Contributions to the phylogeny of the haplolepideous mosses

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Front cover image: illustration of the leucobryoid moss *Octoblepharum albidum* Hedw.

Back cover image: *Octoblepharum albidum* growing luxuriously on the palm *Syagrus coronata* (Mart.) Becc. (licuri) in an Atlantic rain forest area in Diogo, Mata de São João, Bahia, Brazil, 2020

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Chapter 1

General Introduction

What are mosses?

Bryophytes comprise three lineages: hornworts, liverworts, and mosses. Although the monophyly of bryophytes is still debated (Puttick et al., 2018; Wickett et al., 2014), they share a unique trait among the land plants: in all bryophytes the life cycle differs from the other land plants (the vascular plants) in having a dominant, branched gametophyte and short-lived sporophyte (Vanderpoorten & Goffinet, 2009). Among the bryophytes, the mosses, classified as division Bryophyta Schimp. (Frey & Stech, 2009; Goffinet et al., 2009; Goffinet & Buck, 2004; Vanderpoorten & Goffinet, 2009) or subdivision Bryophytina Engl. (Kadereit et al., 2014) can be recognised by their leafy gametophyte, seta (sporangial stalk) elongation prior to spore maturation, and capsules (sporangia) with a columella (see Figure 1) (Frey & Stech, 2009; Vanderpoorten & Goffinet, 2009). Due to these unifying morphological traits the mosses were long recognised as a natural group, which was later confirmed by phylogenetic analyses of molecular data (Qiu et al., 2006). As a species-rich group with ca. 12500 species in ca. 120 families and ca. 860 genera, mosses are morphologically diverse in both their gametophytic and sporophytic characters (Frey & Stech, 2009).

A brief overview of moss classifications

The mosses are currently arranged in eight classes and, within their most speciose class, Bryopsida Pax, seven subclasses (Figure 2; Goffinet & Buck, 2020; Liu et al., 2019), according to morphological features and to the results of molecular phylogenetics (D. Bell et al., 2020; Chang & Graham, 2014; Cox et al., 2004, 2010; Frey & Stech, 2009; Goffinet et al., 2009; Goffinet & Buck, 2020; Liu et al., 2019; One Thousand Plant Transcriptomes Initiative et al., 2019). Moss relationships, as resolved with molecular phylogenetic methods, are largely congruent with some of the sporophyte characters initially adopted to classify mosses. The mode of dehiscence of the capsule for spore release, and the presence or absence and characteristics of one or two rings of filaments or teeth around the opening of the capsule (the peristome, which controls the spore release) were the main characters used in the early moss classification systems (e.g. by Brotherus and Fleischer; cf. Vitt, 1984). In contrast, gametophyte characters used in early classifications seem to be much less congruent with the moss relationships as inferred by molecular phylogenetics. The position of the perichaetia (i.e., archegonia and modified leaves around them), for instance, was used to define major divisions in some classifications (see Vitt, 1984), however only one character state, pleurocarpy (perichaetia produced on lateral, differentiated branches), corresponds to a synapomorphy. In contrast, acrocarpy (perichaetia produced terminally in the main stem) is a plesiomorphic condition and the posteriorly defined cladocarpy (perichaetia produced terminally in lateral

branches) arose multiple times in moss evolution (Goffinet et al., 2009; La Farge-England, 1996; Vanderpoorten & Goffinet, 2009).

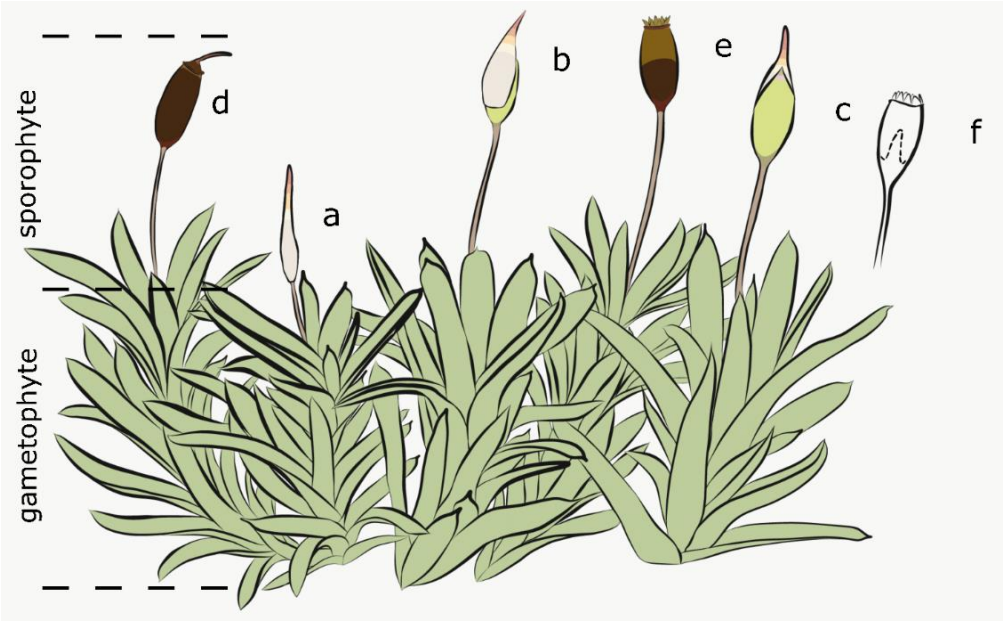

Figure 1. General morphology and diagnostic traits of mosses, as opposed to other lineages of land plants, exemplified by a drawing of Octoblepharum *Hedw.: leafy gametophytes and the short-lived monosporangiate sporophytes in different stages of development:* ***a****: seta still elongating, capsule immature (before meiosis), covered by the calyptra*; **b***: seta elongated, capsule partially enlarged, covered by the calyptra*; **c***: capsule enlarged (after meiosis), with immature spores; **d***: capsule with mature spores and operculum visible after the calyptra* has fallen off; **e***: capsule open, half empty of spores, with peristome visible after the operculum has fallen off. **f***: outlines of the columella (central column of sterile tissue; dashed) and spore sac inside a capsule. *The calyptra, the protective cap that covers the capsule through its development, is not part of the sporophyte, but formed by gametophytic tissue derived from the archegonia.*

Five classes of mosses characteristically do not have peristomes (i.e., are eperistomate or gymnostomous). Those classes differ from one another in the mode of dehiscence of their capsules, among other characters (Frey & Stech, 2009). In Takakiopsida Stech & W. Frey capsules open along a single, spiralled longitudinal slit, in Andreaeopsida J.H. Schaffn. and Andreaeobryopsida Goffinet & W.R. Buck along four longitudinal slits, forming valves, and in Sphagnopsida Schimp. and Oedipodiopsida Goffinet & W.R. Buck via a differentiated lid (the operculum). Furthermore, Sphagnopsida and Andreaeopsida can be distinguished from other moss classes by the presence of a gametophytic stalk to elevate the sporangium (the pseudopodium) instead of the more common sporophytic seta.

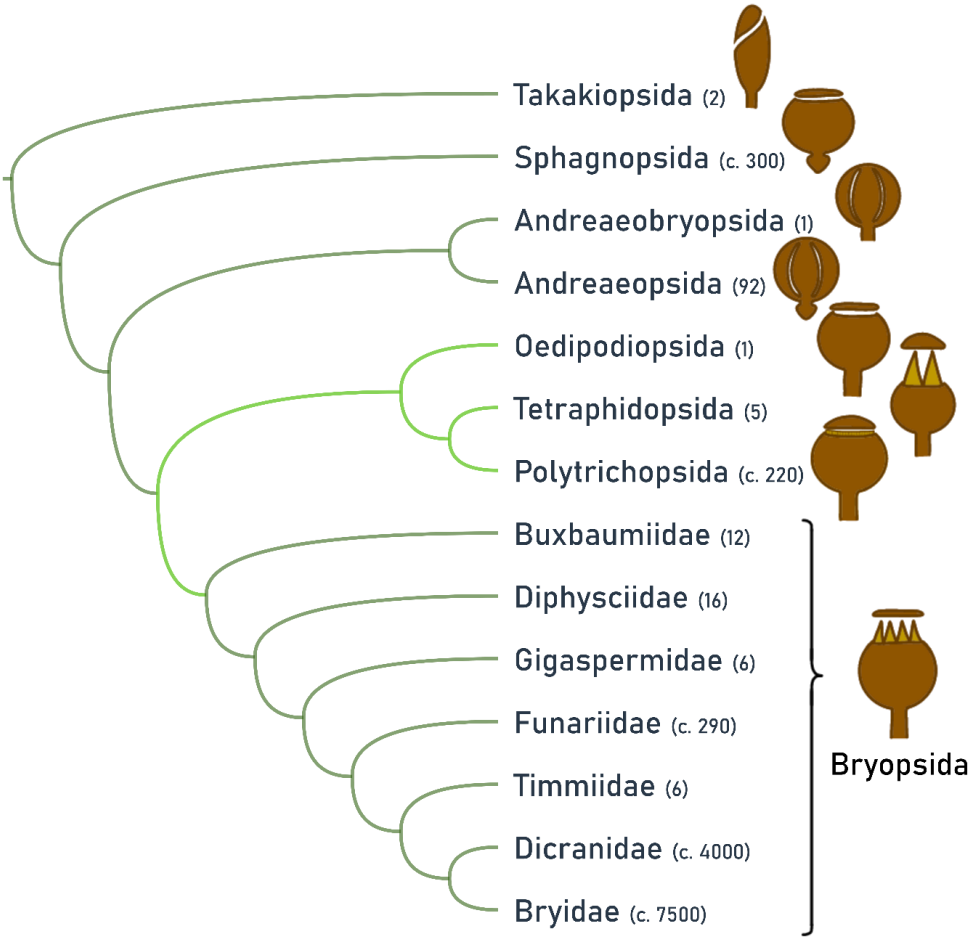

Figure 2. Major moss lineages: relationships, number of species and sporophyte characters for the eight classes. Oedipodiopsida, Tetraphidopsida, Polytrichopsida and Bryopsida form a well-supported clade, however the relationships between these four classes are not yet resolved with confidence (and are marked with light green branches to indicate that).

The three classes of peristomate mosses (with peristome; Tetraphidopsida Goffinet & W.R. Buck, Polytrichopsida Doweld, and Bryopsida) are operculate (with cases of secondary losses of peristome/operculum) (Frey & Stech, 2009; Goffinet et al., 2009). Their higher level classification is based on the peristome morphology (with support of molecular phylogenies; e.g. Chang & Graham, 2014; Liu et al., 2012). Peristome teeth are formed by the outer (OPL), primary (PPL), and inner (IPL) peristomial cell layers of the capsule amphithecium (i.e., the

outer one of two tissues in the embryonic capsule; the inner tissue is called endothecium) (Figure 3c, d), and differences in the ontogeny result in the broad variation observed (Edwards, 1979, 1984). The teeth vary in structure (i.e., formed by entire cells or by cell wall remnants, the peristome plates), number of rings or rows of peristome teeth (one or two, i.e., single or double peristomes), shape, and ornamentation (Goffinet et al., 2009).

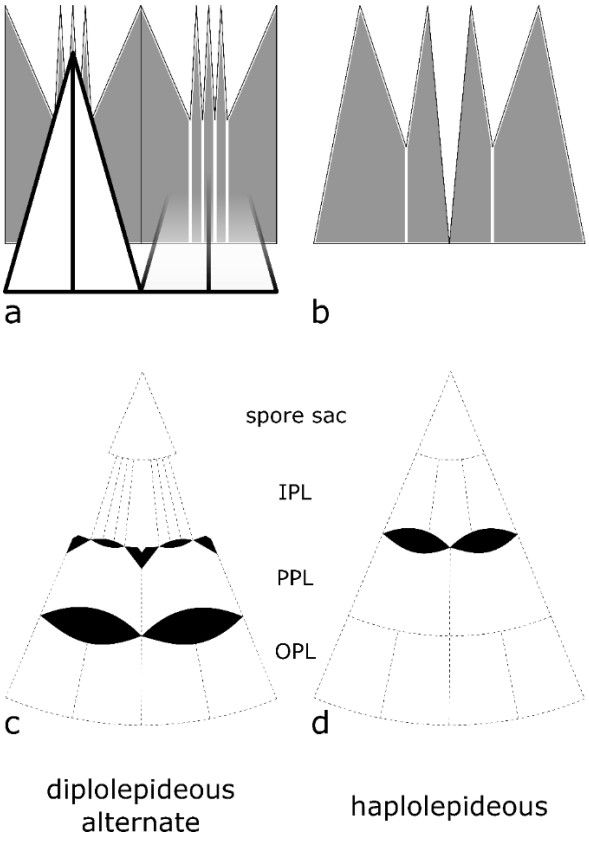

Figure 3. Two examples of arthrodontous peristomes and their cell layers. ***a***, ***b****: views from the outside. The plates forming the peristome teeth are represented with distinct outlines and filling according to which peristomial cell layer they originate from: outer layer (OPL) by thick black lines and white filling, primary layer (PPL) by thin black lines without filling, and inner layer (IPL) by thick white lines with grey filling.* ***c***, ***d****: transverse section of the capsule at the height of the peristome teeth, showing the cell walls which are degraded during maturation of the peristome in dashed lines, and the remaining cell walls which form the plates of the peristome teeth highlighted in solid black. The capsule wall (exothecium) and the OPL, PPL, and IPL originate from the amphithecium, while the columella and spore sac originate from the endothecium.* ***a***, ***c****. Diplolepideous alternate,* Bryum*-type peristome, 4:2:4–12,* ***b***, ***d****. Haplolepideous,* Dicranum*-type peristome, 4:2:3. The peristomial formula OPL:PPL:IPL is derived from the number of cells in each layer in 1/8 of the capsule circumference. All schemes show 1/8 of the capsule circumference.*

The species in Tetraphidopsida and Polytrichopsida have one ring of peristome teeth formed by bundles of entire, elongated cells (nematodontous peristome), the former with four massive, erect teeth, the latter with numerous short teeth and a thin membrane, the epiphragm, closing the mouth of the capsule (Frey & Stech, 2009; Goffinet et al., 2009). The species in Bryopsida have one or two rings of peristome teeth, which are formed by pairs of plates and most frequently are capable of hygroscopic movements (arthrodontous peristome; Figure 3) (Frey & Stech, 2009; Gallenmüller et al., 2018; Goffinet et al., 2009). Some characteristics of the arthrodontous peristome can be described by the peristomial formula, devised by Edwards (1979). It serves as a short descriptor of the number of cell columns in each of the peristomial layers that determine the number of plates which form the outer and inner surfaces of the peristome teeth in the peristome rings. The OPL and PPL contribute to the structure of the outer ring of teeth (the exostome), and the PPL and IPL contribute to the inner ring (the endostome) (see Figure 3).

The vast majority of moss species belong to Bryopsida (ca. 98%), and its seven subclasses can also be recognised based on peristome variation (Frey & Stech, 2009). Buxbaumiidae Doweld, Diphysciidae Ochyra, and Timmiidae Ochyra, some of the least speciose lineages, comprise quite particular peristome types. Their peristomes may be considered evolutionary intermediate stages between nematodontous and arthrodontous peristomes: thus not typically arthrodontous, and resembling in some features the nematodontous peristomes (Edwards, 1984). The Gigaspermidae Stech & W. Frey, the fourth of the least species-rich subclasses of Bryopsida, have either gymnostomous or cleistocarpous (i.e., without a differentiated operculum) capsules.

The remaining subclasses have either haplolepideous or diplolepideous peristome types, patterns which were first described by Philibert (Philibert, 1884; Taylor, 1962). Philibert classified the peristome as haplolepideous or diplolepideous based on the number of columns of plates on the outer surface of each of the outer (or single ring of) peristome teeth: one column of plates in the haplolepideous peristomes and two columns in the diplolepideous peristomes (Goffinet et al., 2009; Taylor, 1962). Philibert remarked that (according to his observations and interpretation) haplolepideous peristomes never had a second ring of teeth, while a second ring of teeth occurred in nearly all diplolepideous moss families (cf. Taylor, 1962). That led to the simplified and incorrect notion that haplolepideous and diplolepideous peristomes differ by the number of rings of peristome teeth and not by the structure of the peristome teeth in terms of peristome plates as defined by Philibert (cf. Taylor, 1962) and later refined by other authors (e.g. Edwards, 1979, 1984).

The Funariidae Ochyra have diplolepideous opposite peristomes (with exostome and endostome teeth opposite to each other), the Bryidae Engl. have diplolepideous alternate peristomes (with the exostome teeth alternate with the endostome teeth), and the Dicranidae Doweld, the study group of this thesis, have haplolepideous (or double-haplolepideous) peristomes (Edwards, 1984; Fedosov et al., 2016a).

The backbone evolutionary relationships of mosses are resolved with high support, based on phylogenetic analyses of different (sets of) molecular markers (Chang & Graham, 2011, 2014; Cox et al., 2004, 2010; Liu et al., 2012, 2019; Magombo, 2003; Newton et al., 2000; Wahrmund et al., 2010). The monophyly of each of the classes and subclasses and their relationships as depicted in Figure 2 are a consensus among the mentioned studies.

The haplolepideous mosses

The haplolepideous mosses (Dicranidae) comprise ca. 30% of the moss diversity, with about 4000 species (Figure 2; Frey & Stech, 2009). Since their characterization by Philibert (1884; cf. Taylor, 1962), the haplolepideous mosses have been considered a natural group, and their monophyly was strongly supported by molecular phylogenetic studies (e.g. Chang & Graham, 2014; Cox et al., 2010; Goffinet & Cox, 2000; Tsubota et al., 2003). Haplolepideous peristomes can be recognised by their peristomial formula 4:2:3 (modified in some taxa), the number 3 in the IPL being caused by an asymmetric pattern of cell divisions (Edwards, 1979). In typical haplolepideous peristomes with 16 (endostome) teeth each tooth has two ventral peristomes plate columns of uneven width, correspondent to one and a half cells of the IPL per tooth (Figure 3; Edwards, 1979; Hedderson et al., 2004; La Farge et al., 2000).

As expected for a group of this size, the haplolepideous mosses comprise a wide range of sporophytic and gametophytic morphological traits (Frey & Stech, 2009; Goffinet et al., 2009; Vitt, 1984). The sporophyte variation is associated with life strategies and optimization of spore dispersal (Goffinet et al., 2011; Vitt, 1984), for example, with a trend of peristome reduction in epiphytic bryophyte groups (Olsson et al., 2009) also observed among the haplolepideous mosses (e.g. in the Calymperaceae Kindb. s.l. clade; Fisher et al., 2007). Some taxa with modified peristomes (i.e., with a fully developed exostome and rudimentary endostome, double peristomes with a fully developed exostome, or reduced to absent forms) were for that reason not at first recognised as belonging in this group (e.g. *Catoscopium* Brid., *Ephemerum* Hampe, *Pseudoditrichum* Steere & Z. Iwats.; Fedosov et al., 2016a; Goffinet et al., 2011; Ignatov et al., 2015; H. A. Miller, 1979; Vitt, 1984). Number and shape of the peristome teeth, their patterns of ornamentation, and thickening of the peristome plates all vary, and were traditionally used to characterise the haplolepideous moss orders (Edwards, 1979; Fedosov et al., 2016a; Frey & Stech, 2009; Shaw, 1985). The capsules vary in shape, orientation, and mode of dehiscence (some are cleistocarpous; Frey & Stech, 2009). The seta varies in length, bending, and even torsion (e.g. in the Leucobryaceae Schimp.; Frahm, 1991).

The gametophyte variation is also sometimes associated with physiological or ecological adaptation (Goffinet et al., 2009; Vanderpoorten & Goffinet, 2009; Vitt, 1984). Most haplolepideous mosses are acrocarpous (Frey & Stech, 2009; Goffinet et al., 2009), however, some groups developed the cladocarpous gametophyte architecture, as *Bryowijkia* Nog. (Cox et al., 2010; Touw, 1993). There are also examples of neoteny within Dicranidae, as in *Micromitrium* Austin and *Ephemerum*, which have gametophytes characteristically little developed and minute (Goffinet et al., 2011). Among other characters, leaf shape, structure of

the leaf costa (the multilayered median part of the leaf), and cell shape, size and wall ornamentation in the leaf lamina (the single layered part of the leaf) vary as well (Frey & Stech, 2009; Goffinet et al., 2009; Vanderpoorten & Goffinet, 2009).

The most common types of costa structure in the haplolepideous mosses (namely the *Dicranum*-type, the *Pottia*-type, and a less specialised one with a homogeneous costa) are characterised by a predominance of chlorophyllose cells (Frey & Stech, 2009). They vary in the specialization of the costa cell layers, which can be differentiated in guide cells, stereids, and epidermal cells, arranged in several ways. Figure 4 (left) shows an example of such costa structure, the genus *Dicranella* (Müll.Hal.) Schimp. A remarkably contrasting pattern is the so-called leucobryoid costa, named after the genus *Leucobryum* Hampe, which is possibly an adaptation to enhance gas exchange (Robinson, 1985, 1990; Vanderpoorten & Goffinet, 2009). Most of the costa cells are enlarged and hyaline (hyalocysts), interconnected by pores, except for 1(−3) layer(s) of small chlorophyllose cells (chlorocysts), which causes these plants to have their characteristic glaucous aspect (Figure 4, right; Goffinet et al., 2008). According to Robinson (1985), large air bubbles extending through the web of hyalocysts would provide the conditions for appropriate gas exchange for the photosynthesis in the chlorocysts, which are enclosed by the leucocysts and thus not directly exposed to air. Furthermore, in the plants with a leucobryoid costa (leucobryoid mosses) the leaf lamina is narrow, sometimes restricted to the leaf base, and the costa occupies most of the leaf width. This highly specialised morphology is so distinct from the morphology of the other haplolepideous mosses that these plants were at first classified together in a single family (Leucobryaceae; Schimper, 1856), despite the differences in their sporophyte characteristics. Earlier molecular phylogenies already indicated that the leucobryoid mosses are polyphyletic, with the exact number of lineages yet to be determined (Cox et al., 2010; Inoue & Tsubota, 2014), and thus have shown that the gametophytic similarities of the leucobryoid lineages are a very curious case of evolutionary convergence.

Figure 4. Two costa types of the haplolepideous mosses. Left: Dicranella varia *(Hedw.) Schimp., with a* Dicranum*-type costa. Left, above: habit; below: leaf in cross section showing a structure formed by different types of chlorophyllose cells, without hyalocysts. Right:* Leucobryum juniperoideum *(Brid.) Müll.Hal., with a leucobryoid costa. Right, above: habit, showing glaucous aspect of the plants; below: leaf in cross section showing a broad costa with layers of enlarged, porous hyalocysts above and below a single layer of small, diamond-shaped chlorocysts. Pictures by Michael Lüth reproduced with permission of the author* (from Lüth, 2020).

Systematics of the haplolepideous mosses

The orders in the Dicranidae were defined mainly by peristome features, especially the patterns of thickening and ornamentation of the peristome plates (e.g. Fleischer, 1900-1923; Vitt, 1984). However, detailed studies of the peristome demonstrated that the existing variability did not fully correspond to the ordinal classification (Edwards, 1979) and the introduction of molecular phylogenetic analyses to moss systematics additionally demonstrated the limited correspondence between the ordinal and family level classification and phylogenetic relationships. In the classification by Goffinet & Buck (2021) there are eight main groups of haplolepideous mosses: the unranked group Protohaplolepidae (Hedderson et al., 2004) plus seven orders (Archidiales Limpr., Bryoxiphiales H.A. Crum & L.E. Anderson,

Dicranales M. Fleisch., Grimmiales M. Fleisch., Pottiales M. Fleisch., Pseudoditrichales Ignatov & Fedosov, and Scouleriales Goffinet & W.R. Buck). A simplified classification for the Dicranidae with only three orders (one of them a broadly circumscribed Dicranales including most protohaplolepideous lineages and Pottiaceae Schimp., among others) was also recently adopted in the literature (Hodgetts et al., 2020).

Higher level molecular phylogenetic studies of (or including) the haplolepideous mosses have been performed since the late 1990s (e.g. N. E. Bell & Newton, 2004; Cox et al., 2010; Fedosov et al., 2015; Fedosov et al., 2016a, 2016b; Goffinet et al., 1998, 2001, 2011; Hedderson et al., 1999, 2004; Hernández-Maqueda, Quandt, Werner, et al., 2008; Ignatov et al., 2015; Inoue & Tsubota, 2014; La Farge et al., 2000, 2002; Newton et al., 2000; O'Brien, 2007; Stech, 1999a, 1999b; Stech et al., 2012; Stech & Frey, 2008; Tsubota et al., 2003, 2004; Wahrmund et al., 2010; Werner et al., 2004, 2007a, 2013), using in their analyses a range of molecular markers from all three genomes – the most widely used being the chloroplast *rbc*L gene and *trn*S-*trn*F region and the mitochondrial *nad*5 intron. These studies resolved a tree of the haplolepideous mosses with two major groups: the protohaplolepideous grade and the core haplolepideous clade (Figure 5). The protohaplolepideous grade comprises a series of species-poor clades which were resolved at the base of the haplolepideous moss tree, including families which previously were either not considered to be haplolepideous mosses (Catoscopiaceae Broth., Drummondiaceae Goffinet, Pseudoditrichaceae Steere & Z. Iwats.), considered to be part of core haplolepideous families (Chrysoblastellaceae Ignatov & Fedosov, Distichiaceae Schimp., Flexitrichaceae Ignatov & Fedosov, Hymenolomataceae Ignatov & Fedosov, Scouleriaceae S.P. Churchill, Timmiellaceae Y. Inoue & H. Tsubota), or (only one case) considered as isolated lineages of uncertain placement within Dicranidae (Bryoxiphiaceae Besch.). Four of these clades were classified in their own orders, existing (Bryoxiphiales) or newly described (Catoscopiales Ignatov & Ignatova, accepted by Frey & Stech (2009), Pseudoditrichales, and Scouleriales), and three others were described as new families, but without a discussion on their ordinal placement (Distichiaceae, Flexitrichaceae, Timmiellaceae).

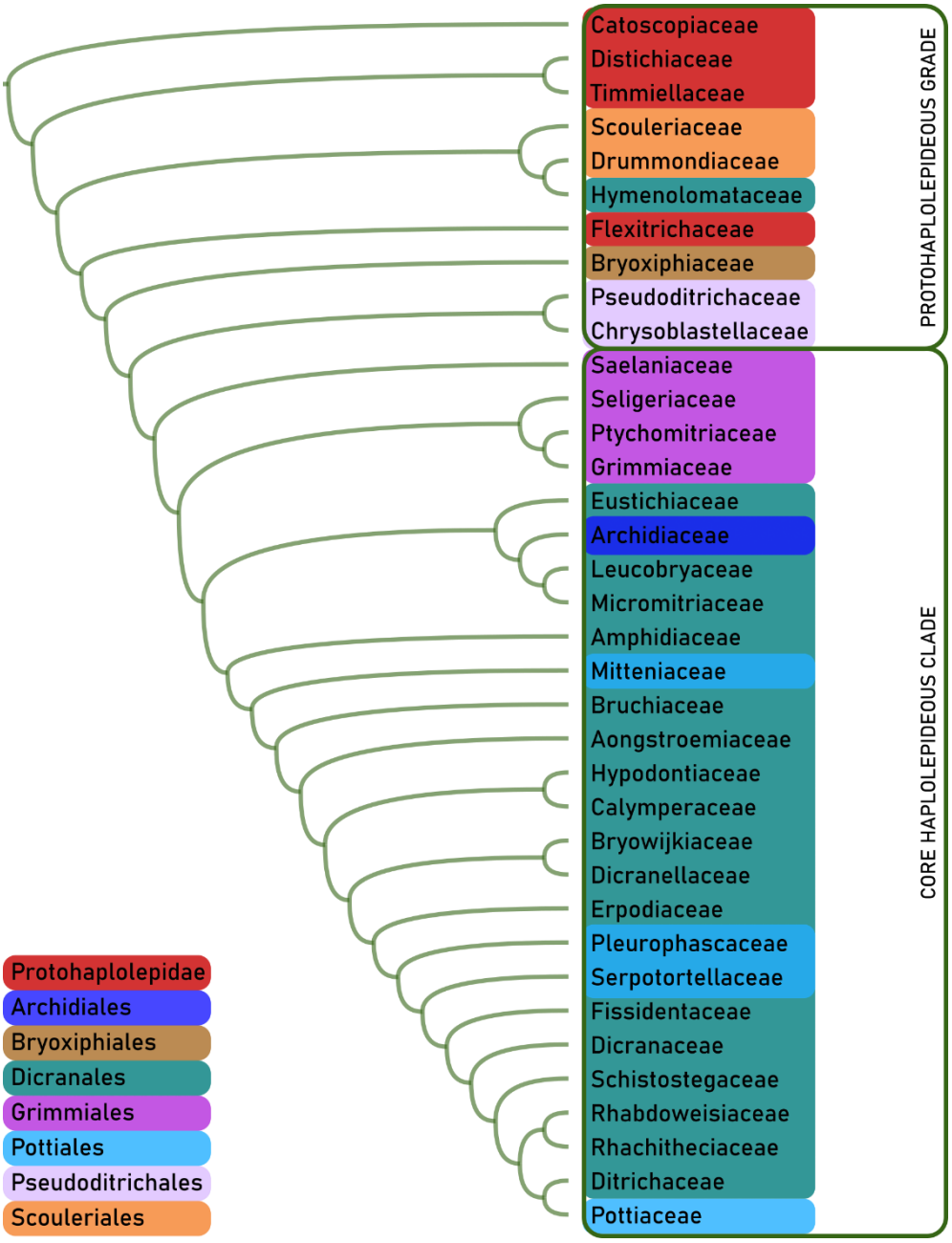

Figure 5. A summary of the supported relationships between haplolepideous moss families.

The core haplolepideous clade (e.g. Cox et al., 2010; Fedosov et al., 2016a; Stech et al., 2012) comprises the majority of the haplolepideous moss species. The traditional orders (Dicranales, Grimmiales, Pottiales, Syrrhopodontales Dixon) are part of this group, as well as some taxa previously not known to belong to the haplolepideous mosses (e.g., *Archidium* Brid., *Ephemerum*, *Mittenia* Lindb.). Most of the new additions were transferred to existing haplolepideous moss orders, except *Archidium* and *Mittenia*, which were placed in Dicranidae in their own previously described orders (in Frey & Stech, 2009; the Mitteniaceae Broth. were placed in Pottiales in Goffinet et al., 2008). Among the three traditional haplolepideous moss orders still accepted in current classifications, Grimmiales was the only monophyletic one, after some changes in its circumscription (Fedosov et al., 2016a; Goffinet & Buck, 2004; Tsubota et al., 2003). The monophyly of Pottiales as presently circumscribed was not supported, since *Hypodontium* Müll.Hal. and *Serpotortella* Dixon were resolved in a clade with the Dicranaceae Schimp. and other Dicranales families (Cox et al., 2010; Fedosov et al., 2016a; Stech et al., 2012). Finally, Dicranales has the most problematic circumscription since its families were scattered all over the haplolepideous tree, and due to the difficulty to characterise it morphologically, given the variation it comprises. As currently circumscribed (Frey & Stech, 2009; Goffinet et al., 2009; Goffinet & Buck, 2021), the orders do not correspond to the haplolepideous peristome types as traditionally defined (cf. Edwards, 1979). While Edwards (1979) described six main peristome types largely correspondent to the haplolepideous moss ordinal classification at that time (one of each Dicranales, Fissidentales M. Fleisch., Grimmiales, Pottiales and Syrrhopodontales, plus the seligerioid type), the same orders (those still accepted in the present) nowadays comprise mixed peristome types (e.g. Dicranales, comprising plants with dicranoid, fissidentoid and syrrhopodontoid peristomes). Moreover, further anatomical and morphological studies of the peristome, in some cases coupled with phylogenetic analyses, revealed that the diversity of the haplolepideous peristome is even greater than as described by Edwards (1979, 1984), for instance with modified peristomes as that of *Pseudoditrichum* (Fedosov et al., 2016a; Shaw, 1984).

Molecular phylogenetic studies also contributed to improving the lower-level classification of the haplolepideous mosses. The largest family, Pottiaceae, was resolved as monophyletic, and except for some rather small additions (e.g. *Ephemerum*; Goffinet & Cox, 2000) and exclusions (e.g. *Luisierella* Thér. & P. de la Varde and *Timmiella* (De Not.) Limpr.; Inoue & Tsubota, 2014), its circumscription remained largely unchanged (Goffinet & Buck, 2004). In contrast, one of the main changes resulting from molecular phylogenies was probably the re-circumscription of the Dicranaceae. The family was historically broadly defined based on a widespread character, the dicranoid haplolepideous peristome, coupled with a rather little specialised gametophyte morphology, with few exceptions (Schimper, 1856). Phylogenetic analyses have shown that the family actually comprised a few clades that are not closely related, which were described/resurrected as separate families (e.g. Aongstroemiaceae De Not., Dicranellaceae Stech; Frey & Stech, 2009; La Farge et al., 2000, 2002; Stech, 1999a; Stech & Frey, 2008).

The re-circumscription of the Dicranaceae also influenced the classification of the leucobryoid mosses. Their classification was revised even before the supporting results of molecular

phylogenetic studies which showed this morphological pattern had multiple origins, however the relationships of the leucobryoid taxa remain to be further investigated. The genus *Octoblepharum* Hedw., most frequently classified in the Calymperaceae, is yet undersampled, and its closest relationships unclear. Further studies are also necessary for the morphologically heterogeneous but molecularly strongly supported Leucobryaceae, which presently comprise both leucobryoid and dicranoid plants, but within which the phylogenetic relationships between the plants with the two morphological patterns were not yet established with confidence.

The monophyly of other (weakly) morphologically circumscribed haplolepideous moss taxa also remains to be tested. Some of these taxa are species-rich and display great gametophytic and sporophytic variability, but remain very little studied, both in terms of taxonomical revisions and molecular phylogenetic studies. One of them is the family Ditrichaceae Limpr., which was resolved as highly polyphyletic in recent studies (Fedosov et al., 2015; Fedosov et al., 2016a). Part of its newly discovered phylogenetic diversity, which extends across the proto- and core haplolepideous mosses, was described as new or resurrected families (e.g., Distichiaceae, Saelaniaceae Ignatov & Fedosov), resulting in a more refined classification also in terms of the morphological diversity of the Ditrichaceae s.l. Nevertheless, the still unresolved/unsupported relationships in the Ditrichaceae s.s. demand further studies and will likely require further taxonomic changes. But there are taxa in an even worse state, as the genus *Dicranella* (Dicranellaceae). With more than 150 species and great morphological variation, the genus has so far been represented in molecular phylogenetic studies by only three species, one of which was found to belong in a different family, the Aongstroemiaceae (Stech, 1999c; Stech et al., 2012).

Aims and outline of the thesis

This thesis aims to infer relationships and clarify circumscriptions of selected haplolepideous mosses, focusing on the taxa formerly classified in the family Dicranaceae and on plants with a leucobroid leaf morphology. The chosen study cases illustrate potential conflicts between the (morphological) circumscription of taxa and their supposed evolutionary relationships. The phylogenetic analyses presented in this manuscript were based on molecular markers of the three genomes: the nuclear ribosomal ITS1-5.8S-ITS2 region, the mitochondrial *nad*5 G1 intron, and the chloroplast regions *trn*S-*trn*F and *atp*B-*rbc*L spacer. The results of the molecular phylogenetic analyses served as a framework for the interpretation of the morphology of the study taxa, allowing the re-evaluation of their circumscriptions in an integrative taxonomic approach.

Chapter 2 focuses on the relationships and circumscription of the leucobryoid genus *Octoblepharum*. The genus was most often placed within the family Calymperaceae, however an alternative taxonomic position in its own family, Octoblepharaceae (Cardot) A. Eddy ex M. Menzel, was proposed. In this thesis, the phylogenetic position of *Octoblepharum* was studied based on its largest sampling so far, to address whether there is morphological and molecular

data support to the classification of the genus in its own family, and to contribute to the understanding of the circumscriptions of its species.

The origin of the leucobryoid morphology within the morphologically heterogeneous family Leucobryaceae remains unclear since relationships within the family were not yet resolved with high support. No study so far targeted the relationships on this level in the Leucobryaceae, and in other studies where the family was represented the sampling of its genera was limited and few molecular markers were applied. In **Chapter 3** phylogenetic analyses were performed to infer the suprageneric relationships within the Leucobryaceae, based on a sampling representing 11 out of its 14 genera (excluded only the rare genera which were never sampled for molecular data), and including ancestral state reconstruction analyses for selected morphological characters. The goal was to evaluate and improve morpho-molecular circumscriptions of the family and its genera. Hypotheses for the evolution of important taxonomic characters were applied to help clarifying the usefulness of such characters in the classification of the family.

Chapter 4 focuses on widespread, morphologically diverse, and yet little studied dicranoid taxa. Families Aongstroemiaceae and Dicranellaceae have been accepted in the latest moss classifications as segregates of the Dicranaceae, but their circumscriptions remain poorly defined. The families and most of their genera, including the type genera *Aongstroemia* Bruch & Schimp. and *Dicranella*, lack thorough taxonomic studies and are little represented in molecular phylogenetic studies. Moreover, the small sampling overlap for the families between different molecular phylogenetic studies greatly restricts the understanding about the phylogenetic relationships in these diverse groups of plants. The sampling for these families was extended with newly sequenced species, especially representing the genus *Dicranella*, and combined all available sequences from other studies to maximise the taxon sampling. This data was analyzed in the context of an alignment containing representatives of 37 out of the 38 haplolepideous families (sensu Frey & Stech, 2009; except the family Viridivelleraceae I.G. Stone which was never sampled for molecular data). Are changes needed to the circumscriptions of Aongstroemiaceae, Dicranellaceae and their genera?

Chapter 2

Tackling relationships and species circumscriptions of *Octoblepharum*, an enigmatic genus of haplolepideous mosses (Dicranidae, Bryophyta)

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Introduction

Octoblepharum was the first genus that was described to accommodate mosses with a so-called leucobryoid morphology. Leucobryoid mosses are easily recognised by the whitish green colour of their gametophytes, which is due to their specific leaf anatomy. The leaves consist mainly of a very wide costa composed of two to several layers of large, hyaline cells (leucocysts or hyalocysts), surrounding one (or up to three) layer(s) of chlorophyllose cells (chlorocysts). Mosses with a leucobryoid morphology belong to the haplolepideous mosses (subclass Dicranidae), which constitute the second largest lineage of Bryophyta with about 4,000 species (Frey & Stech, 2009).

Octoblepharum comprises 18 accepted species (http://www.tropicos.org), including the pantropical *O. albidum* Hedw., the recently described *O. pocsii* Magill & B.H. Allen, recorded for Africa and Asia, and 16 species restricted to the Neotropics (10 species), tropical Africa (3), tropical Asia (2), and Australia (1), respectively (Eddy, 1990; He, 2014; Mägdefrau, 1983; Magill & Allen, 2013; Salazar Allen & Tan, 2010; Townsend, 1963; Yano, 1993). The genus was first included in the family Leucobryaceae (Schimper, 1856), which originally comprised all leucobryoid mosses. After Cardot (1899) separated *Octoblepharum* within the Leucobryaceae as tribe Octoblephareae Cardot, Fleischer (1904) described the family Leucophanaceae M. Fleisch. to include the former Leucobryaceae genera *Arthrocormus* Dozy & Molk., *Exodictyon* Cardot, *Leucophanes* Brid. and *Octoblepharum*, the latter in the subfamily Octoblepharoideae [‘Gruppe Octoblephareae’] (Cardot) M. Fleisch. Andrews (1947) considered the Leucophanaceae genera to belong to the Calymperaceae, based on similarities such as leaf structure, presence of a preperistome, and presence of leaf apex propagula. Finally, Eddy (1990) classified *Octoblepharum* in its own family, stating that ’the combination of gametophyte features, monoecious (autoecious) reproductive system and peculiar peristome structure appears to set *Octoblepharum* apart from Leucobryaceae on the one hand and Calymperaceae on the other’. Since Eddy did not follow the nomenclatural rules, Menzel (1991) validated the name Octoblepharaceae. This checkered taxonomic history is due to the fact that *Octoblepharum* differs from all other leucobryoid genera by its leaf cross section with

triangular chlorocysts in a single layer (Salazar Allen, 1991) as well as by a reduced peristome consisting of eight or 16 entire teeth, which made inferences about relationships difficult (Edwards, 1979).

Although authors such as Enroth (1989, 1990) acknowledged *Octoblepharum* as being anomalous among the leucobryoid Calymperaceae, the name Octoblepharaceae has not been widely used (e.g., Ellis, 2007), and the latest classifications of mosses (Frey & Stech, 2009; Goffinet et al., 2009) still treated *Octoblepharum* as part of the Calymperaceae. One reason may be that molecular phylogenetic data on *Octoblepharum* are still scarce (cf. Stech et al., 2012) and the monophyly of the genus has not yet been tested, since only *O. albidum* was included with one or two specimens in analyses of molecular evolution (Wall & Herbeck, 2003), moss relationships (Hedderson et al., 2004; La Farge et al., 2000; Tsubota et al., 2003, 2004) or the Calymperaceae relationships (as outgroup; Fisher et al., 2007). In these studies, *Octoblepharum albidum* was either resolved as sister to the remaining (well-supported) Calymperaceae with low support (Hedderson et al., 2004; La Farge et al., 2000; Tsubota et al., 2003) or separated from them (Tsubota et al., 2004).

If *Octoblepharum* and the remaining Calymperaceae are indeed sister groups, how they relate to other putatively close lineages, such as the Dicranaceae and the monogeneric Hypodontiaceae M. Stech (Cox et al., 2010; Stech et al., 2012; Tsubota et al., 2004), are still open questions. Only one phylogeny included *Octoblepharum*, the Calymperaceae and the Hypodontiaceae simultaneously (Tsubota et al., 2004), but yielded insufficient resolution for the relationships among these groups.

Furthermore, species delimitations and relationships within *Octoblepharum* are still incompletely known. Half of the currently 18 accepted names represent little known species (Crosby et al., 1999), most of which are poorly described and/or never re-collected after their description. A single study investigated the genetic variation within and between three *Octoblepharum* species based on RAPD markers (Korpelainen & Salazar Allen, 1999), and the hypothesis that *Octoblepharum* is split into two evolutionary lines, one with eight and the other with 16 peristome teeth (Salazar Allen, 1991), is yet to be tested by molecular phylogenetic reconstructions.

Based on an extended dataset of Stech et al. (2012), including DNA sequence markers of all three genomes, the aim of this study was to (i) test the monophyly of *Octoblepharum*, (ii) infer its relationships with the Calymperaceae and *Hypodontium* (Hypodontiaceae) as putative close relatives, to conclude whether the genus should be placed in its own family or not, and (iii) provide a preliminary assessment of species circumscriptions and relationships within *Octoblepharum*.

Material and Methods

Plant material and sequence data sampling

For analyses at suprafamilial level within the haplolepideous mosses, the dataset from Stech et al. (2012), which comprised combined chloroplast *rps*4-*trn*T-*trn*L-*trn*F and *atp*B-*rbc*L sequences of 54 species of the Dicranidae as well as *Timmia austriaca* Hedw. (Timmiidae) and *Encalypta streptocarpa* Hedw. (Encalyptidae Ochyra et al.) as outgroup representatives, was extended by 21 specimens of *Octoblepharum*, and 12 samples of the (other) Calymperaceae. Material of seven species of *Octoblepharum* suitable for DNA sequencing was selected from herbaria L, MO, SING, and UB. The final sampling comprised four species, due to misidentifications or failure to obtain PCR products for some specimens.

Besides the extended taxon sampling, further markers were sequenced for the total dataset, namely mitochondrial *nad*5 as well as plastid *trn*S-*rps*4, to complete the *trn*S-*trn*F region (*trn*S-*rps*4-*trn*T-*trn*L-*trn*F; cf. Hernández-Maqueda et al., 2008). For analyses of relationships within *Octoblepharum*, a subset of the Dicranidae dataset including all *Octoblepharum* specimens as well as *Calymperes erosum* Müll.Hal., *Leucophanes angustifolium* Renauld & Cardot and *Syrrhopodon gardneri* (Hook.) Schwägr. as outgroup representatives was used, to which the nuclear ribosomal ITS1-5.8S-ITS2 region was added as an additional marker with high sequence variability.

Voucher information and GenBank accession numbers are listed in Appendix 1.

DNA extraction, amplification, and sequencing

DNA extractions of newly included specimens were performed with the NucleoSpin® Plant II Kit (Macherey-Nagel). Primers and PCR amplification protocols for all amplified regions are listed in Appendix 2. The PCR amplification mix was prepared with 14.3 µL MilliQ® water (Merck Millipore Corporation), 3 µL Q-solution® (Qiagen), 2.5 µL 10× CoralLoad® PCR buffer (Qiagen), 1 µL $MgCl_2$ (Qiagen), 0.9 µL dNTP, 1 µL of each primer (forward and reverse, ordered from Sigma-Aldrich Co.), 0.3 µL of Taq DNA polymerase (Qiagen), and 1 µL template DNA per sample for each marker except *nad*5, for which replacing Q-solution with MilliQ water yielded better results. PCR products were purified and sequenced at Macrogen Inc. (www.macrogen.com) and BaseClear B.V. (www.baseclear.com) using the amplification primers.

Phylogenetic reconstructions

Sequences were manually aligned in Geneious® v8.0.5 (Biomatters Ltd). Phylogenetic reconstructions were performed under maximum parsimony (MP), maximum likelihood (ML),

and Bayesian inference (BI). In each analysis, gaps were either treated as missing data or coded as informative by simple indel coding (SIC) (Simmons & Ochoterena, 2000) using SeqState (K. Müller, 2004). Evolutionary model testing for ML and BI was performed for each of the two combined datasets, Dicranidae and *Octoblepharum*, in jModelTest 2 (Darriba et al., 2012; Guindon & Gascuel, 2003). According to the Akaike Information Criterion (AIC), the selected model for the Dicranidae dataset was GTR+Γ+I, followed closely by GTR+Γ, and for the *Octoblepharum* dataset it was GTR+Γ. Yang (2006) and other authors (as Jia et al., 2014 and references therein) recommended the use of the model GTR+Γ instead of GTR+Γ+I, with the support of mathematical and biological arguments, and we followed this recommendation in the analyses performed in this study. Since model testing for each partition (ITS, *nad*5, *trn*S-*trn*F, and *atp*B-*rbc*L) separately resulted in the selection of a GTR model (GTR, GTR+Γ, GTR+I, GTR+Γ+I), and maximum parsimony analyses of each partition did not reveal incongruent topologies, all model-based analyses were performed with the combined datasets under the GTR+Γ model.

Maximum parsimony analyses were performed in PAUP® 4.0b10 (Swofford, 2002). Heuristic searches were implemented using random sequence addition with 1,000 replicates and tree bisection-reconnection branch-swapping. Heuristic bootstrap searches were performed with 1,000 replicates and 10 random addition cycles per bootstrap pseudoreplicate with the same options in effect. Maximum likelihood searches and thorough bootstrap analyses were performed with RAxML v8 (Stamatakis, 2014) employing raxmlGUI v1.3.1 (Silvestro & Michalak, 2012). Ten independent ML searches and 1,000 bootstrap replicates were performed within each analysis. Bayesian inference analyses were performed in MrBayes v3.2.6 (Ronquist et al., 2012), on the CIPRES Science Gateway v3.3 (M. A. Miller et al., 2010). Four runs with four chains (5×10^6 or 10^7 generations each) were run simultaneously, with the temperature of the single heated chain set to 0.4. Chains were sampled every 1,000 generations and the respective trees were written to a tree file. After verifying the convergence of runs in Tracer v1.6 (Rambaut et al., 2014), 50% majority rule consensus trees and PP of clades were calculated after the chains converged.

Results

The Dicranidae alignment of combined mitochondrial and chloroplast markers comprised 5,096 positions, of which 2,126 were variable, and 1,513 of the variable positions were parsimony-informative (*nad*5 276, *trn*S-*trn*F 934, and *atp*B-*rbc*L 303 parsimony-informative positions). Simple indel coding added 745 (*nad*5 45, *trn*S-*trn*F 495, and *atp*B-*rbc*L 205) parsimony-informative indels.

The *Octoblepharum* alignment of combined nuclear, mitochondrial, and chloroplast markers comprised 5,374 positions, of which 1,156 were variable, and 684 of the variable positions

were parsimony-informative (ITS 152, *nad*5 74, *trn*S-*trn*F 347, and *atp*B-*rbc*L 111 parsimony informative positions). Simple indel coding added 295 (ITS 147, *nad*5 7, *trn*S-*trn*F 104, and *atp*B-*rbc*L 37) parsimony-informative indels.

Figure 6 shows the consensus tree from Bayesian inference of the Dicranidae dataset with indel coding, with indication of branch support for Bayesian inference, maximum likelihood, and maximum parsimony. Tree topologies for the suprageneric relationships between *Hypodontium* (Hypodontiaceae), the Calymperaceae, and *Octoblepharum* did not differ between phylogenetic analysis methods or datasets with and without indel coding (trees not shown). *Octoblepharum* was resolved as monophyletic with maximum support (Bayesian posterior probability [PP] 1.00, bootstrap support from maximum likelihood [ML BS] and maximum parsimony [MP BS] 100%). The clade of the Calymperaceae sister to *Octoblepharum* received maximum support in all analyses. *Hypodontium* sister to this clade received high support with indels (PP 1.00, ML BS 95%, MP BS 98%), but lower support without indels (PP 0.94, ML BS 77%, MP BS 89%). The Dicranaceae appeared as sister to this clade, also with high support (PP 1.00, ML BS 100%, MP BS 96–98%).

