sensitivity analyses sensu Wheeler 1995). Results obtained with different gap and base substitution costs can be used for comparing the stability of clades in trees based on different gap costs, or, by using for example congruence as an additional measure of optimality between different analyses, for seeking the optimal weighting scheme (see Giribet 2003). Whether this method is appropriate for either purpose is, however, questionable (Frost *et al.* 2001, Grant & Kluge 2003). If parsimony is used as the optimality criterion in search of the best hypothesis of relationships, a priori weighting of indels will increase number of assumptions needed to explain the data. According to this view, the truly parsimonious result is thus obtained only if all characters are given the same weight. I agree in this with above-mentioned authors, although in most of the direct optimization analyses in this thesis, sensitivity analyses were performed (**I**, **III**). This can now be deemed superfluous. However, even in **III,** where sensitivity analyses support differential weighting for indels, the topology obtained with equal weighting is shown as the most parsimonious result (Figs. 1–3 in **III**). In **I** and **V** (results from sensitivity analyses not shown) equal weighting of all characters also led to the most congruent results, and these cladograms are shown as results of direct optimization analyses (Fig. 4 in **I** and Fig. 2 in **V**).

Conclusions

Phylogenetic analyses utilizing DNA sequence data offer a powerful tool, especially in studies of morphologically rather simple organisms. In the case of pleurocarpous mosses, the morphological variation alone contains relatively little information on higher level relationships. Phylogenetic studies including morphology suggest that evolution of most characters is homoplastic even at genus and species level (see **III**), and that certain environmental conditions may lead to parallel or convergent evolution of similar characters (aquatic habitats, Vanderpoorten *et al.* 2002; epiphytism, **V**, Hedenäs 2001,Vanderpoorten *et al.* 2002). This may be due to the relatively simple morphological structures of these mosses which repeatedly results in similar character combinations. Due to these problems with morphological data and rapid radiation of the main pleurocarpous moss lineages (Shaw *et al.* 2003), information from non-coding and relatively rapidly evolving genomic regions offer the only tool for studies on family level relationships. The aim of this thesis was to the study phylogenetic relationships of two pleurocarpous moss families, Brachytheciaceae and Meteoriaceae. For this purpose ITS2, *psb*T-H, and *trn*L-F regions contained enough phylogenetic information to resolve family – genus level relationships in Meteoriaceae and

Brachytheciaceae (**I**, **III**, **V**), and most of the clades in these cladograms are even very well supported. In some other pleurocarpous moss families, however, even these rapidly evolving DNA regions do not solve relationships between genera (Vanderpoorten *et al.* 2001, Quandt *et al.* 2004).

DNA sequence data of the *psb*T-H gene complex offered some new insights into the function and phylogenetic utility of this region, and into the evolution of short DNA inversions (**III**, **IV**). These kinds of inversions in noncoding chloroplast regions are expected to be common (Kelchner & Wendel 1996, Graham *et al.* 2000), but in phylogenetic studies they are probably often overlooked.

Use of non-coding DNA sequence data in phylogenetic analyses demands a special attention to homology between DNA sequences. In this thesis, both direct optimization and analysis of manual alignments, which were constructed using information on the structure and function of DNA, derived congruent phylogenies (**I**, **V**). This thus lends support to the view that despite difficulties in alignment, non-coding DNA with multiple indel events still can be useful for phylogeny reconstruction. Compared to analyses of static manual alignments, the direct optimization method is, however, more objective and aims to search simultaneously for the optimal hypothesis of character transformations and of phylogenetic relationships between taxa. Due to this fact, it enables us to solve the ambiguities in alignments by using the same optimality criterion in search for a character transformation scheme and phylogeny. It is also possible to routinely explore the effect of different homology statements on topology, which is much more laborious with other phylogenetic methods.

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