Figure 7 shows the consensus tree from Bayesian inference of the *Octoblepharum* dataset with indel coding, with indication of branch support for Bayesian inference, maximum likelihood, and maximum parsimony. For the *Octoblepharum* infrageneric relationships, the clades for each of the included species had maximum support in all analyses and datasets, as well as the sister group relationship of *O. cocuiense* Mitt. and *O. pulvinatum* (Dozy & Molk.) Mitt. (PP 1.00, ML and MP BS 100%). However, a difference between analyses was observed in the placement of *O. arthrocormoides* N. Salazar Allen & B.C. Tan, which appeared as sister to the clade formed by *O. cocuiense* and *O. pulvinatum* with high support in the BI and ML analyses (PP 1.00, ML BS 94–100%), whereas in the MP analyses the species was resolved with maximum bootstrap support as sister to a weakly supported clade (MP BS 60–74%) formed by all other *Octoblepharum* samples (trees not shown).


Timmia austriaca
Encalypta streptocarpa
Catoscopium nigritum
Ditrichum flexicaule
Drummondia prorepens
Dicranoweisia crispula
Bryoxiphium norvegicum
Blindia acuta
Ptychomitrium polyphyllum
Schistidium apocarpum
Racomitrium canescens
Eustichia longirostris
Archidium alternifolium
Ochrobryum gardneri
Leucobryum juniperoideum
Campylopus flexuosus
Pilopogon africanus
Atractylocarpus alticaulis
Campylopodiella flagellacea
Brothera leana
Dicranodontium denudatum
Amphidium mougeotii
Amphidium lapponicum
Rhabdoweisia crenulata
Oncophorus wahlenbergii
Oncophorus virens
Cynodontium polycarpum
Oreoweisia bogotensis
Erpodium biseriatum
Aulacopilum cf. abbreviatum
Ceratodon purpureus
Trichodon cylindricus
Pleuridium acuminatum
Syntrichia ruralis
Tortula muralis
Cinclidotus riparius
Diobelonella palustris
Aongstroemia longipes
Cheilothela chloropus
Dicranella heteromalla
Dicranella cerviculata
Microcampylopus khasianus
Leptotrichella flaccidula
Campylopodium medium
Fissidens fontanus
Fissidens bryoides
Leucoloma procerum
Dicranoloma plurisetum
Chorisodontium wallisii
Paraleucobryum longifolium
Dicranum polysetum
DICRANACEAE
Hypodontium dregei
Hypodontium pomiforme
HYPODONTIACEAE
Syrrhopodon incompletus Sint Eustatius 93
Calymperes afzelii Sint Eustatius 95
Calymperes erosum MSCe
Calymperes palisotii Sint Eustatius 94
Calymperes motleyi MS1023
Syrrhopodon gardneri MSSyg
Mitthyridium fasciculatum Malaysia 99
Mitthyridium flavum Malaysia 100
Syrrhopodon prolifer Brazil 96
Syrrhopodon trachyphyllus Australia 98
Arthrocormus schimperi Malaysia 86
Leucophanes molleri Gabon 90
Leucophanes molleri Guinea 92
Leucophanes sp. Netherlands 80b
Leucophanes angustifolium Gabon 89
Leucophanes octoblepharioides China 91
CALYMPERACEAE
Octoblepharum albidum Brazil 32
Octoblepharum albidum Thailand 107
Octoblepharum albidum Java 83
Octoblepharum albidum Australia 77
Octoblepharum albidum Australia 81
Octoblepharum albidum Nicaragua 50
Octoblepharum albidum Netherlands 80a
Octoblepharum albidum Honduras MSOal
Octoblepharum albidum Brazil 20
Octoblepharum cocuiense Ecuador 72
Octoblepharum cocuiense Brazil 109
Octoblepharum cocuiense Brazil 111
Octoblepharum cocuiense Brazil 108
Octoblepharum cocuiense Bolivia 51
Octoblepharum cocuiense Brazil 110
Octoblepharum cocuiense Bolivia 49
Octoblepharum pulvinatum Brazil 112
Octoblepharum pulvinatum French Guiana 75
Octoblepharum pulvinatum Nicaragua 52
Octoblepharum arthrocormoides Malaysia 84
Octoblepharum arthrocormoides Malaysia 85
OCTOBLEPHARACEAE
-/100
100/-
87/-
95/75
89/-
70/-
100/-
-/-
79/-
-/-
71/-
85/-
79/-
98/83
89/96
87/74
86/-
72/-
82/78
81/73
81/81
95/-
0.03

← *Figure 6. Bayesian inference consensus tree of 88 representatives of haplolepideous mosses (Dicranidae) based on mitochondrial and chloroplast DNA sequences (nad5, trnS-trnF region, and atpB-rbcL), with indel coding.* Timmia austriaca *(Timmiidae) and* Encalypta streptocarpa *(Encalyptidae) were used as outgroup representatives. Branch support is indicated for Bayesian inference (BI), maximum likelihood (ML), and maximum parsimony (MP) analyses of the same dataset. Bold branches represent posterior probabilities (PP) ≥ 0.95 for BI and bootstrap (BS) values ≥ 90% for ML and MP. Actual BS values are shown if in the range of < 90% and ≥ 70% for ML and/or MP. BS values below 70% are not shown ("-"). Branch lengths are to scale, except the ones indicated by the symbol "//" (shortened four times).*

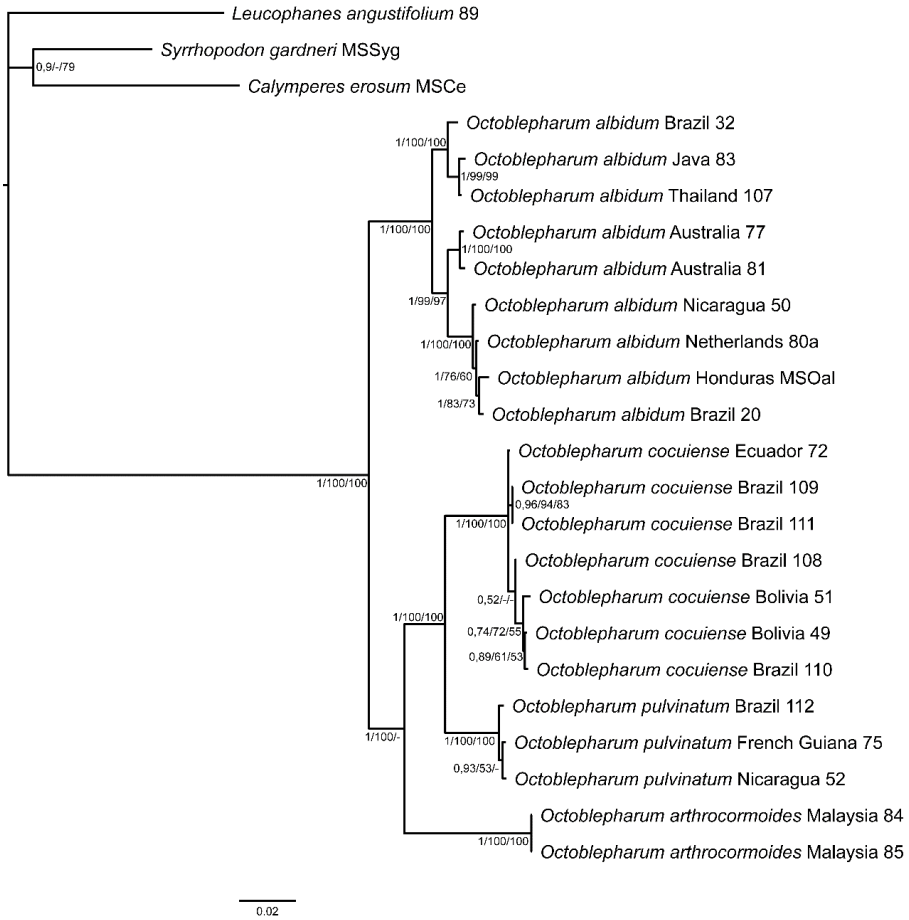

Figure 7. Bayesian inference consensus tree of 21 representatives of Octoblepharum *(Octoblepharaceae) based on nuclear, mitochondrial, and chloroplast DNA sequences (ITS,* nad*5,* trn*S*-trn*F region, and* atp*B*-rbc*L), with indel coding.* Calymperes erosum, Leucophanes angustifolium, *and* Syrrhopodon gardneri *(Calymperaceae) were used as outgroup representatives. Support values are shown for Bayesian inference, maximum likelihood, and maximum parsimony analyses (BI PP/ML BS/MP BS) of the same dataset.*

Discussion

Circumscription and relationships of Octoblepharum

The present study allows the most comprehensive phylogenetic inference of *Octoblepharum* available so far, based on 21 specimens from the Americas, Asia, and Australia, representing four species. Our data strongly support the monophyly of *Octoblepharum*, which could not be assessed in previous studies that included only samples of *O. albidum* (Hedderson et al., 2004; La Farge et al., 2000; Tsubota et al., 2003, 2004; Wall & Herbeck, 2003). The sister group relationship of *Octoblepharum* with the remainder of the Calymperaceae sensu Frey & Stech (2009), which was already resolved with moderate support in La Farge et al. (2000), receives maximal support in all present analyses. *Hypodontium* is resolved sister to this clade with significant support at least in the analyses with indels included. Previous phylogenetic reconstructions either lacked resolution for those relationships, or did not include representatives of all three clades (*Octoblepharum*, other Calymperaceae, and *Hypodontium*; Cox et al., 2010; La Farge et al., 2000; Stech et al., 2012; Tsubota et al., 2004).

Based on the molecular data, we followed Eddy (1990) and Menzel (1991) in classifying *Octoblepharum* in its own family Octoblepharaceae. *Octoblepharum* and the remaining Calymperaceae are sister groups, but high genetic divergence between them is evident. Morphological synapomorphies for *Octoblepharum* are the peculiar leaf shape and leaf anatomy. Leaf shape is a character not commonly highlighted as distinctive in the genus description (e.g., Eddy, 1990; Salazar Allen, 1994). Leaves are ligulate, the entire strap-shaped portion composed exclusively by costa, with a cuspidate to mucronate leaf apex and a distinct sheathing basal hyaline lamina. The alternate position of the hyalocysts of the different layers in costa cross-section can as well be considered a synapomorphy for *Octoblepharum* (see below). Eddy (1990) described *Octoblepharum* (and the Octoblepharaceae) as being monoecious, but his description was based mainly on *O. albidum* (the single species reported in his study for Malesia), and there are Neotropical species of the genus which are dioecious (Salazar Allen, 1991).

Octoblepharum has been retained in the Calymperaceae based on questionable arguments. First, there was a common notion that the leucobryoid Calymperaceae (genera *Arthrocormus*, *Exodictyon*, *Exostratum* L.T.Ellis, and *Leucophanes*) and *Octoblepharum* should be classified together, as in Fleischer (1904) and Andrews (1947), due to the shared leucobryoid morphology. However, the leaf structures of the leucobryoid Calymperaceae genera and *Octoblepharum* have little in common, and Ellis (1985) states that *Octoblepharum* leaves resemble more the ones of the leucobryoid Dicranales (e.g., *Leucobryum*). As is evident from Cardot's (1899) illustrations of the leaf sections, *Octoblepharum* can be distinguished by the above mentioned triangular chlorocysts, while the leucobryoid Calymperaceae show diamond-shaped chlorocysts. In addition, the leucobryoid Calymperaceae genera *Arthrocormus*, *Exodictyon*, and *Exostratum* exhibit additional layers of chlorocysts, not found in any other

leucobryoid genus, and *Leucophanes* has a distinctive bundle of stereids, also absent in all other leucobryoid genera. The close relationship between the leucobryoid Calymperaceae genera is well supported by molecular data (Fisher et al., 2007; present study). They compose a derived clade within the family Calymperaceae and thus are only distantly related to *Octoblepharum*.

Second, some species of *Octoblepharum* may occasionally present gemmae at the leaf tips, very similar in shape to those found among the Calymperaceae (e.g., Andrews, 1947; Harrington & Egunyomi, 1976; Maciel-Silva et al., 2013). However, elongate gemmae at leaf tips are also found in other unrelated moss families, e.g., in the Orthotrichaceae Arn. of subclass Bryidae (Vitt, 2014). For that reason, we do not consider this specific character adequate to support, alone, the circumscription of a family.

Third, some authors included *Octoblepharum* in the Calymperaceae based on peristome characters (Salazar Allen, 1994, and references therein). However, according to Ellis (1985), the presence of a preperistome is the only shared peristome trait between *Octoblepharum* and the remaining Calymperaceae. The *Octoblepharum* peristome cannot be considered as alike that of *Syrrhopodon* Schwägr., i.e., formed by 16 undivided teeth with equally thick inner and outer layers, usually papillose and without trabeculae, without basal membrane and often with a preperistome (Frey & Stech, 2009), since it is not papillose and may have trabeculae (Salazar Allen, 1994). Neither can it be considered as dicranoid, i.e., formed by 16 teeth divided to half, dorsally trabeculate and vertically striate, without a developed basal membrane (Frey & Stech, 2009), since it bears a preperistome and has undivided (even reduced in number) teeth (Salazar Allen, 1994).

Edwards (1979) has briefly described the peristome of *O. albidum* as reduced, with eight simple teeth, the peristome formula 2(−3):2, deviating from the typical haplolepideous formula, and no trabeculae, with dorsal and ventral peristome plates slightly convex. He claimed this reduction would make it difficult to make inferences on *Octoblepharum* relationships. However, the non-reduced *Octoblepharum* peristomes with 16 teeth, not as well described in the literature, show a different combination of characters, and may be more informative regarding relationships. On preliminary examination, the *O. pulvinatum* teeth (specimen French Guiana 75 in Figures 6, 7), for example, are well-developed, with strong trabeculae, vertical striae, no papillae, without the ventral zig-zag line usually seen in other haplolepideous peristomes (absent also in the eight teeth species, but due to their reduction), and differ from the eight teeth pattern as well as from the syrrhopodontoid or dicranoid types. Thus, to compare the Octoblepharaceae and the Calymperaceae based on peristome characters requires further studies on the variability of this structure among the species of *Octoblepharum*. Ellis (1985) may be correct, and the *Octoblepharum* peristome may even represent a fifth main expression of the haplolepideous peristome, diverging from the

dicranoid, seligerioid, syrrhopodontoid, and pottioid ones already named in the literature (Frey & Stech, 2009).

Delimitation and relationships of Octoblepharum *species*

The four included *Octoblepharum* species (*O. albidum*, *O. arthrocormoides*, *O. cocuiense*, and *O. pulvinatum*) are molecularly well-defined according to the present study (Figure 7). The molecular data support the present morphological circumscriptions of these *Octoblepharum* species, and our morphological studies indicate that the available literature allows the correct identification of the specimens. The closely related *O. cocuiense* and *O. pulvinatum* (Figure 7) both possess a peristome with 16 teeth, whereas the peristome of *O. albidum* consists of eight teeth. The sporophyte (and thus peristome) of *O. arthrocormoides* is still unknown. Salazar Allen (1992) suggested the state of eight peristome teeth found in *O. albidum* would be a derived condition, while 16 peristome teeth, the most common number among the Dicranidae, also present in the closest relatives of *Octoblepharum* (*Hypodontium* and most of the Calymperaceae), would most likely be plesiomorphic for the genus. However, a larger sampling of *Octoblepharum* species is necessary to test this hypothesis as the current topology does not provide sufficient evidence. In addition, the placement of *O. arthrocormoides* diverges between BI/ML and MP analyses (cf. Results), either sister to the two included species with 16 peristome teeth (Figure 7) or sister to all other included *Octoblepharum* species in MP trees (trees not shown). Since in the trees for BI and ML the two samples of *O. arthrocormoides* form a very long branch, the topology in MP may be a result of long-branch attraction.

The two specimens of *O. arthrocormoides* studied here are probably the first report after its original description. Their identity was revealed by molecular analyses, since they were originally labelled as *O. albidum*. *Octoblepharum arthrocormoides* is very similar to *O. albidum* in gametophytic features such as colour, size of the leaves and leaf shape. The only literature which compares the two species is the original description of *O. arthrocormoides* by Salazar Allen & Tan (2010). *Octoblepharum arthrocormoides* differs from *O. albidum* in its broken leaf apices, shorter lamina hyalocysts, more hyalocyst layers, and lack of inflated and porate marginal hyalocysts (Salazar Allen & Tan, 2010). According to our observations, the main distinctive feature between these species is the general appearance of the gametophytes, slenderer and with broken leaf apices in *O. arthrocormoides*, and more compact with entire leaves in *O. albidum*. The central lamina hyalocysts are indeed shorter and mostly quadrate in both specimens of *O. arthrocormoides* studied here, as opposed to longer and mostly short-rectangular to rectangular hyalocysts in *O. albidum*. Number of hyalocyst layers is almost overlapping in the specimens included in this study, ranging from 5–7 layers on each side of the chlorocyst layer in *O. arthrocormoides* versus 2–5 in *O. albidum*. In addition, we observed

that the pores between lamina hyalocysts are clearly visible in *O. albidum* but only visible as small dots in *O. arthrocormoides*.

Although all specimens in the well-supported *O. cocuiense* clade exhibit fragile leaves with the pseudocosta characteristic of the species, they display considerable morphological variability. Two of these specimens were previously misidentified as *O. erectifolium* Mitt. ex R.S. Williams. Gametophytically, the two species may be mistaken due to similarities in the fragility of leaves, shape of lamina cells, dentation of upper lamina margins, and in some specimens of *O. cocuiense*, unusually long leaves for the genus (according to descriptions in Salazar Allen, 1994). However, they differ in that *O. erectifolium* shows leaves even longer than the range of variation of *O. cocuiense* (from 2 mm long), lacks a pseudocosta and never shows a purple tone in its leaves. The misidentified specimens in this study were indeed in the larger range of leaf sizes for *O. cocuiense*, but in all other characters they fit well the species description. They did not bear sporophytes, otherwise they would have been easily identified correctly as *O. cocuiense*, since *O. erectifolium* has eight peristome teeth. Two other *O. cocuiense* specimens were mistaken for *O. pulvinatum*. These had a pale green tone, hexagonal basal lamina cells similar to those of some *O. pulvinatum* specimens, and small plant and leaf sizes corresponding to smaller specimens of *O. cocuiense*. These characteristics resemble those of *O. pulvinatum*, which has its name in reference to its also fragile leaves. The main trait which allowed their correct identification as *O. cocuiense* was the presence of a pseudocosta, although in specimens such as these, which lack the strong pink pigmentation, this structure can be quite inconspicuous and is only visible at low magnifications.

Octoblepharum pulvinatum also shows vegetative similarities to species with eight peristome teeth. It resembles *O. albidum*, the Neotropical *O. cylindricum* Schimp. ex Mont., and the recently described Paleotropical *O. pocsii* (He, 2014; Magill & Allen, 2013) in the green colour and lack of a pseudocostal area. It further resembles *O. albidum* and *O. cylindricum* in occasionally showing a pink tone at its leaf bases, and differs from those species in its fragile leaves and quadrate to short hexagonal basal lamina cells (Salazar Allen, 1991). On the other hand, it further resembles *O. pocsii* in its fragile, long leaves, and short basal lamina cells, differing in having shorter leaves, and in occasionally showing a pink tone at its leaf bases (Magill & Allen, 2013).

Octoblepharum albidum, although being well-supported based on the molecular data (Figures 6, 7), displays a considerable intraspecific molecular variability. Furthermore, the three well-supported clades resolved within *O. albidum* based on our preliminary data indicate the presence of geographical structure. One lineage is formed by Neotropical samples (Brazil, Honduras, and Nicaragua), plus a specimen from a glasshouse in the Hortus Botanicus Leiden, which thus is likely of Neotropical origin. The second lineage, sister to the first, includes only Australian samples, and the third lineage, sister to the clade formed by the other two, comprises one Brazilian and two Asian samples, indicating that *O. albidum* populations in Brazil

belong to two different lineages. The three *O. albidum* clades fit the general morphological description of the species. However, as *O. albidum* is a reportedly variable species (Florschütz, 1955; Magill & Allen, 2013; Salazar Allen, 1991, 1994), further studies are necessary to verify if these clades correspond to morphologically distinguishable groups within a species complex.

Chapter 3

Testing hypotheses on suprageneric relationships and morphological evolution in the Leucobryaceae (Bryophyta)

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Introduction

Leucobryoid mosses are haplolepideous mosses (Dicranidae) whose gametophytes exhibit a characteristic whitish green color. This color is caused by a leaf anatomy with a very wide costa composed of two to several layers of large, hyaline cells interconnected by pores (called leucocysts or hyalocysts), embedding 1–3 layers of chlorophyllose cells (chlorocysts).

Schimper (1856) recognised four leucobryoid moss genera (*Arthrocormus*, *Leucobryum*, *Leucophanes* and *Octoblepharum*) in a single family, the Leucobryaceae. The leucobryoid genera *Cladopodanthus* Dozy & Molk. and *Schistomitrium* Dozy & Molk. were not mentioned by Schimper (1856), and three further genera (*Cardotia* Besch. ex Cardot, *Exodictyon* and *Ochrobryum* Mitt.) were described later during the nineteenth century. All these genera share a generally similar habit and leaf structure, but differ in their leaf anatomy (Cardot, 1899; Fleischer, 1904) and peristome characters (e.g., the presence of a preperistome, thickening and ornamentation of inner and outer plates). Based on these differences, Fleischer (1904) separated the leucobryoid genera into two families: the Leucobryaceae, considered more related to the Dicranaceae, and the Leucophanaceae, related to the Calymperaceae. Several other classifications for the aforementioned genera as well as further leucobryoid genera described during the twentieth century (*Carinafolium* R.S. Williams, *Exostratum*, *Holomitriopsis* H. Rob. and *Steyermarkiella* H. Rob.) were proposed (overview in Yamaguchi, 1993). The latest classification of mosses previous to the introduction of molecular phylogenetic studies (Buck & Goffinet, 2000) recognised 11 leucobryoid genera, classified in the Dicranaceae (*Holomitriopsis* and *Steyermarkiella*), the Calymperaceae (*Arthrocormus*, *Exodictyon*, *Exostratum*, *Leucophanes*, and *Octoblepharum*) and the Leucobryaceae (*Cladopodanthus*, *Leucobryum*, *Ochrobryum*, and *Schistomitrium*).

That the leucobryoid genera in the Calymperaceae (and the Octoblepharaceae, cf. Bonfim Santos & Stech, 2017a) were not closely related to those in the Leucobryaceae, as proposed by Fleischer (1904), was confirmed by molecular data (e.g., Bonfim Santos & Stech, 2017a; Cox et al., 2010; Fisher et al., 2007; La Farge et al., 2000). In contrast, molecular phylogenetic

evidence suggested that a number of the Dicranaceae genera belong to the Leucobryaceae (Hedderson et al., 2004; La Farge et al., 2000, 2002; Stech, 1999b, 2004; Tsubota et al., 2003, 2004). These genera display morphologies that are either somewhat leucobryoid (*Brothera* Müll.Hal., *Campylopodiella* Cardot) or dicranoid (e.g., *Atractylocarpus* Mitt., *Campylopus* Brid., *Dicranodontium* Bruch & Schimp., *Pilopogon* Brid.). The typical dicranoid costa has a median band of enlarged deuter cells surrounded by dorsal and ventral layers of stereids, and dorsal and ventral epidermal layers (Frahm, 1991b). This basic structure, however, varies considerably in different genera, e.g., by the absence of epidermal or stereid layers or their further subdivision into more layers. The so-called paraleucobryoid costa, named after the genus *Paraleucobryum* (Limpr.) Loeske (Dicranaceae), is characterised by median chlorocysts (corresponding to the deuter cells) surrounded by ventral and dorsal hyalocysts, thus resembling the costa of *Leucobryum*. The term paraleucobryoid has also been applied to the costa of *Brothera* and *Campylopodiella*, which, however, differs by the presence of stereids. In contrast to the hyalocysts of the leucobryoid costa, the hyalocysts in *Paraleucobryum*, *Brothera* and *Campylopodiella* lack interconnecting pores between cells and never form multiple layers.

In the classification of mosses by Goffinet & Buck (2004) and Goffinet et al. (2008), seven dicranoid genera (*Atractylocarpus*, *Brothera*, *Bryohumbertia* P. de la Varde & Thér., *Campylopodiella*, *Campylopus*, *Dicranodontium* and *Microcampylopus* (Müll.Hal.) Fleisch.) were included in the Leucobryaceae. The classification proposed by Frey & Stech (2009) differs from that presented by Goffinet & Buck (2004) and Goffinet et al. (2008) by placing *Microcampylopus* in the Dicranellaceae, adding *Holomitriopsis*, *Mitrobryum* H. Rob., *Sphaerothecium* Hampe and *Steyermarkiella* (Dicranaceae in Goffinet et al., 2008) to the Leucobryaceae, and incorporating nomenclatural changes at genus level, synonymizing *Bryohumbertia* with *Campylopus* (Stech, 2004) and mistakenly adopting the use of *Atractylocarpus* for the *Campylopodiella* species and of *Metzleria* Schimp. ex Milde for the *Atractylocarpus* species (a misinterpretation of the nomenclatural proposal by Frahm & Isoviita, 1988). The placement of *Microcampylopus* in the Dicranellaceae and of *Holomitriopsis* in the Leucobryaceae was supported by molecular data (Bonfim Santos & Stech, 2017a; Cox et al., 2010; Stech, 2004; Stech et al., 2012). *Mitrobryum*, *Sphaerothecium* and *Steyermarkiella* have not yet been included in molecular phylogenetic studies.

While the Leucobryaceae are molecularly well circumscribed, molecular data to assess generic delimitations and relationships within the family are still limited. Cox et al. (2010) covered 10 out of 14 genera of the Leucobryaceae sensu Frey & Stech (2009), which were resolved in three lineages: a dicranoid lineage comprising *Campylopus* and *Pilopogon* (the sequences named as *Microcampylopus leucogaster* (Müll.Hal.) B.H. Allen in the same clade originate from a collection that actually belongs to *Campylopus*); another dicranoid lineage comprising *Brothera*, *Campylopodiella* and *Dicranodontium*; and a leucobryoid lineage with *Ochrobryum*,

Holomitriopsis, *Leucobryum*, *Cladopodanthus* and *Schistomitrium*. A similar topology including representatives of eight genera was resolved in Stech et al. (2012). However, relationships between the major lineages within the Leucobryaceae were contradictory between these and other molecular studies, and the taxon sampling was too limited to infer generic circumscriptions.

Furthermore, uniting gametophytically heterogeneous genera in the Leucobryaceae considerably obscured the family's morphological circumscription. The evolution of morphological traits within the Leucobryaceae and the suitability of morphological characters to assess generic delimitations and relationships remain insufficiently known, since all revisional work concerning these genera predates molecular phylogenetic studies.

Consequently, the present study aims to (1) test hypotheses of suprageneric relationships and genus circumscriptions in the Leucobryaceae based on phylogenetic analyses of a comprehensive taxon and molecular marker sampling and (2) infer the evolution of morphological characters within the family based on ancestral state reconstructions.

Material and methods

Taxon sampling, DNA extraction and sequencing

The taxon sampling comprised 63 Leucobryaceae specimens (representing 11 out of 14 genera and 45 species) as well as *Archidium alternifolium* (Dicks. ex Hedw.) Schimp. (Archidiaceae), *Eustichia longirostris* (Brid.) Brid. (Eustichiaceae) and *Micromitrium tenerum* (Bruch & Schimp.) Crosby (Micromitriaceae Smyth ex Goffinet & Budke) as outgroup representatives following earlier phylogenetic reconstructions (Fedosov et al., 2016a; Goffinet et al., 2011; Stech et al., 2012). Molecular markers from all three genomes were sequenced: mitochondrial (mt) *nad*5 G1 intron, chloroplast (cp) *trn*S-*trn*F region and *atp*B-*rbc*L spacer and nuclear (nr) ribosomal ITS1-5.8S-ITS2 (ITS) region. Sequences were obtained from GenBank and newly sequenced specimens from herbaria L, MO, SING and UB. Voucher information and GenBank accession numbers are listed in Appendix 1.

Procedures for DNA extraction, amplification and sequencing followed Bonfim Santos & Stech (2017a). Sequences were manually aligned in Geneious® v8.0.5 (Biomatters Ltd.), using the alignment from Stech et al. (2012) as a starting point.

Phylogenetic reconstructions

Three alignments were analyzed. The first alignment comprised the combined mitochondrial and chloroplast markers for all Leucobryaceae specimens, with *Archidium alternifolium*, *Eustichia longirostris* and *Micromitrium tenerum* as outgroup representatives. The second and

third alignments represented two major clades within the Leucobryaceae, the *Dicranodontium* and the leucobryoid clades, respectively, and included additionally ITS, which was in parts unalignable across the whole family due to high sequence variability. Two samples of *Ochrobryum gardneri* (Müll.Hal.) Mitt. were used as outgroup representatives, based on the analyses of the first alignment and alignability of the ITS sequences. The third major lineage within the Leucobryaceae, the *Campylopus* clade, has been extensively studied elsewhere (Stech, 2004; Stech et al., 2010; Stech & Dohrmann, 2004; Stech & Wagner, 2005) and therefore was not studied in detail in this work. Alignment lengths as well as numbers of variable and parsimony-informative positions are provided in Table 1.

Table 1. Length, number of variable and parsimony informative positions per alignment.

	ITS	5.8S	*nad*5	cd	sp	*rps*4	*atp*B	total
Leucobryaceae alignment								
Total positions	-	-	864	182	1163	613	675	3497
% variable positions	-	-	15,28	6,04	33,53	21,70	25,04	23,88
% parsimony-informative positions	-	-	08,91	2,75	23,39	16,15	15,11	15,87
***Dicranodontium* clade alignment**								
Total positions	1324	157	860	182	1028	609	562	4722
% variable positions	17,75	0	4,54	2,20	10,02	4,11	7,30	9,47
% parsimony-informative positions	09,82	0	2,56	1,10	06,62	2,30	4,27	5,51
Leucobryoid clade alignment								
Total positions	1510	157	858	182	1041	613	555	4916
% variable positions	24,37	3,19	4,66	4,40	22,38	10,93	14,41	16,29
% parsimony-informative positions	14,83	0,64	2,80	2,20	16,81	7,99	11,17	10,96

Table 2. Partitioning scheme and nucleotide substitution models selected with PartitionFinder per alignment.

partition name	region	positions	RAxML model	MrBayes model
Leucobryaceae alignment				
nad5	*nad*5 G1 intron	1-864	GTR+G	HKY+G
cd	*trn* coding sequences cpDNA	865-875, 1916-1987, 2334-2368, 2685-2735, 2810-2822	GTR+I+G	K80+I+G
sp	spacers and introns *trn*S-*trn*F region	876-943, 1557-1915, 1988-2333, 2369-2684, 2736-2809	GTR+G	GTR+G
rps4	*rps*4 gene	944-1556	GTR+I+G	GTR+I+G
atpBrbcL	*atp*B-*rbc*L spacer	2823-3497	GTR+G	GTR+G
***Dicranodontium* clade alignment**				
ITS	ITS1 and ITS2	1-663, 821-1481	GTR+G	SYM+G
5.8S	5.8S	664-820	-	JC
nad5	*nad*5 G1 intron	1482-2341	GTR+G	HKY+G
cd	*trn* coding sequences cpDNA	2342-2352, 3310-3381, 3688-3722, 4029-4079, 4148-4160	-	K80+I
sp + atpBrbcL	spacers and introns cpDNA	2353-2405, 3015-3309, 3382-3687, 3723-4028, 4080-4147, 4161-4722	GTR+G	GTR+G
rps4	*rps*4 gene	2406-3014	GTR+I+G	GTR+I+G
5.8S + cd	5.8S + *trn* coding sequences cpDNA	664-820, 2342-2352, 3310-3381, 3688-3722, 4029-4079, 4148-4160	GTR+I+G	-
Leucobryoid clade alignment				
ITS	ITS1 and ITS2	1-895, 1053-1667	GTR+G	SYM+G
5.8S + cd	5.8S + *trn* coding sequences cpDNA	896-1052, 2526-2536, 3510-3581, 3881-3915, 4226-4276, 4349-4361	GTR+G	K80+I
nad5	*nad*5 G1 intron	1668-2525	GTR+G	HKY+I
sp	spacers and introns *trn*S-*trn*F region	2537-2590, 3204-3509, 3582-3880, 3916-4225, 4277-4348	GTR+I+G	GTR+I+G
rps4	*rps*4 gene	2591-3203	GTR+I+G	GTR+I+G
atpBrbcL	*atp*B-*rbc*L spacer	4362-4916	GTR+I+G	GTR+I+G

Phylogenetic reconstructions were performed under maximum likelihood (ML) using RAxML v.8 (Stamatakis, 2014) and Bayesian inference (BI) using MrBayes v.3.2.6 (Ronquist et al., 2012), both on the CIPRES Science Gateway v.3.3 (M. A. Miller et al., 2010). Analyses were run for the markers separated per genome (mt, cp, nr) and for the complete alignments (mt + cp or mt + cp + nr, respectively), to check for incongruence and to infer how each genome contributed to the resolution and clade support. Gaps were treated as missing data. Selection of partitioning schemes and evolutionary model testing were performed in Partition-Finder v1.1.1 (Lanfear et al., 2012) for the models that can be implemented in RAxML (GTR) and MrBayes (GTR and several of its nested models), respectively, with or without a gamma-distributed rate variation among sites (Γ) and a proportion of invariable sites (*I*). Model parameters were estimated independently for each partition. The resulting best partitioning schemes and evolutionary models according to the Akaike information criterion (AIC) were implemented in the ML and BI analyses (Table 2). In RAxML, a single type of rate heterogeneity pattern (either + Γ, + *I* or + Γ + *I*) can be applied for all partitions per analysis; thus, we implemented GTR + Γ in all ML analyses. For all maximum likelihood analyses, rapid bootstrapping with 1000 iterations was performed. For Bayesian inference, four runs with four chains (5 × 106 generations each) were run simultaneously, with the temperature of the single heated chain set to 0.4. Chains were sampled every 1000 generations, and the respective trees were written to tree files. After verifying the convergence of runs in Tracer v1.6 (Rambaut et al., 2014), fifty percent majority rule consensus trees and PP of clades were calculated, discarding the burn-in phase (25%).

The Shimodaira-Hasegawa (SH) test (Goldman et al., 2000; Shimodaira & Hasegawa, 1999) was applied to compare selected alternative phylogenetic hypotheses for the Leucobryaceae and the *Dicranodontium* clade. For the Leucobryaceae, the ML tree (topology as in Figure 8) was compared with the hypotheses of (1) the sister group relationship of the *Campylopus* clade and the *Dicranodontium* clade (as resolved in Stech, 2004), (2) the sister group relationship of the *Campylopus* clade and the leucobryoid clade (Tsubota et al., 2004), (3) a monophyletic *Leucobryum* (as resolved in the analyses of the leucobryoid clade alignment) and (4) a clade formed by *Cladopodanthus*, *Holomitriopsis*, *Ochrobryum* and *Schistomitrium,* a relationship suggested by Eddy (1990) and Robinson (1990). For the *Dicranodontium* clade, the ML tree (topology as in Figure 9) was compared with the hypotheses of (1) a monophyletic *Campylopodiella*, (2) a monophyletic *Dicranodontium*, (3) a monophyletic *Dicranodontium* including *D. subporodictyon* Broth., (4) a monophyletic *Dicranodontium* including *Atractylocarpus intermedius* (B.H. Allen) J.-P. Frahm and (5) a monophyletic *Dicranodontium* including both *D. subporodictyon* and *A. intermedius*, all hypotheses based on the taxonomic literature (Allen, 1992a; Allen & Ireland, 2002; Frahm, 1997; P. Müller & Frahm, 1987). Constraint trees were used as an input to ML with RAxML. The resulting trees with branch length values and corresponding alignment were loaded into PAUP* v.4.0b10 (Swofford, 2002), where these trees were compared with the respective unconstrained topologies using

the SH test with 10,000 bootstrap replicates and the resampling estimated log-likelihood method (RELL).

The NeighborNet algorithm (Bryant & Moulton, 2004) implemented in SplitsTree4 (Huson & Bryant, 2006) was applied to the Leucobryaceae alignment for visualization of the data in a phylogenetic network. Missing data obscured the network patterns; thus, all specimens with missing data for an entire molecular marker were removed from the alignment.

Ancestral state reconstructions

Maximum likelihood ancestral state reconstructions were performed with Mesquite v.3.2 (Maddison & Maddison, 2017) under the Markov k-state model (Lewis, 2001). The analyzed characters for the Leucobryaceae were leucobryoid morphology (0 absent, 1 present), seta orientation when young or moist (0 straight, 1 twisted, 2 cygneous), capsule orientation (0 orthotropous, 1 homotropous) and calyptra shape (0 cucullate, 1 mitrate, 2 reduced). The analyzed characters for the *Dicranodontium* clade were the ventral costa layer (0 differentiated in ventral epidermis and stereid band below, 1 stereid band, 2 hyalocysts), dorsal costal stereids (0 in groups, 1 in a continuous band, 2 absent), cell type in the dorsal epidermal layer of costa (0 chlorocysts, 1 stereids, 2 hyalocysts), occurrence of pitted basal lamina cells (0 absent, 1 present) and seta orientation when young or moist (0 straight, 1 twisted, 2 cygneous). For both alignments, the character evolution analyses were performed on the ML tree and on the constrained ML trees representing plausible alternative hypotheses according to the SH test results (Leucobryaceae: sister group relationship of the *Campylopus* clade and the *Dicranodontium* clade, sister group relationship of the *Campylopus* clade and the leucobryoid clade, and *Leucobryum* monophyletic; *Dicranodontium* clade: *Campylopodiella* and *Dicranodontium* reciprocally monophyletic).

Results

Phylogenetic reconstructions

Figure 8 shows consensus trees from Bayesian inference of the concatenated mitochondrial and chloroplast markers for the Leucobryaceae, all concatenated markers for the *Dicranodontium* clade and all concatenated markers for the leucobryoid clade, respectively. Branch support values are indicated for Bayesian inference (posterior probabilities, PP) and maximum likelihood analyses (bootstrap support, BS). Trees resulting from analyses of subsets of data are shown in Appendix 3, and relevant aspects of their topologies and branch support patterns are mentioned below. The trees obtained from analyses of markers from each genome separately differed in resolution and branch support, but did not reveal statistically supported incongruence (i.e., no PP ≥ 0.95 or BS ≥ 70% for the conflicting branches in both

incongruent topologies). They were also congruent with the trees resulting from the combined analyses, except for the BI analyses of the leucobryoid clade (see below).

The Leucobryaceae genera included in this study were split in three well-supported clades (Figure 8), the *Campylopus* clade (PP 1, BS 100%), which comprised the dicranoid genera *Campylopus* (PP 1, BS 100%) and *Pilopogon* (PP 1, BS 98%), the *Dicranodontium* clade (PP 1, BS 100%), which comprised the remaining dicranoid genera (*Atractylocarpus*, *Brothera*, *Campylopodiella*, *Dicranodontium*) and the leucobryoid clade (PP 1, BS 99%), which comprised the leucobryoid genera (*Cladopodanthus*, *Holomitriopsis*, *Leucobryum*, *Ochrobryum*, *Schistomitrium*). Within the leucobryoid clade, *Cladopodanthus* and *Schistomitrium* were both monophyletic and formed sister clades with maximum support (PP 1, BS 100%). They appeared in these analyses as sister to *Leucobryum sanctum* (Nees ex Schwägr.) Hampe (PP 1, BS 82%). The *Leucobryum*/*Cladopodanthus*/*Schistomitrium* clade was monophyletic (PP 1, BS 100%) and sister to *Holomitriopsis* (PP 1, BS 95%). *Ochrobryum* was monophyletic (PP 1, BS 100%) and sister to the clade formed by all other leucobryoid genera. The sister group relationship of the *Dicranodontium* clade and the leucobryoid clade was not significantly supported by BI, but well supported by ML (BS 92%), while in the analyses of the chloroplast markers separately it was supported by both methods (PP 0.99, BS 91%). Analyses of the chloroplast markers resulted in trees with the same topology and similar branch support as the analyses of all markers combined, whereas in analyses of *nad*5 alone relationships within the main Leucobryaceae clades were unresolved or weakly supported (Appendix 3).

Figure 8. Bayesian inference consensus tree of 63 Leucobryaceae representatives based on mitochondrial and chloroplast DNA sequences (nad5, trnS-trnF region and atpB-rbcL). Archidium alternifolium *(Archidiaceae Schimp.),* Eustichia longirostris *(Eustichiaceae Broth.) and* Micromitrium tenerum *(Micromitriaceae) were used as outgroup representatives. Branch support is indicated for Bayesian inference (BI) and maximum likelihood (ML) analyses of the same alignment. Bold branches represent posterior probabilities (PP) ≥ 0.95. Actual bootstrap (BS) values are shown if ≥ 70% .* →


Eustichia longirostris Bolivia MS1020
Micromitrium tenerum United States 69
Archidium alternifolium Germany MSAra
Pilopogon laevis Venezuela MSPla
Pilopogon guadalupensis Costa Rica MSPg
Pilopogon africanus Rwanda MSPa
Pilopogon gracilis GenBank
Campylopus introflexus GenBank
Campylopus introflexus Brazil/Trindade Island TJH31
Campylopus pilifer Costa Rica MSC012
Campylopus flexuosus Germany MSCf
Campylopus sp. Malawi 55
Campylopus subcomosus Vietnam 59
Campylopus subcomosus Vietnam 63
Dicranodontium subporodictyon UK, Scotland MS18
Campylopodiella flagellacea Panama MSCfl
Brothera leana China MSBl
Brothera leana Japan 42
Atractylocarpus intermedius Honduras 40
Atractylocarpus longisetus Colombia 41
Atractylocarpus alticaulis Rwanda MSAa
Dicranodontium denudatum Japan 6
Dicranodontium denudatum var. glabrum Spain 21
Dicranodontium denudatum France MSDd
Campylopodiella stenocarpa Nicaragua 57
Dicranodontium pulchroalare Costa Rica 44
Dicranodontium porodictyon United States 61
Ochrobryum gardneri Honduras MS1012
Ochrobryum subulatum Bolivia 66
Ochrobryum gardneri Brazil 31
Holomitriopsis laevifolia GenBank
Leucobryum sanctum Indonesia 121
Schistomitrium sparei Malaysia 115
Schistomitrium brevi-apiculatum GenBank
Cladopodanthus heterophyllus Malaysia 117
Cladopodanthus speciosus GenBank
Leucobryum bowringii China 119
Leucobryum bowringii Thailand 128
Leucobryum crispum GenBank
Leucobryum giganteum Brazil 18
Leucobryum sp. Brazil 19
Leucobryum babetii Gabon 127
Leucobryum martianum Bolivia 5
Leucobryum subobtusifolium Bolivia 17
Leucobryum subobtusifolium Brazil 13
Leucobryum subobtusifolium Brazil 15
Leucobryum candidum GenBank
Leucobryum aduncum Java 125
Leucobryum chlorophyllosum GenBank
Leucobryum javense Thailand 129
Leucobryum seemannii GenBank
Leucobryum boninense GenBank
Leucobryum javense China 126
Leucobryum scabrum Japan 11
Leucobryum madagassum Madagascar 39
Leucobryum polakowskyi Caribbean Netherlands 118
Leucobryum antillarum Bolivia 36
Leucobryum albidum United States 8
Leucobryum juniperoideum China 4
Leucobryum glaucum GenBank
Leucobryum juniperoideum Bhutan 27
Leucobryum juniperoideum Bhutan 28
Leucobryum juniperoideum Bhutan 29
Leucobryum juniperoideum Bhutan 30
Leucobryum juniperoideum China 3
Leucobryum juniperoideum France MSLj
0.02

Within the *Dicranodontium* clade (Figure 9), *Atractylocarpus* was monophyletic (PP 0.97, BS 82%) and the two *Brothera leana* (Sull.) Müll.Hal. samples formed a clade with maximum support (PP 1, BS 100%), whereas *Campylopodiella* was not monophyletic, with a well-supported sister group relationship between *Brothera leana* and *Campylopodiella flagellacea* (Müll.Hal.) J.-P. Frahm & Isov. (PP 1, BS 95%). *Dicranodontium* was not monophyletic either, since the *D. pulchroalare* Broth./*D. porodictyon* Cardot & Thér. clade (PP 1, BS 100%) was sister to the weakly supported *Brothera/Campylopodiella* clade (PP 0.96). *Dicranodontium subporodictyon* was well supported within this clade as sister to the *Atractylocarpus* clade (PP 0.97, BS 80%). Resolution and branch support were highest in the separate ITS analyses, followed by the cp markers, and *nad*5 provided the least resolution (Appendix 3).

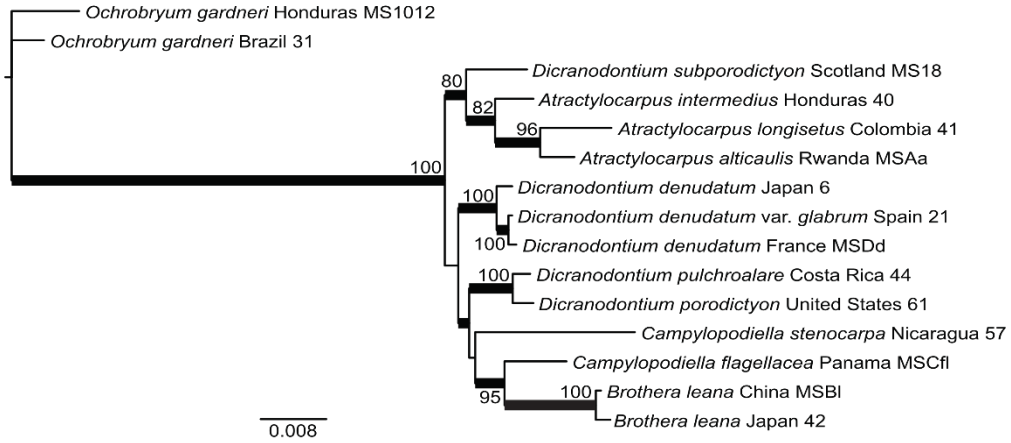


Figure 9. Bayesian inference consensus tree of 13 representatives of the Dicranodontium *clade of the Leucobryaceae based on nuclear, mitochondrial and chloroplast DNA sequences (ITS,* nad*5,* trn*S*-trn*F region and* atp*B*-rbc*L). Two samples of* Ochrobryum gardneri *(Leucobryaceae) were used as outgroup representatives. Branch support is indicated for Bayesian inference (BI) and maximum likelihood (ML) analyses of the same alignment. Bold branches represent posterior probabilities (PP) ≥ 0.95. Actual bootstrap (BS) values are shown if ≥ 70% .*

For the leucobryoid clade, the results shown in Figure 10 mostly agreed with those in Figure 8, except for the relationships between the *Cladopodanthus*/*Schistomitrium* clade and *Leucobryum* species. In the analyses of the leucobryoid clade (Figure 10), the *Cladopodanthus*/*Schistomitrium* clade (PP 1, BS 100%) and the monophyletic *Leucobryum* (PP 0.99) were sister groups (PP 1, BS 95%), while as shown in Figure 8, *Cladopodanthus* and *Schistomitrium* were nested within *Leucobryum*, causing *Leucobryum* to be paraphyletic. Bayesian inference analyses of the leucobryoid clade for each genome did not resolve the same relationships as all markers combined, but repeated the topology as seen in Figure 8.

Maximum likelihood analyses for all markers combined recovered a monophyletic *Leucobryum* as well, but with low support, and thus did not represent an incongruence in relation to the analyses per genome. Chloroplast markers were the most informative for the relationships within the leucobryoid clade, followed by *nad*5, and ITS provided the least resolution (Appendix 3).

The SH test applied to the Leucobryaceae alignment (Table 3) did not reject the two alternative hypotheses of sister group relationships between the three main Leucobryaceae clades, nor the hypothesis of *Leucobryum* being monophyletic (as in Figure 10) as significantly less likely than the unconstrained ML topology (as in Figure 8). The alternative topology with *Cladopodanthus*, *Holomitriopsis*, *Ochrobryum* and *Schistomitrium* forming one clade was rejected. The SH test applied to the *Dicranodontium* clade alignment (Table 3) did not reject the hypotheses of the monophyly of *Campylopodiella* and of *Dicranodontium*, but the hypotheses of a broader circumscription of *Dicranodontium*, including *D. subporodictyon* and/or *Atractylocarpus intermedius*, were rejected.

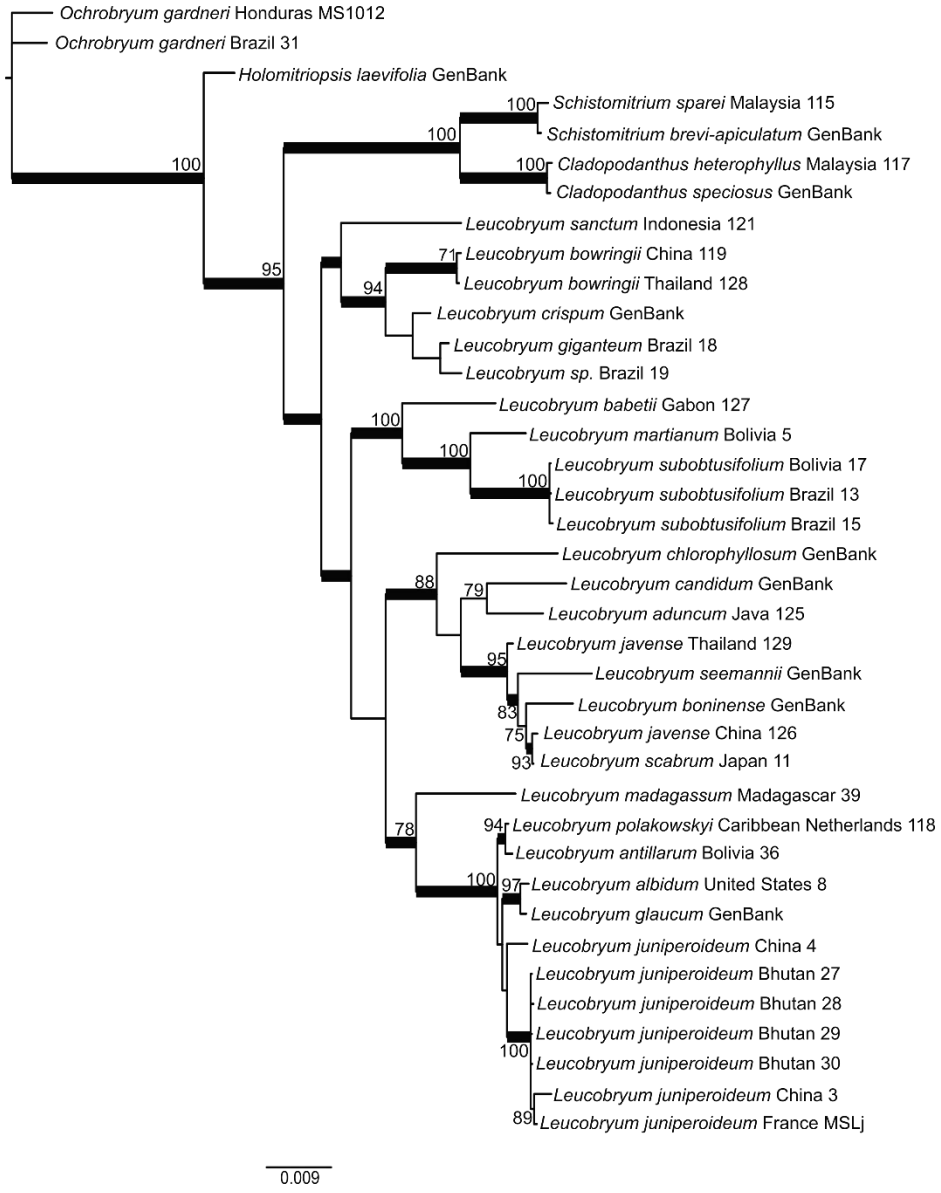


Figure 10. Bayesian inference consensus tree of 36 representatives of the leucobryoid clade of the Leucobryaceae based on nuclear, mitochondrial and chloroplast DNA sequences (ITS, nad*5,* trn*S*-trn*F region and* atp*B*-rbc*L*). Two samples of* Ochrobryum gardneri *(Leucobryaceae) were used as outgroup representatives. Branch support is indicated for Bayesian inference (BI) and maximum likelihood (ML) analyses of the same alignment. Bold branches represent posterior probabilities (PP) ≥ 0.95. Actual bootstrap (BS) values are shown if ≥ 70% .*

Table 3. Results from the SH tests applied to the Leucobryaceae alignment and to the Dicranodontium *clade alignment.*

Constrained topology	Diff lnL	*P*
Leucobryaceae alignment		
Campylopus clade and *Dicranodontium* clade monophyletic	0.81090	0.7011
Campylopus clade and leucobryoid clade monophyletic	0.81090	0.7010
Leucobryum monophyletic	6.91759	0.5329
Cladopodanthus, *Holomitriopsis*, *Ochrobryum* and *Schistomitrium* monophyletic	61.38622	0.0010*
***Dicranodontium* clade aligment**		
Campylopodiella monophyletic	14.32059	0.3515
Dicranodontium monophyletic	3.65772	0.7721
Dicranodontium monophyletic including *D. subporodictyon*	57.95291	0.0014*
Dicranodontium monophyletic including *Atractylocarpus intermedius*	30.23904	0.0499*
Dicranodontium monophyletic including *D. subporodictyon* and *A. intermedius*	53.40964	0.0037*

* Statistically worse trees at $P < 0.05$.

As a graphic representation of the distances between the aligned sequences, the phylogenetic network for the Leucobryaceae alignment (Figure 13) shows alternative relationships between Leucobryaceae representatives are possible than those resolved by our analyses. By far, most of the sequence divergence represented in the graph is found after the early splits between the three main Leucobryaceae clades, while distances in this initial evolution of the family are quite small and allow for the three alternative topologies of sister group relationships. Additionally, the network also shows the uncertainty regarding the relationships between representatives of *Leucobryum* and the genera *Cladopodanthus* and *Schistomitrium*.

Ancestral state reconstructions

Ancestral state reconstructions of the Leucobryaceae resolved the leucobryoid morphology as a derived character which originated in the most recent common ancestor (MRCA) of the leucobryoid clade (Figure 11a). The cygneous seta originated at least twice, in the MRCA of the

genus *Campylopus* and within the *Dicranodontium* clade (Figure 11b). According to this analysis, the twisted seta originated in the MRCA of the *Dicranodontium* clade. However, due to the low resolution of the relationships within the *Dicranodontium* clade in the analyses for the entire family (Figure 8), character evolution in this clade is better interpreted in the analyses for the *Dicranodontium* clade (Figures 9, 12). Homotropous capsules originated twice, in the MRCAs of *Campylopus* and *Leucobryum*, and reversed to the plesiomorphic state in the MRCA of *Cladopodanthus* and *Schistomitrium* (Figure 11c). The mitrate calyptra originated twice, in the MRCAs of *Ochrobryum* and *Cladopodanthus*/*Schistomitrium* (Figure 11d). The reconstructions under the alternative hypotheses differed only for the capsule orientation under the hypothesis of a monophyletic *Leucobryum*, which would have originated twice, in the MRCAs of *Campylopus* and *Leucobryum*, without reversals to the plesiomorphic state (Appendix 4).

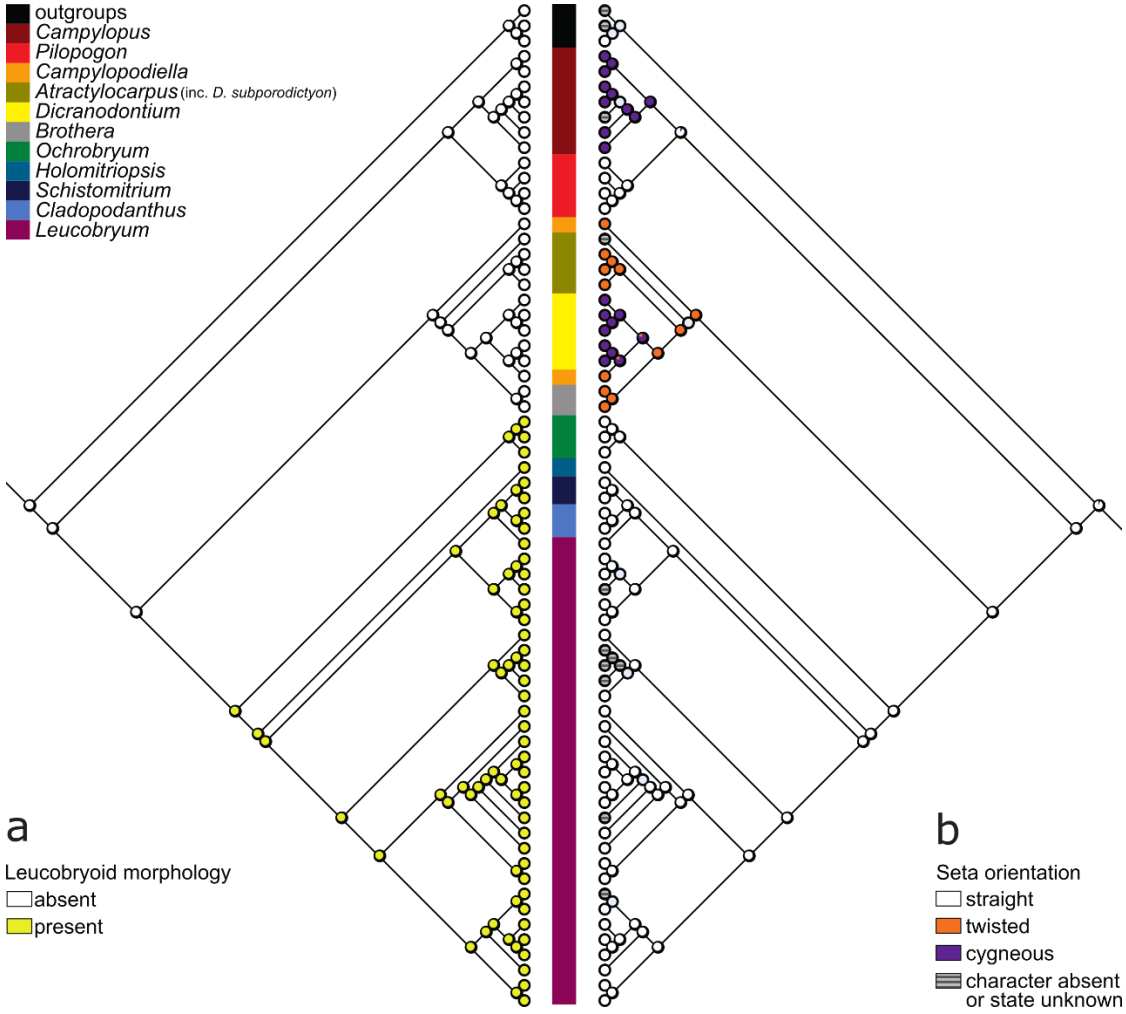
For the *Dicranodontium* clade, the analysis based on the ML tree (Figure 12a–e, left) resolved the ventral costa layers forming a stereid band as originating independently within *A. intermedius* and in the ancestors of *C. flagellacea* and *C. stenocarpa* (Wilson) P. Müll. & J.-P. Frahm. The hyalocyst layer in the ventral costa originated in the MRCA of *Brothera* (Figure 12a). The isolated stereid groups originated either twice, in the MRCA of *Brothera*/*Campylopodiella flagellacea* and in the ancestral of *C. stenocarpa*, or less likely only once, in the previous node (Figure 12b). The stereids forming the dorsal epidermis originated in the MRCA of *Atractylocarpus*/*D. subporodictyon*, while hyalocysts originated either twice, in the MRCA of *Brothera*/*Campylopodiella flagellacea* and in the ancestor of *C. stenocarpa*, or less likely only once in the previous node (Figure 12c). Pitted basal lamina cells most likely originated in the MRCA of *Atractylocarpus*/*D. subporodictyon* (Figure 12d). The cygneous seta originated in the MRCA of the entire *Dicranodontium* clade and was modified to an erect and twisted seta in the *Campylopodiella*/*Brothera* clade and in the MRCA of *A. longisetus* (Hook.) E.B. Bartram/*A. alticaulis* (Broth.) R.S. Williams (Figure 12e). The analyses based on the hypothesis of both *Campylopodiella* and *Dicranodontium* monophyletic (Figure 12f–j, right) differed in that the dorsal stereids in isolated groups would have originated in the MRCA of *Brothera*/*Campylopodiella* (Figure 12g), as the hyalocysts in the dorsal epidermis (Figure 12h) and the twisted seta (Figure 12j).

Discussion

In line with earlier phylogenetic studies (Cox et al., 2010; Fedosov et al., 2015; Fedosov et al., 2016a; Hedderson et al., 2004; La Farge et al., 2000, 2002; Stech, 2004; Tsubota et al., 2003, 2004), the analyses of the present dataset resolved three well-supported lineages within the Leucobryaceae: the dicranoid *Campylopus* clade, the dicranoid *Dicranodontium* clade and the leucobryoid clade. The latter is characterised by the leucobryoid costa as synapomorphic

character (Figure 11a). The twisted seta could represent a synapomorphy for the *Dicranodontium* clade in the ancestral state reconstruction of the Leucobryaceae (Figure 11b). However, *Atractylocarpus intermedius* and *Dicranodontium* present a cygneous seta, which more likely represents the ancestral character state in the analyses of the *Dicranodontium* clade separately (Figure 12e, j). The evolution of a cygneous seta occurred twice in the Leucobryaceae, possibly from different ancestral states, namely from a twisted seta in *Dicranodontium* and from a straight seta in *Campylopus* (Figure 11b). The other morphological characters analyzed here (capsule orientation and calyptra shape) changed character states within the *Campylopus* and/or leucobryoid clades, and thus do not represent synapomorphies for either clade. Contrary to what was suggested by Robinson (1990), *Leucobryum* is a derived genus and its homotropous capsules are not a plesiomorphic trait shared with *Campylopus*, but evolved independently in both genera (Figure 11c). Thus, the orthotropous capsules of the remaining leucobryoid genera do not represent evidence of shared ancestry, and neither does the mitrate calyptra found in *Ochrobryum* and in the *Cladopodanthus*/*Schistomitrium* clade (Figure 11d).

*Figure 11. Maximum likelihood character evolution analyses for the occurrence of leucobryoid morphology (**a**), seta orientation (**b**), capsule orientation (**c**) and calyptra shape (**d**) for the Leucobryaceae alignment, under the phylogenetic hypothesis represented by the unconstrained maximum likelihood tree.* →

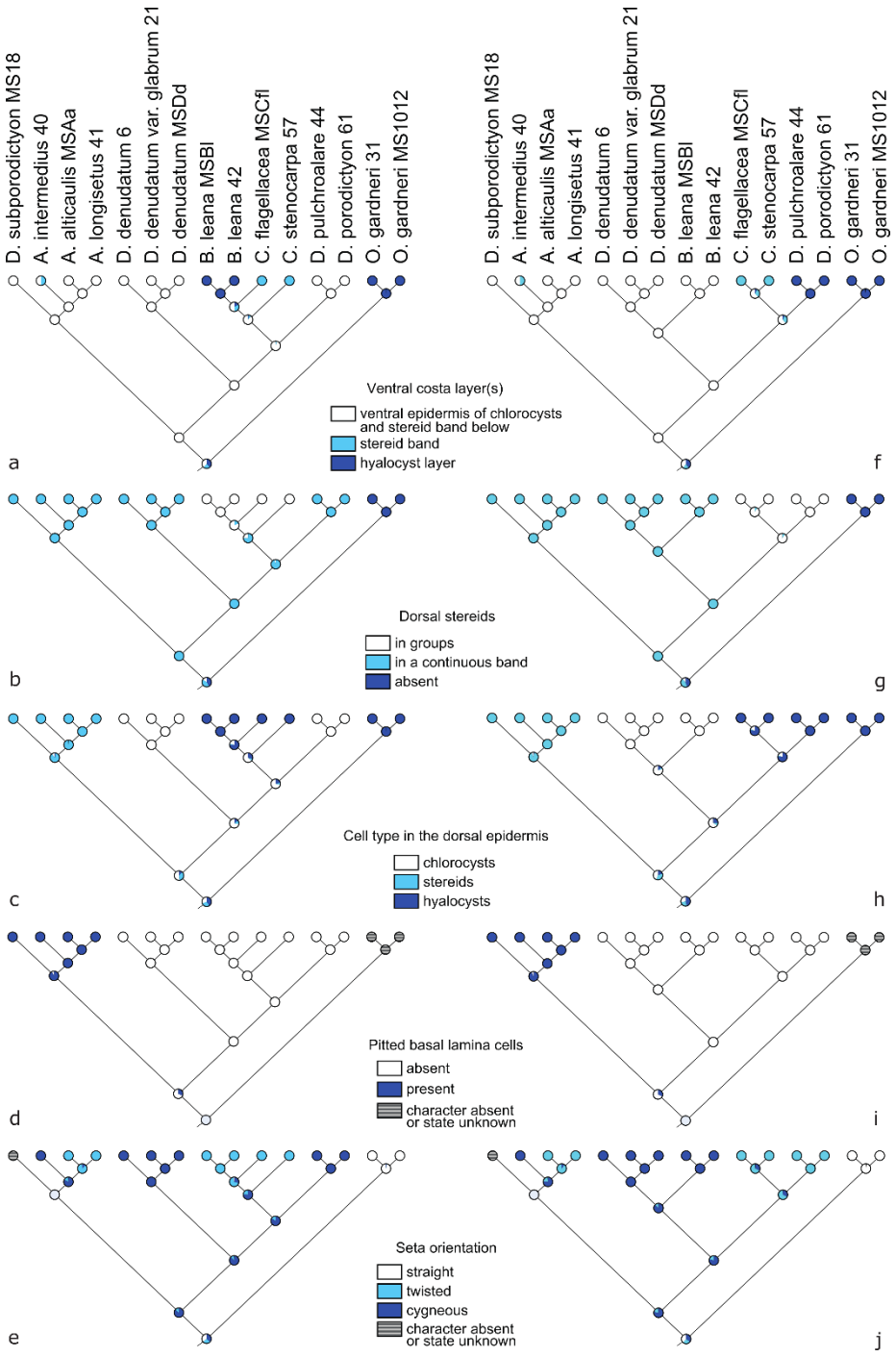

outgroups
Campylopus
Pilopogon
Campylopodiella
Atractylocarpus (inc. D. subporodictyon)
Dicranodontium
Brothera
Ochrobryum
Holomitriopsis
Schistomitrium
Cladopodanthus
Leucobryum
a
Leucobryoid morphology
absent
present
b
Seta orientation
straight
twisted
cygneous
character absent or state unknown

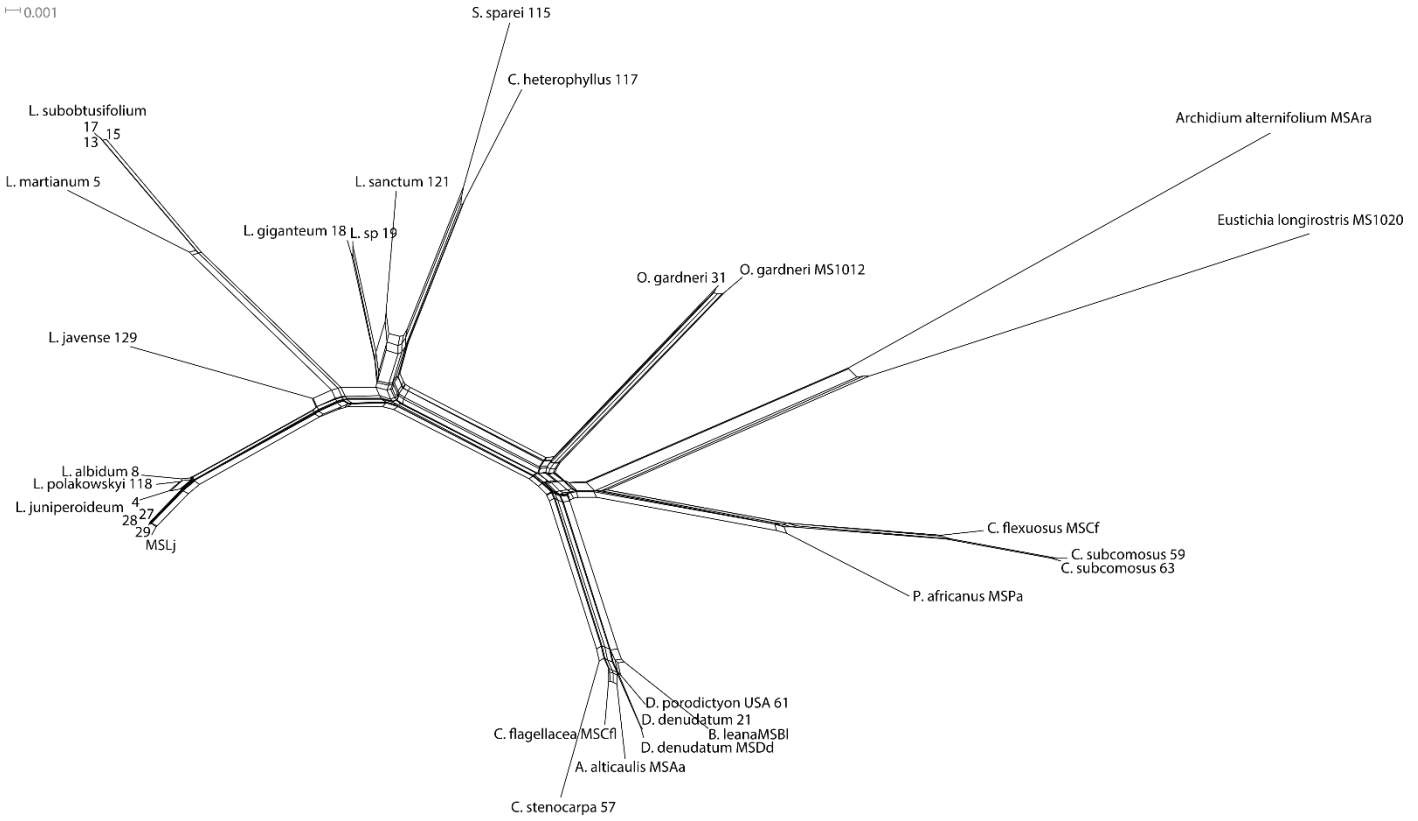
c
Capsule orientation
orthotropous
homotropous
character absent
or state unknown
d
Calyptra shape
cucullate
mitrate
reduced
character absent
or state unknown

Earlier phylogenetic analyses could not resolve the relationships between the three major Leucobryaceae clades (Hedderson et al., 2004) or supported different sister group relationships: *Campylopus* clade and leucobryoid clade (Tsubota et al., 2004), *Campylopus* clade and *Dicranodontium* clade (one tree in Stech, 2004) and, most frequently, *Dicranodontium* clade and leucobryoid clade (Cox et al., 2010; Fedosov et al., 2015, 2016a, La Farge et al., 2000, 2002; Stech et al., 2012; Tsubota et al., 2003, 2004; and another tree in Stech, 2004). Three studies (Cox et al., 2010; La Farge et al., 2002; Tsubota et al., 2003, 2004) recovered the latter topology with ≥ 70% bootstrap support or ≥ 0.95 Bayesian posterior probability. Our results corroborate these studies, since our analyses also recovered the sister group relationship of the *Dicranodontium* and leucobryoid clades, supported in ML analyses for the total alignment (Figure 8) and in both BI and ML analyses for the chloroplast markers (Appendix 3). However, the three possible alternative topologies of sister group relationships could not be rejected based on the SH test performed on our data (Table 3, Leucobryaceae alignment). Relationships between the major lineages within the Leucobryaceae thus remain somewhat uncertain even with a larger taxon and marker sampling than in previous studies. The patterns observed in the phylogenetic network (Figure 13) indicate that the early evolution of the Leucobryaceae may have been an event of rapid radiation, with the least sequence divergence occurring until the split of the three main Leucobryaceae lineages. The shorter the branches, e.g., the least substitutions the branches represent, the harder it is to reconstruct the relationships associated with them, and this phylogenetic network puts in evidence this challenge in the phylogenetic reconstruction for the early evolution of the Leucobryaceae.

We found the *Dicranodontium* clade to have the lowest sequence variability for the molecular markers applied in this study (except for ITS), resulting in short branch lengths in the phylogenetic reconstructions (Figures 8, 10). This clade is also the least species-rich (ca. 30 species, Frey & Stech, 2009). Its species have the narrowest habitat range: temperate to subtropical and montane/alpine tropical regions, mostly in moist forest habitats (except some *Atractylocarpus* only found above the tree line) and absent from tropical lowlands (Frahm, 1991b; Padberg & Frahm, 1985), and the least variation in costa structure. The latter ranges from a typically dicranoid costa in *Dicranodontium* to variations with reduced numbers of cell types or modifications on the ventral side (hyalocysts in *Brothera*, stereids in *Campylopodiella*, Figure 12a, f) and the dorsal side (stereids in *Atractylocarpus*, hyalocysts in *Brothera*/*Campylopodiella*, Figure 12c, h). Dorsal stereids remain present, albeit partly in reduced numbers, in all genera, either in groups or as a continuous stereid band (Figure 12b,g).

*Figure 12. Maximum likelihood character evolution analyses for the ventral costa layer (**a**, **f**), dorsal stereids (**b**, **g**), cell type in the dorsal costa epidermis (**c**, **h**), occurrence of pitted basal lamina cells (**d**, **i**) and seta orientation (**e**, **j**) for the* Dicranodontium *clade alignment, under two different phylogenetic hypotheses: the unconstrained maximum likelihood tree (**a**–**e**, left) and the constrained maximum likelihood tree with* Campylopodiella *and* Dicranodontium *monophyletic (**f**–**j**, right).* →


D. subporodictyon MS18
A. intermedius 40
A. alticaulis MSAa
A. longisetus 41
D. denudatum 6
D. denudatum var. glabrum 21
D. denudatum MSDd
B. leana MSBl
B. leana 42
C. flagellacea MSCfl
C. stenocarpa 57
D. pulchroalare 44
D. porodictyon 61
O. gardneri 31
O. gardneri MS1012
Ventral costa layer(s)
ventral epidermis of chlorocysts and stereid band below
stereid band
hyalocyst layer
a
f
Dorsal stereids
in groups
in a continuous band
absent
b
g
Cell type in the dorsal epidermis
chlorocysts
stereids
hyalocysts
c
h
Pitted basal lamina cells
absent
present
character absent or state unknown
d
i
Seta orientation
straight
twisted
cygneous
character absent or state unknown
e
j


0.001
S. sparei 115
C. heterophyllus 117
L. subobtusifolium
17
13
15
L. martianum 5
L. sanctum 121
L. giganteum 18
L. sp 19
Archidium alternifolium MSAra
Eustichia longirostris MS1020
O. gardneri 31
O. gardneri MS1012
L. javense 129
L. albidum 8
L. polakowskyi 118
L. juniperoideum
4
27
28
29
MSLj
C. flexuosus MSCf
C. subcomosus 59
C. subcomosus 63
P. africanus MSPa
D. porodictyon USA 61
D. denudatum 21
B. leanaMSBl
D. denudatum MSDd
C. flagellacea MSCfl
A. alticaulis MSAa
C. stenocarpa 57

← *Figure 13. NeighborNet phylogenetic network of 30 Leucobryaceae representatives based on nuclear, mitochondrial and chloroplast DNA sequences (ITS,* nad*5,* trn*S*-trn*F region and* atp*B*-rbc*L). The outgroup representatives* Archidium alternifolium *(Archidiaceae) and* Eustichia longirostris *(Eustichiaceae) are included in the graph.*

The *Campylopus* and leucobryoid clades are similar in containing more molecular variation than the *Dicranodontium* clade, indicated by their long branch lengths (Figures 8, 10). The largest number of Leucobryaceae species (ca. 160, Frey & Stech, 2009), the broadest distribution and habitat range (from latitude 70°N to 65°S, from sea level to 4800 m a.s.l.; Frahm, 1991) and the greatest variety of modifications of the dicranoid costa structure are found in the *Campylopus* clade. *Campylopus* and *Pilopogon* species may either have the basic dicranoid costa structure or be modified in various ways. Part of the species have a ventral epidermis of chlorocysts or stereids, with the dorsal epidermis ribbed (with protruding cells) or forming lamellae up to seven cells high (e.g., Gama et al., 2016), or with all costa layers but the deuter cells reduced to stereids. Other *Campylopus* species have ventral hyalocysts that may cover more than half of the costa section, with the dorsal epidermis consisting of smaller cells (smooth, ribbed or forming lamellae) or also of hyalocysts (Frahm, 1991b; Frahm, 1983). The leucobryoid clade is also species-rich (ca. 110 species, Frey & Stech, 2009). Its species are concentrated in the tropics (with the exception of some *Leucobryum* species), with maximum diversity in tropical and subtropical rainforests (Eddy, 1990). The costa in this clade is highly modified, although rather invariable when compared to the *Campylopus* clade. Its leucobryoid pattern varies solely in the number of ventral and dorsal hyalocyst layers.

The findings discussed above indicate that patterns of molecular variation, species richness, geographical distribution, ecological amplitude and of variation in costa structure covary in the three main lineages of the Leucobryaceae. The two lineages with the most modified morphologies are also the most molecularly variable, species diverse, and occupy the broadest distributions and widest variety of habitats. Thus, it can be hypothesised that costa structure modifications, by allowing an improved exploitation of the available ecological niches and environment resources, could have triggered higher phylogenetic diversity in the *Campylopus* and leucobryoid clades. Within the *Campylopus* clade, the variety of modified costa forms may be a response to (or perhaps the cause of) the broad ecological spectrum of the genus and may represent different optimization strategies for photosynthesis, water uptake, water storage and mechanical fixation (Frahm, 1985). The rather invariable leucobryoid costa, in contrast, seems to be most successful in quite distinct environments, possibly representing a strategy to optimise gas exchange and water balance in the damp habitat of tropical forests (Robinson, 1985, 1990). It is not, however, restricted to the Leucobryaceae, but appears as a derived state in at least two other families of haplolepideous mosses, the Calymperaceae (genera *Arthrocormus*, *Exodictyon*, *Exostratum* and *Leucophanes*) and the Octoblepharaceae

(*Octoblepharum*) (Bonfim Santos & Stech, 2017a; Cox et al., 2010; Fisher et al., 2007; La Farge et al., 2000), which occur mainly in tropical rainforests as well.

An evolutionary history with species-rich long branch clades and short branch clades with much lower species diversity was also observed in the flowering plant family Annonaceae Juss. (Richardson et al., 2004). However, later studies of the Annonaceae have shown that differences in species numbers could not be attributed to diversification rate shifts, nor could the observed rate shifts be correlated with key morphological innovations (Erkens et al., 2012). Whether this is the case also in the Leucobryaceae remains to be tested.

The presently estimated phylogenetic relationships raise doubts concerning the delimitation of some of the genera in the *Dicranodontium* clade (Figure 9) and about the monophyly of *Leucobryum* (Figures 8, 10). Within the *Dicranodontium* clade, the genus *Atractylocarpus* is molecularly well supported (this study) and distinguished from the other genera by its leaves gradually tapering into a long subula, the position of rhizoid initials and pitted basal lamina cells (Frahm, 1991; Padberg & Frahm, 1985; Figure 12d, i). According to our results, its circumscription should include *Dicranodontium subporodictyon*. This species received much attention due to its peculiar disjunct distribution pattern (British Columbia/Canada, Scotland/UK, Sikkim/India and Yunnan Province/China). Its systematic position remained unclear because of incompatible gametophytic characters with each of the three genera in which it was placed, i.e., *Campylopus*, *Dicranodontium* (Leucobryaceae) and *Dicranum* Hedw. (Dicranaceae), aggravated by the fact that its sporophytes are still unknown (Allen & Ireland, 2002; Chien & Tong, 1992; Corley & Wallace, 1974; Frahm, 1997). The present molecular data unequivocally support a placement of *D. subporodictyon* in the *Dicranodontium* clade, where it appears as sister to the *Atractylocarpus* species included in our study. This relationship could have been predicted since the species displays the diagnostic characters of *Atractylocarpus* (long subulate leaves, incrassate, pitted lamina cells and position of rhizoid initials), which seem to have been overlooked or misinterpreted in previous studies. Consequently, we propose a new combination here (see Taxonomic treatment). The monospecific *Brothera* can be recognised by the absence of ventral stereids and the presence of hyalocysts in its costa (Frahm, 1991b; P. Müller & Frahm, 1987).

The delimitations of *Campylopodiella* and *Dicranodontium*, however, are less clear. Both genera were resolved as paraphyletic (Figure 9), although the SH test results did not reject the hypotheses of their monophyly (see Table 3). As far as morphological characters are concerned, the cygneous seta, as discussed above, does not represent a synapomorphy for the genus *Dicranodontium*, but also occurs in *Atractylocarpus*, while the twisted seta is shared by *Atractylocarpus* and *Campylopodiella* (Figure 12e, j), and elongate upper lamina cells occur in all genera of the *Dicranodontium* clade (P. Müller & Frahm, 1987; Padberg & Frahm, 1985). *Dicranodontium*, however, is the only genus of this clade with a typical dicranoid costa. Considering the uncertainty regarding generic limits in the *Dicranodontium* clade, we do not

yet propose major changes in the classification. In case future studies support the paraphyly of *Campylopodiella* and *Dicranodontium*, a broader circumscription of a morphologically variable *Dicranodontium* may be adopted, which would be separated from *Atractylocarpus* by the characters listed above. Although *Brothera* and *Campylopodiella* have distinctive morphological characters in relation to *Dicranodontium*, those could be interpreted as "budding" diversification (Vanderpoorten & Long, 2006).

In the leucobryoid clade, all genera but *Leucobryum* share a costa mostly formed by two hyalocyst layers at leaf base, hypocentric chlorocysts (closer to the dorsal surface in transverse section, due to a difference in the depth of the ventral and dorsal hyalocyst layers), capsules that are orthotropous and symmetrical, entire peristome teeth (except *Ochrobryum*, peristome absent) and a mitrate calyptra (except *Holomitriopsis*, cucullate) (Allen, 1992b; Eddy, 1990; Magill, 1993; Robinson, 1965). *Leucobryum*, in contrast, is characterised by irregularly subdivided hyalocysts forming three to several layers at some portions of the costa at leaf base, asymmetrical, homotropous to orthogonal, curved and gibbose capsules, peristome teeth divided to the middle and a cucullate calyptra (Yamaguchi, 1993). *Leucobryum subobtusifolium* (Broth.) B.H. Allen, a species which was originally placed in *Ochrobryum*, but transferred to *Leucobryum* based on the presence of apical clusters of brood leaves instead of globose propagules (its sporophyte is unknown) (Allen, 1992b), indeed belongs to the latter genus according to the present molecular analyses.

The conclusion by Eddy (1990) and Robinson (1990) that *Cladopodanthus*, *Holomitriopsis*, *Ochrobryum* and *Schistomitrium* are closely related and should be separated from *Leucobryum* in the family Schistomitriaceae A. Eddy (1990), however, is rejected by the present molecular data (Figures 8, 10, Table 3). Our results suggest that the shared character states of these genera are either convergences (as the mitrate calyptra) or retained ancestral character states (as the orthotropous capsules), while at least part of the distinguishing traits of *Leucobryum* correspond to apomorphic character states within the leucobryoid clade.

The close relationship between *Cladopodanthus*/*Schistomitrium* and *Leucobryum* supported here is in agreement with results of previous studies (Cox et al., 2010; La Farge et al., 2000; Tsubota et al., 2004). However, our study provided conflicting results regarding the relationships between these genera. While the family-level analyses with *nad*5 and chloroplast markers support the sister group relationship of *Cladopodanthus*/*Schistomitrium* and the Asian species *L. sanctum*, and thus resolve *Leucobryum* as paraphyletic, the clade-level analyses (with additionally ITS) recover either this same topology (separate analyses of markers per genome, supported for mitochondrial and chloroplast markers) or the monophyly of *Leucobryum* (for all markers combined, Figure 10, supported for BI only). The results of the SH test (Table 3) show that the available data do not support a preference for either hypothesis. Since morphology indicates that *Leucobryum* is monophyletic, the contradictory topology could be caused by plesiomorphic molecular characters shared by *Leucobryum*

species (*L. bowringii* Mitt., *L. crispum* Müll.Hal., *L. giganteum* Müll.Hal. and *L. sanctum*) as well as *Cladopodanthus* and *Schistomitrium*. On the other hand, the phylogenetic network (Figure 13) puts in evidence the uncertainty of these relationships and may indicate the occurrence of non-tree-like patterns in the evolution of these taxa. Hybridization and introgression are phenomena shown to be related to the origin of some species and genera in bryophytes (see Natcheva & Cronberg, 2004); thus, their possible role in shaping the patterns found in the Leucobryaceae cannot be disregarded.

Taxonomic treatment

Atractylocarpus subporodictyon (Broth.) Bonfim Santos & Stech, **comb. nov.** ≡ *Dicranodontium subporodictyon* Broth., Symb. Sin. 4: 20. 1929. ≡ *Campylopus subporodictyon* (Broth.) B.H. Allen & Ireland, Lindbergia 27: 76. 2002. ≡ *Dicranum subporodictyon* (Broth.) C. Gao & T. Cao, Bryobrothera 1: 218. 1992.—TYPE: China, "NW-Y.: An nassen Granitfelsen der wtp. St. im birm. ons. bei Schutsche am Dijou-djiang (e Irrawadi-Oberlauf), 27°54', 2000 m. 7. VII. 1916" *Handel-Mazzetti 9433* (holotype: H-BR; isotype: H).

Chapter 4

Phylogenetic inferences reveal deep polyphyly of Aongstroemiaceae and Dicranellaceae within the haplolepideous mosses (Dicranidae, Bryophyta)

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Introduction

The classification of mosses (Bryophyta) has changed considerably during the last two decades based on molecular phylogenetic inference. By identifying homology and convergence in morphological characters, molecular data has helped to tackle main challenges resulting from the traditional morphology-based moss classifications, such as different interpretations of the significance of gametophytic versus sporophytic traits, and the presence of morphologically ill-defined genera, families and orders that frequently changed their circumscription through time (Carvalho-Silva et al., 2017; Huttunen et al., 2018; and references therein). On the other hand, low molecular diversity and short branch lengths, probably resulting from rapid radiation, hampered assessing suprafamilial relationships, at least in the largest moss lineage, the pleurocarpous mosses (Huttunen et al., 2012; Shaw et al., 2003).

Haplolepideous mosses (subclass Dicranidae) form the second largest lineage of mosses with ca. 4000 species, corresponding to 30% of the currently recognised moss diversity (Frey & Stech, 2009). Over the last 20 years, molecular phylogenetic reconstructions have indicated the need for revising morphology-based classifications. Early molecular studies already resulted in significant rearrangements within the subclass at the suprafamilial level. Examples are the split of the Dicranaceae in its traditional sense (cf. Brotherus, 1909, 1924) into several families (La Farge et al., 2002; Stech, 1999b; Stech & Frey, 2008) and the transfer of several families and genera from other subclasses to the Dicranidae (Goffinet et al., 1998, 2001; Hedderson et al., 2004; La Farge et al., 2000; Stech, 1999a; Tsubota et al., 2003). Subsequent molecular analyses (Bonfim Santos & Stech, 2017a; Cox et al., 2010; Fedosov et al., 2015; Fedosov et al., 2016a, 2016b; Goffinet et al., 2011; Ignatov et al., 2015; Inoue & Tsubota, 2014; Krug, 2017; Liu et al., 2019; Stech et al., 2012) added support to a division of Dicranidae into a paraphyletic assemblage of ‘protohaplolepideous’ taxa, an intermediate grade or differently supported clade, and a ‘core’ clade comprising the largest portion of the haplolepideous mosses, which only partially correspond to the existing ordinal classifications (cf. Stech et al., 2012).

Despite new insights from molecular data, analyses of a broader sampling from all major Dicranidae lineages, along with detailed studies of morphological and ecological evolution (Huttunen et al., 2018), are necessary for a revised classification. The importance of including understudied haplolepideous taxa in molecular analyses was recently exemplified by phylogenetic reconstructions of the morphologically diverse family Ditrichaceae, which turned out to be highly polyphyletic (Fedosov et al., 2015; Fedosov et al., 2016a). Three new families were established to accommodate part of the Ditrichaceae, namely Chrysoblastellaceae, Saelaniaceae, and Flexitrichaceae (Fedosov et al., 2016a). The latter study also shed new light on the evolution of the peristome as one of the main sporophytic characters for moss classification (Edwards, 1979). In addition to the different types of haplolepideous peristomes occurring in the Dicranidae (Frey & Stech, 2009; Ignatov et al., 2015; Shaw et al., 1989), with a single row of teeth (endostome) around the capsule mouth, Fedosov et al. (2016a) described the double-opposite peristome, with a developed exostome and endostome elements opposite the exostome teeth. The latter type occurs in the protohaplolepideous genus *Pseudoditrichum* and, albeit rather strongly reduced, in the likewise protohaplolepideous taxa *Catoscopium*, *Chrysoblastella* R.S. Williams, *Distichium* Bruch & Schimp. and *Flexitrichum flexicaule* (Schwägr.) Ignatov & Fedosov.

The circumscription of the Aongstroemiaceae and Dicranellaceae, and generic delimitations within them, are among the major problems remaining in the Dicranidae classification. The two families were resurrected or newly circumscribed, respectively, as segregates of the former Dicranaceae s.l. based on molecular data (see Stech & Frey, 2008). Aongstroemiaceae at present comprises five genera (*Aongstroemia*, *Aongstroemiopsis* M. Fleisch., *Dichodontium* Schimp., *Diobelonella* Ochyra, and *Polymerodon* Herzog) with 14 species, and Dicranellaceae comprises five genera (*Bryotestua* Thér. & P. de la Varde, *Campylopodium* (Müll.Hal.) Besch., *Dicranella*, *Leptotrichella* (Müll.Hal.) Lindb., and *Microcampylopus*) with about 230 species (Frey & Stech, 2009).

The families Aongstroemiaceae and Dicranellaceae, as well as their respective types *Aongstroemia* and *Dicranella*, have rather weak morphological circumscriptions (Frey & Stech, 2009). *Aongstroemia* currently has seven species (cf. Crosby et al., 1999; Frey & Stech, 2009; *Tropicos.org*) characterised by julaceous gametophytes with proximally concave leaves that are tightly appressed to the stem (in contrast to the more or less patent leaves in *Dicranella*). However, the species vary greatly in other morphological features (overall leaf shape, costa length, lamina cell shape, presence of gemmae in the leaf axils, presence of stomata on the capsule wall, presence of annulus, presence of peristome, shape of the peristome teeth; Allen, 1994; Crum, 1994; Drugova, 2010; Eckel, 2007), and some were considered to more closely resemble species from other genera (*Astomiopsis* Müll.Hal. and *Bryomanginia* Thér. from the Ditrichaceae; Allen, 1994).

One of the main problems concerning the circumscription of *Dicranella* started when Mitten (1869) and Bescherelle (1872) separated a number of species from *Dicranella* in the newly described genera *Anisothecium* Mitt. and *Microdus* Schimp. *Anisothecium* is distinguished from *Dicranella* by its peristome teeth that are attached to a somewhat higher basal membrane (Allen, 1994; Crum, 2007), while *Microdus* is considered to differ from *Dicranella* by its undivided, lightly papillose to nearly smooth and sometimes rudimentary peristome teeth (Ochyra, 1997). Different opinions about whether *Anisothecium* and *Microdus* should be kept separate, or included in *Dicranella*, have persisted until recently (*Anisothecium*: Allen, 1994; Crosby et al., 1999; Goffinet et al., 2008 *vs.* Crum, 2007; Frey & Stech, 2009; *Microdus*: Crosby et al., 1999; Frey & Stech, 2009; Goffinet et al., 2008 *vs.* Crum, 2007). *Microdus* was recognised as a synonym of *Leptotrichella*, and as the later name has priority (cf. Ochyra, 1997), the genus will be further referred to as *Leptotrichella* in this article. *Dicranella* s.l. comprises just under 220 accepted species, divided differently across the segregate genera in recent publications, e.g., 40 accepted species in *Anisothecium*, 162 in *Dicranella*, and 11 in *Leptotrichella* (as *Microdus*) in Crosby et al. (1999) vs. 158 in *Dicranella* (including *Anisothecium*), and 60 in *Leptotrichella* in Frey & Stech (2009). Not surprisingly, *Dicranella* s.l. comprises broad ranges in many morphological characters, e.g., rhizoidal gemmae (tubers), occurrence of sheathing leaf bases, differentiation of perichaetial leaves, color of the seta, inclination and shape of the capsule, presence of an annulus, and height of the basal membrane of the peristome (see, e.g., Nyholm, 1987; Risse, 1986; Smith, 2004).

So far, only two species of *Aongstroemia* and three of *Dicranella* have been included in molecular phylogenetic reconstructions, and already the analysis of such a small part of the (morphological) diversity of both genera indicates that they may not be monophyletic. Aongstroemiaceae and Dicranellaceae appeared as clearly separate in analyses that included the type species of *Aongstroemia*, *A. longipes* (Sommerf.) Bruch & Schimp., and of *Dicranella*, *D. heteromalla* (Hedw.) Schimp. (Bonfim Santos & Stech, 2017a; Stech, 1999b; Stech et al., 2012). In contrast, *Aongstroemia filiformis* (P. Beauv.) Wijk & Margad., occupied different phylogenetic positions, either together with genera of Ditrichaceae (Cox et al., 2010; Fedosov et al., 2015; as *A. jamaicensis* Müll.Hal.) or in the same clade as *D. heteromalla* (Fedosov & al., 2016a). *Dicranella cerviculata* (Hedw.) Schimp. was resolved as closely related to *D. heteromalla* in the Dicranellaceae, whereas *D. palustris* (Dicks.) Crundw. ex E.F. Warb. was resolved as more closely related to *Aongstroemia* and *Dichodontium* (La Farge et al., 2002; Stech, 1999b; Stech et al., 2012; Stech & Frey, 2008) and placed into Aongstroemiaceae based on molecular and morphological characters (cf. Frey & Stech, 2009; Ryszard Ochyra et al., 2003; Stech, 1999b, 1999c).

Furthermore, several genera presently classified in other families (cf. Frey & Stech, 2009) possibly belong to the Aongstroemiaceae or Dicranellaceae, namely *Bryowijkia* (Bryowijkiaceae Stech & W. Frey), *Cladophascum* Dixon (Bruchiaceae Schimp.), *Hygrodicranum*

Cardot and *Trichodontium* (Dixon) Fife (Dicranaceae), and three genera of Ditrichaceae (*Chrysoblastella* R.S. Williams, *Eccremidium* Wilson, *Garckea* Müll.Hal.) (Cox et al., 2010; Fedosov et al., 2015; Fedosov et al., 2016a; Goffinet et al., 2011; Inoue & Tsubota, 2014; La Farge et al., 2002; Stech & Frey, 2008; Tsubota et al., 2003, 2004). *Chrysoblastella chilensis* (Mont.) Reimers was resolved in different positions in the phylogenetic trees based on different samples, either among the protohaplolepideous lineages (Cox et al., 2010; Fedosov et al., 2016a) or as sister to *Dicranella* (Inoue & Tsubota, 2014), indicating at least one misidentified specimen or contamination. *Microcampylopus* was erroneously resolved as part of the Leucobryaceae (Cox et al., 2010; Stech, 1999b) based on specimens later verified as belonging to *Campylopus* (see Bonfim Santos & Stech, 2017b) and *Pilopogon* (M. Stech pers. obs.).

The present study is intended to provide a baseline for future research on the phylogenetic relationships and circumscriptions of the Aongstroemiaceae and Dicranellaceae by summarizing the available knowledge and providing new phylogenetic analyses of published and newly generated molecular data. Specific goals of this study are to assess whether (i) Aongstroemiaceae and Dicranellaceae are molecularly distinct, (ii) *Aongstroemia* and *Dicranella* are monophyletic, and (iii) the current circumscriptions of the Aongstroemiaceae, Dicranellaceae, and their types are in line with their estimated phylogenetic relationships. Furthermore, the Dicranidae phylogeny will be reviewed by discussing the results of the present study in the context of earlier phylogenetic reconstructions.

Material and Methods

Taxon sampling, DNA extraction and sequencing

The sampling comprised DNA sequences of 168 specimens representing 117 species of haplolepideous mosses and all haplolepideous families except Viridivelleraceae, which has not yet been included in molecular analyses. Taxon and specimen selection were based on published phylogenetic reconstructions of the Dicranidae and the classification of Frey & Stech (2009). The latter listed species number per genus and adopted a narrower circumscription of the Dicranaceae, with the Aongstroemiaceae and Dicranellaceae regarded as separate families, in contrast to the most recent online classification (Goffinet & Buck, 2021). Thirteen species of *Dicranella*, namely *D. campylophylla* (Taylor) A. Jaeger, *D. cardotii* (R.Br.bis) Dixon, *D. cerviculata*, *D. crispa* (Hedw.) Schimp., *D. curvipes* (Lindb.) Ignatov, *D. grevilleana* (Brid.) Schimp., *D. heteromalla*, *D. howei* Renauld & Cardot, *D. rufescens* (With.) Schimp., *D. schreberiana* (Hedw.) Hilf. ex H.A. Crum & L.E. Anderson, *D. staphylina* H.Whitehouse, *D. subulata* (Hedw.) Schimp., and *D. varia*, as well as *Kiaeria riparia* (H. Lindb.) M.F.V.Corley (*Dicranella riparia* (H. Lindb.) Mårtensson & Nyholm) and three species of Aongstroemia, namely *A. filiformis*, *A. longipes*, and *A. orientalis* Mitt., were included.

Sequences of mitochondrial (*nad*5 G1 intron) and chloroplast markers (two parts of the *trn*S-*trn*F region: *trn*S-*rps*4 spacer/*rps*4 gene and *trn*L gene/*trn*L-*trn*F spacer; Hernández-Maqueda et al., 2008) were in part obtained from previous studies (Bonfim Santos & Stech, 2017a, 2017b; Cox et al., 2010; Fedosov et al., 2016a; La Farge et al., 2002; O'Brien, 2007; Stech, 1999b, 2004; Stech et al., 2012). The loci choice was based on the availability of sequences from these studies and considerations on marker variability. For example, the nuclear ribosomal ITS region was not included (following Bonfim Santos & Stech, 2017b) since the internal transcribed spacers are largely unalignable at family and suprafamilial levels in the Dicranidae.

Additionally, 208 new sequences of the target loci were generated either from specimens obtained from the herbaria L, MW and SP, either using DNA extracts from concluded (DNA barcoding of the Dutch bryophytes) or ongoing (Russian bryophyte flora) studies or from newly extracted DNA. These represented *Dicranella* (45 specimens), *Aongstroemia* (8), other taxa that are considered morphologically or phylogenetically close to Aongstroemiaceae or Dicranellaceae (7; of genera *Bryowijkia*, *Campylopodium*, *Campylopus*, *Dichodontium*, *Hygrodicranum*, *Kiaeria* I. Hagen, and *Microcampylopus*), and 11 representatives of other underrepresented haplolepideous lineages (*Blindia* Bruch & Schimp., *Dicranum*, *Distichium*, *Ditrichum* Hampe, *Erpodium* (Brid.) Brid., *Flexitrichum* Ignatov & Fedosov, *Platyneuron* (Cardot) Broth., *Rhamphidium* Mitt., *Trematodon* Michx.). *Encalypta streptocarpa* (Encalyptidae) and *Timmia austriaca* (Timmiidae) were included as outgroup representatives, based on their positions in previous published reconstructions (Cox et al., 2010; Tsubota et al., 2004).

Voucher information and GenBank accession numbers are listed in Appendix 1, with indications of the newly generated sequences and of the sequences that are missing from the dataset (either due to unsuccessful sequencing from our vouchers or because the sequences were not available for the vouchers from published phylogenetic studies included in our analyses).

In some cases, vouchers of specimens included in earlier phylogenies were requested on loan for morphological study, in particular when these were resolved in incongruent positions, indicating possible misidentification. The most prominent case concerns *Chrysoblastella chilensis*, which was resolved as sister to *Dicranella heteromalla* (specimen *R.D. Seppelt 26697*, HIRO; Inoue & Tsubota, 2014) or as part of the protohaplolepideous mosses, either as a separate lineage (specimen *Buck 39507*, DUKE; Cox et al., 2010) or as sister to *Pseudoditrichum* (Pseudoditrichaceae) in Fedosov et al. (2016a), the latter combining *nad*5 from *Buck 39507* and *rps*4 and *rbc*L from *R.D. Seppelt 26697*.

Procedures for DNA extraction, amplification and sequencing followed Bonfim Santos & Stech (2017a) and Fedosov et al. (2016a, 2016b). Sequences were manually aligned in Geneious®

v8.0.5 (Biomatters Ltd.; https://www.geneious.com), using the alignment from Bonfim Santos & Stech (2017a) as a starting point.

Phylogenetic reconstructions

Phylogenetic reconstructions were performed under maximum likelihood (ML) using RAxML v.8 (Stamatakis, 2014) and Bayesian inference (BI) using MrBayes v.3.2.6 (Ronquist et al., 2012), both on the CIPRES Science Gateway v.3.3 (M. A. Miller et al., 2010). Analyses were run for each marker separately to check for supported incongruence (conflicting topologies with >70% maximum likelihood bootstrap support or >0.95 Bayesian posterior probability, assessed by visual comparison of the respective trees) and for the concatenated alignment of all markers. Gaps were treated as missing data. Evolutionary model testing was performed in PartitionFinder v1.1.1 (Lanfear et al., 2012) for the models that can be implemented in RAxML (GTR) and MrBayes (GTR and several of its nested models), respectively, both with or without a gamma-distributed rate variation among sites (Γ) and/or a proportion of invariable sites (I). According to the Akaike information criterion (AIC), the selected evolutionary models by both tests (one for each RAxML and MrBayes implemented models) were GTR+Γ for the *nad*5 G1 intron and GTR+Γ+I for the *trn*S-*rps*4 spacer/*rps*4 gene and *trn*L gene/*trn*L-*trn*F spacer, which were implemented in the BI analyses. In RAxML a single type of rate heterogeneity pattern (either +Γ, +I or +Γ+I) can be applied for all partitions per analysis; thus, we implemented GTR+Γ in the ML analysis of the concatenated markers. In the concatenated marker analyses, model parameters were independently estimated for each partition. For all maximum likelihood analyses, rapid bootstrapping with the majority-rule criterion automatic halt (autoMRE) was performed. For Bayesian inferences, four runs with four chains (5×10^6 generations each) were run simultaneously, with the temperature of the single heated chain set to 0.4. Chains were sampled every 1000 generations, and the respective trees were written to tree files. After verifying the convergence of runs in Tracer v1.6 (Rambaut et al., 2014), 50 percent majority-rule consensus trees and posterior probabilities of clades were calculated, discarding the burn-in phase (25%).

The Shimodaira-Hasegawa (SH) test (Goldman et al., 2000; Shimodaira & Hasegawa, 1999) was applied to test phylogenetic hypotheses related to the monophyly of *Dicranella*. In test 1, the ML tree (topology as in Figure 14) was compared with selected hypotheses obtained from the literature for the circumscriptions of *Aongstroemia*, *Dicranella*, and related genera. These hypotheses are listed in Table 4 along with the results. The generic placement of the species included in this study according to each hypothesis, as well as the constraint applied to each analysis, are provided in Appendix 5. In test 2 (Table 5), the ML tree was compared with alternative hypotheses of relationships between *Dicranella* representatives that were resolved in unsupported places in the ML tree, namely (1) the sister-group relationship of the *D.*

crispa/*D. subulata* clade and the *D. rufescens* clade, (2) the sister-group relationship of the *D. crispa*/*D. subulata* clade and the *D. staphylina* clade, (3) the sister-group relationship of the *D. staphylina* clade and the *D. rufescens* clade, and (4) all the *Dicranella* clades of uncertain placement (*D. staphylina*, *D. crispa* /*D. subulata*, *D. rufescens*) forming a clade. Constraint trees were used as an input to ML analyses with RAxML. The resulting trees with branch length values and corresponding alignment were loaded into PAUP* v.4.0b10 (Swofford, 2002), where these trees were compared with the respective unconstrained topologies using the SH test with 10,000 bootstrap replicates and the resampling estimated log-likelihood (RELL) method.

Table 4. Results from the SH test of selected hypotheses for the circumscriptions of Aongstroemia, Dicranella*, and related genera.*

Constrained topology	Diff lnL	*P*
Dicranella sensu Frey & Stech (2009)	367.77408	0.0000*
Dicranella sensu Crum (2007)	555.31960	0.0000*
Dicranella sensu Crosby et al. (1999)	378.05984	0.0000*
Dicranella sensu Frey & Stech (2009) with the inclusion of *Kiaeria riparia* (cf. Nyholm, 1987)	404.65213	0.0000*
Dicranella sensu Crum (2007) with the inclusion of *Kiaeria riparia* (cf. Nyholm, 1987)	592.33283	0.0000*
Dicranella sensu Crum (2007) with the inclusion of *Kiaeria riparia* (cf. Nyholm, 1987) and exclusion of *Diobelonella palustris* (Dicks.) Ochyra (cf. Stech 1999c, Ochyra et al., 2003)	584.39542	0.0000*
Aongstroemia sensu Crosby et al. (1999)	399.13529	0.0000*
Aongstroemia monophyletic with the exclusion of *A. orientalis* (suggested to be closely related to Ditrichaceae genera; cf. Allen, 1994)	192.13965	0.0000*
Dichodontium and *Diobelonella palustris* forming a clade (*D. palustris* included in *Dichodontium* cf. Stech, 1999c)	2.55658	0.8423

The test was applied to the haplolepideous moss alignment of the concatenated molecular markers (mitochondrial *nad*5 G1 intron, and plastid *trn*S/*rps*4 gene and *trn*L gene-*trn*L-*trn*F spacer). * Statistically worse trees at $P < 0.05$.

Table 5. Results from the SH test of four alternative hypotheses of relationships between the Dicranella *clades of unsupported placement in the maximum likelihood tree presented in this study.*

Constrained topology	Diff lnL	*P**
D. crispa/*D. subulata* sister to *D. rufescens*	2.04326	0.7089
D. crispa/*D. subulata* sister to *D. staphylina*	7.20512	0.4403
D. rufescens sister to *D. staphylina*	6.88519	0.4401
Dicranella staphylina, *D. crispa*, *D. subulata*, *D. rufescens* forming a monophyletic group	7.02972	0.4498

The test was applied to the haplolepideous moss alignment of the concatenated molecular markers mitochondrial *nad*5 G1 intron, and plastid *rps*4 gene and *trn*L-*trn*F spacer.

* This test resulted in no statistically worse trees at $P < 0.05$.

Results

The alignment lengths for the *nad*5 G1 intron, *trn*S-*rps*4 spacer/*rps*4 gene, and *trn*L gene/*trn*L-*trn*F spacer were 967, 750, and 831 bp, respectively.

Figure 14 shows the single optimal maximum likelihood (ML) tree calculated from the concatenated mitochondrial and chloroplast markers, with indication of ML bootstrap support (BS) and posterior probabilities (PP) from Bayesian inference. No supported incongruences for the higher-level relationships discussed here were observed between the combined analysis and analyses run for each DNA region separately (Appendix 6). Some incongruences regarding relationships at the infrafamilial or infrageneric level were detected between the separate analyses (e.g., relationships between specimens of *Dicranella curvipes* and *D. heteromalla*).

Most relationships of the protohaplolepideous lineages, from *Catoscopium* to *Bryoxiphium* Mitt., were statistically supported in the phylogeny (Figure 14). These taxa were separated from a clade comprising *Dicranella staphylina*, the protohaplolepideous *Pseudoditrichum mirabile* Steere & Z. Iwats., and the remaining haplolepideous taxa with 84% BS and PP 1. Backbone relationships within the latter clade were poorly supported, except for the clade comprising *Amphidium* Schimp. and the core haplolepideous moss families, with a support value of PP 0.99.

The type species of *Aongstroemia*, *A. longipes*, and the conserved type of *Dicranella*, *D. heteromalla*, were resolved in separate clades. Five *Dicranella* species were resolved as more closely related to the type of *Aongstroemia* than to the type of *Dicranella*. Of these, *D. campylophylla*, *D. grevilleana*, and *D. schreberiana* formed a well-supported clade (BS 98%, PP

1) with *A. longipes* and two *Hygrodicranum* species (*H. bolivianum* Herzog, *H. herrerai* R.S. Williams). The clade including *A. longipes* and the above mentioned *Dicranella* and *Hygrodicranum* taxa was sister to *Dichodontium* (BS 77%). This larger clade was sister to *Diobelonella palustris* (Dicks.) Ochyra (BS 98%, PP 1). One further *Dicranella* species, *D. cardotii*, for which only *nad*5 G1 intron sequences were obtained, was also resolved within the Aongstroemiaceae (BS 97%, PP 1; Appendix 6). *Dicranella howei* and *D. varia* formed a clade (BS 87%, PP 0.95) that was resolved as sister to the clade formed by the Aongstroemiaceae genera (*Aongstroemia* s.str., *Dichodontium*, *Diobelonella*) plus the above mentioned *Dicranella* and *Hygrodicranum* species (BS 74%).

Dicranella heteromalla and the Asian *D. curvipes* (BS 96%, PP 1) formed a clade (BS 100%, PP 1) that was resolved as sister to *D. cerviculata* (BS 100%, PP 1). These three species formed the *Dicranella* s.str. clade (BS 100%, PP 1). The clade comprising species of the other Dicranellaceae genera (*Campylopodium*, *Leptotrichella*, *Microcampylopus*), as well as *Aongstroemia filiformis*, *Garckea phascoides* Müll.Hall. and *Trichodontium falcatum* (R.Br. bis) Fife (BS 100%, PP 0.95), was resolved as sister to *Dicranella* s.str. (BS 100%, PP 1), together forming the Dicranellaceae clade (BS 100%, PP 1). *Cladophascum gymnomitrioides* (Dixon) Dixon (Bruchiaceae) and *Eccremidium floridanum* H.A. Crum (Ditrichaceae) were sister species (BS 100%, PP 1), and together resolved as sister to the above described Dicranellaceae clade (BS 100%, PP 0.99). A clade formed by the two *Bryowijkia* species (BS 100%, PP 1) was sister to the *Cladophascum*-*Eccremidium*-Dicranellaceae clade (BS 100%, PP 1), and this larger clade including *Bryowijkia* was in turn sister to the specimen of *Chrysoblastella chilensis* labelled MS Cc (BS 74%, PP 1). The specimen of *Ditrichum* sp. labelled *Buck 39507* (as *Chrysoblastella chilensis* in Cox et al., 2010) was resolved as sister to a specimen identified as *Ditrichum* cf. *cylindricarpum* (Müll.Hal.) F. Muell. (BS 100, PP 1) within the protohaplolepideous grade.

A third *Aongstroemia* species, *A. orientalis*, and other three clades with *Dicranella* specimens, namely *D. staphylina*, *D. crispa*/D. *subulata*, and *D. rufescens*, all with significant support, did not belong to either the Aongstroemiaceae or Dicranellaceae. *Aongstroemia orientalis* was resolved as sister to *Astomiopsis amblyocalyx* Müll.Hall. (Ditrichaceae) (BS 100%, PP 1) within a clade including other Ditrichaceae and representatives of the Pottiaceae (BS 85%, PP 1). *Dicranella staphylina* showed affinities with the protohaplolepideous taxa (see above), while *D. crispa*/*D. subulata* and *D. rufescens* were resolved (without support) as the two clades closest to the core haplolepideous clade.

Kiaeria (*Dicranella*) *riparia* was resolved within the Rhabdoweisiaceae Limpr. clade, sister to the clade formed by *Arctoa fulvella* (Dicks.) Bruch & Schimp., *Glyphomitrium daviesii* (Dicks. ex With.) Brid., and *Oncophorus integerrimus* Hedenäs (BS 84%, PP 0.99).

None of the resolved clades corresponds to the genus *Anisothecium* as circumscribed in the consulted literature (cf. Appendix 5). The species of *Leptotrichella* included in our analyses (*L.*

flaccidula (Mitt.) Ochyra) was resolved as separate from any of the clades containing *Dicranella* species.

The SH test 1 rejected all the selected hypotheses of circumscriptions for *Aongstroemia*, *Dicranella* and related genera obtained from the literature (see Appendix 5) except the hypothesis of *Dichodontium flavescens* (Dicks.) Lindb., *D. pellucidum* (Hedw.) Schimp. and *Diobelonella palustris* forming a monophyletic group (Table 4). The SH test 2 did not reject any of the tested alternative hypotheses for the relationships between the three clades of *Dicranella* s.l. representatives of uncertain placement in the ML tree (*D. staphylina*, *D. crispa*/*D. subulata*, *D. rufescens*) (Table 5).

Discussion

Phylogeny of the haplolepideous mosses

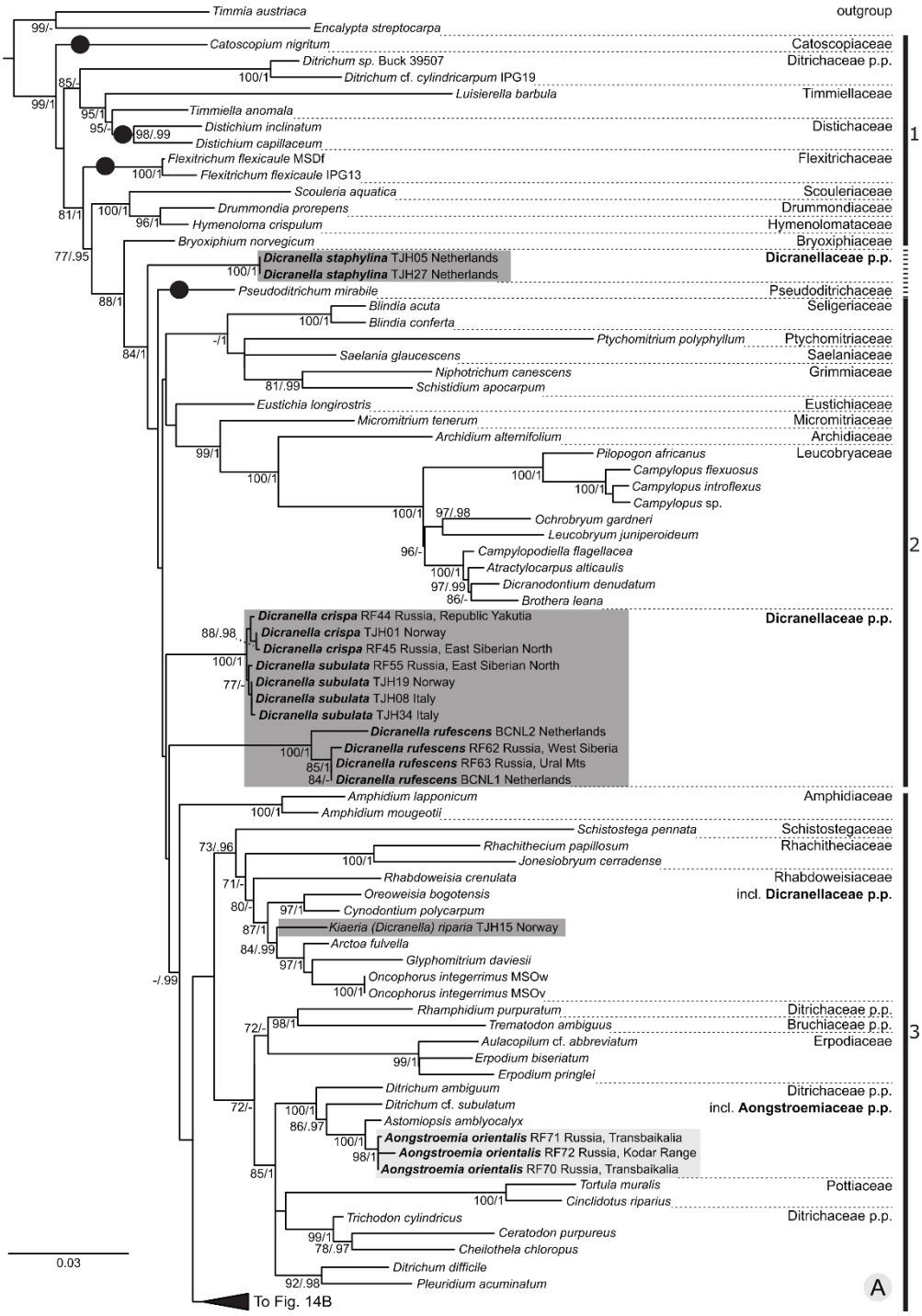
Relationships of the major lineages in Dicranidae (paraphyletic assemblage of 'protohaplolepideous' taxa, an intermediate grade or clade, and a 'core' clade comprising the largest portion of the haplolepideous mosses) are generally concordant in all recent phylogenies (e.g. Bonfim Santos & Stech, 2017a; Cox et al., 2010; Fedosov et al., 2015; Fedosov et al., 2016a; Inoue & Tsubota, 2014; Liu et al., 2019; Stech et al., 2012; present study). As was previously shown for Ditrichaceae (Fedosov et al., 2015; Fedosov et al., 2016a), adding species from underrepresented genera (*Aongstroemia*, *Dicranella*, but also *Ditrichum*, see below) sheds new light on the phylogenetic diversity in the haplolepideous mosses. Based on the obtained topologies and morphological evidence, several of the newly discovered lineages should probably be recognised as separate genera and, in some cases, families. However, still only a small percentage of the species diversity of large genera such as *Dicranella*, *Ditrichum*, and *Leptotrichella* have been analysed. More extensive molecular phylogenetic reconstructions may resolve currently unsupported relationships with more confidence and may result in an even higher number of separate lineages that need to be addressed taxonomically. Nevertheless, the present results provide a new, more robust framework on which subsequent studies can build to eventually present a fully revised taxonomy of the Dicranidae.

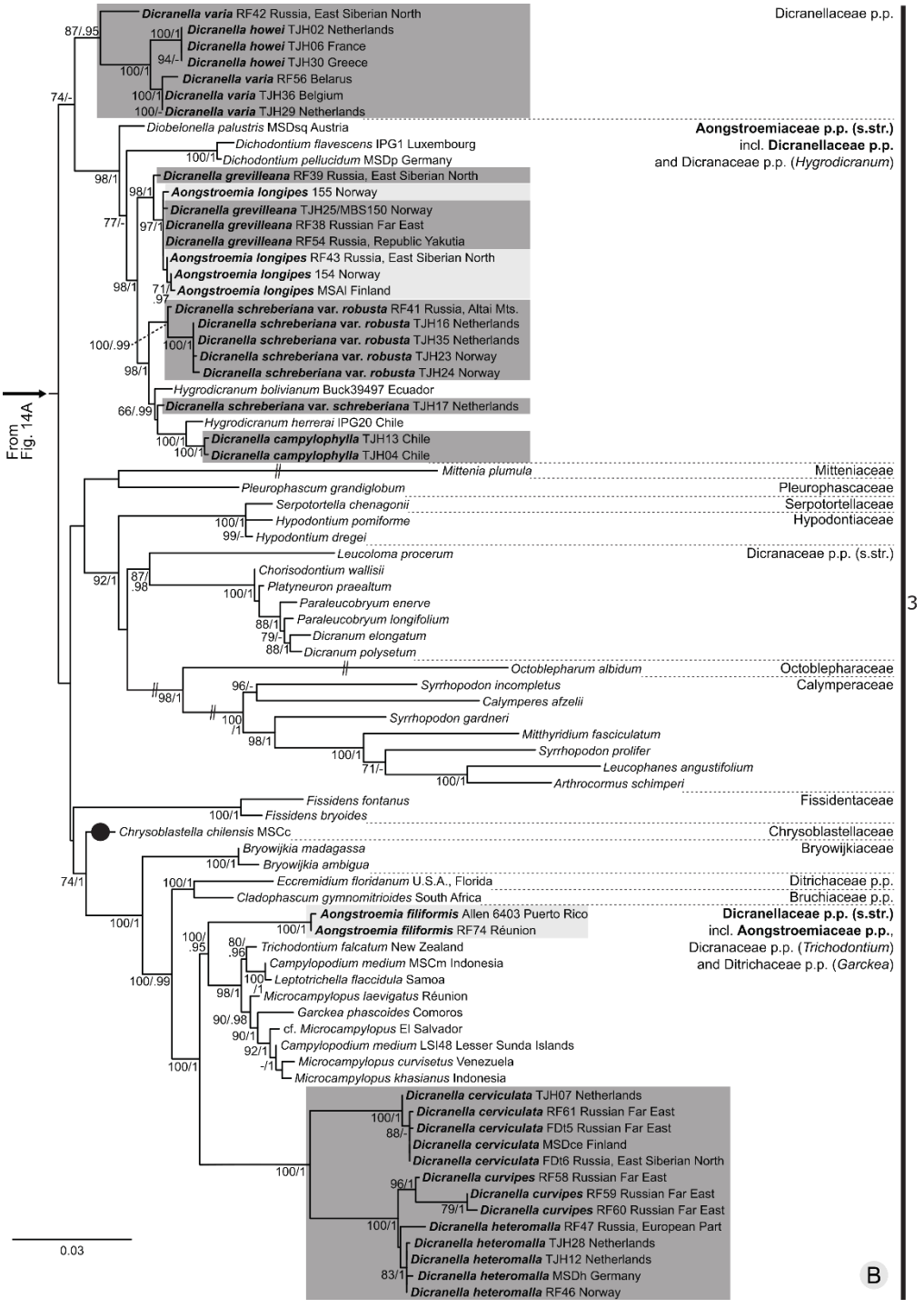
Apart from incomplete taxon sampling, the low and varying support for the backbone in the present and other Dicranidae phylogenies hampers inferences of relationships. Such low resolution was ascribed to a rapid radiation in the evolutionary history in the pleurocarpous Hypnales (M. Fleisch.) W.R. Buck & Vitt (Huttunen et al., 2012; Shaw et al., 2003), which may have occurred in the haplolepideous mosses as well (Cox et al., 2010). Molecular dating indicated that Dicranidae diversified within the last approximately 130 million years (Laenen et al., 2014), which is in accordance with the oldest reliable fossil evidence from the Cretaceous (older fossils that may represent haplolepideous species do exist but their affinities are less

clear; cf. discussion in Savoretti et al., 2018). As part of the first shift in diversification rate in mosses in the Cretaceous (Laenen et al., 2014), the evolution of the main haplolepideous lineages may thus be an example supporting the 'shadow of angiosperm' hypothesis, as a response to an explosive increase in the structural diversity of flowering plants (Laenen et al., 2014; Schmidt et al., 2010). However, low clade support may also result from using too few markers or markers with little variation and/or considerable homoplasy. Most backbone phylogenetic studies of Dicranidae were based on more markers but fewer taxa (e.g. Chang & Graham, 2014; Ignatov et al., 2015), but comparative analyses of possible correlations between (lack of) clade support, taxon sampling, and marker characteristics are still missing.

The present data show that the diversity of the protohaplolepideous grade is still incompletely known. A protohaplolepideous lineage with a ditrichoid morphology (specimens *Ditrichum* sp. *Buck 39507 p.p.* and *D.* cf. *cylindricarpum* IPG19) was discovered, adding to the polyphyly of *Ditrichum*. A detailed morphological and molecular study of this clade will be performed separately. Additionally, our phylogenetic analyses support the classification of *Chrysoblastella chilensis* in its own family, the Chrysoblastellaceae, which is, however, not closely related to the protohaplolepideous Pseudoditrichaceae and should be removed from the Pseudoditrichales, in contrast to the findings of Fedosov et al. (2016a).

Figure 14. Maximum likelihood tree of Dicranidae representatives, with Encalypta streptocarpa *(Encalyptidae) and* Timmia austriaca *(Timmiidae) as outgroup. The phylogenetic reconstruction was based on a concatenated dataset of mitochondrial* nad*5 intron and chloroplast* trn*S*-rps*4 and* trn*L*-trn*F regions, using the GTR+ Γ substitution model. Branch lengths are to scale, except those indicated by "//" (shortened to 50% of their original length). Maximum likelihood bootstrap support values ≥70% and posterior probabilities ≥0.95 from Bayesian inference are shown at the branches. Names of families resolved as para- or polyphyletic based on their latest circumscription are followed by 'p.p.'. Lineages representing the polyphyletic genera* Aongstroemia *and* Dicranella *are highlighted with lighter or darker grey boxes, respectively, and the respective family clades including the type species are indicated by 's.str.'. Vertical lines and numbers on the right indicate the main haplolepideous groups distinguished based on molecular data:* ***1****, protohaplolepideous grade;* ***2****, intermediate grade;* ***3****, core haplolepideous clade. The yet ambiguous transition between 1 and 2 is indicated by a dashed line (see text for details). Black circles indicate lineages with double opposite peristomes (complete in* Pseudoditrichum*, reduced in the other lineages).* →


outgroup
Timmia austriaca
Encalypta streptocarpa
Catoscopium nigritum
Catoscopiaceae
Ditrichum sp. Buck 39507
Ditrichum cf. cylindricarpum IPG19
Ditrichaceae p.p.
Luisierella barbula
Timmiellaceae
Timmiella anomala
Distichium inclinatum
Distichium capillaceum
Distichaceae
Flexitrichum flexicaule MSDf
Flexitrichum flexicaule IPG13
Flexitrichaceae
Scouleria aquatica
Scouleriaceae
Drummondia prorepens
Drummondiaceae
Hymenoloma crispulum
Hymenolomataceae
Bryoxiphium norvegicum
Bryoxiphiaceae
Dicranella staphylina TJH05 Netherlands
Dicranella staphylina TJH27 Netherlands
Dicranellaceae p.p.
Pseudoditrichum mirabile
Pseudoditrichaceae
Blindia acuta
Blindia conferta
Seligeriaceae
Ptychomitrium polyphyllum
Ptychomitriaceae
Saelania glaucescens
Saelaniaceae
Niphotrichum canescens
Schistidium apocarpum
Grimmiaceae
Eustichia longirostris
Eustichiaceae
Micromitrium tenerum
Micromitriaceae
Archidium alternifolium
Archidiaceae
Pilopogon africanus
Campylopus flexuosus
Campylopus introflexus
Campylopus sp.
Ochrobryum gardneri
Leucobryum juniperoideum
Leucobryaceae
Campylopodiella flagellacea
Atractylocarpus alticaulis
Dicranodontium denudatum
Brothera leana
Dicranella crispa RF44 Russia, Republic Yakutia
Dicranella crispa TJH01 Norway
Dicranella crispa RF45 Russia, East Siberian North
Dicranella subulata RF55 Russia, East Siberian North
Dicranella subulata TJH19 Norway
Dicranella subulata TJH08 Italy
Dicranella subulata TJH34 Italy
Dicranella rufescens BCNL2 Netherlands
Dicranella rufescens RF62 Russia, West Siberia
Dicranella rufescens RF63 Russia, Ural Mts
Dicranella rufescens BCNL1 Netherlands
Dicranellaceae p.p.
Amphidium lapponicum
Amphidium mougeotii
Amphidiaceae
Schistostega pennata
Schistostegaceae
Rhachithecium papillosum
Jonesiobryum cerradense
Rhachitheciaceae
Rhabdoweisia crenulata
Oreoweisia bogotensis
Cynodontium polycarpum
Rhabdoweisiaceae
incl. Dicranellaceae p.p.
Kiaeria (Dicranella) riparia TJH15 Norway
Arctoa fulvella
Glyphomitrium daviesii
Oncophorus integerrimus MSOw
Oncophorus integerrimus MSOv
Rhamphidium purpuratum
Ditrichaceae p.p.
Trematodon ambiguus
Bruchiaceae p.p.
Aulacopilum cf. abbreviatum
Erpodium biseriatum
Erpodium pringlei
Erpodiaceae
Ditrichum ambiguum
Ditrichum cf. subulatum
Astomiopsis amblyocalyx
Ditrichaceae p.p.
incl. Aongstroemiaceae p.p.
Aongstroemia orientalis RF71 Russia, Transbaikalia
Aongstroemia orientalis RF72 Russia, Kodar Range
Aongstroemia orientalis RF70 Russia, Transbaikalia
Tortula muralis
Cinclidotus riparius
Pottiaceae
Trichodon cylindricus
Ceratodon purpureus
Cheilothela chloropus
Ditrichaceae p.p.
Ditrichum difficile
Pleuridium acuminatum
0.03
To Fig. 14B
A
1
2
3


Dicranellaceae p.p.
Dicranella varia RF42 Russia, East Siberian North
Dicranella howei TJH02 Netherlands
Dicranella howei TJH06 France
Dicranella howei TJH30 Greece
Dicranella varia RF56 Belarus
Dicranella varia TJH36 Belgium
Dicranella varia TJH29 Netherlands
Aongstroemiaceae p.p. (s.str.)
incl. Dicranellaceae p.p.
and Dicranaceae p.p. (Hygrodicranum)
Diobelonella palustris MSDsq Austria
Dichodontium flavescens IPG1 Luxembourg
Dichodontium pellucidum MSDp Germany
Dicranella grevilleana RF39 Russia, East Siberian North
Aongstroemia longipes 155 Norway
Dicranella grevilleana TJH25/MBS150 Norway
Dicranella grevilleana RF38 Russian Far East
Dicranella grevilleana RF54 Russia, Republic Yakutia
Aongstroemia longipes RF43 Russia, East Siberian North
Aongstroemia longipes 154 Norway
Aongstroemia longipes MSAI Finland
Dicranella schreberiana var. robusta RF41 Russia, Altai Mts.
Dicranella schreberiana var. robusta TJH16 Netherlands
Dicranella schreberiana var. robusta TJH35 Netherlands
Dicranella schreberiana var. robusta TJH23 Norway
Dicranella schreberiana var. robusta TJH24 Norway
Hygrodicranum bolivianum Buck39497 Ecuador
Dicranella schreberiana var. schreberiana TJH17 Netherlands
Hygrodicranum herrerai IPG20 Chile
Dicranella campylophylla TJH13 Chile
Dicranella campylophylla TJH04 Chile
From Fig. 14A
Mittenia plumula
Mitteniaceae
Pleurophascum grandiglobum
Pleurophascaceae
Serpotortella chenagonii
Serpotortellaceae
Hypodontium pomiforme
Hypodontiaceae
Hypodontium dregei
Leucoloma procerum
Dicranaceae p.p. (s.str.)
Chorisodontium wallisii
Platyneuron praealtum
Paraleucobryum enerve
Paraleucobryum longifolium
Dicranum elongatum
Dicranum polysetum
Octoblepharum albidum
Octoblepharaceae
Syrrhopodon incompletus
Calymperaceae
Calymperes afzelii
Syrrhopodon gardneri
Mitthyridium fasciculatum
Syrrhopodon prolifer
Leucophanes angustifolium
Arthrocormus schimperi
Fissidens fontanus
Fissidentaceae
Fissidens bryoides
Chrysoblastella chilensis MSCc
Chrysoblastellaceae
Bryowijkia madagassa
Bryowijkiaceae
Bryowijkia ambigua
Eccremidium floridanum U.S.A., Florida
Ditrichaceae p.p.
Cladophascum gymnomitrioides South Africa
Bruchiaceae p.p.
Aongstroemia filiformis Allen 6403 Puerto Rico
Aongstroemia filiformis RF74 Réunion
Dicranellaceae p.p. (s.str.)
incl. Aongstroemiaceae p.p.,
Dicranaceae p.p. (Trichodontium)
and Ditrichaceae p.p. (Garckea)
Trichodontium falcatum New Zealand
Campylopodium medium MSCm Indonesia
Leptotrichella flaccidula Samoa
Microcampylopus laevigatus Réunion
Garckea phascoides Comoros
cf. Microcampylopus El Salvador
Campylopodium medium LSI48 Lesser Sunda Islands
Microcampylopus curvisetus Venezuela
Microcampylopus khasianus Indonesia
Dicranella cerviculata TJH07 Netherlands
Dicranella cerviculata RF61 Russian Far East
Dicranella cerviculata FDt5 Russian Far East
Dicranella cerviculata MSDce Finland
Dicranella cerviculata FDt6 Russia, East Siberian North
Dicranella curvipes RF58 Russian Far East
Dicranella curvipes RF59 Russian Far East
Dicranella curvipes RF60 Russian Far East
Dicranella heteromalla RF47 Russia, European Part
Dicranella heteromalla TJH28 Netherlands
Dicranella heteromalla TJH12 Netherlands
Dicranella heteromalla MSDh Germany
Dicranella heteromalla RF46 Norway
0.03
3
B

When including all available *Chrysoblastella chilensis* accessions as separate samples in preliminary analyses, they were resolved in very distant positions: *Buck 39507* and our voucher IPG19 formed the protohaplolepideous lineage mentioned above, whereas *R.D. Seppelt 26697* (not included in Figure 14) was resolved closely related to Bryowijkiaceae and Dicranellaceae s.str., sister to our voucher MSCc (herbarium B; published as *Cheilothela chloropus* (Brid.) Lindb. in Stech et al., 2012; Bonfim Santos & Stech, 2017a). None of these vouchers was sister to *Pseudoditrichum mirabile* as resolved in Fedosov et al. (2016a). Morphological identification of the available specimens revealed that voucher MSCc matches the description of *Chrysoblastella chilensis*, IPG19 was identified as *Ditrichum* cf. *cylindricarpum*, and *Buck 39507* turned out to be a mixed voucher of an undetermined *Ditrichum* species (likely the plant sequenced in Cox et al., 2010) and a true plant of *C. chilensis*. The morphological description of Chrysoblastellaceae in Fedosov et al. (2016a), which is in line with other descriptions of the genus *Chrysoblastella* (e.g. Buck, 1981), was based on a third specimen that has not yet been included in phylogenetic analyses (*Ireland & Bellolio 32976*; NY, duplicate MHA).

The present results have important implications for the evolution of the double-opposite peristome, indicating that this peristome type is not confined to the protohaplolepideous mosses, but evolved independently in the core haplolepideous mosses as well, or appeared as a rudimentary plesiomorphic trait. Furthermore, the present study, together with other phylogenies (e.g., Carter et al., 2014; Fedosov et al., 2021; Goffinet et al., 2011), suggests multiple losses of the peristome (capsules gymnostomous or cleistocarpous) during Dicranidae evolution, which occurred in the protohaplolepideous mosses (e.g., *Scouleria* Hook. p.p., *Bryoxiphium*), the intermediate grade or clade (e.g., Micromitriaceae) and the core haplolepideous clade (e.g., *Amphidium*, *Schistostega* D. Mohr, Rhabdoweisiaceae p.p., the *Astomiopsis-Aongstroemia orientalis* clade, Pottiaceae p.p., *Pleurophascum* Lindb., and the *Cladophascum-Eccremidium* clade).

Phylogeny of the Aongstroemiaceae, Dicranellaceae, and their types

The Aongstroemiaceae and the Dicranellaceae were resolved in our analyses as separate families within the core haplolepideous clade. Their present circumscriptions, however, are not in line with the inferred phylogenetic relationships, for two reasons. Firstly, *Aongstroemia* and *Dicranella* are polyphyletic according to the present data, and secondly, species of other genera were resolved within the Aongstroemiaceae and Dicranellaceae clades, as discussed in the following sections. A similar result was obtained before for the morphologically weakly delimited *Ditrichum* and the Ditrichaceae (Fedosov et al., 2015; Fedosov et al., 2016a). Only three out of the 13 species included species of *Dicranella* actually belong in the Dicranellaceae. The other 10 species are either resolved closer to the type of *Aongstroemia*, *A. longipes* (Aongstroemiaceae), or form clades not closely related to any of the currently recognised

families. Likewise, the three sampled *Aongstroemia* species are each resolved in a different core haplolepideous family (i.e., *A. longipes* in the Aongstroemiaceae, *A. filiformis* in the Dicranellaceae, and *A. orientalis* in the Ditrichaceae). The position of *Kiaeria* (*Dicranella*) *riparia* in the Rhabdoweisiaceae is confirmed by the detailed phylogenetic analysis of the latter family in Fedosov et al. (2021).

None of the tested circumscriptions of *Aongstroemia* and *Dicranella* from the literature are supported by our data (SH test: Table 4, Appendix 5). *Anisothecium* (e.g. sensu Crosby et al., 1999) is not supported as a separate genus from *Dicranella*, since the analysed *Dicranella* species placed in *Anisothecium* (e.g. by Crosby et al., 1999; *Dicranella campylophylla*, *D. grevilleana*, *D. rufescens*, *D. schreberiana*, *D. staphylina*, *D. varia*; cf. Appendix 5) are divided into different and not closely related clades as well. Based on the single species included, *Leptotrichella* (sensu Ochyra, 1997) is supported as separate from all *Dicranella* lineages.

Current circumscriptions of *Aongstroemia* and *Dicranella* originated from classifications published in the 19th century and, in fact, are based on plesiomorphic characters (stegocarpous capsules with a well-developed dicranoid peristome) and highly homoplastic traits, which likely originated independently in several lineages of pioneer mosses (small and slender plants, julaceous appearance or, in contrast, linear to subulate leaves), but ignored morphological characters of higher taxonomic value (e.g., presence of an annulus, shape of rhizoid tubers, etc.). Similarly, molecular phylogenetic approaches revealed numerous cases of deep polyphyly of traditionally circumscribed genera in pleurocarpous mosses, for instance *Calliergon* (Sull.) Kindb., *Drepanocladus* (Müll.Hal.) G. Roth, and *Hygrohypnum* Lindb. (Vanderpoorten et al., 2002b, 2002a) as well as *Hypnum* Hedw. (Câmara et al., 2018; Kučera et al., 2019; Schlesak et al., 2018), and these results were immediately followed by corresponding taxonomical solutions.

The re-evaluation of the broad morphological variation in *Dicranella* and *Aongstroemia* based on the current sampling revealed that the different molecular lineages resolved in our analyses possess distinctive (combinations of) morphological characters, as described in the following sections. In particular, the taxonomic significance of the morphology of rhizoid tubers (rhizoid-borne vegetative propagules) for *Dicranella* s.l., first suggested by Risse (1986) but not considered in subsequent studies, was supported by our molecular results. We provide tentative morphological descriptions for the Aongstroemiaceae and *Dicranella* clades based on the sampled specimens and the literature, as a basis for further study and taxonomical consequences. A densely sampled phylogeny coupled with extensive morphological study, as a follow up of the present research, may identify informative morphological characters to circumscribe Aongstroemiaceae and Dicranellaceae.

Revised circumscription of Dicranella *s.str. and the Dicranellaceae*

The clade referred here as *Dicranella* s.str. comprises the conserved type species, *D. heteromalla* (cf. Margadant & Geissler, 1995), *D. cerviculata*, and the Asian *D. curvipes*. Based on these three species, *Dicranella* s.str. would be recognised by the wide (⅓–½ of the leaf width at base) and excurrent costae and yellow setae, combined with stem leaves that are not sheathing but perichaetial leaves with sheathing bases that suddenly contracts into long, narrow subulas, capsules that are inclined to horizontal, asymmetric, curved, and furrowed to sulcate when dry, with annuli poorly differentiated (Nyholm, 1987; Smith, 2004), and the absence of rhizoid tubers (Correns, 1899; Risse, 1986; Whitehouse, 1966). *Dicranella cerviculata* has entire to slightly serrulate leaf apices, weakly delimited costae and strumose capsules, while *D. heteromalla* has distinctly serrulate leaf margins from the apex up to midleaf, strong costae, and capsules not strumose (Nyholm, 1987). *Dicranella curvipes*, distinct from *D. heteromalla* by their cygneous setae, was described as *D. heteromalla* var. *curvipes* Lindb. (Lindberg, 1872) and recently raised to the species level by Ignatov et al. (2006). The present molecular data do not unequivocally separate *D. curvipes* from *D. heteromalla* (Appendix 6), and further study is needed to assess the taxonomic status of *D. curvipes.*

Analyses by Cox et al. (2010) and Fedosov et al. (2016a) had already shown a close relationship of *Aongstroemia filiformis* (= *A. jamaicensis*, cf. Allen, 1994), *Cladophascum* (Bruchiaceae), *Eccremidium floridanum* (Ditrichaceae) and the type of *Garckea*, *G. phascoides* (Ditrichaceae). However, Cox et al. (2010) did not include any *Dicranella* species, whereas in Fedosov et al. (2016a) a specimen of *Dicranella heteromalla* was part of a clade containing the same vouchers of *A. filiformis*, *E. floridanum*, and *G. phascoides*. According to the present results, Dicranellaceae comprise the taxa listed above together with *Trichodontium* and the core genera already included in the family in Frey & Stech (2009) (*Campylopodium*, *Dicranella* s.str., *Leptotrichella*, *Microcampylopus*).

Aongstroemia filiformis differs morphologically from the type of *Aongstroemia*, *A. longipes* (see below), by its larger leaves (4–6 mm vs. 0.5–1 mm) that are abruptly subulate from oblong leaf bases (vs. scale-like to ovate-lanceolate in *A. longipes*), excurrent costae (vs. subpercurrent to percurrent or only rarely excurrent), laminal cells that are short rectangular at the leaf base to linear-vermicular at the apex (vs. elongate, irregularly hexagonal, rhomboid or rectangular, and shorter at the apex), capsules with stomata, and peristomes that are divided above into two or three prongs (vs. divided, perforated or entire) (Allen, 1994; Crum, 1994; Eckel, 2007a).

Based on the phylogenetic relationships resolved here, the Dicranellaceae would include plants with three different sporophytic morphologies, with either (1) long setae and emergent to exserted, peristomate capsules (in the initially included genera, plus *A. filiformis* and *Trichodontium*); (2) short setae and immersed, peristomate capsules (in *Garckea*); and (3) short setae and eperistomate capsules (immersed in *Cladophascum*, most commonly laterally

emergent and pendulous but sometimes erect and immersed in *Eccremidium*) (Buck, 2007; Crum, 1994; Sim, 1926). The family thus would include at least two lineages with independent sporophyte reduction (*Garckea* and *Cladophascum*/*Eccremidium*), and the present results add to the understanding of the relationships of these lineages as inferred in previous studies (Cox et al., 2010; Fedosov et al., 2015; Fedosov et al., 2016a). Gametophytically, all Dicranellaceae taxa share long lanceolate leaves (in *Cladophascum*, at least the perichaetial leaves) with a strong costa (Frey & Stech, 2009), which are not very distinctive among the haplolepideous mosses. Dicranellaceae are markedly morphologically distinct from their well-supported sistergroup *Bryowijkia* (Bryowijkiaceae), which has cladocarpous, profusely branched plants, plicate leaves with differentiation between stem and branch leaves, and microstomous capsules (Frey & Stech, 2008; Vitt & Buck, 1984).

Revised circumscription of Aongstroemia *s.str. and the Aongstroemiaceae*

Based on the present results, the Aongstroemiaceae clade comprises *Aongstroemia longipes*, *Dichodontium*, and *Diobelonella* (as in Frey & Stech, 2009) as well as five species presently placed in *Dicranella* (three of which have been previously combined under *Aongstroemia* by Carl Müller (1849): *D. campylophylla*, *D. grevilleana*, and *D. varia*) and two *Hygrodicranum* species. Within this clade, there was less molecular support for the sister-group relationship of the *Dicranella* lineage composed by *D. howei* and *D. varia* to the Aongstroemiaceae s.str. clade. *Dicranella howei* and *D. varia* have in common with the Aongstroemiaceae s.str. clade peristome teeth that are vertically pitted-striolate at base. *Dicranella howei* and *D. varia* further resemble part of the taxa in the Aongstroemiaceae s.str. in their inclined, ovoid, asymmetric, gibbous capsules that remain smooth when dry. On the contrary, they differ from *Dichodontium* by the presence of rhizoid tubers, from *Aongstroemia* and *Diobelonella* by the irregular instead of spherical shape of the tubers (although *Aongstroemia* does not always present tubers), and further from *Aongstroemia* by having undifferentiated stem and perichaetial leaves without sheathing bases (Crum, 2007; Eckel, 2007a; Renauld & Cardot, 1893; Smith, 2004; Whitehouse, 1966). With or without the inclusion of *D. howei* and *D. varia* as part of the Aongstroemiaceae, the family remains morphologically heterogeneous and without distinctive characters that separate it from other haplolepideous moss families.

The current characterization of *Aongstroemia* based on the possession of julaceous gametophytes (see Eckel, 2007) does not hold, since the three included *Aongstroemia* species belong to different families, whereas the *Dicranella* and *Hygrodicranum* species that were resolved as closely related to *A. longipes* do not have julaceous gametophytes. The re-circumscribed *Aongstroemia* would be recognised by stem leaves with a broad sheathing base that is abruptly contracted to a short- to long-pointed, spreading to squarrose leaf apex (the latter also present in well-developed *A. longipes* plants, according to Drugova, 2010),

rectangular lamina cells, spherical rhizoid tubers (if present) without protruding cells, capsules erect to inclined, symmetric to asymmetric, oval/obloid, straight to curved and sometimes slightly strumose, on a straight, erect, red to darkened seta (Smith, 2004; Whitehouse, 1966). The leaves of *A. longipes* may have originated from a reduction of the apex, eliminating the subulate awn and thus resulting in the ovate-lanceolate leaf shape and julaceous habit. A broad range of lengths of the leaf awn is not unusual among haplolepideous mosses, for instance, in some species of the genus *Ditrichum* (see the complex *D. lineare* (Sw.) Lindb./*D. plumbicola* Crundw.; Atherton et al., 2010; Frahm et al., 2008). The close relationship of *Aongstroemia longipes* and *Dicranella grevilleana*, which cannot be separated with the present molecular markers, may indicate that the gametophyte morphology of *A. longipes* represents a unique derived state within the clade. Capsule morphology markedly differs between *A. longipes* (capsules ovoid, symmetric, erect, smooth) and *D. grevilleana* (capsules curved, asymmetric, inclined, furrowed when dry), although in their sister clade morphological transitions exist within single species: In both *D. campylophylla* and *D. schreberiana* capsule shape ranges between that of *A. longipes* and *D. grevilleana* (Ochyra et al., 2008; Smith, 2004). Nevertheless, *A. longipes* and *D. grevilleana* should be maintained as separate species unless evidence to the contrary arises from further phylogenetic analyses.

Further problems of species delimitation to be addressed in subsequent studies concern *Dicranella varia*/*D. howei* and *D. schreberiana*. The former two species were regarded as conspecific by some authors (e.g., Crum, 2007) but not yet formally synonymised (cf. Tropicos, 2020). The results of our phylogenetic analyses support the monophyly of *D. howei* but not of *D. varia*. The split of *D. varia* into a clade of European samples sister to *D. howei*, and a single specimen from Siberia, together with differences between the type specimen of *D. howei* from California and Mediterranean material (Crundwell & Nyholm, 1977), and the occurrence of intermediate forms between the two species, support the need of further study. The same holds for the two varieties of *D. schreberiana* included in this study, which were resolved in separate positions, with *D. schreberiana* var. *schreberiana* resolved as more closely related to *Hygrodicanum* and *D. campylophylla* than to *D. schreberiana* var. *robusta* (Schimp. ex Braithw.) H.A. Crum & L.E. Anderson.

The clade formed by *D. campylophylla*, *D. cardotii*, *D. schreberiana*, *Hygrodicranum bolivianum* and *H. herrerai* (Figure 14, Appendix 6) is the only one in our analysis to include former *Dicranella* species with mamillose or papillose lamina cells. Moreover, this clade includes species with a bistratose lamina, also found in the *D. howei*/*D. varia* clade, and in the genus *Dichodontium* (Smith, 2004). A regularly to irregularly bistratose lamina (homogeneously two-layered or with an interrupted, irregular second layer) is the main diagnostic character of *Hygrodicranum*, which comprises three aquatic species (Cook et al., 1974) never collected with sporophytes, but is found in some *Dicranella* species (as *D. campylophylla* and *D. cardotii*) as well. Earlier molecular analyses of aquatic, especially rheophytic, pleurocarpous mosses have

already shown that the character of bi- to multistratose laminae was taxonomically overrated (e.g., Spitale & Petraglia, 2010; Stech & Frahm, 2000). A similar example from the haplolepideous mosses is *Fissidens grandifrons* Brid., which was earlier classified in its own genus *Pachyfissidens* (Müll.Hal.) Limpr. (Limpricht, 1887: 454). The present data indicate that *Hygrodicranum* does not deserve recognition as a separate genus, but the type, *H. falklandicum* Cardot, is yet to be included in phylogenetic analyses.

The separation of *Diobelonella* from *Dichodontium* (Ochyra et al., 2003; as opposed to Stech, 1999c) is supported by our molecular results and morphology. While *Dichodontium* has short, thick-walled, coarsely-papillose or mamillose distal lamina cells, irregularly dentate upper leaf margins, and strong costae with two stereid bands, *Diobelonella* has prosenchymatous, thin-walled, entirely smooth distal lamina cells, entire to crenulate leaf margins, and weak costae with a single stereid band (Ochyra et al., 2003). Furthermore, *Diobelonella palustris* has spherical rhizoid tubers similar to those of *Dicranella campylophylla*, *D. grevilleana*, and *D. schreberiana*, although the tubers of the latter three species do not have protruding cells (Ochyra et al., 2003; Risse, 1986; Whitehouse, 1966). *Dichodontium* is not reported to have typical rhizoid tubers but bears ellipsoid or clavate multicellular gemmae on filamentous branches on the leaf axils (Eckel, 2007b; Smith, 2004). On the other hand, the results of the SH test (Table 4, Appendix 5) do not reject the hypothesis of *Dichodontium* and *Diobelonella palustris* forming a single clade.

Dicranella *and* Aongstroemia *segregates outside Dicranellaceae and Aongstroemiaceae*

Regarding species so far still considered in *Dicranella*, the precise placement of three remaining supported *Dicranella* clades (*D. staphylina*, *D. crispa*/*D. subulata* and *D. rufescens*), intermediate between the protohaplolepideous and the main haplolepideous clade, is still unclear. The SH test rejected the hypothesis of the monophyly of a clade formed by all *Dicranella* species included in this study but did not exclude the hypothesis that these three *Dicranella* clades form a monophyletic group or that any two of the three clades are sister groups (Table 5). Nevertheless, considering that these clades can each be recognised by a combination of morphological features, but have little in common, they might be considered as different genera and families.

Dicranella staphylina is a very small species known from cultivated fields across North America and Europe. The epithet is based on the characteristic rhizoid tubers shaped like bunches of grapes (from the Greek *staphyle*), which are regularly found (Miguel Velasco, 1986; Whitehouse, 1969, 2001). In addition to the tubers, plants of *D. staphylina* can be recognised by bright green color, stems ramified only at base, and stem leaves not sheathing, often with a recurved margin at the base (Nyholm, 1987; Whitehouse, 1969). Its sporophytes are little known, which is a common phenomenon among tuber-bearing moss species (Whitehouse,

1966). In fact, the only report of this life phase corresponded to 10 immature sporophytes and lacked information about some features as annulus and basal membrane (Arts, 1985). Characters that could be inferred from the perichaetial leaves (differentiated, with a sheathing base and abruptly contracted into the spreading apex) and the immature sporophytes (seta yellow to orange, capsules erect, symmetrical, smooth, with few stomata, and peristome teeth bifid to the middle; Arts, 1985), are little informative for the relationships with other haplolepideous mosses and yet to be confirmed based on mature sporophytes. Nevertheless, the phylogenetic position of *D. staphylina* (represented here by Dutch specimens), branching off early in the haplolepideous moss tree, although without support (Figure 14), indicates that it was assigned to *Dicranella* based on rather superficial gametophytic similarities.

The clade composed of *Dicranella crispa* and *D. subulata* can be recognised by having an oblong leaf base gradually narrowed into a long subulate apex (abruptly so in *D. subulata* perichaetial leaves), percurrent to excurrent costa filling most of the subula, and capsules +/- erect, +/- symmetric, striate to furrowed when dry, with well-differentiated annulus formed by 2–3 rows of widened cells (Crum, 2007; Nyholm, 1987). It is morphologically close to the *Dicranella schreberiana* clade, but the latter differs in the very broad, abruptly narrowed ('quadrate') and tightly clasping sheathing base in stem leaves, the most frequently inclined to horizontal and slightly asymmetric to gibbous capsules (Nyholm, 1987; Smith, 2004), spherical rhizoid tubers (Whitehouse, 1966) and not or poorly differentiated annulus. Tubers in *D. subulata* resemble the other basal lineage corresponding to *D. rufescens* and are considered structurally homologous to rhizoids (different from, e.g., those of *D. campylophylla*, considered to develop from a tuber initial cell; cf. Risse, 1986). *Dicranella crispa* and *D. subulata* are molecularly (Figure 14) and morphologically distinct from each other. *Dicranella crispa* has a squarrose leaf apex from the sheathing base and an erect capsule, while *D. subulata* has leaves +/- erect spreading or secund (only perichaetial leaves with sheathing base) and capsules sometimes slightly inclined and asymmetric (Nyholm, 1987; Smith, 2004).

Dicranella rufescens, which has also been combined into the genera *Anisothecium*, *Aongstroemia*, and *Dicranum*, differs from all other *Dicranella* lineages included in this study by two characters: its peristome with a high basal membrane, contrasting to the short basal membranes up to three cells high in the other lineages, and the red color of its stems (Hallingbäck et al., 2006). *Dicranella rufescens* is morphologically close to *D. humilis* R. Ruthe. The latter species shares with *D. rufescens* the red coloration of the stem but differs by inclined, slightly curved and asymmetric capsules (upright, straight, symmetric in *D. rufescens*) (Hallingbäck et al., 2006). Additionally, Kučera (2004) describes that *D.* rufescens has exothecial walls that are always equally thickened, while *D. humilis* has sometimes weaker transverse walls, even though this character does not seem to be a stable distinguishing trait. Among our specimens originally labelled as *D. rufescens,* BCNL1 was sterile, and BCNL2 had the typical capsules of *D. rufescens*. RF63, however, was initially identified as *D. humilis*, based

on its slightly inclined capsules and slightly different thickness of the longitudinal and transverse exothecial cell walls.

Based on the absence of peristome teeth, Allen (1994) considered some species of *Aongstroemia* to resemble the Ditrichaceae genera *Astomiopsis* and *Bryomanginia*. As predicted based on morphology, *Aongstroemia orientalis* was found to be closely related to *Astomiopsis amblyocalyx* (Ditrichaceae) and should probably be transferred to that genus. *Aongstroemia orientalis* and also *A. julacea* (Hook.) Mitt. (the latter not yet included in molecular phylogenetic analyses) differ from *Aongstroemia* as defined here (see above) not only by eperistomate capsules but also in having gemmae in the leaf axils (Allen, 1994; Drugova, 2010). The other three currently accepted *Aongstroemia* species not yet included in molecular phylogenetic studies, *A. appressa* Hampe ex Müll.Hal., *A. gayana* (Mont.) Müll.Hal., and *A. subcompressa* Hampe ex Müll Hal. are little known, and their affinities are unclear.

Chapter 5

Summary and Conclusions

The haplolepideous mosses (Dicranidae) are the second largest lineage of mosses, with about 4000 species representing ca. 30% of the moss diversity (Frey & Stech, 2009). The group is named after its haplolepideous peristome, which differs from other moss peristomes by its cell pattern described by the peristomial formula 4:2:3 (Edwards, 1979). Haplolepideous moss species can be found in a wide range of habitats and their gametophytic and sporophytic morphology varies greatly. The monophyly of the Dicranidae is well supported by the results of several studies (e.g., Cox et al., 2010; Liu et al., 2019). Since the late 1990's several molecular phylogenetic studies increased the understanding about relationships and circumscriptions within the Dicranidae, but also contributed to identify many problems which remain to be tackled. Dicranidae ordinal classification is not in line with our knowledge about phylogenetic relationships and is not supported by the morphology. At lower taxonomic levels it was shown that some morphological patterns that were used to define families do not correspond to monophyletic groups. At the same time there are many families and genera with weak morphological circumscriptions of which the monophyly remains to be tested.

In this thesis, the systematics and relationships of selected haplolepideous mosses, namely the leucobryoid mosses and some families and genera segregated from the former Dicranaceae s.l., were studied using molecular phylogenetic methods. Sequences of the nuclear ribosomal ITS region, the mitochondrial *nad*5 intron, and the chloroplast *trn*S-*trn*F region and *atp*B-*rbc*L spacer were obtained from new DNA extractions of available specimens (from herbaria or ongoing projects), from additional sequencing of previously sequenced specimens, and from previous studies through GenBank (e.g. Cox et al., 2010; La Farge et al., 2002; Stech et al., 2012). Those represented 37 out of the 38 haplolepideous moss families in our phylogenetic analyses. Maximum parsimony, maximum likelihood, and Bayesian inference were used for the phylogenetic reconstructions. Ancestral state reconstructions, phylogenetic network analysis (NeighborNet), and relationship hypothesis testing (Shimodaira-Hasegawa test) were performed to contribute to the interpretation of the results of the phylogenetic reconstructions. By means of a literature revision and re-evaluation of morphological characters the morphological circumscriptions of taxa were evaluated and improved, in line with the molecular phylogenetic results.

What are leucobryoid mosses? How are they related to other haplolepideous mosses?

The leucobryoid mosses are 11 haplolepideous genera whose leaves in cross section have layers of large, porous hyalocysts encircling small chlorocysts, which results in their whitish colours. This remarkable modified anatomy led to their initial classification as a single family, the Leucobryaceae (Schimper, 1856). However, phylogenetic reconstructions have shown that these plants are not all closely related to each other (Cox et al., 2010; Fisher et al., 2007; Stech et al., 2012). In this thesis, the relationships of leucobryoid mosses were further investigated (**Chapters 2**, **3**).

The pantropical leucobryoid genus *Octoblepharum* (Calymperaceae s.l. or Octoblepharaceae) was studied based on an extended sampling relative to previous studies, with additional species and a larger set of molecular markers (**Chapter 2**). *Octoblepharum*, the other Calymperaceae s.l. genera, and the Hypodontiaceae were grouped in a clade which shows high genetic divergence compared to the remainder of the haplolepideous moss tree (Figure 6). This clade thus comprises two distinct lineages of leucobryoid mosses that are separated from each other by a grade of several non-leucobryoid clades: the monophyletic *Octoblepharum* sister to the Calymperaceae s.str. clade, and the derived *Leucophanes*-clade within the Calymperaceae s.str., including *Arthrocormus* and *Leucophanes* (**Chapter 2**), as well as *Exodictyon* and *Exostratum* (Fisher et al., 2007; Tsubota et al., 2004).

The Leucobryaceae were studied in detail for the first time in their current circumscription based on molecular data (**Chapter 3**), i.e., excluding some leucobryoid taxa (*Octoblepharum* and the *Leucophanes*-clade) and including non-leucobryoid taxa in the family (Frey & Stech, 2009; Goffinet & Buck, 2004). The Leucobryaceae comprise three well-supported clades, two consisting of dicranoid genera segregated from the Dicranaceae s.l., and one consisting of leucobryoid plants (Cox et al., 2010; Tsubota et al., 2004; **Chapter 3**). The relationships between these clades, however, are not strongly supported. A phylogenetic network (NeighborNet) helped visualizing why: short branches between the earliest splits correspond to little genetic divergence, and consequently, too little information to allow resolving those relationships with confidence. That pattern suggests that the early radiation of the family may have been a rapid event. Within the three main clades high genetic divergence, high species richness, broad distribution range, and modification of the basic dicranoid leaf structure seem to be directly connected to each other.

Despite their superficial gametophytic similarity, each of the three lineages of leucobryoid Dicranidae (*Octoblepharum*, the *Leucophanes*-clade within Calymperaceae s.str., and the leucobryoid clade of Leucobryaceae) shows a different type of leucobryoid morphology of the gametophyte, each with its own synapomorphic characters (**Chapters 2**, **3**). Further differences between the three leucobryoid lineages can be found in the more stable sporophytic

characters. Leucobryoid mosses are species-rich, widespread lineages, and their evolutionary success might be a result of the adaptive nature of their specialised morphology.

What characterises the family Octoblepharaceae? Are species circumscriptions within this monogeneric family supported by molecular data?

In the past, *Octoblepharum* has been classified in the Calymperaceae s.l, or in its own family Octoblepharaceae (Eddy, 1990; Menzel, 1991). The latter classification was supported by the considerable genetic divergence observed between this genus and the Calymperaceae s.str. (**Chapter 2**). Despite its morphological resemblance to the *Leucophanes*-clade, these two lineages of leucobryoid plants differ in their leaf structure in cross section (e.g., chlorocysts are triangular in *Octoblepharum* but diamond-shaped in the *Leucophanes*-clade) and are not directly related. Moreover, the leaf shape in *Octoblepharum* is unique among mosses: ligulate leucobryoid leaves with a cuspidate to mucronate apex and with a hyaline lamina that is restricted to a distinct sheathing base.

The four *Octoblepharum* species included in this research are well circumscribed according to the results of molecular phylogenetic analyses (**Chapter 2**), which corroborate the morphological species circumscriptions from the literature, especially the work by Noris Salazar Allen (e.g. Salazar Allen, 1991, 1994). *Octoblepharum arthrocormoides*, a recently described and a yet little-known species, was recognised after the phylogenetic analysis of herbarium material previously labelled as *O. albidum*. *Octoblepharum albidum*, by its turn, a pantropical, common species, was shown to comprise great intraspecific molecular variability.

Is the current systematics of the Leucobryaceae compatible with molecular phylogenies?

Several genera within Leucobryaceae can be considered well defined, since their morphological circumscriptions were not in conflict with the results of the molecular phylogenetic reconstructions (**Chapter 3**). This is not the case, however, for the dicranoid genera *Atractylocarpus*, *Campylopodiella*, and *Dicranodontium*, and for the most species-rich leucobryoid genus, *Leucobryum*. Relationships of the genera *Mitrobryum*, *Sphaerothecium*, and *Steyermarkiella*, which are rare and could not be included in this study, remain to be studied.

Ancestral state reconstructions contributed to delimit the genus *Atractylocarpus*, which includes *A. subporodictyon*. This species was formally transferred from *Dicranodontium* to *Atractylocarpus* with support of molecular analyses. Morphologically, *Atractylocarpus* can be characterised by pitted basal laminal cells in this revised circumscription. The cygneous seta

was not supported as a distinctive trait for the genus *Dicranodontium* but resolved as an ancestral trait for the entire *Dicranodontium* clade (*Atractylocarpus*, *Brothera*, *Campylopodiella*, and *Dicranodontium*). *Campylopodiella* and *Dicranodontium* were resolved as polyphyletic, albeit without strong support. These two genera and the monospecific genus *Brothera* are all morphologically similar and difficult to separate from each other.

Most genus level relationships within the leucobryoid clade are well supported, except for those between the Asian *Cladopodanthus* and *Schistomitrium* and the widespread *Leucobryum*. While the clade comprising *Cladopodanthus* and *Schistomitrium* was nested within *Leucobryum* in analyses at the family level (without ITS), it formed a sister clade to *Leucobryum* in some analyses of the leucobryoid clade only, a hypothesis not rejected by the Shimodaira-Hasegawa tests.

What are *Aongstroemia* and *Dicranella*? What strategies can be applied to improve the circumscription of such poorly characterised genera?

Aongstroemia and *Dicranella* are examples of the many widespread, morphologically diverse, and poorly circumscribed haplolepideous moss genera. The same holds for the families Aongstroemiaceae and Dicranellaceae, segregates from Dicranaceae s.l. Both require revision due to taxonomical problems and are little represented in molecular phylogenetic analyses. Their circumscription and relationships were studied in this thesis with analyses of all known major lineages of Dicranidae, including all available sequence data for *Aongstroemia*, *Dicranella*, and other closely related genera (**Chapter 4**). Both *Aongstroemia* and *Dicranella* were resolved as polyphyletic, and thus their current circumscription was found not to correspond to natural groups. *Dicranella*, previously understood as one morphologically diverse genus, in fact represents many evolutionary lineages spread across the haplolepideous tree, each of them representing a smaller and more homogeneous compartment of the total morphological diversity. Upon further investigation, some characters considered important for their classification turned out not to be, whereas other, previously overlooked characters were found to be important, which may largely explain the problematic circumscription of both genera.

Aongstroemia, a small genus with species narrowly distributed across the globe, was characterised by plants with julaceous stems. However, the three species (with julaceous stems) studied here all belong to separate, not closely related lineages. Sporophytic and leaf characters do differ between each of the three species and are thus more useful characters for the classification than the leaf arrangement on the stem.

For *Dicranella*, a widespread genus with over 200 species, many proposals for the classification of its species were suggested through time, involving different combinations of other genus

names based on different sets of morphological characters. There are many typification problems, and many species are insufficiently characterised. All in all, the genus concept of *Dicranella* seems to correspond to a vague idea of small, dicranoid haplolepideous mosses which lack the distinctive characters of other genera. *Dicranella* species were resolved in six clades across the Dicranidae phylogeny, from the protohaplolepideous to the core haplolepideous lineages (**Chapter 4**). As in *Aongstroemia*, these clades correspond to subdivisions of the broad morphological variation within *Dicranella* s.l. and are well circumscribed based on combinations of characters, e.g., from leaves and capsules. An interesting finding is that an already described but yet understudied character, namely the morphology of rhizoidal tubers, seems quite informative for the relationships of *Dicranella* species in different clades.

What is a double haplolepideous peristome? In which taxa is this peristome type found and how are those taxa distributed across the phylogeny of the haplolepideous mosses?

The systematics and relationships of arthrodontous mosses with less typical, modified or reduced, to completely absent peristomes have always been subject of discussion, including multiple transfers of respective taxa between the major lineages within Bryopsida. While most Dicranidae have a haplolepideous peristome with a single row of teeth, a few species-poor haplolepideous taxa have a peristome with two opposite rows of teeth. These double-opposite haplolepideous peristomes occur in different variations, with equally developed rows of teeth or a reduced inner or outer row of teeth, but can still be described by the haplolepideous peristome formula 4:2:3 (Edwards, 1979; Fedosov et al., 2016a). Yet, such peristomes often prompted the classification of the respective taxa outside the Dicranidae. Some of these taxa were recently studied based on an integrative taxonomic approach (e.g. Fedosov et al., 2016a).

The phylogenetic analyses performed to elucidate the relationships of *Aongstroemia* and *Dicranella* contributed to the systematics of the double-peristomate haplolepideous mosses as well, since the sampling specifically included haplolepideous families which were resolved as either polyphyletic or in incongruent positions between different previous studies (**Chapter 4**). According to the results of this research, double-peristomate families not only occur within the protohaplolepideous grade (Catoscopiaceae, Distichiaceae, Flexitrichaceae, Pseudoditrichaceae), but are represented also among the core haplolepideous mosses (Chrysoblastellaceae), which implies the independent evolution of this peristome type in the different major haplolepideous lineages.

In the protohaplolepideous grade, both the single and double-peristomate lineages consist of only a few species, contrasting with the speciose core-haplolepideous clade of (almost all)

single-peristomate plants. Whether the protohaplolepideous taxa represent “experiments” with different types of (peristome) morphologies early in the evolution of the Dicranidae, or remnants of once more speciose lineages, remains unknown. The continuous additions of taxa to the protohaplolepideous grade based on molecular data at least suggests that the evolution and diversity of the “basal” Dicranidae is more complex than previously known, and yet incompletely understood.

Future studies

This thesis resulted in a better understanding of the circumscriptions and relationships of the haplolepideous mosses, in particular the leucobryoid genera and other taxa formerly classified in Dicranaceae s.l. which were insufficiently represented in previous molecular phylogenetic analyses. Moreover, in the phylogenetic reconstructions of **Chapter 4** for the first time all haplolepideous moss families for which molecular data are currently available (37 out of 38 families) were included. These analyses thus represent the most complete overview of this lineage of mosses, combining existing and new data.

Molecular inferences were based on a number of standard molecular markers, from all three genomes and with different levels of variability. This approach allowed to carry out phylogenetic analyses at different taxonomic levels, detect possible incongruence between genomes, and easily incorporate sequences from GenBank, ensuring comparability with results of previous studies. The taxonomic sampling was clearly expanded compared to earlier studies, however, some genera remained underrepresented. This was due to unsuccessful DNA extraction of some specimens, to the need of sequencing additional markers from specimens included in previous studies (resulting in lower capacity to include new specimens), and to the naturally limited representation of rare and narrowly distributed taxa in biological collections. Nevertheless, the focus on utilizing available specimens from herbaria or other ongoing projects, rather than, for example, attempting to collect new material, was a cost-effective strategy to construct a phylogenetic framework of the studied taxa . Based on that framework, future studies with targeted collection efforts and DNA sequencing of missing taxa will allow verifying and extending the results presented here.

For instance, since the majority of the *Dicranella* species still remains molecularly unstudied, it would be one high priority task to sequence more species of this genus, to infer to which of the newly discovered lineages (or possibly other, yet unknown lineages) they belong, as a basis for a taxonomic revision. In the case of *Octoblepharum,* the species studied here are well circumscribed, but the results concerning the pantropical *O. albidum* suggest there is a geographical signal in its molecular variation, and further studies of morpho-molecular variation within *O. albidum*, based on a larger number of specimens, might reveal (cryptic) speciation. For the Leucobryaceae, a more extensive DNA sequence sampling would be

desirable to clarify the relationships between its three well-supported main clades. In general, ancestral state reconstructions performed on extended molecular phylogenies may contribute to the much-needed re-evaluation of the weak morphological circumscriptions of numerous haplolepideous moss taxa.

The still small number of molecular markers included in this study restricted the overall resolution and branch support of some phylogenetic reconstructions, in particular the backbone of the Dicranidae. Although Sanger sequencing of individual markers will probably be replaced at some point by high-throughput sequencing techniques, in particular SMRT (single molecule real-time sequencing), phylogenetic analyses based on individual DNA markers are still common. Such data can still contribute to the progress of systematics and be further applied to answer open questions about circumscriptions, biogeography, and morphological evolution of the haplolepideous mosses. Nevertheless, tackling problems such as the evolution at higher taxonomic levels in the Dicranidae, but also delimiting species in taxonomically complex genera such as *Campylopus* (e.g., Gama et al., 2017) will certainly benefit from genomic approaches such as Hybrid Capture-based sequencing (HybSeq). The large-scale sequencing of hundreds of DNA markers achieved by that method would most probably allow to infer phylogenetic relationships and patterns of morphological evolution with more confidence. As a large, monophyletic group with multiple challenges (e.g., relationships of the protohaplolepideous taxa, polyphyly of traditionally circumscribed families and genera, taxonomically complex, species-rich genera), the Dicranidae would lend themselves well as a case study for employing new sequencing approaches in bryophytes.

Samenvatting en Conclusies

De haplolepide bladmossen (Dicranidae) zijn de op een na grootste afstammingslijn binnen de bladmossen, met ongeveer 4000 soorten die 30% van de diversiteit van de bladmossen uitmaken (Frey & Stech, 2009). De groep is vernoemd naar haar haplolepide peristoom, dat verschilt van de peristomen van andere bladmossen door het cellenpatroon dat met de peristoomformule 4:2:3 kan worden beschreven (Edwards, 1979). Haplolepide bladmossen worden in veel verschillende habitats aangetroffen en hun gametofytische en sporofytische morfologie vertoont een brede variatie. De monofylie van de Dicranidae wordt goed ondersteund door de resultaten van meerdere studies (bv. Cox et al., 2010; Liu et al., 2019). Sinds eind jaren 90 hebben meerdere moleculaire fylogenetische studies bijgedragen aan ons begrip van de verwantschappen en omgrenzing van taxa binnen de Dicranidae. Deze studies hebben echter ook de problemen aangetoond die nog aangepakt moeten worden. De classificatie van de Dicranidae op orde-niveau komt niet overeen met onze kennis over fylogenetische verwantschappen en wordt niet ondersteund door de morfologie. Op lagere taxonomische niveaus werd aangetoond dat sommige morfologische patronen die gebruikt werden om families te definiëren niet overeenkomen met monofyletische groepen. Tegelijkertijd zijn er veel families en genera met een zwakke morfologische omschrijving waarvan de monofylie nog moet worden getest.

In dit proefschrift zijn de systematiek en verwantschappen van meerdere taxa binnen de haplolepide bladmossen bestudeerd, te weten de leucobryoïde bladmossen en een aantal families en genera die werden afgesplitst van de eerdere Dicranaceae s.l., gebruik makend van moleculaire fylogenetische methoden. Sequenties van de nucleaire ribosomale ITS regio, het mitochondriale *nad*5 intron, en de chloroplast *trn*S-*trn*F regio en *atp*B-*rbc*L spacer werden verkregen uit nieuwe DNA extracten van beschikbare specimens (uit herbaria of lopende projecten), door aanvullend sequensen van reeds eerder gesequenste specimens, en uit eerdere studies via GenBank (bv. Cox et al., 2010; La Farge et al., 2002; Stech et al., 2012). Deze representeerden 37 van de 38 haplolepide mossenfamilies in onze fylogenetische analyses. Maximale parsimonie, maximum likelihood, en Bayesiaanse methodes zijn gebruikt voor de fylogenetische reconstructies. Voorouderlijke kenmerkreconstructies, fylogenetische netwerkanalyse (NeighborNet) en testen van verwantschapshypotheses (Shimodaira-Hasegawa test) werden uitgevoerd om een bijdrage te leveren aan de interpretatie van de uitkomsten van de fylogenetische reconstructies. Door middel van een revisie van de literatuur en herwaardering van morfologische kenmerken, werd de morfologische omschrijving van de taxa geëvalueerd en verbeterd, in lijn met de moleculaire fylogenetische resultaten.

Wat zijn leucobryoïde bladmossen? Hoe zijn zij verwant aan andere haplolepide bladmossen?

De leucobryoïde bladmossen zijn 11 haplolepide genera wiens bladeren in doorsnede lagen vertonen van grote, poreuze hyalocyten die kleine chlorocyten omsluiten, waardoor hun witachtige kleur tot stand komt. Deze opmerkelijke gemodificeerde morfologie leidde tot hun oorspronkelijke classificatie als één enkele familie, de Leucobryaceae (Schimper, 1856). Fylogenetische reconstructies hebben echter aangetoond dat deze planten niet allemaal nauw verwant zijn aan elkaar (Cox et al., 2010; Fisher et al., 2007; Stech et al., 2012). In dit proefschrift zijn de verwantschappen van de leucobryoïde bladmossen verder onderzocht (**Hoofdstukken 2, 3**).

Het pantropische leucobryoïde genus *Octoblepharum* werd bestudeerd op basis van een bredere bemonstering vergeleken met eerdere studies, met additionele soorten en een grotere set van moleculaire markers (**Hoofdstuk 2**). *Octoblepharum*, de andere genera van de Calymperaceae s.l. en de Hypodontiaceae werden gegroepeerd in een clade die hoge genetische divergentie vertoont vergeleken met de rest van de stamboom van de haplolepide bladmossen (Figuur 6). Deze clade bevat dus twee verschillende lijnen van leucobryoïde bladmossen, die van elkaar gescheiden zijn door een grade van meerdere niet-leucobryoïde clades: het monofyletische *Octoblepharum* in een zustergroep relatie met de Calymperaceae s.str. clade, en de afgeleide *Leucophanes*-clade binnen de Calymperaceae s.str., inclusief *Arthrocormus* en *Leucophanes* (**Hoofdstuk 2**) evenals *Exodictyon* en *Exostratum* (Fisher et al., 2007; Tsubota et al., 2004).

De Leucobryaceae zijn voor het eerst in detail bestudeerd (**Hoofdstuk 3**) in hun huidige omschrijving, d.w.z. exclusief sommige leucobryoïde taxa (*Octoblepharum* en de *Leucophanes*-clade) en inclusief niet-leucobryoïde taxa (Frey & Stech, 2009; Goffinet & Buck, 2004). De Leucobryaceae omvatten drie goed ondersteunde clades, twee met dicranoïde planten en één met leucobryoïde planten (Cox et al., 2010; Tsubota et al., 2004; **Hoofdstuk 3**). Hun onderlinge verwantschappen worden echter niet sterk ondersteund. Een fylogenetisch netwerk (NeighborNet) hielp om zichtbaar te maken waarom dit zo was: korte takken tussen de vroegste afsplitsingen corresponderen met lage genetische divergentie, en bijgevolg te weinig informatie om deze verwantschappen met vertrouwen te kunnen oplossen. Binnen de drie hoofdtakken lijken hoge genetische divergentie, grote soortenrijkdom, een breed verspreidingsgebied en modificatie van de basale dicranoide bladstructuur direct met elkaar in verband te staan.

Ondanks hun oppervlakkige gametofytische gelijkenis, vertoont elk van de drie leucobryoïde afstammingslijnen binnen de Dicranidae (*Octoblepharum*, de Leucophanes-clade binnen de Calymperaceae s.str., en de leucobryoïde clade van de Leucobryaceae) een andere type leucobryoïde morfologie van de gametofyt, elk met eigen synapomorfieën (**Hoofdstukken 2**,

3). Verdere verschillen tussen de drie leucobryoïde lijnen zijn te vinden in de stabielere sporofytische kenmerken. Leucobryoïde bladmossen vormen soortenrijke, wijdverspreide afstammingslijnen, en hun evolutionair succes zou het resultaat van de adaptieve aard van hun gespecialiseerde morfologie kunnen zijn.

Wat kenmerkt de familie Octoblepharaceae? Worden soortomschrijvingen in deze monogenerische familie ondersteund door moleculaire data?

Vroeger werd *Octoblepharum* ofwel in de Calymperaceae s.l. ofwel in een eigen familie Octoblepharaceae (Eddy, 1990; Menzel, 1991) geplaatst. Het laatste werd ondersteund door de behoorlijke genetische divergentie die werd opgemerkt tussen dit genus en de Calymperaceae s.str. (**Hoofdstuk 2**). Ondanks de morfologische gelijkenis aan de *Leucophanes* clade verschillen de twee lijnen van leucobryoïde planten in hun bladstructuur in dwarsdoorsnede (bijvoorbeeld zijn de hyalocyten in *Octoblepharum* driehoekig maar in de *Leucophanes* clade ruitvormig) en zijn ze niet nauw verwant. Bovendien is de bladvorm van *Octoblepharum* uniek binnen de bladmossen: lintvormige leucobryoïde bladeren met een korte of langere stekelpuntige bladtop en een hyaline bladschijf die weliswaar duidelijk, maar alleen aan de bladbasis in de vorm van een bladschede aanwezig is.

De vier soorten van *Octoblepharum* die in de analyses zijn meegenomen (**Hoofdstuk 2**), zijn goed omschreven volgens de resultaten van de moleculaire fylogenetische analyses, welke de morfologische soortomgrenzingen in de literatuur ondersteunen, met name het werk van Noris Salazar Allen (bv. Salazar Allen, 1991, 1994). De pas onlangs beschreven en weinig bekende soort *Octoblepharum arthrocormoides* werd alleen herkend door middel van de moleculaire analyse van herbarium materiaal dat eerder was gelabeld als *O. albidum*. Voor *Octoblepharum albidum* zelf, een pantropische, algemene soort, werd een grote intraspecifieke moleculaire variatie aangetoond.

Is de huidige systematiek van de Leucobryaceae in overeenstemming met moleculaire fylogenieën?

Een aantal genera in de Leucobryaceae kunnen als goed gedefinieerd worden beschouwd, omdat hun morfologische omschrijvingen niet in strijd waren met de resultaten van de moleculaire fylogenetische reconstructies (**Hoofdstuk 3**). Dit geldt echter niet voor de dicranoïde genera *Atractylocarpus*, *Campylopodiella* en *Dicranodontium*, en voor het meest soortenrijke leucobryoïde genus *Leucobryum*. De verwantschapsrelaties van de genera *Microbryum*, *Sphaerothecium* en *Steyermarkiella* blijven onduidelijk omdat zij vanwege hun zeldzaamheid niet in dit onderzoek meegenomen konden worden.

Voorouderlijke kenmerkreconstructies ondersteunen de afbakening van *Atractylocarpus*, inclusief *A. subporodictyon*. Deze soort werd formeel overgebracht van *Dicranodontium* naar *Atractylocarpus*, ondersteund door de moleculaire analyses. Deze nieuwe classificatie ondersteunt dat *Atractylocarpus* morfologisch gekenmerkt wordt door poreuze basale bladcellen. Bovendien werd aangetoond dat de zwanehalsachtig gekromde seta geen onderscheidend kenmerk van *Dicranodontium* is, maar een oorspronkelijk kenmerk van de gehele *Dicranodontium* clade. *Campylopodiella* en *Dicranodontium* bleken polyfyletisch te zijn, alhoewel zonder sterke ondersteuning. Morfologisch zijn deze twee genera en het monotypische genus *Brothera* nauw verwant en moeilijk van elkaar te onderscheiden.

De meeste verwantschappen op genus-niveau binnen de leucobryoïde clade zijn goed ondersteund, met uitzondering van de verwantschap tussen de Aziatische genera *Cladopodanthus* en *Schistomitrium* en de wijdverspreide *Leucobryum*. Terwijl de clade van *Cladopodanthus* en *Schistomitrium* in de analyses op familie-niveau (zonder ITS) binnen *Leucobryum* was genest, vormde deze een zuster clade van *Leucobryum* in sommige analyses van de leucobryoïde clade, een hypothese die niet kan worden verworpen door de Shimodaira-Hasegawa test.

Wat zijn *Aongstroemia* en *Dicranella*? Welke benaderingen kunnen worden gebruikt om de omschrijving van soortgelijke morfologisch zwa ondersteunde genera te verbeteren?

Aongstroemia en *Dicranella* zijn voorbeelden van de vele wijdverspreide, morfologisch diverse en slecht omschreven genera van de haplolepide bladmossen. Hetzelfde geldt voor de families Aongstroemiaceae en de Dicranellaceae, welke eerder zijn afgesplitst van de Dicranaceae s.l. Zij moeten allebei worden gereviseerd vanwege taxonomische problemen, en zijn weinig vertegenwoordigd in moleculaire fylogenetische analyses. Hun omschrijving en verwantschappen werden in dit proefschrift bestudeerd door middel van analyses van alle bekende hoofdlijnen van de Dicranidae, inclusief alle beschikbare sequentiedata van *Aongstroemia*, *Dicranella*, en andere nauw verwante genera (**Hoofdstuk 4**). Zowel *Aongstroemia* als *Dicranella* bleken polyfyletisch te zijn; hun huidige omschrijving correspondeert dus niet met natuurlijke groepen. *Dicranella* werd eerder beschouwd als één morfologisch divers genus, maar omvat in feite meerdere afstammingslijnen verspreid over de fylogenie van de Dicranidae, die elk een kleiner, meer homogeen deel van de totale morfologische diversiteit vertegenwoordigen. Nader onderzoek toonde aan dat sommige kenmerken die vroeger werden beschouwd als belangrijk voor de classificatie dat in feite niet zijn, terwijl andere kenmerken die voorheen over het hoofd werden gezien wel belangrijk zijn. Dit verklaart voor een groot deel de problematische omschrijving van beide genera.

Aongstroemia, een klein genus met soorten die wereldwijd voorkomen maar nauwe verspreidingsgebieden kennen, werd gekenmerkt door planten met wormvormig bebladerde

stengels. De drie soorten met wormvormige stengels die hier zijn bestudeerd, behoren echter allemaal tot verschillende, niet nauw met elkaar verwante afstammingslijnen. Bladkenmerken en sporofytische kenmerken verschillen wel tussen de drie soorten en zijn dus bruikbaarder voor de classificatie dan de rangschikking van de bladeren aan de stengel.

Dicranella, een wijdverspreid genus met meer dan 200 soorten, kende door de tijd heen veel voorstellen om de soorten in te delen, waarbij verschillende combinaties van andere genusnamen werden gebruikt, gebaseerd op verschillende sets van morfologische kenmerken. Er zijn veel problemen met de typificatie en veel soorten zijn ontoereikend gedefinieerd. Alles bij elkaar genomen, lijkt het genusconcept van *Dicranella* overeen te komen met een vaag idee van kleine, dicranoïde haplolepide bladmossen die de onderscheidende kenmerken van andere genera missen. *Dicranella*-soorten werden aangetroffen in zes clades verspreid over de hele fylogenie van de Dicranidae, van de protohaplolepide tot de core haplolepide lijnen (**Hoofdstuk 4**). Net zoals in *Aongstroemia* komen deze clades overeen met de onderverdeling van de brede morfologische variatie binnen *Dicranella* s.l. en zijn ze goed omschreven op basis van combinaties van kenmerken, bijvoorbeeld van de bladeren en kapsels. Een interessante bevinding is dat een eerder al beschreven, maar tot nu toe onvoldoende bestudeerd kenmerk, namelijk de morfologie van de tubers aan de rizoïden, nogal informatief lijkt te zijn wat betreft de verwantschappen van *Dicranella*-soorten in de verschillende clades.

Wat is een dubbel haplolepid peristoom? In welke taxa komt dit type peristoom voor en hoe zijn deze taxa verdeeld over de fylogenie van de haplolepide bladmossen?

De systematiek en verwantschappen van arthrodonte bladmossen met minder typische, gemodificeerde of gereduceerde tot helemaal afwezige peristomen zijn altijd al onderwerp van discussie geweest, en de betreffende taxa zijn in het verleden veelvuldig tussen de hoofdgroepen binnen de Bryopsida verplaatst. Hoewel de meeste Dicranidae een enkelvoudig haplolepide peristoom hebben, hebben enkele soortenarme taxa een peristoom met twee rijen van tegenoverstaande tanden. Deze haplolepide peristomen met tegenoverstaande tanden komen in meerdere varianten voor, met gelijkmatig ontwikkelde rijen van tanden of een gereduceerde binnenste of buitenste rij van tanden, maar kunnen nog steeds worden beschreven door de haplolepide peristoomformule 4:2:3 (Edwards, 1979; Fedosov et al., 2016a). Toch hebben dit soort peristomen vaak geleid tot een indeling van de betreffende taxa buiten de Dicranidae. Sommige van deze taxa zijn recent bestudeerd op basis van een integratieve taxonomische aanpak (e.g. Fedosov et al., 2016a).

De fylogenetische analyses om de verwantschappen van *Aongstroemia* en *Dicranella* op te helderen leverden ook een bijdrage aan de systematiek van de haplolepide bladmossen met

een dubbel peristoom omdat de bemonstering specifiek was gericht op taxa die in eerdere studies polyfyletisch of in tegenstrijdige posities geplaatst bleken te zijn (**Hoofdstuk 4**). Volgens de moleculaire data komen families met een dubbel peristoom niet alleen binnen de protohaplolepide grade voor (Catoscopiaceae, Distichiaceae, Flexitrichaceae, Pseudoditrichaceae) maar ook in de kerngroep van de haplolepide bladmossen (Chrysoblastellaceae), wat de onafhankelijke evolutie van dit type peristoom in de verschillende hoofdlijnen van de haplolepide bladmossen impliceert.

In de protohaplolepide grade omvatten de evolutionaire lijnen waarin zowel soorten met enkele als soorten met dubbele peristomen voorkomen slechts weinig recente soorten, terwijl de clade van de kerngroep van de haplolepide bladmossen juist soortenrijk is en nagenoeg alleen soorten met een enkel peristoom omvat. Of de protohaplolepide taxa als "experimenten" met verschillende (peristoom) morfologieën vroeg in de evolutie van de Dicranidae moeten worden beschouwd, of overblijfselen zijn van ooit soortenrijke lijnen, blijft onbekend. Dat er gebaseerd op moleculair onderzoek voortdurend taxa aan de protohaplolepide grade worden toegevoegd, laat ten minste vermoeden dat die evolutie en diversiteit van de "basale" Dicranidae complexer is dan tot nu toe was bekend, en nog onvoldoende is begrepen.

Toekomstige studies

Dit proefschrift heeft geleid tot een beter begrip van de omschrijvingen en verwantschappen van de haplolepide bladmossen, met name van de leucobryoïde genera en van andere taxa die eerder werden geplaatst in de Dicranaceae s.l., en die in eerdere moleculaire fylogenetische analyes ontoereikend vetegenwoordigd waren. Bovendien zijn in de analyses in **Hoofdstuk 4** voor de eerste keer alle families van de haplolepide bladmossen vertegenwoordigd waarvan moleculaire data beschikbaar waren (37 van de 38 families). Deze analyses geven dus het meest complete overzicht van deze groep van bladmossen tot nu toe, waarbij zowel bestaande en nieuw verkregen data werden gecombineerd.

De moleculaire inzichten zijn gebaseerd op een aantal gebruikelijke moleculaire markers, afkomstig van de drie genomen en met verschillende mate van variatie. Door deze aanpak was het mogelijk om analyses op verschillende taxonomische niveaus uit te voeren, mogelijke incongruentie tussen genomen te ontdekken, en sequenties van GenBank makkelijk op te nemen, waardoor de vergelijkbaarheid met resultaten van eerdere studies gewaarborgd werd. De taxonomische bemonstering werd aanzienlijk uitgebreid vergeleken met eerdere studies, echter blijft een aantal genera ondervertegenwoordigd. De redenen hiervoor zijn dat de DNA extracties van sommige monsters mislukt waren, het sequensen van additionele markers van reeds in eerdere studies gebruikte exemplaren (waardoor er minder capaciteit was om nieuwe monsters te sequensen) en de beperkte beschikbaarheid van taxa in biologische collecties die

zeldzaam zijn of een nauw verspreidingsgebied kennen. Desondanks was het gebruiken van al beschikbare monsters van herbaria of andere lopende projecten, in plaats van de poging om nieuw materiaal te verzamelen, een kostenefficiënte strategie om een fylogenetisch raamwerk van de bestudeerde taxa te maken. Opbouwend op dat raamwerk kunnen toekomstige studies de hier getoonde resultaten verifiëren en verdiepen, en daarvoor nog ontbrekende taxa gericht verzamelen en sequensen.

Een taak van hoge prioriteit zou bijvoorbeeld zijn om meer soorten van het genus *Dicranella* te sequensen, gezien het feit dat de meeste soorten nog steeds niet moleculair geanalyseerd zijn. Daarmee zou bepaald kunnen worden tot welke van de nieuw ontdekte (of mogelijk andere, nog steeds onbekende) afstammingslijnen deze soorten horen, als basis voor een taxonomische revisie. Wat het genus *Octoblepharum* betreft, zijn de hier bestudeerde soorten goed gekenmerkt, maar duiden onze resultaten op een geografisch signaal in de moleculaire variatie binnen de pantropische soort *O. albidum*. Verder onderzoek naar de morfologische en moleculaire variatie binnen *O. albidum*, gebaseerd op een groter aantal monsters, zou mogelijk (cryptische) soortvorming onthullen. Een bredere moleculaire bemonstering is eveneens wenselijk om de verwantschappen tussen de drie clades van de Leucobtyaceae op te helderen. Voorouderlijke kenmerkreconstructies, toegepast op uitgebreide moleculaire fylogenieën, zouden zeker bijdragen aan de hard nodige herwaardering van de vage morfologische omschrijvingen van talrijke haplolepide mostaxa.

De algemene resolutie en ondersteuning van de takken van sommige fylogenetische reconstructies, in het bijzonder van het ruggengraat van de Dicranidae, werd beperkt door het nog steeds kleine aantal moleculaire merkers dat in dit onderzoek werd gebruikt. Ook al zal het sequensen volgens de Sanger methode op een gegeven moment waarschijnlijk worden vervangen door technieken met hoge doorvoer, vooral SMRT (single molecule real time sequencing), zijn fylogenetische analyses op basis van individuele DNA merkers nog steeds gangbaar. Dit soort gegevens kan nog steeds een bijdrage leveren aan de voortgang van de systematiek, en kan verder worden gebruikt om open vragen omtrent de omschrijving, biogeografie, en morfologische evolutie van de haplolepide bladmossen te beantwoorden.

Desondanks zullen genoom-gebaseerde methoden zoals Hybrid Capture-Based Sequencing (HybSeq) heel nuttig zijn om problemen op te helderen zoals, bijvoorbeeld, de evolutie van de Dicranidae op hoger taxonomisch niveau, of het onderscheiden van soorten in taxonomisch ingewikkelde genera zoals Campylopus (e.g., Gama et al. 2017). Het grootschalig sequensen van meerdere honderden DNA merkers met deze methode zal het hoogstwaarschijnlijk mogelijk maken om de fylogenetische verwantschappen en de morfologische evolutie van de haplolepide bladmossen met meer zekerheid op te lossen. Als grote, monofyletische groep die veelvuldige uitdagingen vertoont (zoals de verwantschappen van de protohaplolepide taxa, de polyfylie van traditionele families en genera, en de taxonomisch ingewikkelde genera met veel

soorten), lenen de Dicranidae zich bij uitstek voor een casestudy om nieuwe sequencing methoden op de bryofyten toe te passen.

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Appendices

Appendix 1. Voucher information of the specimens analysed in this thesis and GenBank accession numbers for their sequences.[1, 2, 3, 4]

Species or infraspecific taxon	DNA voucher	Herbarium	Collector data	*nad*5	*trn*S-*rps*4	*rps*4-*trn*T	*trn*T-*trn*L	*trn*L-*trn*F	*atp*B-*rbc*L	*ITS1*	*ITS2*	Chapter
Funariidae: Encalyptaceae												
Encalypta streptocarpa	GenBank+	MUB	*Aedo 14348*	-	-	-	-	HM148898	-	-	-	2, 4
Encalypta streptocarpa	MS 0794+	L	*Stech B060412.2*	KX580428	KX580513	EU186541	EU186541	EU186541	EU186582	-	-	2, 4
Timmiidae: Timmiaceae												
Timmia austriaca	GenBank+	DUKE	*Schofield 98363*	-	-	-	-	AF229892	-	-	-	2, 4
Timmia austriaca	MS 0807+	L	*Stech B970831.4*	KX580441	KX580565	EU186543	EU186543	EU186543	EU186584	-	-	2, 4
Amphidiaceae												
Amphidium lapponicum	MS 1365+	L	*Ignatov 14.6.1989*	-	-	JQ690740	JQ690740	JQ690740	-	-	-	2, 4
Amphidium lapponicum	MS Ala+	herb. W. Frey	*Kürschner 1-4647*	KX580409	KX580480	-	-	-	JQ690698	-	-	2, 4
Amphidium mougeotti	MS Am	BONN	*Frahm s.n.*	KX580430	KX580481	AF127187	AF127187	AF127187	AY159894	-	-	2, 4

[1] The format in which the DNA voucher codes are presented varies slightly throughout this thesis. When only one voucher of a taxon was included, DNA codes were not always given in the Chapter in question. In some cases, codes are accompanied by the country of origin. Lastly, the DNA voucher prepared by me, the author of this thesis, appear also without the prefix MBS (numbers only).

[2] The DNA voucher codes marked by the prefix MS followed by numbers are from DNA extractions by Prof. W. Frey's group, of which Michael Stech was a member. The DNA voucher codes marked by the prefix MS and followed by letters are DNA extractions from Stech's PhD thesis.

[3] The symbol + indicates vouchers that had their sequences concatenated with sequences from another voucher of the same species (also indicated with +) for some of the analyses of this thesis.

[4] The genera *Hygrodicranum* and *Trichodontium* were listed within Aongstroemiaceae and Dicranellaceae, respectively, following the results of the phylogenetic analyses presented in Chapter 4. On the other hand, all species from the polyphyletic *Aongstroemia* and *Dicranella* were kept in their respective families Aongstroemiaceae and Dicranellaceae, even though the phylogenetic analyses resolved many of those species in other clades.

Species or infraspecific taxon	DNA voucher	Herbarium	Collector data	*nad*5	*trn*S-*rps*4	*rps*4-*trn*T	*trn*T-*trn*L	*trn*L-*trn*F	*atp*B-*rbc*L	*ITS1*	*ITS2*	Chapter
Aongstroemiaceae												
Aongstroemia filiformis	GenBank	DUKE	*Allen 6403*	AY908869	AY908094	-	-	-	-	-	-	4
Aongstroemia filiformis	MBS 157	MA	*Schäfer-Verwimp & Verwimp s.n. 25/05/1998*	-	-	-	-	MN178046	-	-	-	4
Aongstroemia filiformis	RF 74	MA	*Muñoz & Churchill 98-345*	MN177981	MN187469	-	-	MN178047	-	-	-	4
Aongstroemia longipes	MBS 154	herb. H.J. During	*Brand s.n.*	MN177982	MN187470	-	-	MN178048	-	-	-	4
Aongstroemia longipes	MBS 155	herb. H.J. During	*During 147089*	MN177983	-	-	-	MN178049	-	-	-	4
Aongstroemia longipes	MS Al	L	*Stech B970828.2*	KX580399	KX580482	AF135091	AF135091	AF135091	JQ690700	-	-	2, 4
Aongstroemia longipes	RF 43	MW	*Fedosov 30.VIII. 2007* (MW 9002156)	MN177984	MN187471	-	-	MN178050	-	-	-	4
Aongstroemia orientalis	RF 70	LE	*Czernyadjeva 27-10*	MN177985	MN187472	-	-	MN178051	-	-	-	4
Aongstroemia orientalis	RF 71	LE	*Afonina A3610*	MN177986	MN187473	-	-	MN178052	-	-	-	4
Aongstroemia orientalis	RF 72	LE	*Afonina 2013*	MN177987	MN187474	-	-	MN178053	-	-	-	4
Dichodontium flavescens	IPG 01	L	*Siebel 2012.223*	-	MN187479	-	-	MN178059	-	-	-	4
Dichodontium pellucidum	MS Dp	L	*Haisch s.n.*	MN177991	MN187480	-	-	MN178060	-	-	-	4
Diobelonella palustris	MS Dsq	BONN	*Frahm s.n.*	KX580424	KX580510	AF135090	AF135090	AF135090	JQ690699	-	-	2, 4
Hygrodicranum bolivianum	GenBank	DUKE	*Buck 39497*	AY908904	AY908115	-	-	-	-	-	-	4
Hygrodicranum herrerai	IPG 20	L	*Stech 15-028*	MN178037	MN187531	-	-	MN178113	-	-	-	4
Archidiaceae												
Archidium alternifolium	MS Ara	BONN	*Frahm s.n.*	KX580403	KX580483	AF135114	AF135114	AF135114	EU186597	-	-	2, 3, 4
Bruchiaceae												
Cladophascum gymnomitrioides	GenBank	MO	*Perold 2475*	AY908871	AY908097	-	-	-	-	-	-	4
Trematodon ambiguus	IPG 07	L	*Nieuwkoop 2008094*	MN178045	-	-	-	MN178118	-	-	-	4
Bryowijkiaceae												
Bryowijkia ambigua	GenBank	BM	*Ellis 901*	AY908873	AY908100	-	-	-	-	-	-	4

Species or infraspecific taxon	DNA voucher	Herbarium	Collector data	*nad5*	*trnS-rps4*	*rps4-trnT*	*trnT-trnL*	*trnL-trnF*	*atpB-rbcL*	*ITS1*	*ITS2*	Chapter
Bryowijkia madagassa	MBS 148	L	*Magill & al. 9975*	-	MN187476	-	-	MN178055	-	-	-	4
Bryoxiphiaceae												
Bryoxiphium norvegicum	MS 1008[+]	L	*Stech 04-242*	KX580391	KX580491	JQ690736	JQ690736	-	-	-	-	2, 4
Bryoxiphium norvegicum	MS Bn[+]	B	*Koponen 36664*	-	-	-	-	AF135101	EU186590	-	-	2, 4
Calymperaceae												
Arthrocormus schimperi	MBS 086	MO	*P.E.S. Câmara 991*	-	KX580485	-	-	KX580484	KX580570	-	-	2, 4
Calymperes afzelii	MBS 095	L	*Stech 15-163*	KX580416	KX580492	KX580492	KX580492	KX580492	KX580571	-	-	2, 4
Calymperes erosum	GenBank[+]	?	*Streimann 64635a*	-	-	-	-	DQ238541	-	-	-	2
Calymperes erosum	MS Ce[+]		*Capesius s.n.* (sterile culture)	KX580451	KX580493	JQ690739	JQ690739	-	JQ690702	KX580478	KX580478	2
Calymperes motleyi	GenBank[+]	?	*Streimann 64209*	-	-	-	-	DQ238533	-	-	-	2
Calymperes motleyi	MS 1023[+]	L	*Streimann 54137*	KX580450	KX580494	JQ690738	JQ690738	-	JQ690701	-	-	2
Calymperes palisotii	MBS 094	L	*Stech 15-119*	KX580415	KX580495	KX580495	KX580495	KX580495	KX580572	-	-	2
Leucophanes angustifolium	MBS 089	MO	*Allen 31133*	KX580461	KX580522	KX580522	KX580522	KX580522	KX580575	KX580477	KX580477	2, 4
Leucophanes molleri	MBS 090	MO	*Allen 31128*	KX580463	KX580523	KX580523	KX580523	KX580523	KX580576	-	-	2
Leucophanes molleri	MBS 092	MO	*Allen 30304*	KX580422	KX580524	KX580524	KX580524	KX580524	KX580577	-	-	2
Leucophanes octoblepharioides	MBS 091	MO	*He 40834*	KX580462	KX580525	KX580525	KX580525	KX580525	KX580578	-	-	2
Leucophanes sp.	MBS 080b	L	*Ho & Kruijer 04-210*	-	-	KX580526	KX580526	KX580526	KX580579	-	-	2
Mitthyridium fasciculatum	MBS 099	SING	*Ho 12-385*	KX580452	KX580528	KX580528	KX580528	KX580528	KX580580	-	-	2, 4
Mitthyridium flavum	MBS 100	SING	*Ho 12-324*	KX580453	-	KX580529	KX580529	-	-	-	-	2
Syrrhopodon gardneri	MS Syg	BSB (B)	Bryotrop project 7904	KX580411	KX580560	AF135087	KX580561	AF135087	JQ690703	KX580464	KX580464	2, 4
Syrrhopodon incompletus	MBS 093	L	*Stech 15-096*	KX580448	KX580562	KX580562	KX580562	KX580562	KX580573	-	-	2, 4
Syrrhopodon prolifer	MBS 096	L	*Pinheiro & al. 221*	KX580454	KX580563	KX580563	KX580563	KX580563	KX580574	-	-	2, 4

Species or infraspecific taxon	DNA voucher	Herbarium	Collector data	*nad5*	*trnS-rps4*	*rps4-trnT*	*trnT-trnL*	*trnL-trnF*	*atpB-rbcL*	*ITS1*	*ITS2*	Chapter
Catoscopiaceae												
Catoscopium nigritum	GenBank[+]	H	*Virtanen 2020*	-	-	-	-	AF497128	-	-	-	2, 4
Catoscopium nigritum	MS 0808[+]	L	*Stech B970828.13*	KX580408	KX580499	EU186545	EU186545	-	EU186592	-	-	2, 4
Chrysoblastellaceae												
Chrysoblastella chilensis	MS Cc	B	*Churchill & al. 13415*	KX580439	KX580501	AF135097	AF135097	AF135097	JQ690710	-	-	2, 4
Dicranaceae												
Chorisodontium wallisii	MS Cws	BONN	*Frahm & Gradstein 300*	KX580398	KX580502	AF135071	KX580503	AF135071	JQ690704	-	-	2, 4
Dicranoloma plurisetum	MS 0430	CHR	*Frey & Pfeiffer 98-T99*	-	-	DQ462606	DQ462606	DQ462606	-	-	-	2
Dicranum elongatum	MBS 143	herb. H.N. Siebel	*Siebel 2014.578*	MN178030	MN187524	-	-	MN178105	-	-	-	4
Dicranum polysetum	MS Dip	L	*Stech B970518.1*	KX580429	KX580509	AF129587	AF129587	AF129587	AY159895	-	-	2, 4
Leucoloma procerum	MS Lp	BONN	*Magill & Pócs 11222*	KX580442	-	AF135072	AF135072	AF135072	JQ690705	-	-	2, 4
Paraleucobryum enerve	MS Pe	herb W. Frey	*Kürschner s.n.*	MN178042	MN187536	-	-	AF135075/ AF136083	-	-	-	4
Paraleucobryum longifolium	MS Pl	L	*Stech B891114.1*	KX580438	KX580555	AF135076	AF135076	AF135076	JQ690706	-	-	2, 4
Platyneuron praealtum	IPG 11	L	*Stech 15-005*	MN178043	MN187537	-	-	MN178116	-	-	-	4
Dicranellaceae												
Campylopodium medium	GenBank	DUKE	*Withey 506*	AY908794	AY908095	-	-	-	-	-	-	4
Campylopodium medium	MBS 048	L	*Schmutz SVD 6706*	-	MN187477	-	-	MN178056	-	-	-	4
Campylopodium medium	MS Cm	BONN	*Eggers CEL2/3*	KX580401	KX580497	AF135088	AF135088	AF135088	JQ690707	-	-	2, 4
Dicranella campylophylla	TJH 04	L	*Stech 15-007*	MN177992	MN187481	-	-	MN178061	-	-	-	4
Dicranella campylophylla	TJH 13	L	*Stech 15-006*	MN177993	MN187482	-	-	MN178062	-	-	-	4
Dicranella cardotii	TJH 22	L	*Streimann 59516*	MN177994	-	-	-	-	-	-	-	4
Dicranella cerviculata	FDt 5	MW	*Neshataeva & al. 27.VIII.2009* (MW 9030767)	MN177995	MN187483	-	-	MN178063	-	-	-	4
Dicranella cerviculata	FDt 6	MW	*Fedosov 14.VIII.2011* (MW 9036970)	MN177996	MN187484	-	-	MN178064	-	-	-	4

Species or infraspecific taxon	DNA voucher	Herbarium	Collector data	*nad5*	*trnS-rps4*	*rps4-trnT*	*trnT-trnL*	*trnL-trnF*	*atpB-rbcL*	*ITS1*	*ITS2*	Chapter
Dicranella cerviculata	MS Dce	L	*Stech B970824.1*	KX580402	KX580505	AF129597	AF129597	AF129597	EU186591	-	-	2, 4
Dicranella cerviculata	RF 61	MW	*Fedosov 12.VIII.2010* (MW 9030770)	MN177997	MN187485	-	-	MN178065	-	-	-	4
Dicranella cerviculata	TJH 07	L	*Aptroot 69861*	-	MN187486	-	-	MN178066	-	-	-	4
Dicranella crispa	MBS 156	herb. H.J. During	*During 137346*	-	-	-	-	MN178067	-	-	-	4
Dicranella crispa	RF 44	MW	*Ignatov & Ignatova 2.VIII.2015* (MW 9074733)	MN177998	MN187487	-	-	MN178068	-	-	-	4
Dicranella crispa	RF 45	MW	*Fedosov 27.VII.2015* (MW 9007542)	MN177999	MN187488	-	-	MN178069	-	-	-	4
Dicranella crispa	TJH 01	L	*Siebel 2014.765*	MN178000	MN187489	-	-	MN178070	-	-	-	4
Dicranella curvipes	RF 58	MW	*Ignatov & Teleganova 11.VIII.2006* (MW 9030958)	MN178001	MN187490	-	-	MN178071	-	-	-	4
Dicranella curvipes	RF 59	MW	*Ignatov 21.VIII.2007* (MW 9030952)	MN178002	MN187491	-	-	MN178072	-	-	-	4
Dicranella curvipes	RF 60	MW	*Ignatov & Ignatova 18.VIII.2013* (MW 9030946)	-	MN187492	-	-	MN178073	-	-	-	4
Dicranella grevilleana	MBS 150 / TJH 25	L	*Siebel 2012.291*	MN178006	MN187496	-	-	MN178077	-	-	-	4
Dicranella grevilleana	RF 38	MW	*Ignatov & Teleganova 21.VIII.2006* (MW 9030841)	MN178003	MN187493	-	-	MN178074	-	-	-	4
Dicranella grevilleana	RF 39	MW	*Fedosov 30.VI.2005* (MW 9030839)	MN178004	MN187494	-	-	MN178075	-	-	-	4
Dicranella grevilleana	RF 54	MW	*Ignatov & Ignatova 16.VII.2015* (MW 9074884)	MN178005	MN187495	-	-	MN178076	-	-	-	4
Dicranella heteromalla	MS 1000	L	*Stech 08-380*	-	-	JQ690737	JQ690737	-	-	-	-	2
Dicranella heteromalla	MS Dh	L	*Stech B960905.1*	KX580413	KX580506	-	-	AF129596	KX580569	-	-	2, 4
Dicranella heteromalla	RF 46	MW	*Ignatov & Ignatova 2.IV.2006* (MW 9030851)	-	MN187497	-	-	MN178078	-	-	-	4
Dicranella heteromalla	RF 47	MW	*Kokoshnikova 19.IX.2007* (MW 9030879)	MN178007	MN187498	-	-	MN178079	-	-	-	4
Dicranella heteromalla	TJH 12	L	*Zwarts s.n. 12-1-2011* (L 0873198)	MN178008	MN187499	-	-	MN178080	-	-	-	4
Dicranella heteromalla	TJH 28	L	*Buter s.n. 15-6-2010* (L 0873082)	MN178009	MN187500	-	-	MN178081	-	-	-	4
Dicranella howei	TJH 02	L	*Siebel 2014.155*	MN178010	MN187501	-	-	MN178082	-	-	-	4
Dicranella howei	TJH 06	L	*Bijlsma 12266*	-	MN187502	-	-	MN178083	-	-	-	4
Dicranella howei	TJH 30	L	*Nieuwkoop 2015559*	MN178011	MN187503	-	-	MN178084	-	-	-	4

Species or infraspecific taxon	DNA voucher	Herbarium	Collector data	*nad5*	*trnS-rps4*	*rps4-trnT*	*trnT-trnL*	*trnL-trnF*	*atpB-rbcL*	*ITS1*	*ITS2*	Chapter
Dicranella rufescens	BCNL 1	L	*Pellicaan s.n.* (L 0873203)	MN178012	MN187505	-	-	MN178086	-	-	-	4
Dicranella rufescens	BCNL 2	L	*Smulders 08151*	-	MN187504	-	-	MN178085	-	-	-	4
Dicranella rufescens	RF 62	MW	*Pisarenko 12.IX.2004* (MW 9030986)	MN178013	MN187506	-	-	MN178087	-	-	-	4
Dicranella rufescens	RF 63	MW	*Bezgodov 19.IX.1998* (MW 9030966)	MN178014	MN187507	-	-	MN178088	-	-	-	4
Dicranella schreberiana var. *schreberiana*	TJH 17	L	*Aptroot 69819*	MN178015	MN187508	-	-	MN178089	-	-	-	4
Dicranella schreberiana var. *robusta*	RF 41	MW	*Ignatov & Ignatova 16.VIII.2012* (MW 9031017)	MN178016	MN187509	-	-	MN178090	-	-	-	4
Dicranella schreberiana var. *robusta*	TJH 16	L	*Nieuwkoop 2012060*	MN178017	MN187510	-	-	MN178091	-	-	-	4
Dicranella schreberiana var. *robusta*	TJH 23	L	*Siebel 2014.732*	MN178018	MN187511	-	-	MN178092	-	-	-	4
Dicranella schreberiana var. *robusta*	TJH 24	L	*Siebel 2014.697*	-	MN187512	-	-	MN178093	-	-	-	4
Dicranella schreberiana var. *robusta*	TJH 35	L	*Siebel 2015.561*	MN178019	MN187513	-	-	MN178094	-	-	-	4
Dicranella staphylina	TJH 05	L	*Aptroot 69818*	MN178020	MN187514	-	-	MN178095	-	-	-	4
Dicranella staphylina	TJH 27	L	*Siebel 2013.451*	MN178021	MN187515	-	-	MN178096	-	-	-	4
Dicranella subulata	RF 55	MW	*Fedosov 16.VII.2015* (MW 9007554)	MN178022	MN187516	-	-	MN178097	-	-	-	4
Dicranella subulata	TJH 08	L	*Siebel 2015.313*	MN178023	MN187517	-	-	MN178098	-	-	-	4
Dicranella subulata	TJH 19	L	*Siebel 2014.610*	MN178024	MN187518	-	-	MN178099	-	-	-	4
Dicranella subulata	TJH 34	L	*Siebel 2015.357*	MN178025	MN187519	-	-	MN178100	-	-	-	4
Dicranella varia	RF 42	MW	*Fedosov 3.VIII.2013* (MW 9031184)	MN178026	MN187520	-	-	MN178101	-	-	-	4
Dicranella varia	RF 56	MW	*Seregin & al. 9.X.2006* (MW 9031125)	MN178027	MN187521	-	-	MN178102	-	-	-	4
Dicranella varia	TJH 29	L	*Siebel 2015.531*	MN178028	MN187522	-	-	MN178103	-	-	-	4
Dicranella varia	TJH 36	L	*Siebel 2015.440*	MN178029	MN187523	-	-	MN178104	-	-	-	4
Leptotrichella flaccidula	MS Mf	B	*Schultze-Motel 3209*	KX580400	KX580520	AF136637	AF136637	AF136637	JQ690709	-	-	2, 4
Microcampylopus curvisetus	MS Mcu	L	*Schäfer-Verwimp & Verwimp 12351*	MN178040	MN187534	-	-	AY545565	-	-	-	4
Microcampylopus khasianus	MS Mk	L	*Schäfer-Verwimp & Verwimp 20891*	KX580412	KX580527	AY545564	AY545564	AY545564	JQ690708	-	-	2, 4

Species or infraspecific taxon	DNA voucher	Herbarium	Collector data	*nad5*	*trnS-rps4*	*rps4-trnT*	*trnT-trnL*	*trnL-trnF*	*atpB-rbcL*	*ITS1*	*ITS2*	Chapter
Microcampylopus laevigatus	MS Ml2	L	*Greven & Khoeblal 4000/12*	MN178041	MN187535	-	-	MN178115	-	-	-	4
cf. *Microcampylopus*	MS 1031	ITIC, dupl. L	*Búcaro s.n.*	MN178039	MN187533	-	-	MN178114	-	-	-	4
Trichodontium falcatum	GenBank	NY	*Streimann 51155*	-	AF435304	-	-	AF435353	-	-	-	4
Ditrichaceae												
Astomiopsis amblyocalyx	GenBank	DUKE	*Cárdenas 3953*	AY908857	AY908072	-	-	-	-	-	-	4
Ceratodon purpureus	GenBank[+]	?	from McDaniel et al., 2007	-	-	-	-	-	EU053087	-	-	2
Ceratodon purpureus	MS Cp[+]		N.N. (sterile culture)	KX580395	KX580500	AF135096	AF135096	AF135096	-	-	-	2, 4
Cheilothela chloropus	GenBank	DUKE	*Werner & Ros 14024*	AY908861	AY908124	-	-	-	-	-	-	4
Distichium capillaceum	MS Dc	L	*Stech B970828.1*	MN178031	MN187525	-	-	MN178106	-	-	-	4
Distichium inclinatum	IPG 06	L	*Zwarts 2309*	MN178032	-	-	-	MN178107	-	-	-	4
Ditrichum ambiguum	MS Tc	B	*Düll 337/2e*	KX580440	KX580567	AF135099	AF135099	AF135099	JQ690711	-	-	2, 4
Ditrichum cf. *cylindricarpum*	IPG 19	L	*Stech 15-019*	MN178033	MN187526	-	-	MN178108	-	-	-	4
Ditrichum cf. *subulatum*	TJH 32	herb. H.N. Siebel	*Siebel 2013.070 p.p.*	MN178034	MN187528	-	-	MN178110	-	-	-	4
Ditrichum difficile	MBS 035	L	*Inturias & Carreño 121*	-	MN187527	-	-	MN178109	-	-	-	4
Ditrichum sp.	GenBank	DUKE	*Buck 39507*	AY908789	AY908165	-	-	-	-	-	-	4
Eccremidium floridanum	GenBank	DUKE	*Allen 7505*	AY908872	AY908098	-	-	-	-	-	-	4
Garckea phascoides	GenBank	MO	*Magill & Pocs 11583*	AY908870	AY908096	-	-	-	-	-	-	4
Glyphomitrium daviesii	GenBank	NY	*Buck 14830*	AY908895	AY908082	-	-	-	-	-	-	4
Pleuridium acuminatum	MS 0168	herb. W. Frey	*Frey 1-4991*	KX580426	KX580557	EU186546	EU186546	EU186546	EU186596	-	-	2, 4
Rhamphidium purpuratum	MBS 135	L	*Stech 08-392*	MN178044	MN187538	-	-	MN178117	-	-	-	4
Trichodon cylindricus	GenBank	NY	*Vitt 35814*	AY908863	AY908125	-	-	-	-	-	-	4
Drummondiaceae												
Drummondia prorepens	MS 1011	L	*Allen 6192*	-	KX580512	JQ690728	JQ690728	JQ690728	KX580568	-	-	2, 4

Species or infraspecific taxon	DNA voucher	Herbarium	Collector data	*nad5*	*trnS-rps4*	*rps4-trnT*	*trnT-trnL*	*trnL-trnF*	*atpB-rbcL*	*ITS1*	*ITS2*	Chapter
Erpodiaceae												
Aulacopilum cf. *abbreviatum*	MS 1018	L	*Sleath 1043/31*	KX580446	KX580487	JQ690730	JQ690730	JQ690730	JQ690712	-	-	2, 4
Erpodium biseriatum	MS 1015	L	*Streimann & Pócs 55051*	KX580445	KX580514	JQ690729	JQ690729	JQ690729	-	-	-	2, 4
Erpodium pringlei	IPG 15	ITIC, dupl. L	*Búcaro 684*	MN178035	MN187529	-	-	MN178111	-	-	-	4
Eustichia longirostris	MS 1020	L	*Churchill & al. 22547*	KX580435	KX580515	JQ690731	JQ690731	JQ690731	JQ690713	-	-	2, 3, 4
Fissidentaceae												
Fissidens bryoides	MS Fb	BSB (B)	*Darmer 13107*	KX580431	KX580516	AF135105	AF135105	AF135105	EU186586	-	-	2, 4
Fissidens fontanus	MS Of	L	*Haapasaari 22.8. 1997*	KX580449	KX580517	AF135107	AF135107	AF135107	EU186585	-	-	2, 4
Flexitrichaceae												
Flexitrichum flexicaule	GenBank*	NY	*Bartlett 15091*	-	-	-	-	-	DQ397160	-	-	2
Flexitrichum flexicaule	IPG 13	L	*Hovenkamp s.n.*	MN178036	MN187530	-	-	MN178112	-	-	-	4
Flexitrichum flexicaule	MS Df*	L	*Stech B890430.2*	KX580389	KX580511	AF135095	AF135095	AF135095	-	-	-	2, 4
Grimmiaceae												
Niphotrichum canescens	MS 0872/Rc	L	*Kortselius 2008.11.0002*	KX580444	-	JQ690732	JQ690732	JQ690732	JQ690714	-	-	2, 4
Schistidium apocarpum	MS Sa	L	*Stech B970226.2*	KX580392	KX580559	AF127185	AF127185	AF127185	EU186588	-	-	2, 4
Hymenolomataceae												
Hymenoloma crispulum	MS Dcr	L	*Stech B970828.2*	KX580390	KX580508	AF135074	AF135074	AF135074	JQ690724	-	-	2, 4
Hypodontiaceae												
Hypodontium dregei	MS 1016	L	L 0472355	KX580414	KX580518	JQ690733	JQ690733	JQ690733	JQ690715	-	-	2, 4
Hypodontium pomiforme	MS 1017	L	*Viviers 105*	KX580443	KX580519	JQ690734	JQ690734	JQ690734	JQ690716	-	-	2, 4
Leucobryaceae												
Atractylocarpus alticaulis	MS Aa	BONN	*Frahm 8070*	KX580404	KX580486	AF129592	AF129592	AF129592	JQ690717	KY618935	KY618935	2, 3, 4
Atractylocarpus intermedius	MBS 040	L	*Allen 11485*	-	KY619059	-	KY619019	KY619019	KY618973	KY618936	KY618936	3

Species or infraspecific taxon	DNA voucher	Herbarium	Collector data	*nad*5	*trn*S-*rps*4	*rps*4-*trn*T	*trn*T-*trn*L	*trn*L-*trn*F	*atp*B-*rbc*L	*ITS1*	*ITS2*	Chapter
Atractylocarpus longisetus	MBS 041	L	*Ireland 23654*	-	KY619040	KY619040	-	KY619015	KY618974	KY618937	KY618937	3
Atractylocarpus subporodictyon	MS 0018	B	*Frahm 2011825*	-	KY619054	-	-	KY619017	KY618986	KY618946	KY618946	3
Brothera leana	MBS 042	L	*Mizutani 16232*	-	KY619031	KY619031	KY619031	KY619031	KY618975	KY618938	KY618938	3
Brothera leana	MS Bl	B	*Koponen 37142*	KX580434	KX580490	AF135077	AF135077	AF135077	JQ690719	KY618939	KY618939	2, 3, 4
Campylopodiella flagellacea	MS Cfl	BONN	*Allen 9172*	KX580423	KX580496	AF135078	AF135078	AF135078	JQ690718	KY618940	KY618940	2, 3, 4
Campylopodiella stenocarpa	MBS 057	MO	*Magill 14925*	KY619075	KY619033	KY619033	KY619033	KY619033	KY618976	KY618941	KY618941	3
Campylopus flexuosus	MS Cf	L	*Stech B960905.2*	KX580406	KX580498	AF129593	AF129593	AF129593	AY159919	-	-	2, 3, 4
Campylopus introflexus	GenBank	DUKE	*Shaw 10490*	AY908906	AY908128	-	-	-	-	-	-	3
Campylopus introflexus	MS C10	L	*Streimann 49976*	MN177989	MN187478	-	-	MN178057	-	-	-	4
Campylopus introflexus	TJH 31	UB	*Faria 67*	KY618929	KY619055	-	-	KY619018	KY618978	-	-	3
Campylopus pilifer	MS C12	BONN	*Arts CR 21/12*	-	-	-	AF442658	AF442645	AY159930	-	-	3
Campylopus sp.	MBS 055	MO	*O'Shea M 7388a p.p.*	-	KY619030	KY619030	KY619030	KY619030	KY618977	-	-	3
Campylopus sp.	MBS 151	SP	*Peralta & al. s.n.*	MN177990	-	-	-	MN178058	-	-	-	4
Campylopus subcomosus	MBS 059	MO	*He & Nguyen 42864*	KY618930	KY619046	KY619046	KY619046	KY619046	KY618979	-	-	3
Campylopus subcomosus	MBS 063	MO	*He & Nguyen 42964*	KY619070	KY619045	KY619045	KY619045	KY619045	KY618980	-	-	3
Cladopodanthus heterophyllus	MBS 117	SING	*Ho 12-208*	KY619072	KY619041	KY619041	KY619041	KY619041	KY618981	KY618947	KY618947	3
Cladopodanthus speciosus	GenBank	NY	*Tan 14 Apr 1991*	AY908912	AY908132	-	-	-	-	-	-	3
Dicranodontium denudatum	MBS 006	L	*Higuchi s.n.*	-	KY619044	KY619044	-	KY619016	KY618983	KY618943	KY618943	3
Dicranodontium denudatum	MS Dd	L	*Frahm s.n.*	KX580432	KX580507	AF129591	AF129591	AF129591	JQ690720	KY618948	KY618948	2, 3, 4
Dicranodontium denudatum var. glabrum	MBS 021	B	*Frahm Sp – 047*	KY619065	KY619053	KY619053	KY619053	KY619053	KY618982	KY618942	KY618942	3
Dicranodontium porodictyon	MBS 061	MO	*Wood & Espaniola 11270*	KY619083	KY619020	KY619020	KY619020	KY619020	KY618984	KY618944	KY618944	3
Dicranodontium pulchroalare	MBS 044	L	*Lyon 71*	-	KY619034	KY619034	KY619034	KY619034	KY618985	KY618945	KY618945	3
Holomitriopsis laevifolia	GenBank	DUKE	*Leisner 23093*	AY908915	AY908135	-	-	-	-	-	-	3

Species or infraspecific taxon	DNA voucher	Herbarium	Collector data	*nad5*	*trnS-rps4*	*rps4-trnT*	*trnT-trnL*	*trnL-trnF*	*atpB-rbcL*	*ITS1*	*ITS2*	Chapter
Leucobryum aduncum	MBS 125	L	*Veldkamp & Roos 8716 E*	-	KY619021	-	KY619011	KY619011	-	KY618949	KY618949	3
Leucobryum albidum	MBS 008	MO	*Allen 29900*	KY619062	KY619058	KY619058	KY619058	KY619058	KY618987	KY618950	KY618950	3
Leucobryum antillarum	MBS 036	L	*Linneo & Soto 1709*	-	KY619035	-	-	KY619014	KY618988	KY618951	KY618951	3
Leucobryum babetii	MBS 127	L	*Magill & Crosby 8486*	KY619076	KY619032	KY619032	-	KY619013	-	-	KY618931	3
Leucobryum boninense	GenBank	MAK	B119207	-	AB740050	-	-	AB742381	-	AB763354	AB763354	3
Leucobryum bowringii	MBS 119	L	*Koponen & al. 53153*	KY619073	KY619037	KY619037	KY619037	KY619037	-	KY618952	KY618952	3
Leucobryum bowringii	MBS 128	SING	*Printarakul 2649*	KY619078	KY619027	-	KY619012	KY619012	KY618989	-	KY618932	3
Leucobryum candidum	GenBank	HIRO	*Yamaguchi 22948*	-	AB740058	-	-	AB742389	-	AB285170	AB285170	3
Leucobryum chlorophyllosum	GenBank	HIRO	HIRO140820	-	AB740060	-	-	AB742391	-	AB763361	AB763361	3
Leucobryum crispum	GenBank	DUKE	*Buck 39451*	AY908914	AY908134	-	-	-	-	-	-	3
Leucobryum giganteum	MBS 018	L	*Stech PA24*	KY619080	KY619024	KY619024	KY619024	KY619024	KY618990	KY618953	KY618953	3
Leucobryum glaucum	GenBank	HIRO	*Yamaguchi 18774*	-	AB740062	-	-	AB742393	-	AB125292	AB125292	3
Leucobryum javense	MBS 126	L	*Koponen & al. 51759*	-	KY619038	KY619038	KY619038	KY619038	KY618991	KY618954	KY618954	3
Leucobryum javense	MBS 129	SING	*Printarakul 2804*	KY619079	KY619026	KY619026	KY619026	KY619026	KY618992	KY618955	KY618955	3
Leucobryum juniperoideum	MBS 003	L	*Koponen & al. 49983*	-	KY619039	KY619039	KY619039	KY619039	KY618997	KY618960	KY618960	3
Leucobryum juniperoideum	MBS 004	L	*Koponen & al. 55911*	KY619074	KY619036	KY619036	KY619036	KY619036	KY618998	KY618961	KY618961	3
Leucobryum juniperoideum	MBS 027	L	*G. & S. Miehe 98-384-20*	KY619066	KY619052	KY619052	KY619052	KY619052	KY618993	KY618956	KY618956	3
Leucobryum juniperoideum	MBS 028	L	*G. & S. Miehe 99-234-35*	KY619068	KY619048	KY619048	KY619048	KY619048	KY618994	KY618957	KY618957	3
Leucobryum juniperoideum	MBS 029	L	*G. & S. Miehe 99-212-35*	KY619067	KY619050	KY619050	KY619050	KY619050	KY618995	KY618958	KY618958	3
Leucobryum juniperoideum	MBS 030	L	*G. & S. Miehe 99-228-2*	-	KY619049	KY619049	KY619049	KY619049	KY618996	KY618959	KY618959	3
Leucobryum juniperoideum	MS Lj	L	*Frahm s.n.*	KX580405	KX580521	AF135084	AF135084	AF135084	JQ690722	KY618962	KY618962	2, 3, 4
Leucobryum madagassum	MBS 039	L	*Phillipson 4040*	-	KY619029	KY619029	KY619029	KY619029	KY618999	KY618963	KY618963	3
Leucobryum martianum	MBS 005	L	*Churchill & Arroyo 21617*	KY619063	KY619056	KY619056	KY619056	KY619056	KY619000	-	-	3

Species or infraspecific taxon	DNA voucher	Herbarium	Collector data	*nad5*	*trnS-rps4*	*rps4-trnT*	*trnT-trnL*	*trnL-trnF*	*atpB-rbcL*	*ITS1*	*ITS2*	Chapter
Leucobryum polakowskyi	MBS 118	L	*Stech s.n.* (Sint Eustatius 03-2015 62)	KY619082	KY619022	KY619022	KY619022	KY619022	KY619001	KY618972	KY618972	3
Leucobryum sanctum	MBS 121	L	*de Kok M 7A*	KY619064	KY619051	KY619051	KY619051	KY619051	KY619002	KY618964	KY618964	3
Leucobryum scabrum	MBS 011	L	*Higuchi s.n.*	-	KY619043	KY619043	KY619043	KY619043	KY619003	KY618965	KY618965	3
Leucobryum seemannii	GenBank	HIRO	*Yamaguchi 17078*	-	AB740091	-	-	AB742422	-	AB285183	AB285183	3
Leucobryum sp.	MBS 019	L	*Stech PA30*	KY619081	KY619023	KY619023	KY619023	KY619023	KY619004	KY618966	KY618966	3
Leucobryum subobtusifolium	MBS 013	L	*Abdo 52*	KY619061	KY619060	KY619060	KY619060	KY619060	KY619006	KY618968	KY618968	3
Leucobryum subobtusifolium	MBS 015	L	*Pinheiro & al. 78*	KY619077	KY619028	KY619028	KY619028	KY619028	KY619007	KY618969	KY618969	3
Leucobryum subobtusifolium	MBS 017	L	*Churchill & Arroyo 21616*	KY618928	KY619057	KY619057	KY619057	KY619057	KY619005	KY618967	KY618967	3
Ochrobryum gardneri	MBS 031	UB	*Gonzaga & al. 7*	KY619069	KY619047	KY619047	KY619047	KY619047	KY619008	KY618970	KY618970	3
Ochrobryum gardneri	MS 1012	L	*Allen 13706*	KX580447	KX580530	JQ690735	JQ690735	JQ690735	JQ690721	KY618971	KY618971	2, 3, 4
Ochrobryum subulatum	MBS 066	MO	*Sanjines 3082*	-	KY619025	KY619025	KY619025	KY619025	KY619009	-	-	3
Pilopogon africanus	MS Pa	BONN	*Frahm 8079*	KX580433	KX580556	AF129595	AF129595	AF129595	JQ690723	-	-	2, 3, 4
Pilopogon gracilis	GenBank	MO	*Breedlove 66830*	AY908907	AY908137	-	-	-	-	-	-	3
Pilopogon guadalupensis	MS Pg	BONN	*Arts CR 03/07*	-	-	-	AF442662	AF442626	AY159904	-	-	3
Pilopogon laevis	MS Pla	BONN	*Frahm s.n.*	-	-	-	AF442661	AF442625	AY159905	-	-	3
Schistomitrium brevi-apiculatum	GenBank	NY	*Koponen 35844*	AY908913	AY908133	-	-	-	-	-	-	3
Schistomitrium sparei	MBS 115	SING	*Ho 12-207*	KY619071	KY619042	KY619042	KY619042	KY619042	KY619010	-	KY618934	3
Micromitriaceae												
Micromitrium tenerum	MBS 069	MO	*Dibble 22372A*	KY930573	KY930574	-	-	KY930575	KY930576	-	-	3, 4
Mitteniaceae												
Mittenia plumula	GenBank	UC	*Streimann s.n.*	-	AY857782	-	-	AY857819	-	-	-	4
Octoblepharaceae												
Octoblepharum albidum	MBS 020	L	*Stech 12-013*	KX580437	KX580534	KX580534	KX580534	KX580534	KX580584	KX580474	KX580474	2, 4

Species or infraspecific taxon	DNA voucher	Herbarium	Collector data	*nad5*	*trnS-rps4*	*rps4-trnT*	*trnT-trnL*	*trnL-trnF*	*atpB-rbcL*	*ITS1*	*ITS2*	Chapter
Octoblepharum albidum	MBS 032	UB	*Faria 754*	KX580436	KX580533	KX580533	KX580533	KX580533	KX580583	KX580471	KX580471	2
Octoblepharum albidum	MBS 050	MO	*Magill 14263*	KX580418	KX580538	KX580538	KX580538	KX580538	KX580588	KX580472	KX580472	2
Octoblepharum albidum	MBS 077	L	*Streimann & Pócs 64211*	KX580455	KX580531	KX580531	KX580531	KX580531	KX580581	KX580476	KX580476	2
Octoblepharum albidum	MBS 080a	L	*Ho & Kruijer 04-010*	-	KX580537	KX580537	KX580537	KX580537	KX580587	KX580473	KX580473	2
Octoblepharum albidum	MBS 081	L	*Streimann 48609*	-	KX580532	KX580532	KX580532	KX580532	KX580582	KX580475	KX580475	2
Octoblepharum albidum	MBS 083	L	*Veldkamp & Roos 8716D*	-	KX580535	KX580535	KX580535	KX580535	KX580585	KX580470	KX580470	2
Octoblepharum albidum	MBS 107	SING	*Printarakul P.N. 1884*	-	KX580539	KX580539	KX580539	KX580539	KX580589	KX580469	KX580469	2
Octoblepharum albidum	MS Oal	B	*Davidse & al. 35012*	KX580417	KX580536	KX580536	KX580536	AF136093	KX580586	-	AF144122	2
Octoblepharum arthrocormoides	MBS 084	L	*Klazenga 2162*	-	KX580541	-	-	KX580540	KX580590	-	-	2
Octoblepharum arthrocormoides	MBS 085	L	*Klazenga 2334*	-	KX580542	KX580542	KX580542	KX580542	KX580591	-	-	2
Octoblepharum cocuiense	MBS 049	MO	*Linneo & Soto 1715*	KX580459	KX580543	KX580543	KX580543	KX580543	KX580592	KX580466	KX580466	2
Octoblepharum cocuiense	MBS 051	MO	*Huaylla & Jimenez 1270*	-	KX580544	KX580544	KX580544	KX580544	KX580593	KX580479	KX580479	2
Octoblepharum cocuiense	MBS 072	MO	*Churchill & al. 24447*	KX580457	KX580548	KX580548	KX580548	KX580548	KX580597	-	-	2
Octoblepharum cocuiense	MBS 108	UB	*Câmara & al. 2152*	KX580458	-	-	-	-	-	-	-	2
Octoblepharum cocuiense	MBS 109	UB	*Sousa & al. 679*	KX580456	KX580545	KX580545	KX580545	KX580545	KX580594	-	-	2
Octoblepharum cocuiense	MBS 110	UB	*Câmara & al. 3772*	KX580420	KX580546	KX580546	KX580546	KX580546	KX580595	KX580465	KX580465	2
Octoblepharum cocuiense	MBS 111	UB	*Sousa & al. 655*	KX580419	KX580547	KX580547	KX580547	-	KX580596	-	-	2
Octoblepharum pulvinatum	MBS 052	MO	*R.E. Magill 14331*	KX580460	KX580551	KX580551	KX580551	KX580551	KX580600	-	KX611155	2
Octoblepharum pulvinatum	MBS 075	MO	*Croat 103358*	KX580427	KX580550	KX580550	KX580550	KX580550	KX580599	KX580468	KX580468	2
Octoblepharum pulvinatum	MBS 112	UB	*Sousa & al. 1048*	KX580421	KX580549	KX580549	KX580549	KX580549	KX580598	KX580467	KX580467	2
Pleurophascaceae												
Pleurophascum grandiglobum	GenBank	NY	*Streimann 51183*	AY908961	AY908101	-	-	-	-	-	-	4
Pottiaceae												

Species or infraspecific taxon	DNA voucher	Herbarium	Collector data	*nad5*	*trnS-rps4*	*rps4-trnT*	*trnT-trnL*	*trnL-trnF*	*atpB-rbcL*	*ITS1*	*ITS2*	Chapter
Cinclidotus riparius	MS 0207	L	*Stech B920517.4*	KX580410	-	EU186544	EU186544	EU186544	EU186587	-	-	2, 4
Syntrichia ruralis	GenBank*	JEPS	from Oliver et al., 2010	-	FJ546412	FJ546412	FJ546412	FJ546412	FJ546412	-	-	2
Syntrichia ruralis	GenBank*	MO	*Shevock & York 16918*	AY908705	-	-	-	-	-	-	-	2
Tortula muralis	MS Tm	L	*Stech B970226.3*	KX580388	KX580566	AF135108	AF135108	AF135108	AY159892	-	-	2, 4
Pseudoditrichaceae												
Pseudoditrichum mirabile	GenBank	MW	*Fedosov 13-3-1028*	KR026964	KR026959	-	-	-	-	-	-	4
Ptychomitriaceae												
Ptychomitrium polyphyllum	MS 0798	L	*Stech 04-040*	-	EU186542	EU186542	EU186542	EU186542	EU186583	-	-	2, 4
Rhabdoweisiaceae												
Arctoa fulvella	GenBank	DUKE	*Schofield 102571*	AY908894	AY908075	-	-	-	-	-	-	4
Cynodontium polycarpum	MS Cyp	L	*Stech B930721.2*	KX580397	KX580504	AF129599	AF129599	AF129599	EU186595	-	-	2, 4
Kiaeria riparia	TJH 15	L	*Siebel 2014.647*	MN178038	MN187532	-	-	-	-	-	-	4
Oncophorus integerrimus	MS Ov	L	*Stech B960801.1*	KX580393	KX580552	AF129598	AF129598	AF129598	EU186593	-	-	2, 4
Oncophorus integerrimus	MS Ow	L	*Stech B970828.3*	KX580394	KX580553	AF135094	AF135094	AF135094	JQ690725	-	-	2, 4
Oreoweisia bogotensis	MS Obo	B	*Philippi P-275*	KX580425	KX580554	AF129600	AF129600	AF129600	JQ690726	-	-	2, 4
Rhabdoweisia crenulata	MS Rhc	BONN	*Frahm s.n. 18.10.97*	KX580396	KX580558	AF127181	AF127181	AF127181	EU186594	-	-	2, 4
Rhachitheciaceae												
Jonesiobryum cerradense	GenBank	NY	*Yano 4677*	AY908901	AY908120	-	-	-	-	-	-	4
Rhachithecium papillosum	GenBank	herb. B. Goffinet	*Pocs & Lye 97123A*	AY908963	AF306978	-	-	-	-	-	-	4
Saelaniaceae												
Saelania glaucescens	GenBank	NY	*Hedderson 8339*	AY908924	AY908148	-	-	-	-	-	-	4
Schistostegaceae												

Species or infraspecific taxon	DNA voucher	Herbarium	Collector data	*nad5*	*trnS-rps4*	*rps4-trnT*	*trnT-trnL*	*trnL-trnF*	*atpB-rbcL*	*ITS1*	*ITS2*	Chapter
Schistostega pennata	GenBank*	?	from Beckert et al., 1999	AJ224856	-	-	-	-	-	-	-	4
Schistostega pennata	GenBank*	RNG	*Hedderson s.n.*	-	AF265359	-	-	-	-	-	-	4
Scouleriaceae												
Scouleria aquatica	GenBank	RNG	*Hedderson 5811*	AY312887	AF023780	-	-	AF023723	-	-	-	4
Seligeriaceae												
Blindia acuta	MS Ba	L	*Frahm s.n.*	KX580407	KX580488	KX580488	KX580489	AF135109	JQ690727	-	-	2, 4
Blindia contecta	IPG 17	L	*Stech 12-032*	MN177988	MN187475	-	-	MN178054	-	-	-	4
Serpotortellaceae												
Serpotortella chenagonii	GenBank	MO	*Orban 9424/CA*	AY908878	AY908113	-	-	-	-	-	-	4
Timmiellaceae												
Luisierella barbula	GenBank	herb. B. Goffinet	*Nash 313*	AY908975	AY908155	-	-	-	-	-	-	4
Timmiella anomala	GenBank	BUF	*Weber 1978*	AY908958	AY908163	-	-	-	-	-	-	4

Appendix 2. Primers and PCR amplification protocols for the mitochondrial, chloroplast and nuclear DNA markers used in this thesis.

Region	Primers	PCR amplification protocol
mitochondrial		
*nad*5	*nad*5_4F[1]/*nad*5_3R[1]	96°C 90" [96°C 45" 60°C 1' 72°C 1']$_{35}$ 72°C 7'
	*nad*5_4F[1]/*nad*5_2220R[2]	96°C 90" [96°C 45" 60°C 1' 72°C 1']$_{35}$ 72°C 7'
	*nad*5_Ki/*nad*5_Li[3]	96°C 90" [96°C 45" 64°C 1' 72°C 1']$_{35}$ 72°C 7'
chloroplast		
*trn*S-*rps*4	*trn*S-F[4]/*rps*5[5]	94°C 3' [94°C 15" 50°C 30" 72°C 1']$_{35}$ 72°C 7'
*rps*4-*trn*T	*rps*4-166F/A-Rbryo[6]	94°C 2' [94°C 1' 52°C 1' 72°C 90"]$_{35}$ 72°C 5'
*trn*T-*trn*L	A-Fbryo/P6/7[6]	94°C 2' [94°C 1' 52°C 1' 72°C 90"]$_{35}$ 72°C 5'
*trn*L-*trn*F	$C_{(M)}$[7]/$F_{(M)}$[8]	94°C 5' [94°C 1' 50°C 1' 72°C 1']$_{34}$ 72°C 2'
*atp*B-*rbc*L	*rbc*L_F/*atp*B_R[9]	94°C 4' [94°C 30" 45°C+1°C/cycle 50" 70°C 75"]$_{10}$ [94°C 30" 55°C 50"+1"/cycle 70°C 75"]$_{25}$ 70°C 7'
nuclear		
ITS1	18F/5.8R[10]	94°C 5' [94°C 45" 45°C 45" 72°C 1']$_{35}$ 72°C 4'
ITS2	5.8F/25R[10]	94°C 5' [94°C 45" 45°C 45" 72°C 1']$_{35}$ 72°C 4'

[1] (Buck et al., 2005)

[2] (Câmara & Shaw, 2013)

[3] (Beckert et al., 1999)

[4] (Souza-Chies et al., 1997)

[5] (Nadot et al., 1994)

[6] (Hernández-Maqueda, Quandt, Werner, et al., 2008)

[7] (Taberlet et al., 1991)

[8] (Frey et al., 1999)

[9] (Gama et al., 2015)

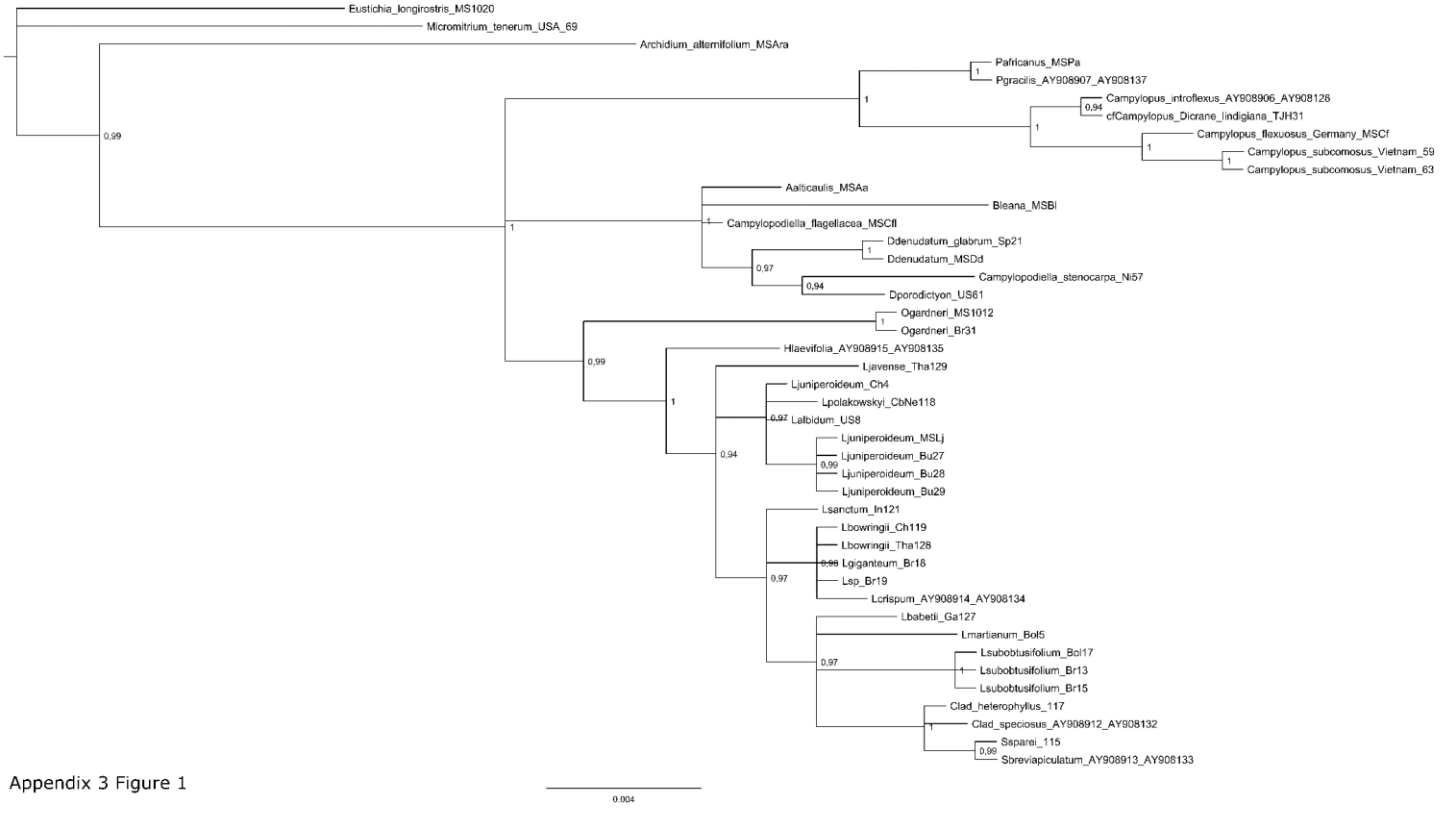
[10] (Stech & Frahm, 1999)

Appendix 3. Trees of Leucobryaceae representatives resulting from analyses of subsets of markers for each alignment. Figures with uneven numbers are Bayesian inference consensus trees, and figures with even numbers are maximum likelihood trees (next pages, pp. 124–139).

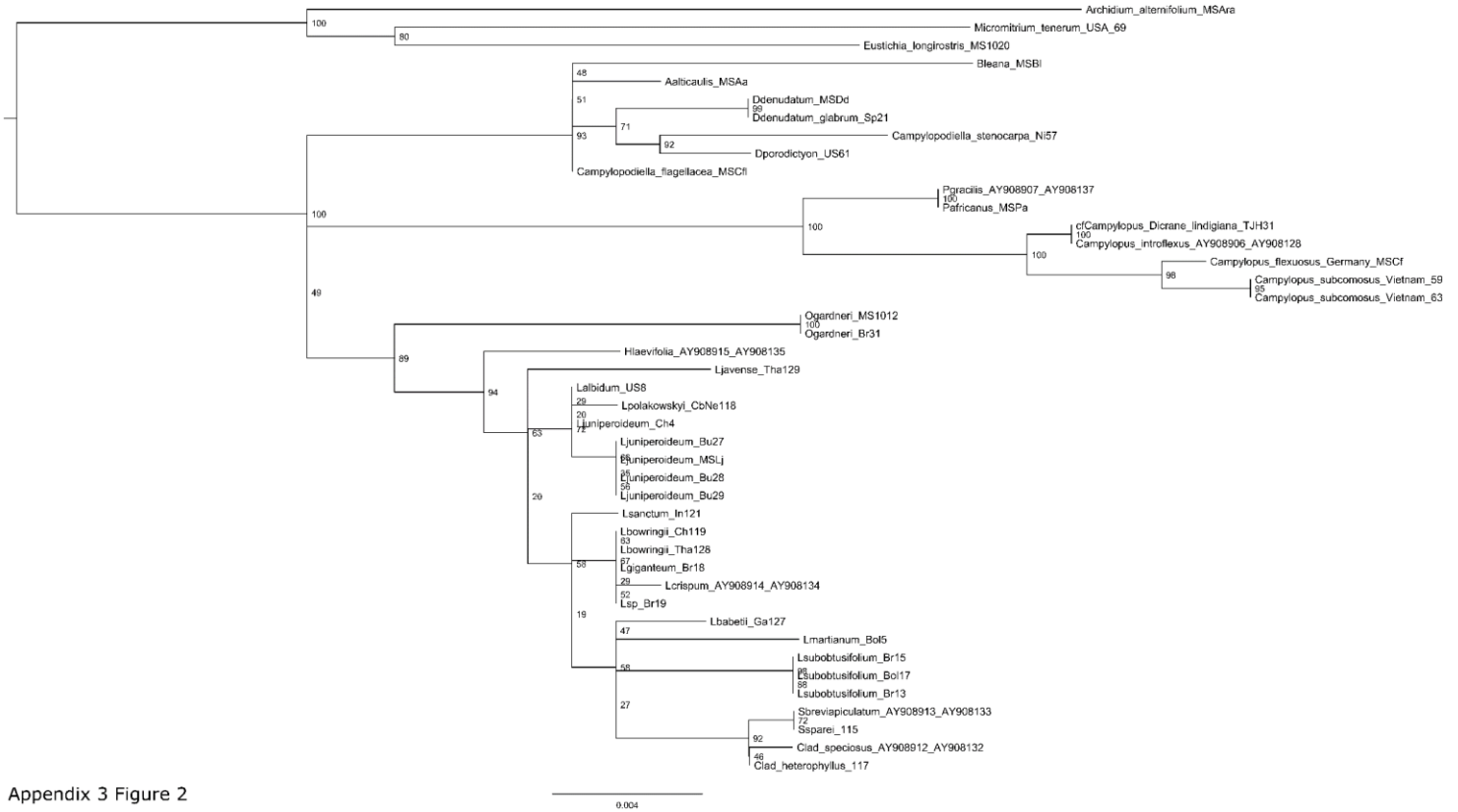
Figures 1–4. Trees for the Leucobryaceae alignment with Archidium alternifolium*,* Eustichia longirostris *and* Micromitrium tenerum *as outgroup representatives. 1, 2. Mitochondrial marker* nad*5. 3, 4. Chloroplast markers.*

Figures 5–10. Trees for the Dicranodontium *clade alignment with two samples of* Ochrobryum gardneri *as outgroup representatives. 5, 6. Mitochondrial marker* nad*5. 7, 8. Chloroplast markers. 9, 10. Nuclear markers ITS1, 5.8S and ITS2.*

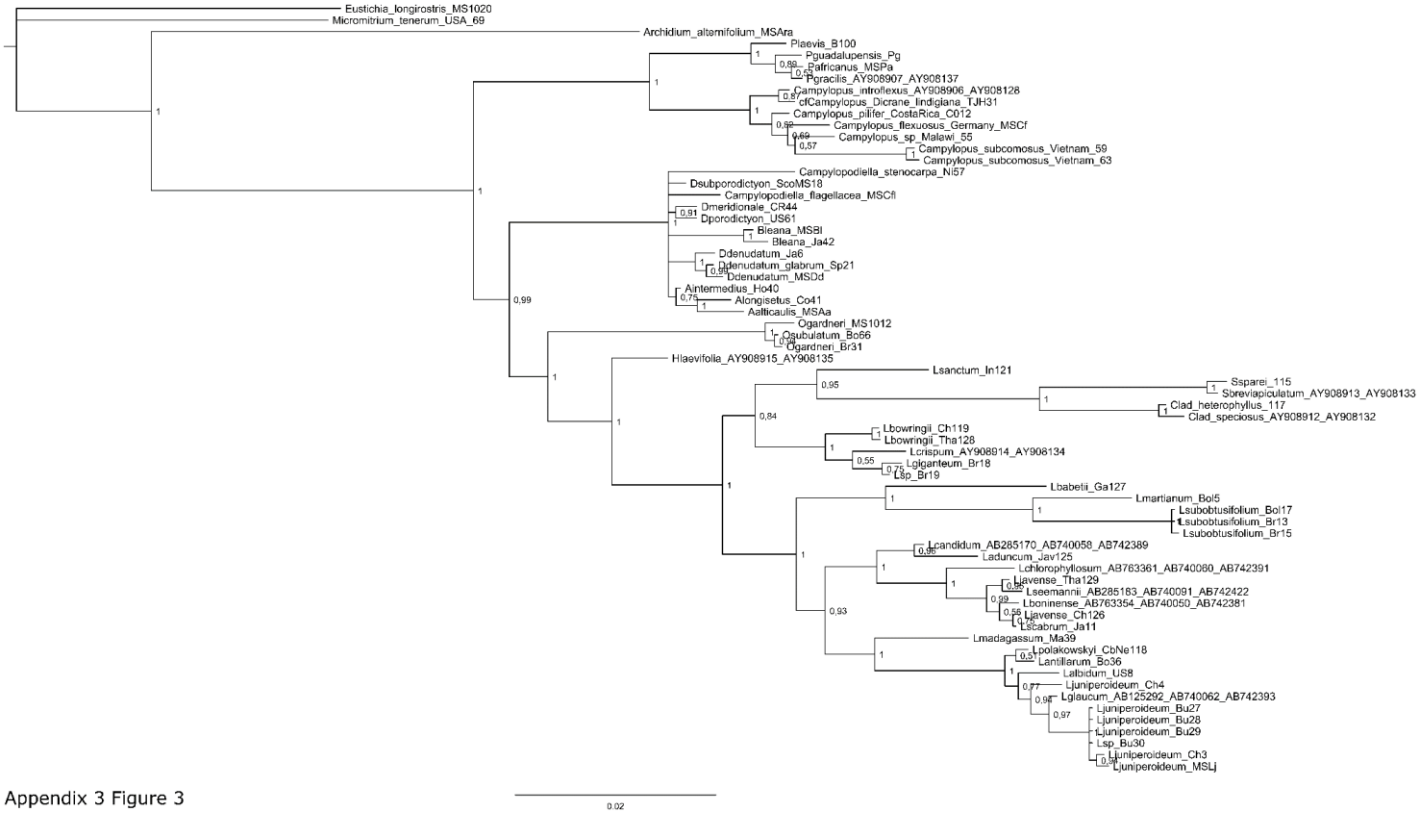
Figures 11–16. Trees for the leucobryoid clade alignment with two samples of Ochrobryum gardneri *as outgroup representatives. 11, 12. Mitochondrial marker* nad*5. 13, 14. Chloroplast markers. 15, 16. Nuclear markers ITS1, 5.8S and ITS2.*



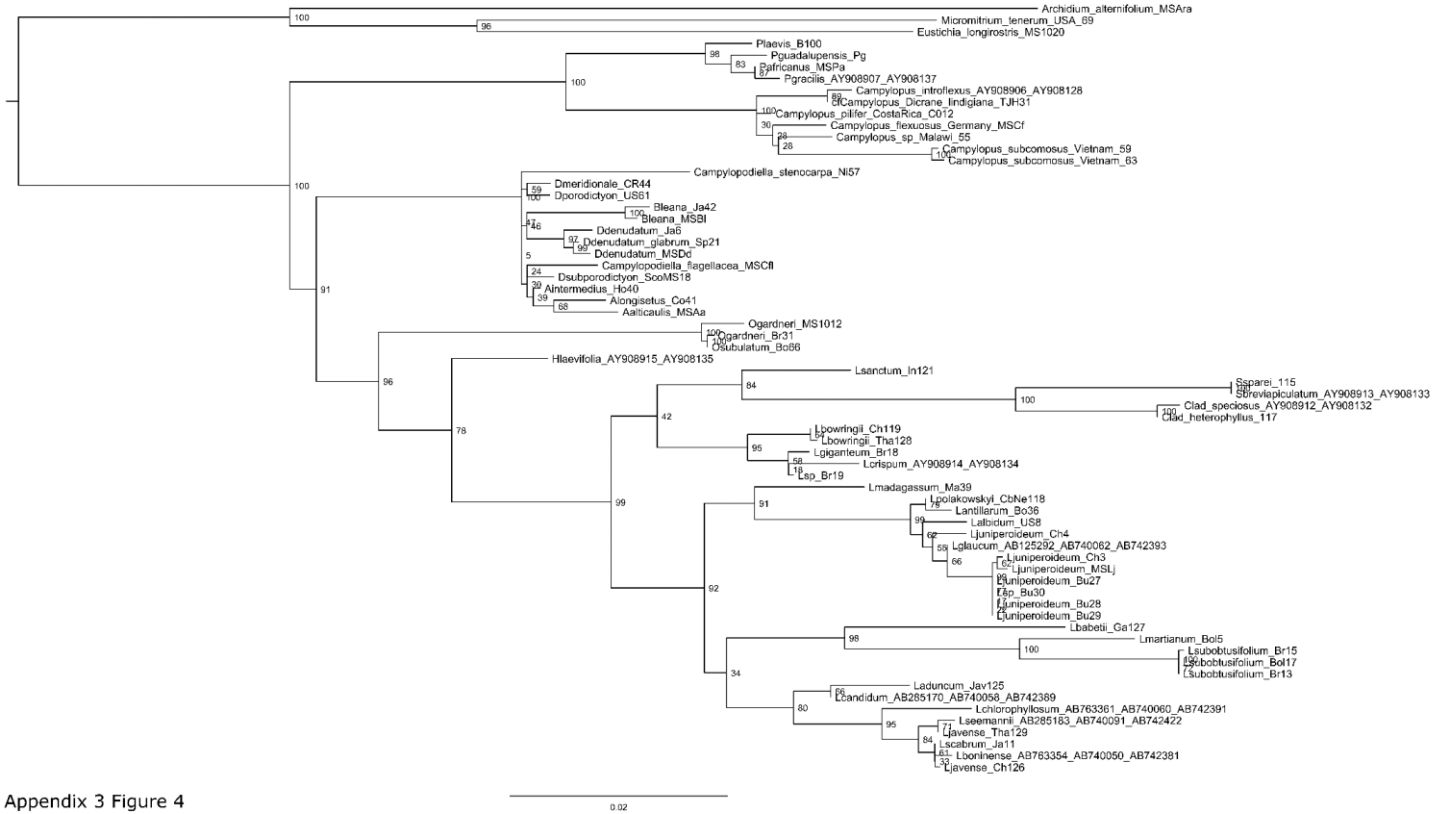
Appendix 3 Figure 1



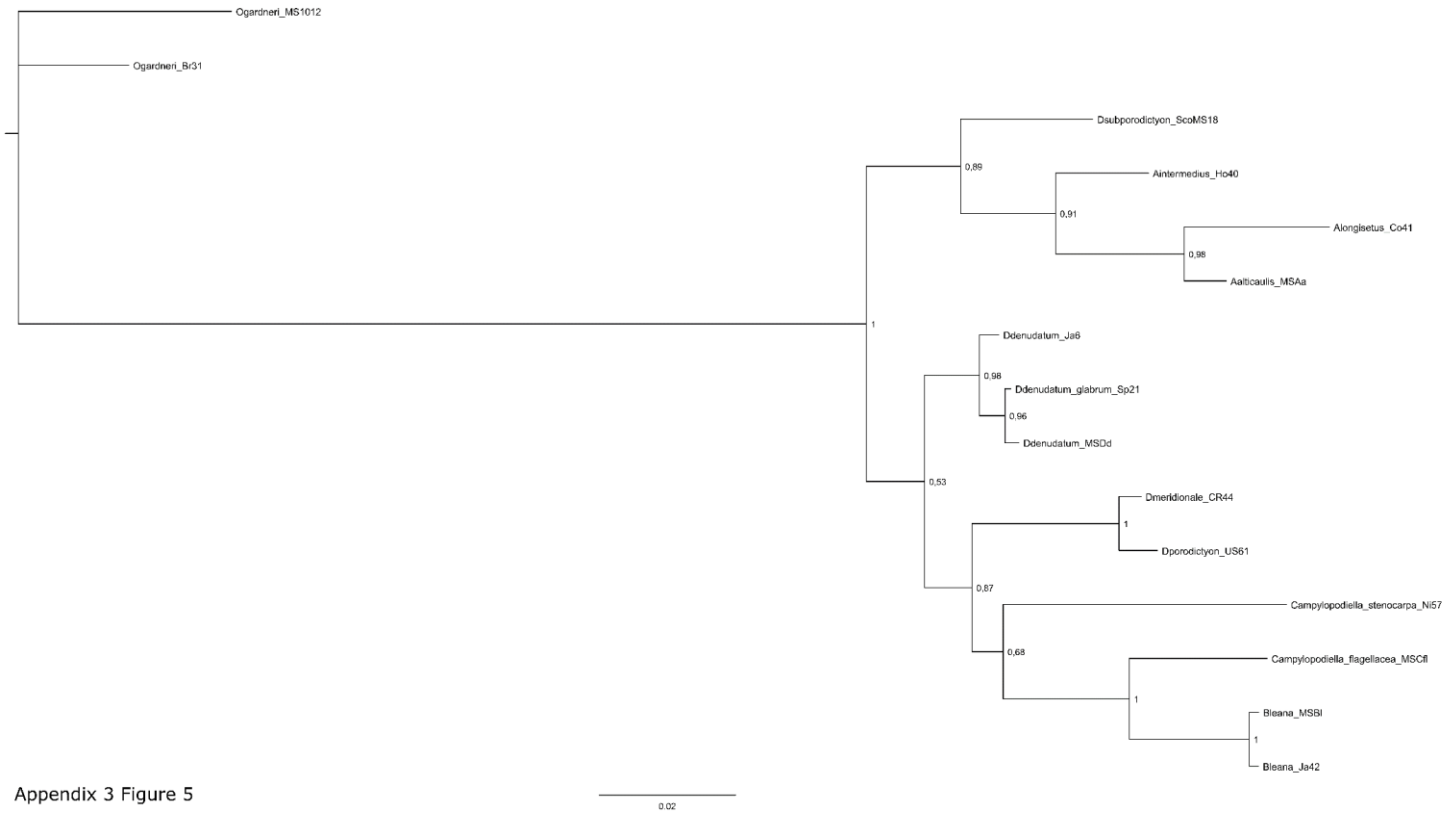
Appendix 3 Figure 2



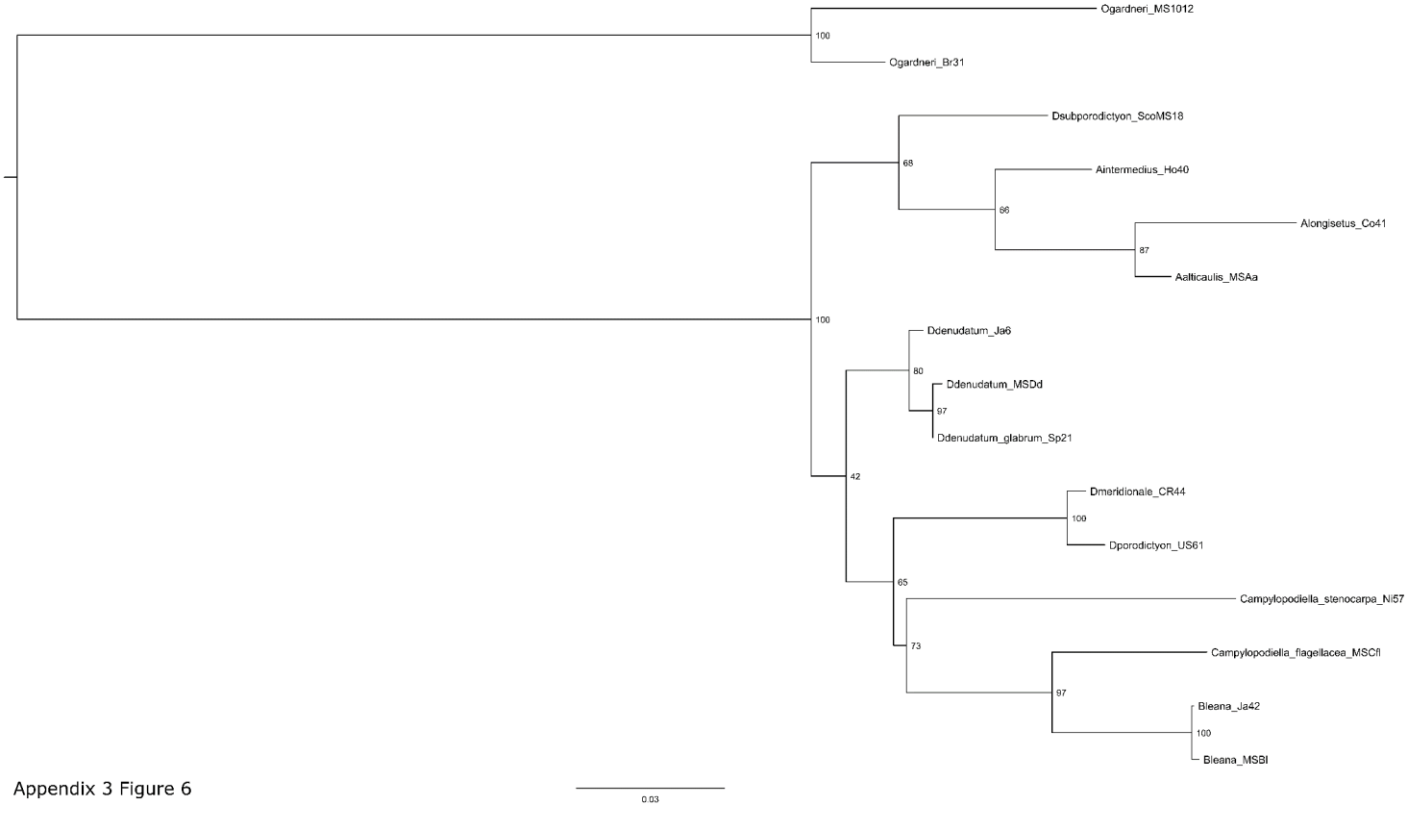
Appendix 3 Figure 3



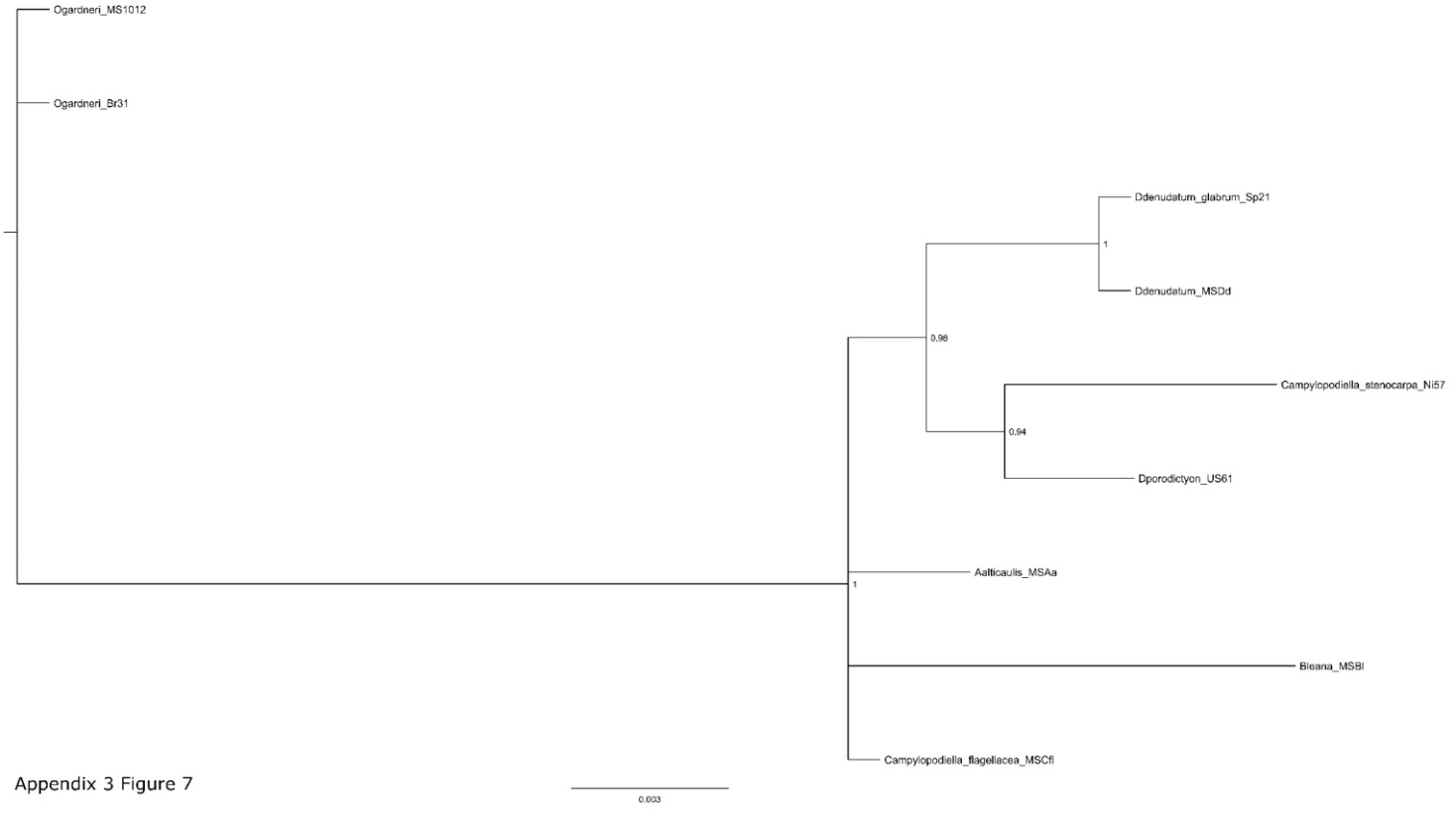
Appendix 3 Figure 4



Appendix 3 Figure 5



Appendix 3 Figure 6



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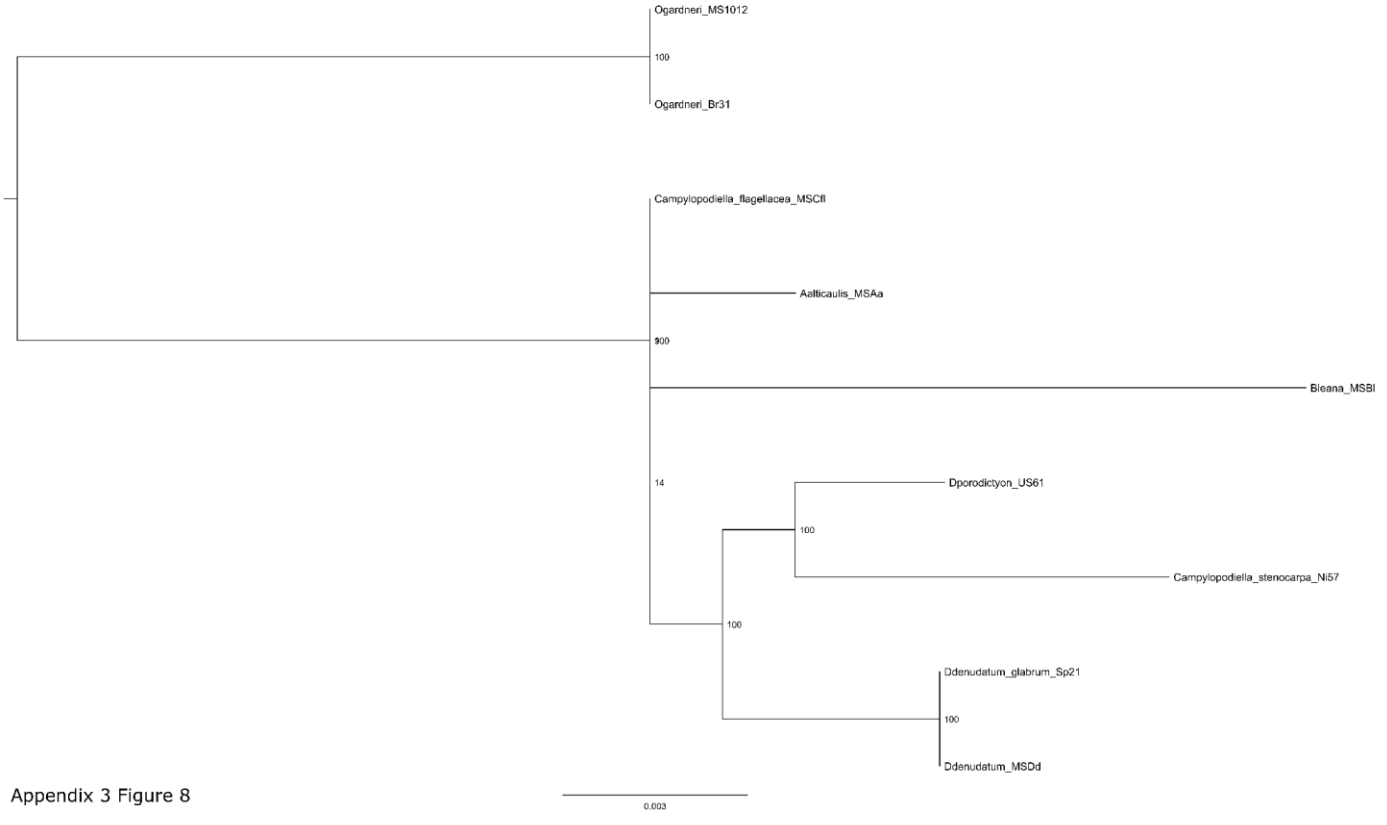
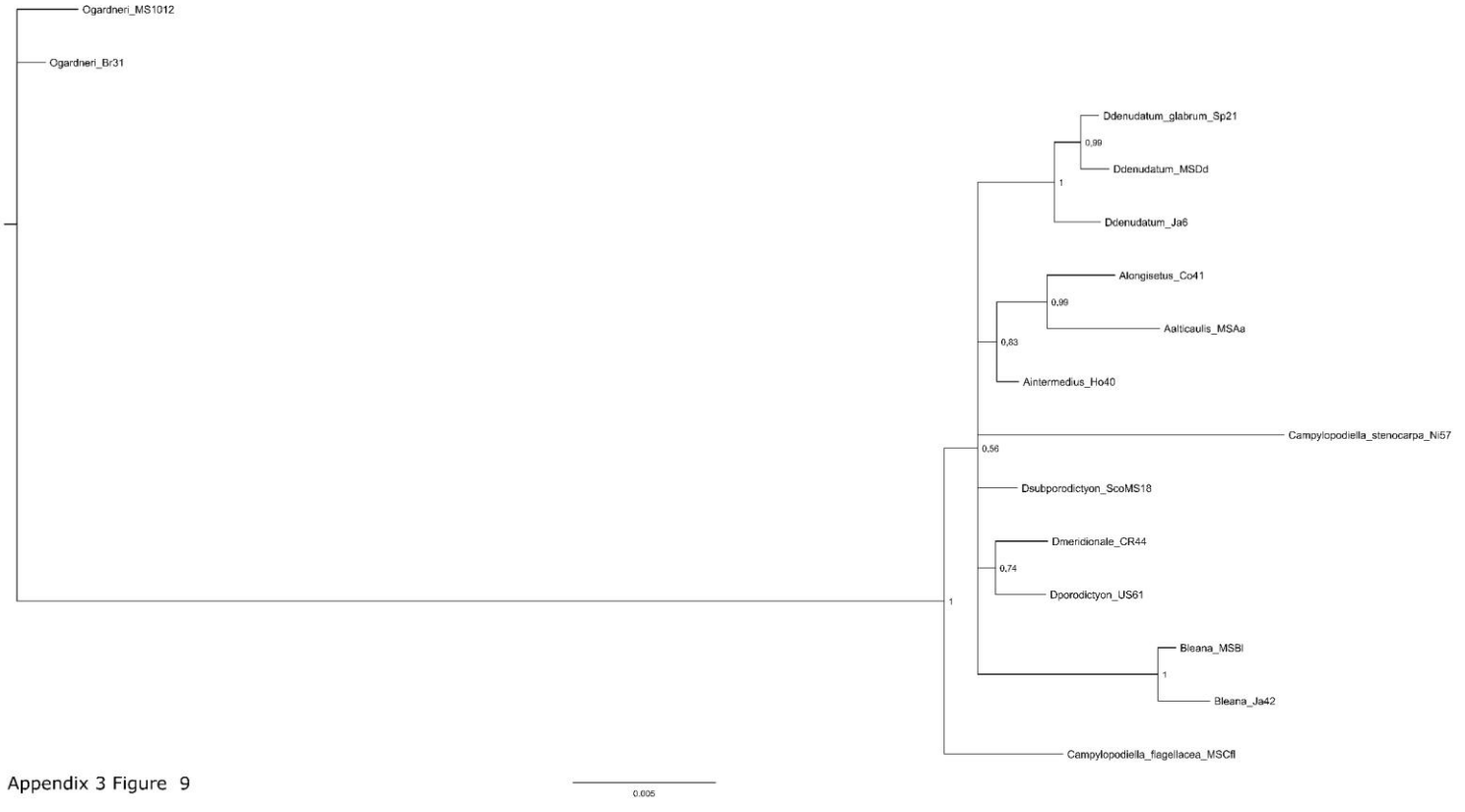
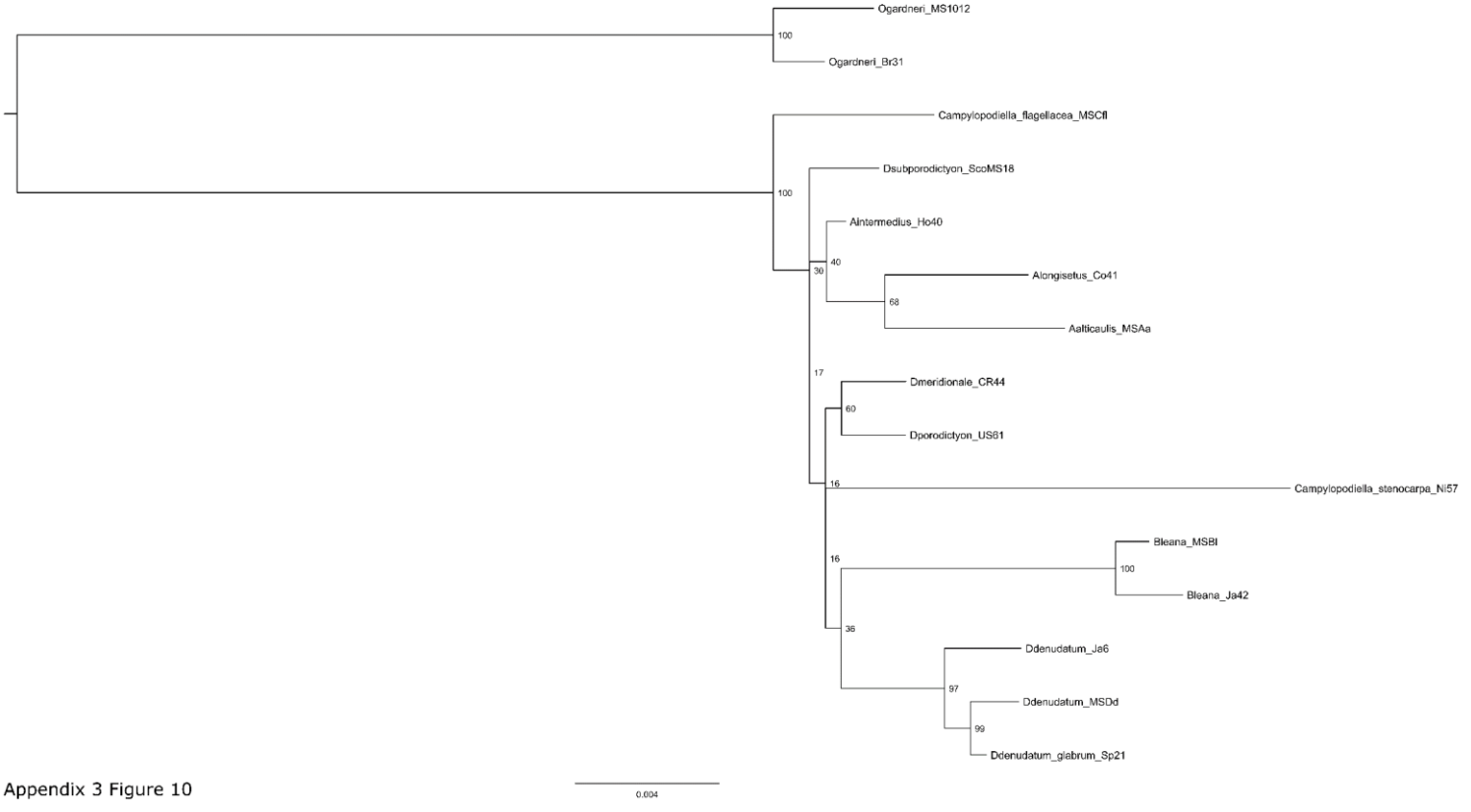


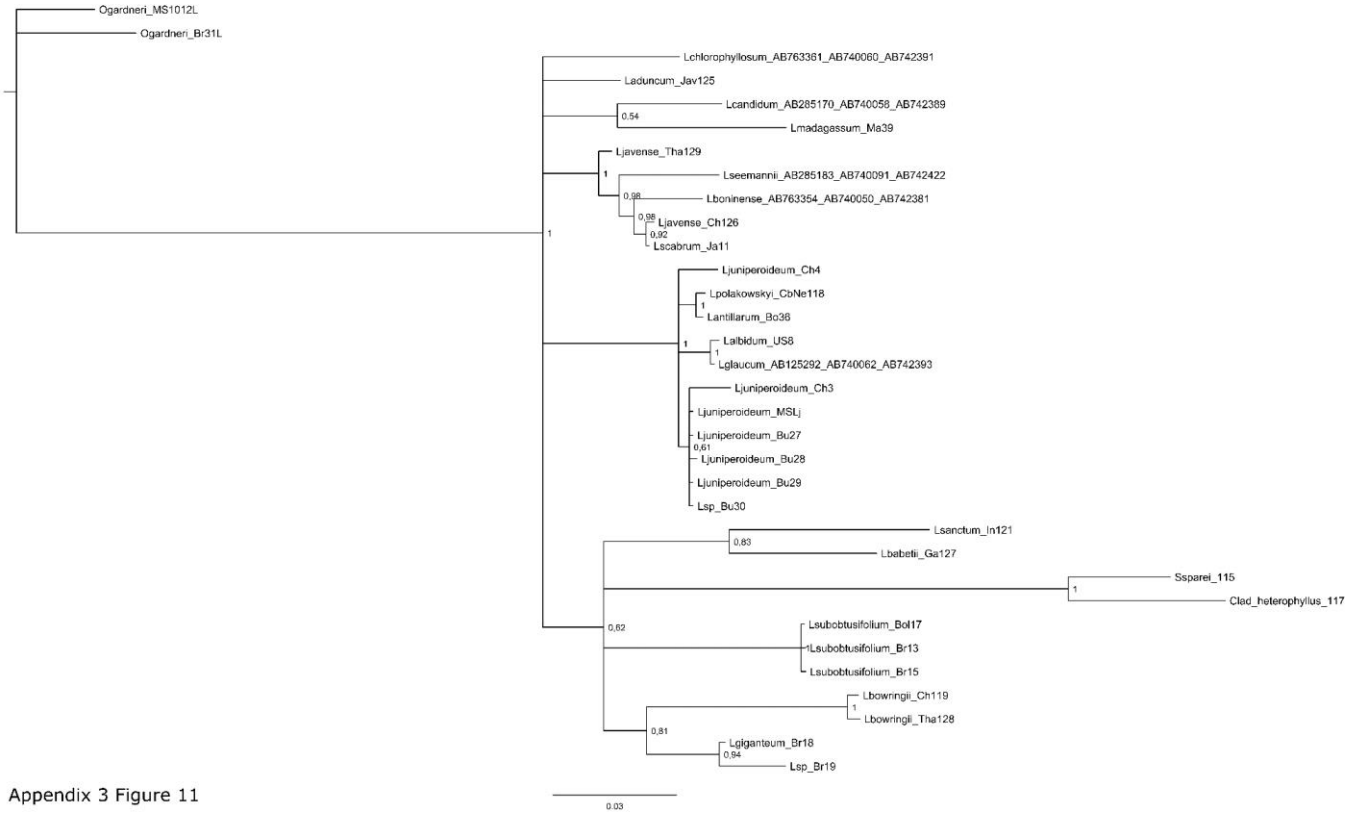
Appendix 3 Figure 8



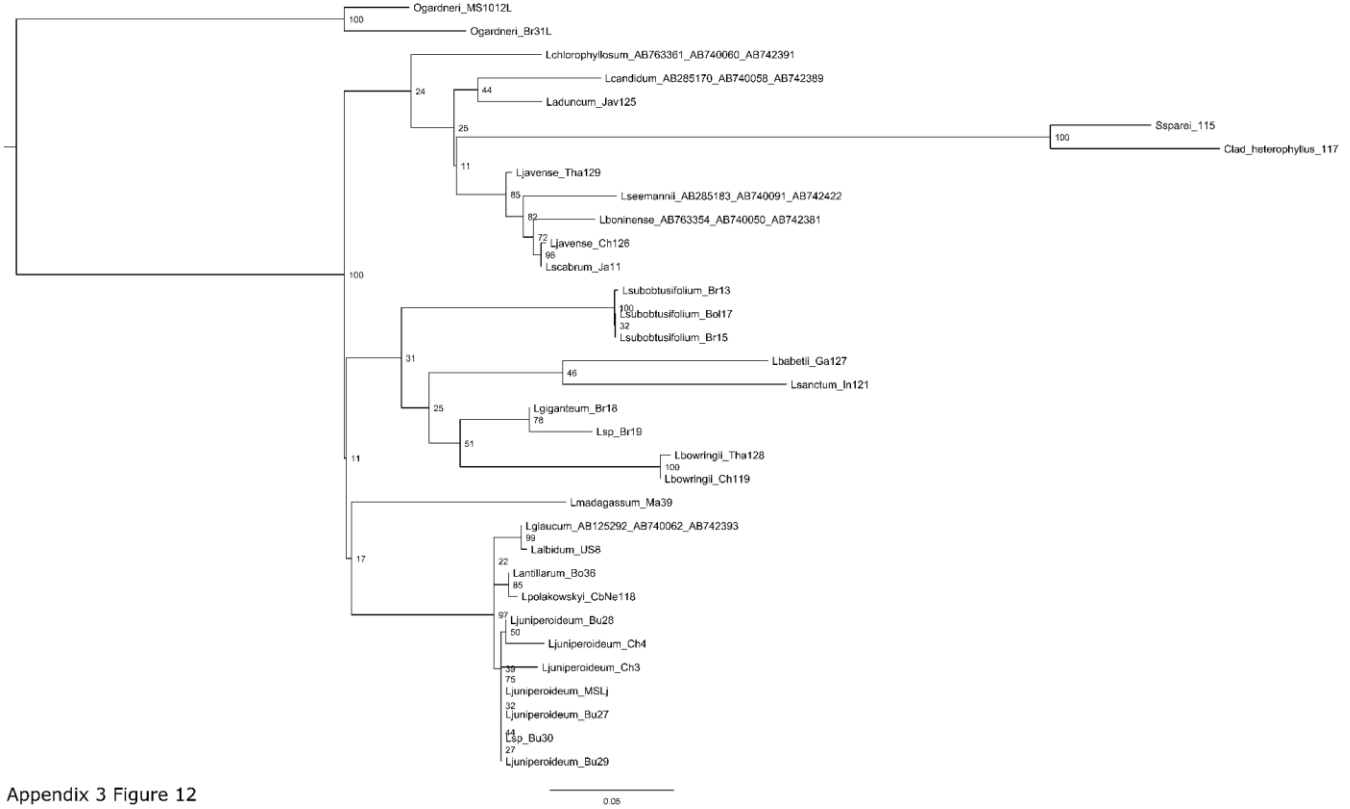
Appendix 3 Figure 9



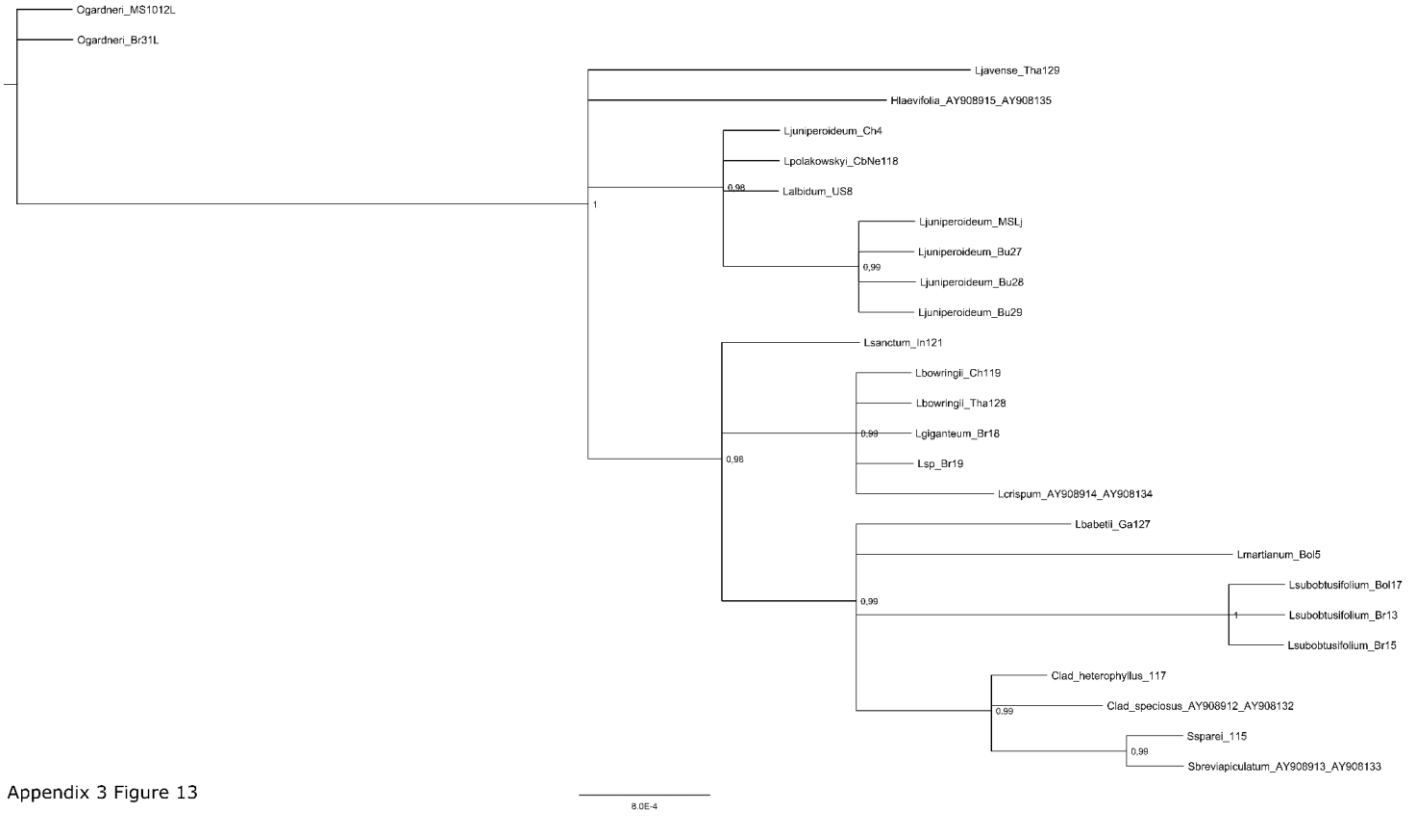
Appendix 3 Figure 10



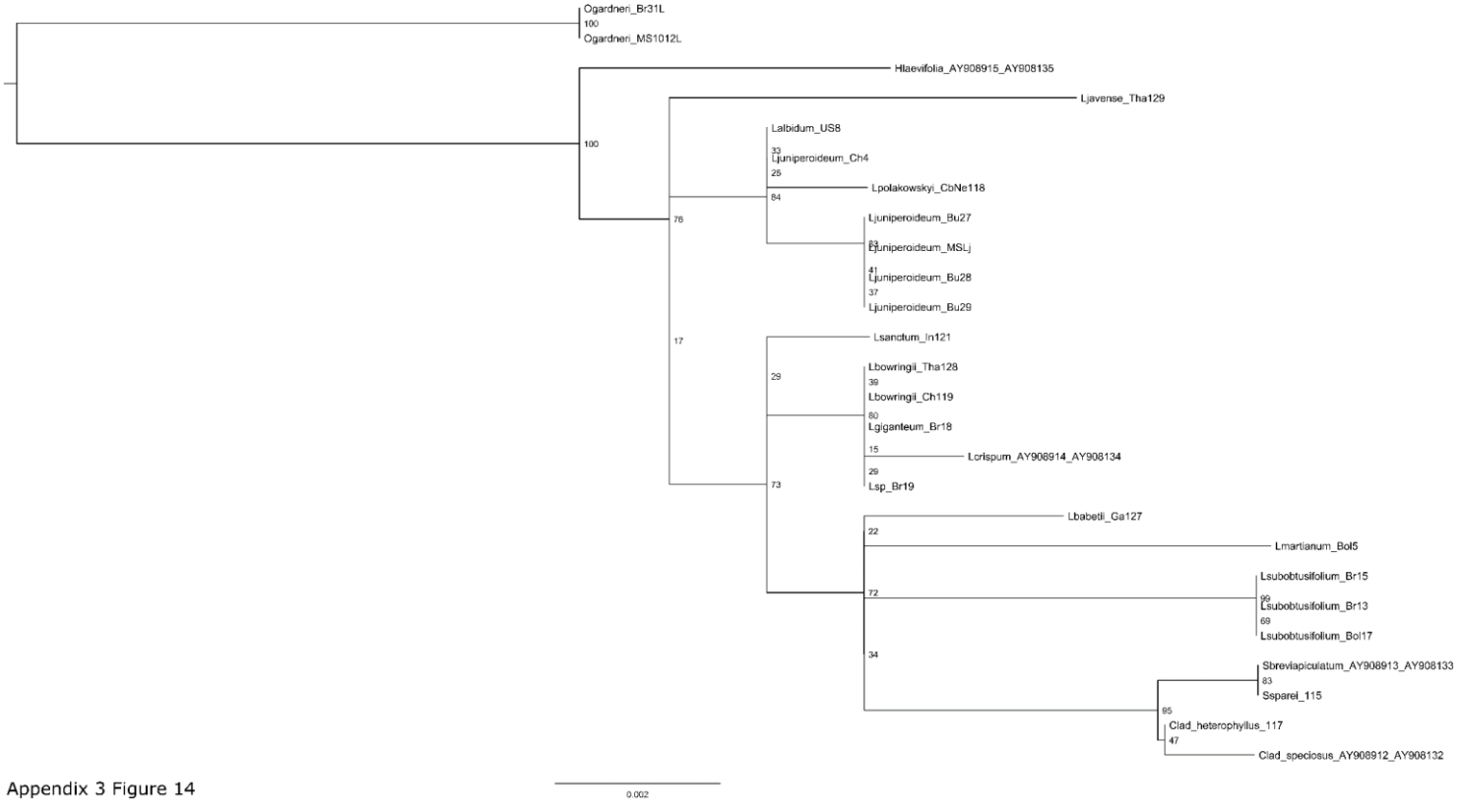
Appendix 3 Figure 11



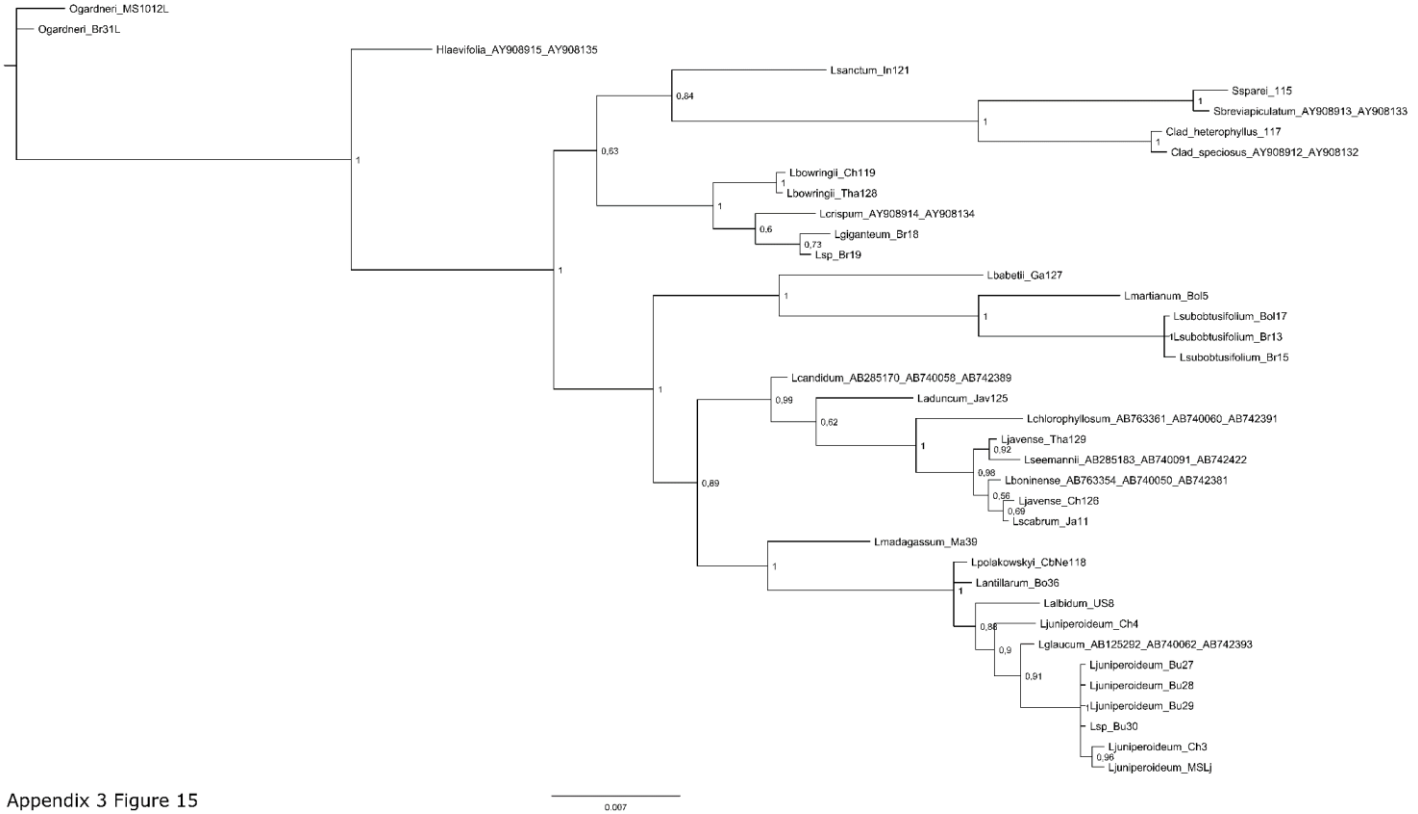
Appendix 3 Figure 12



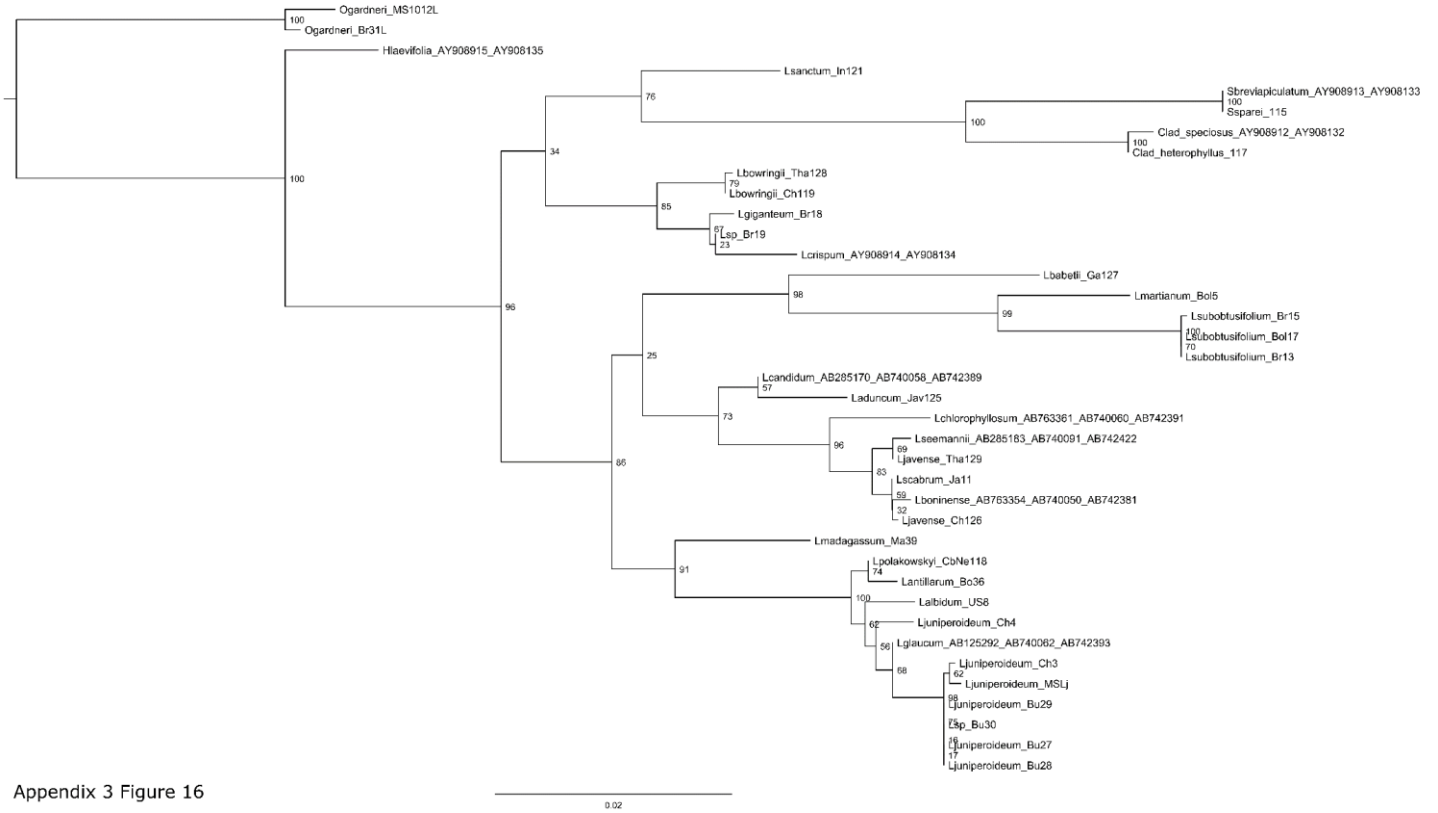
Appendix 3 Figure 13



Appendix 3 Figure 14

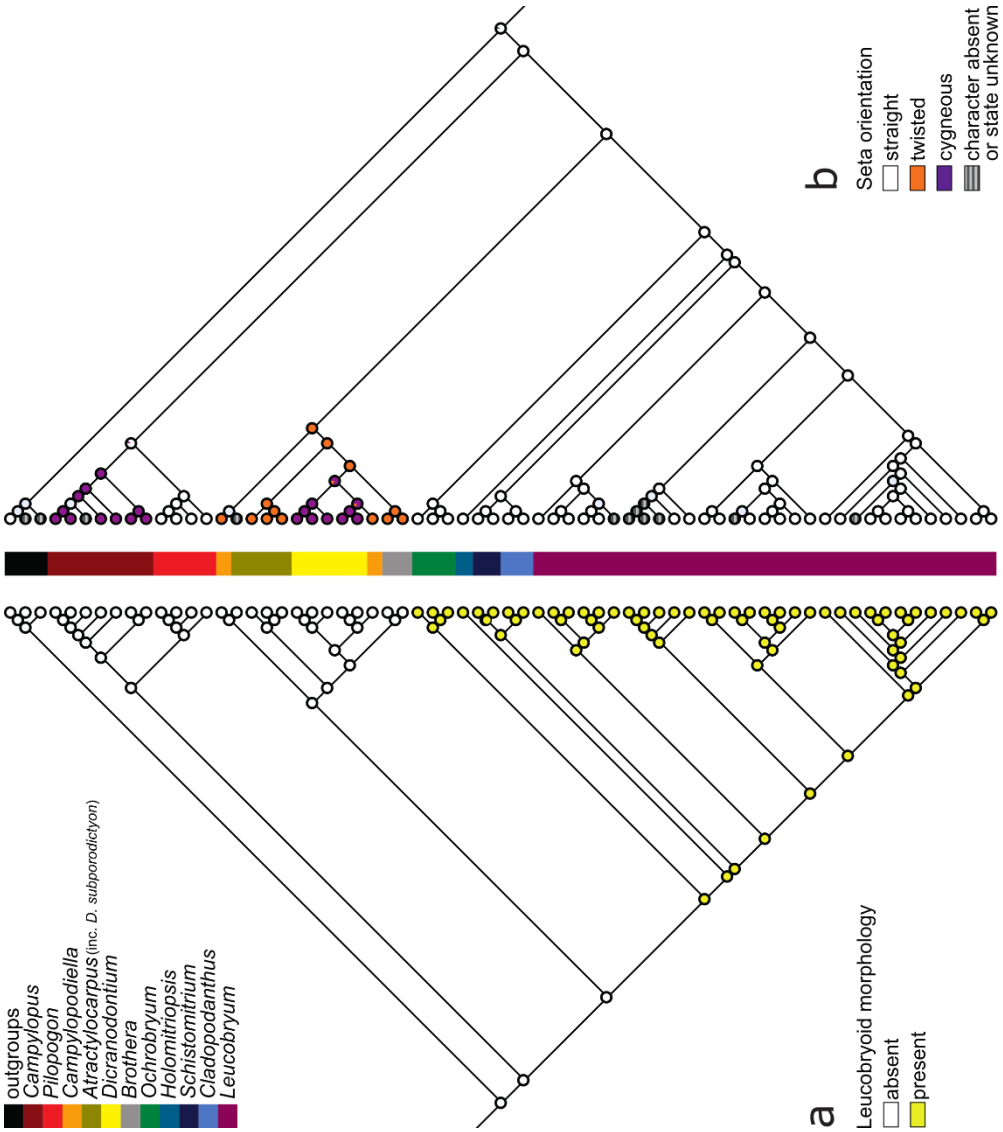


Appendix 3 Figure 15



Appendix 3 Figure 16

*Appendix 4. Maximum likelihood character evolution analyses of Leucobryaceae representatives for the occurrence of leucobryoid morphology (**a**), seta orientation (**b**), capsule orientation (**c**), and calyptra shape (**d**), under the phylogenetic hypothesis represented by the constrained ML tree with* Leucobryum *monophyletic.*



c
Capsule orientation
orthotropous
homotropous
character absent
or state unknown
d
Calyptra shape
cucullate
mitrate
reduced
character absent
or state unknown

Appendix 5. Selected hypotheses for the circumscriptions of Aongstroemia, Dicranella*, and related genera, tested in the SH test 1 of Chapter 3. Species that change genus placement between hypotheses are marked with different colors to facilitate recognition of differences.*

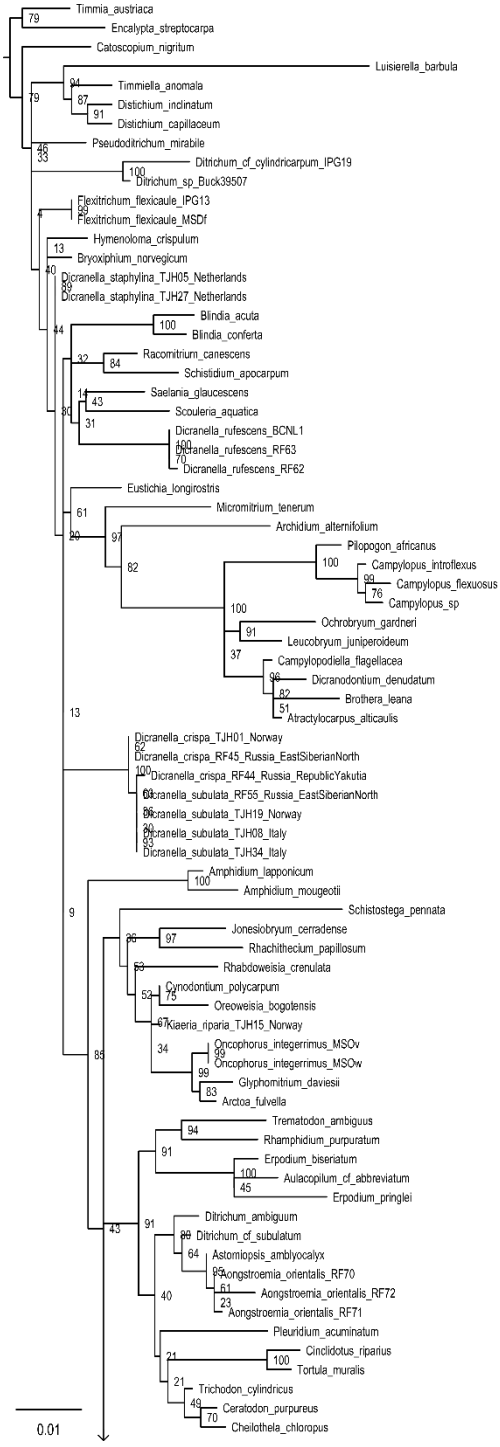
Constrained topology	Accepted genera relevant to the constraint with (approximate) number of species	Species included in the sampling per considered genus (taxa highlighted in bold indicate those set as a monophyletic group in the applied constraint)
Dicranella sensu Frey & Stech (2009)	*Dicranella* (157)	***D. campylophylla*, *D. cardotii*, *D. cerviculata*, *D. crispa*, *D. curvipes*, *D. grevilleana*, *D. heteromalla*, *D. howei*, *D. rufescens*, *D. schreberiana*, *D. staphylina*, *D. subulata*, *D. varia*** (13)
	Leptotrichella (60)	*L. flaccidula* (1)
	Diobelonella (1)	*D. palustris* (1)
	Kiaeria (6)	*K. riparia* (1)
Dicranella sensu Crum (2007)	*Dicranella* (ca. 220)	***D. campylophylla*, *D. cardotii*, *D. cerviculata*, *D. crispa*, *D. curvipes*, *D. flaccidula*, *D. grevilleana*, *D. heteromalla*, *D. howei*, *D. palustre*, *D. rufescens*, *D. schreberiana*, *D. staphylina*, *D. subulata*, *D. varia*** (15)
	Kiaeria (6)	*K. riparia* (1)
Dicranella sensu Crosby et al. (1999)	*Anisothecium* (40)	*A. campylophyllum*, *A. grevilleanum*, *A. palustre*, *A. rufescens*, *A. schreberianum*, *A. staphylinum*, *A. varium* (7)
	Dicranella (162)	***D. cardotii*, *D. cerviculata*, *D. crispa*, *D. curvipes*, *D. flaccidula*, *D. heteromalla*, *D. howei*, *D. subulata*** (8)
	Kiaeria (6)	*K. riparia* (1)

Constrained topology	Accepted genera relevant to the constraint with (approximate) number of species	Species included in the sampling per considered genus (taxa highlighted in bold indicate those set as a monophyletic group in the applied constraint)
Dicranella sensu Frey & Stech (2009) with the inclusion of *Kiaeria riparia* (cf. Nyholm, 1987)	*Dicranella* (158)	***D. campylophylla*, *D. cardotii*, *D. cerviculata*, *D. crispa*, *D. curvipes*, *D. grevilleana*, *D. heteromalla*, *D. howei*, *D. riparia*, *D. rufescens*, *D. schreberiana*, *D. staphylina*, *D. subulata*, *D. varia*** (14)
	Leptotrichella (60)	*L. flaccidula* (1)
	Diobelonella (1)	*D. palustris* (1)
	Kiaeria (5)	none
Dicranella sensu Crum (2007) with the inclusion of *Kiaeria riparia* (cf. Nyholm, 1987)	*Dicranella* (ca. 220)	***D. campylophylla*, *D. cardotii*, *D. cerviculata*, *D. crispa*, *D. curvipes*, *D. flaccidula*, *D. grevilleana*, *D. heteromalla*, *D. howei*, *D. palustre*, *D. riparia*, *D. rufescens*, *D. schreberiana*, *D. staphylina*, *D. subulata*, *D. varia*** (16)
	Kiaeria (5)	none
Dicranella sensu Crum (2007) with the inclusion of *Kiaeria riparia* (cf. Nyholm, 1987) and exclusion of *Diobelonella palustris* (cf. Ochyra et al., 2003; Stech, 1999)	*Dicranella* (ca. 220)	***D. campylophylla*, *D. cardotii*, *D. cerviculata*, *D. crispa*, *D. curvipes*, *D. flaccidula*, *D. grevilleana*, *D. heteromalla*, *D. howei*, *D. riparia*, *D. rufescens*, *D. schreberiana*, *D. staphylina*, *D. subulata*, *D. varia*** (15)
	Diobelonella (1)	*D. palustris* (1)
	Kiaeria (5)	none

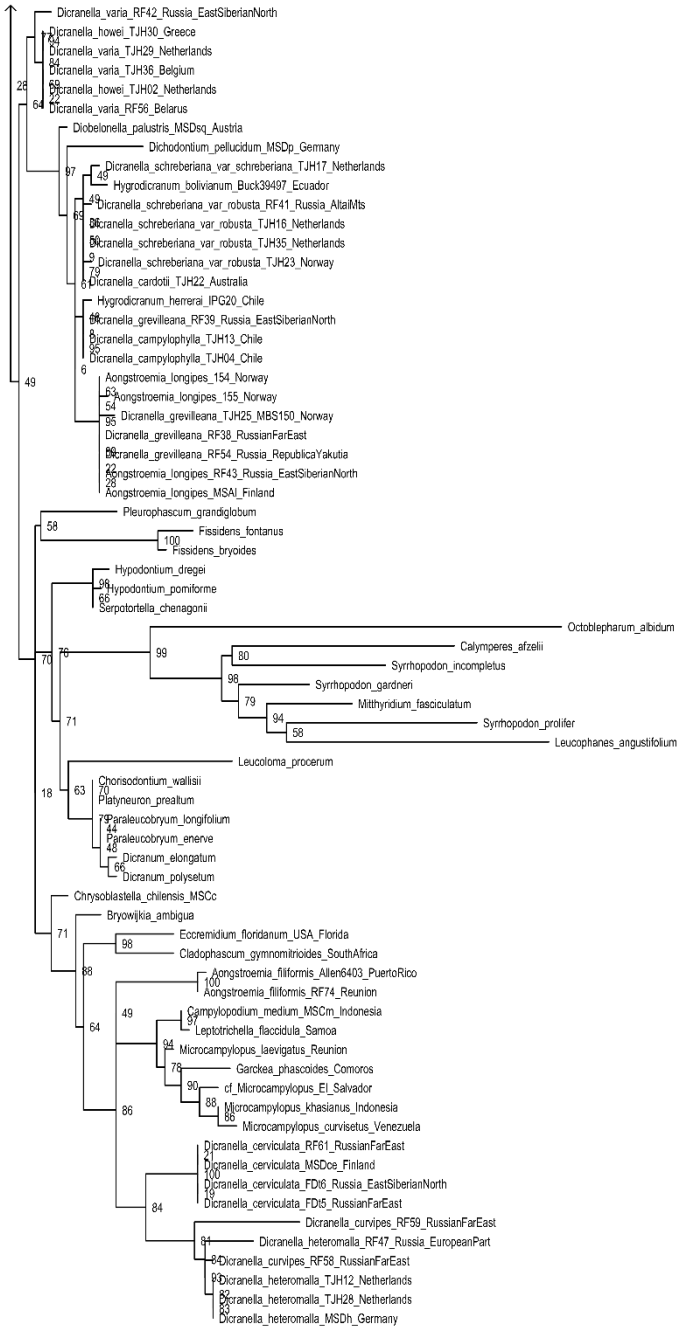
Constrained topology	Accepted genera relevant to the constraint with (approximate) number of species	Species included in the sampling per considered genus (taxa highlighted in bold indicate those set as a monophyletic group in the applied constraint)
Aongstroemia sensu Crosby et al. (1999)	*Aongstroemia* (7)	***A. filiformis*, *A. longipes*, *A. orientalis*** (3)
	Astomiopsis (6)	*A. amblyocalyx* (1)
	Bryomanginia (1)	none
Aongstroemia monophyletic with the exclusion of *A. orientalis* (suggested to be closely related to Ditrichaceae genera; cf. Allen, 1994)	*Aongstroemia* (5)	***A. filiformis*, *A. longipes*** (2)
	Astomiopsis/*Bryomanginia* (9)	*A. amblyocalyx*, *A. orientalis* (2)
Dichodontium and *Diobelonella palustris* forming a clade (*D. palustris* included in *Dichodontium* cf. Stech, 1999)	*Dichodontium* (3)	***D. flavescens*, *D. palustre*, *D. pellucidum*** (3)
	Diobelonella (0 – not accepted)	-

Appendix 6. Maximum likelihood trees (Figures 1, 3 and 5) and Bayesian inference trees (Figures 2, 4 and 6) of haplolepideous moss representatives, with Encalypta streptocarpa *(Encalyptaceae) and* Timmia austriaca *(Timmiaceae) as outgroup representatives, based on different molecular markers: Figures 1, 2. Mitochondrial marker* nad*5 intron. Figures 3, 4. Chloroplast marker* trn*S*-rps*4 spacer/*rps*4 gene. Figures 5, 6. Chloroplast marker* trn*L*-trn*F (next pages, pp. 146–157).*

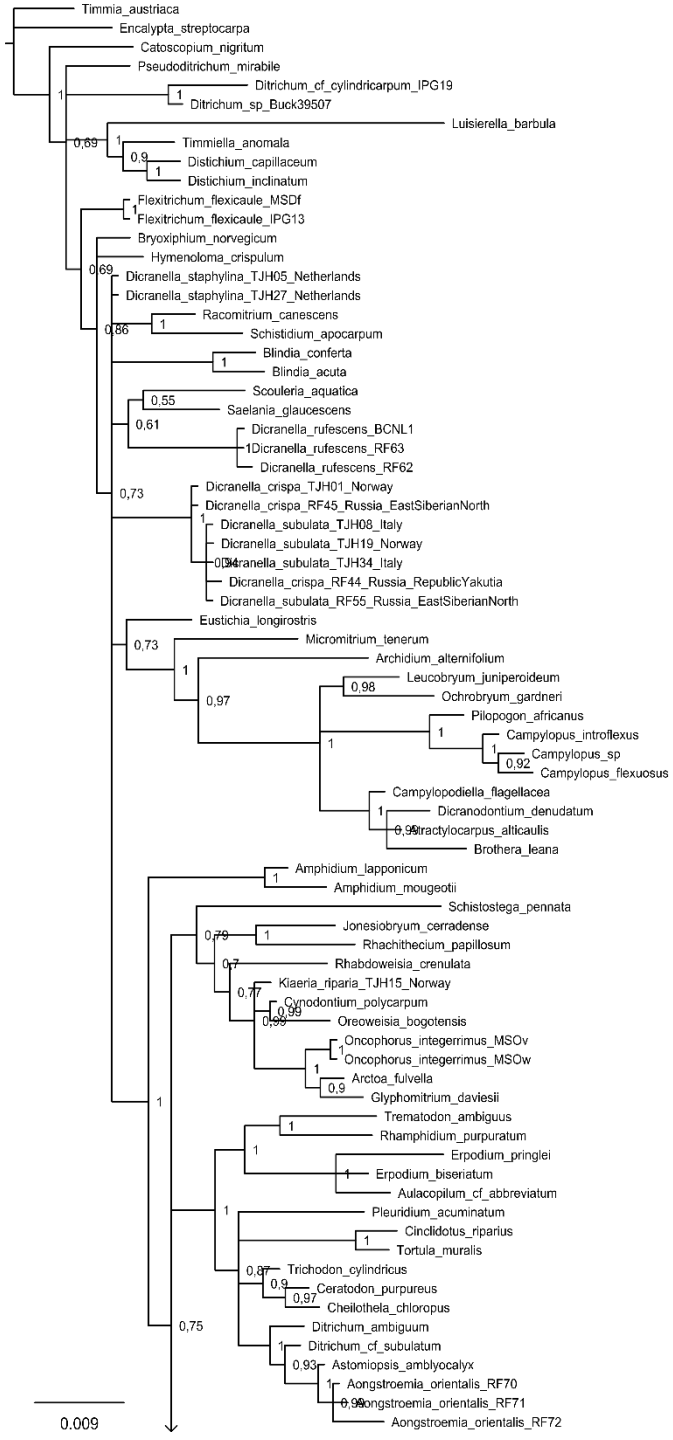
Appendix 6 Figure 1a



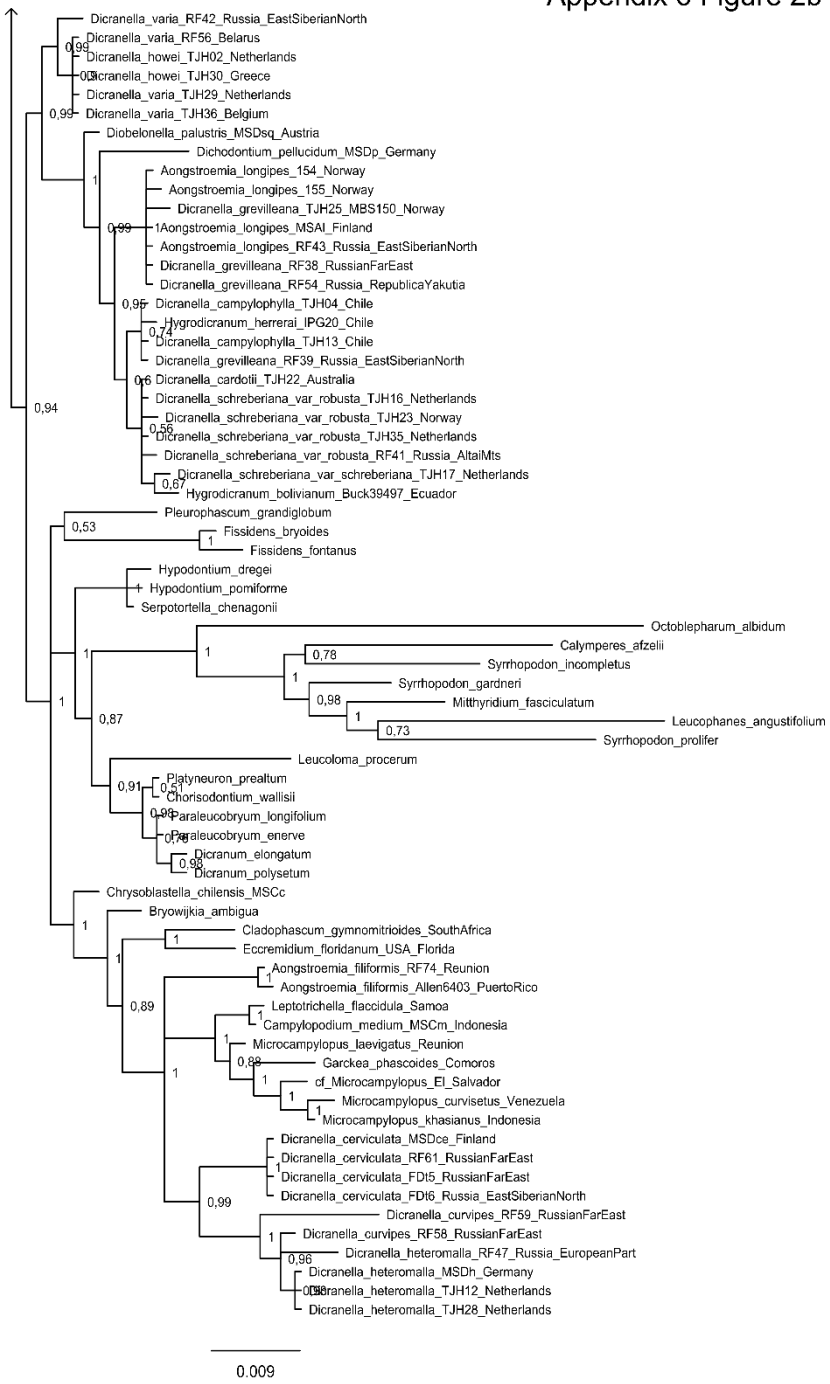
Appendix 6 Figure 1b



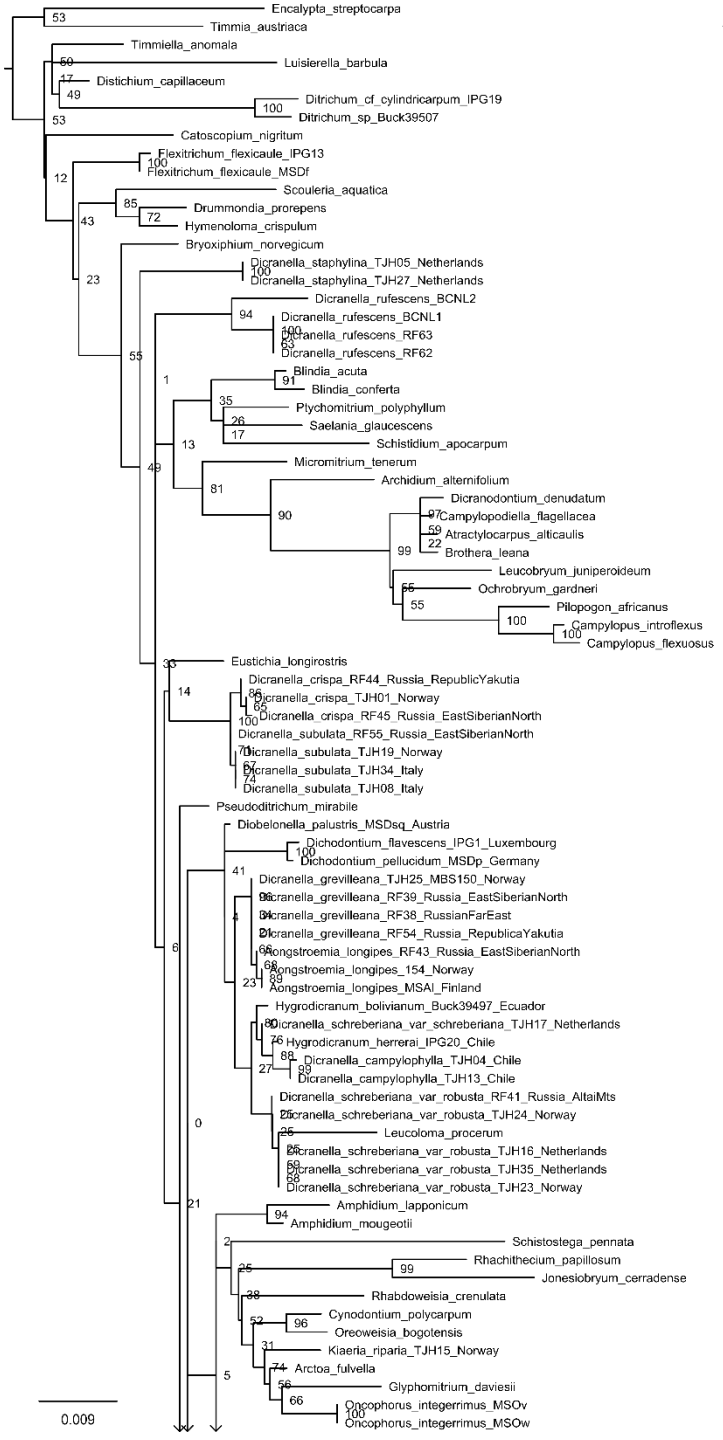
Appendix 6 Figure 2a



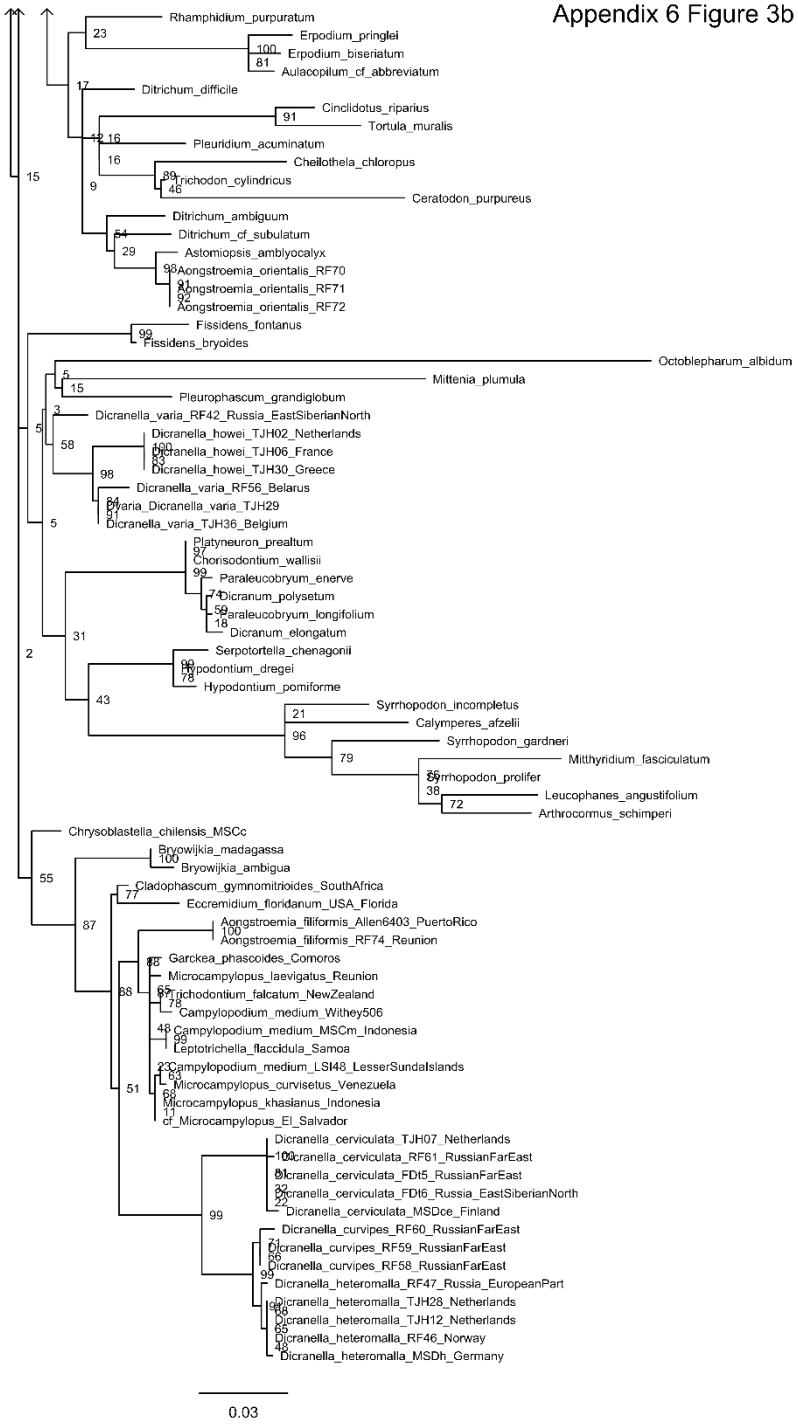
Appendix 6 Figure 2b



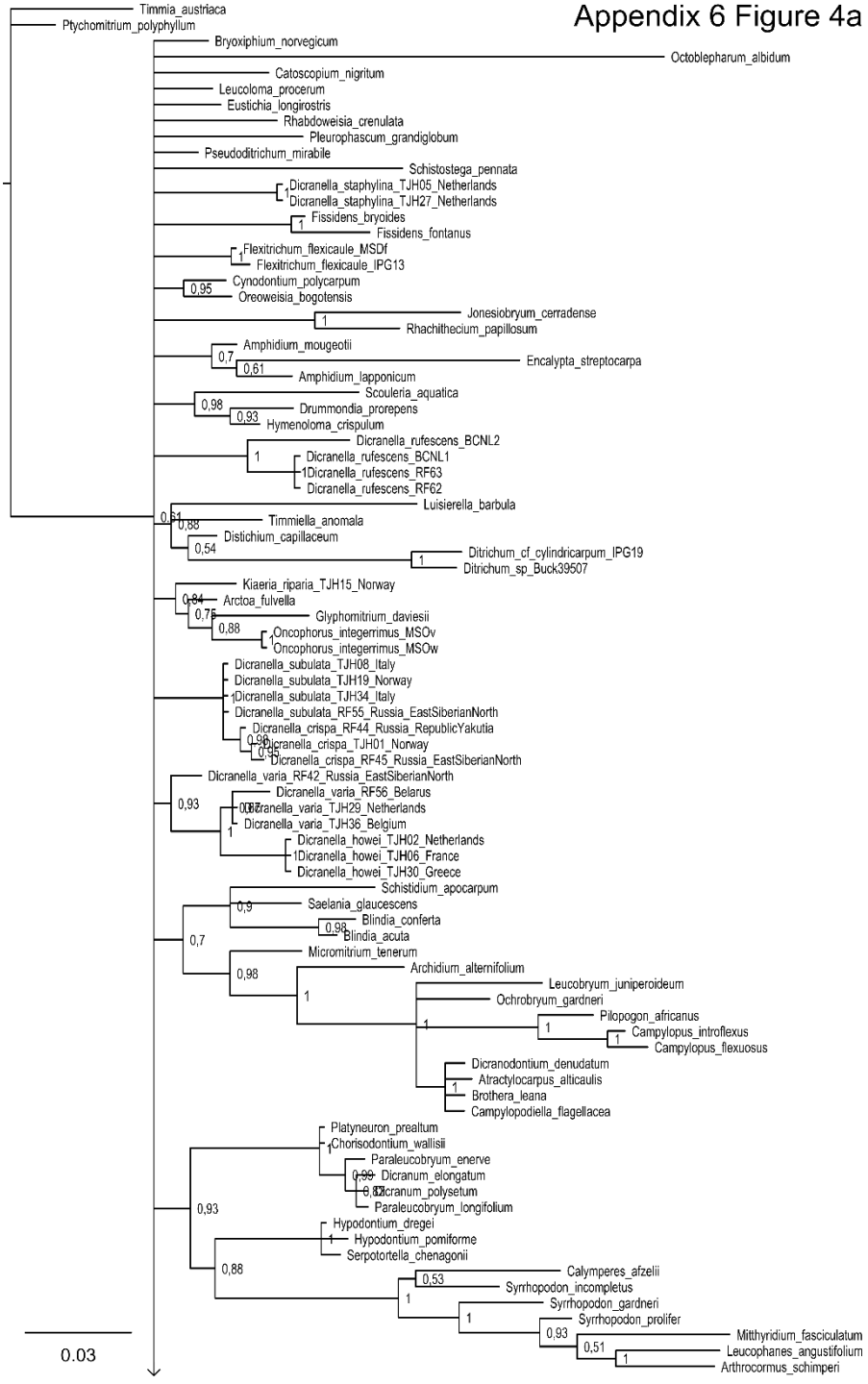
Appendix 6 Figure 3a



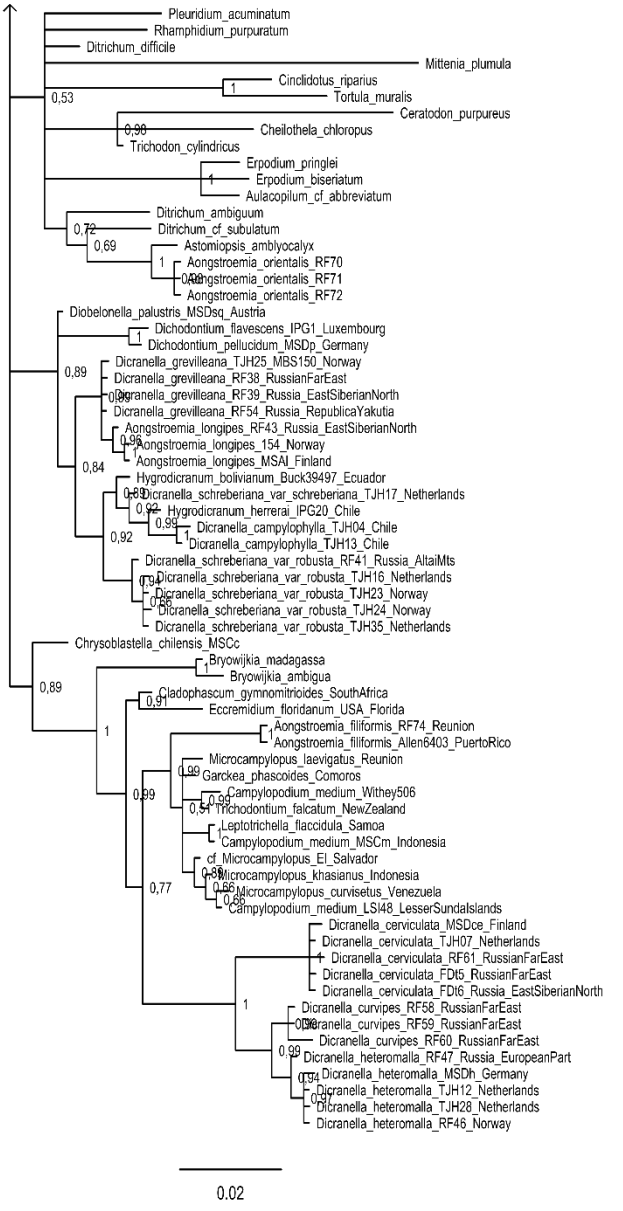
Appendix 6 Figure 3b



Appendix 6 Figure 4a

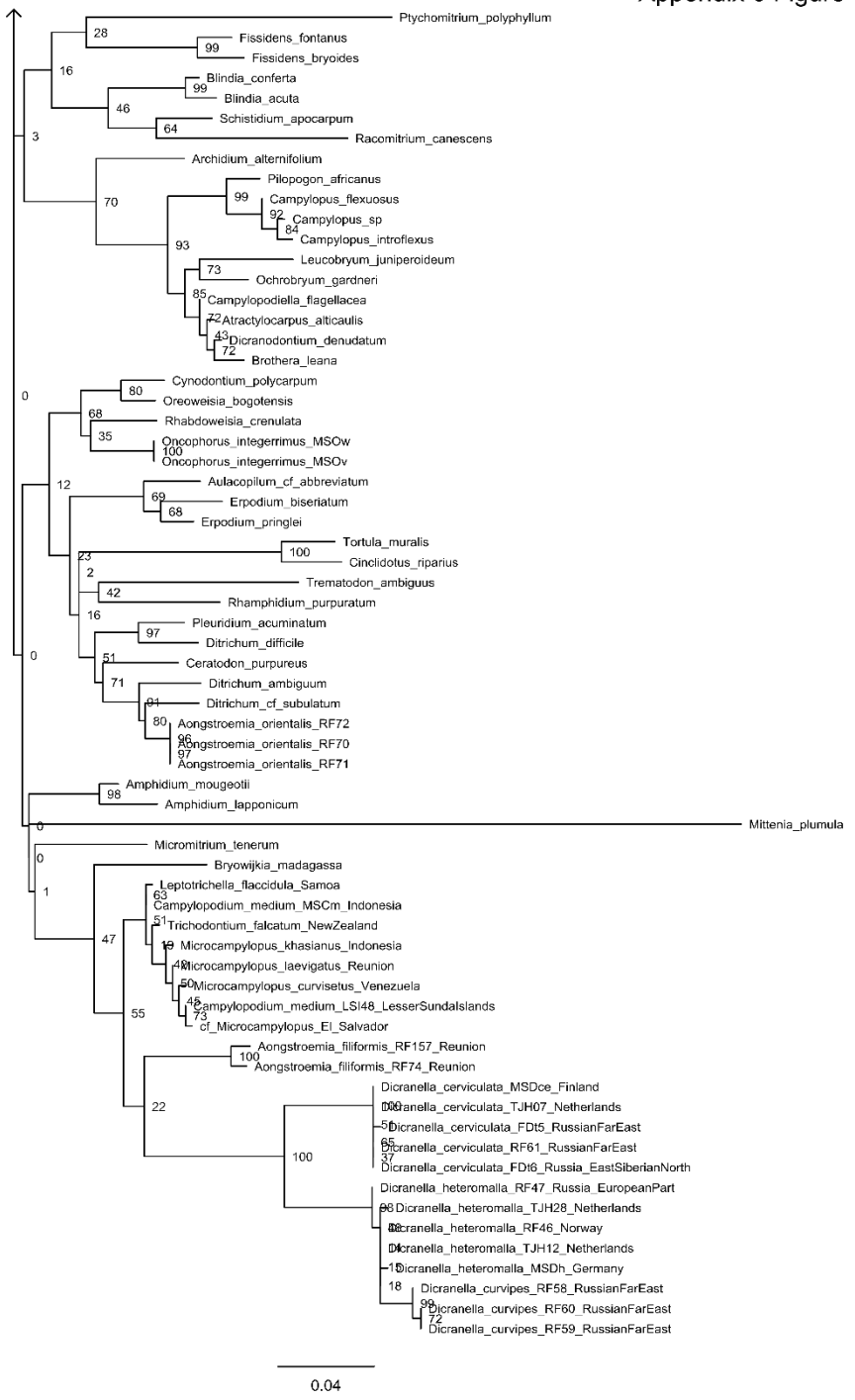


Appendix 6 Figure 4b



Appendix 6 Figure 5a

Appendix 6 Figure 5b



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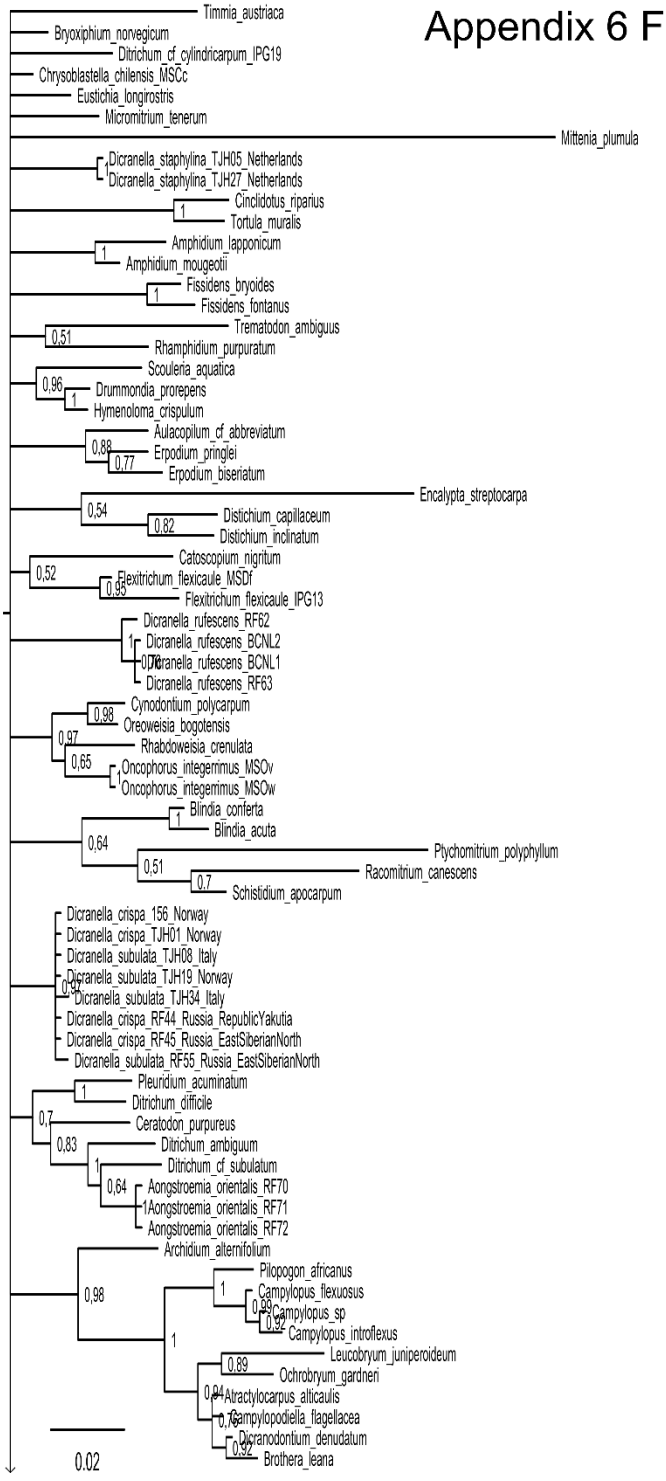
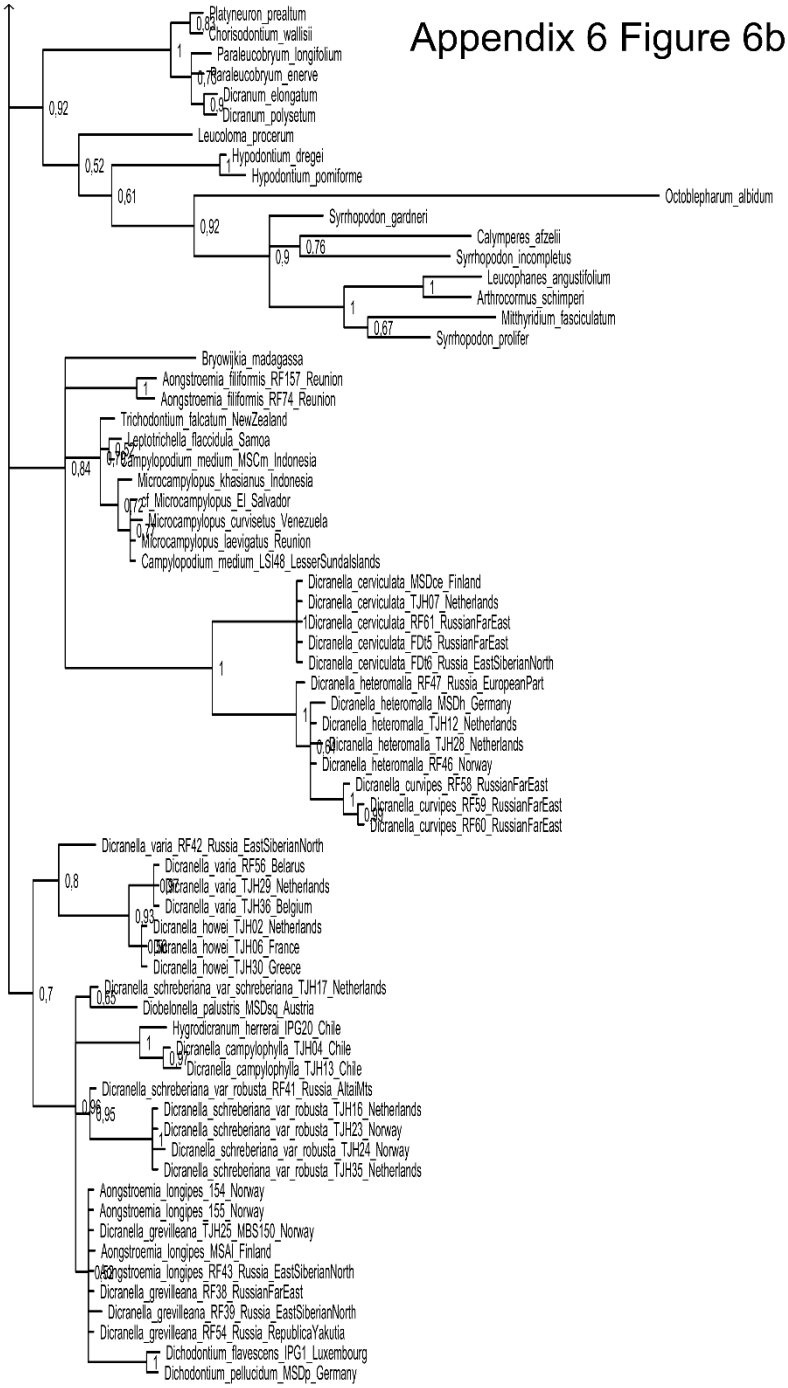


Appendix 6 Figure 6b



Curriculum Vitae

Marina Bonfim Santos was born on 21 October 1987 in Salvador, Brazil. She was raised in Bahia in close contact with nature, which greatly motivated her decision to pursue a bachelor's degree in Biological Sciences at the Federal University of Bahia (UFBA), which she obtained in 2009.

She discovered the world of the bryophytes during the second year of her undergraduate studies, when she started a research internship at the Brioflora lab. She studied the haplolepideous moss genus *Campylopus* in Bahia State, and later, during her master's studies, in the whole Northeast Brazil, under the supervision of Prof.dr. Cid José Passos Bastos. She obtained a master's degree in Botany from the State University of Feira de Santana (UEFS) in 2011. During her study, Marina contributed to inventories of bryophytes in different regions of Bahia. After her study, Marina worked as a researcher at herbarium HUEFS of the State University of Feira de Santana and, later, as a temporary teacher to Biology students at the Federal University of Bahia, which was a life changing experience as she discovered her passion for education.

Marina's interest on the moss genus *Campylopus* throughout this period and her desire to incorporate phylogenetic methods to her research led her to contact Dr. Michael Stech, who became her supervisor in the next stage of her training. In October 2013 she moved to Leiden to work on her PhD project on phylogenetic relationships of haplolepideous mosses (Dicranidae) and the evolution of the leucobryoid morphology at the Naturalis Biodiversity Center and Leiden University. She presented her results at conferences in Canada, Spain, the United Kingdom, and Marina's home country, Brazil. She also had the opportunity to supervise bachelor's and master's projects on topics linked to her own research. She moved back to Brazil in 2018, where she finished writing her thesis. After her graduation, Marina intends to use her experiences in research and education in projects dedicated to teaching Sciences to the Brazilian youth.

List of publications

Papers

Bonfim Santos, M., Fedosov, V., Hartman, T., Fedorova, A., Siebel, H., Stech, M. (2021). Phylogenetic inferences reveal deep polyphyly of Aongstroemiaceae and Dicranellaceae within the haplolepideous mosses (Dicranidae, Bryophyta). *Taxon 70*(2): 246–262.

Fedosov, V. E., Fedorova, A. V., Larraín, J., **Bonfim Santos, M.**, Stech, M., Kučera, J., Brinda, J. C., Tubanova, D. Y., von Konrat, M., Ignatova, E. A., & Ignatov, M. S. (2021). Unity in diversity: phylogenetics and taxonomy of Rhabdoweisiaceae (Dicranales, Bryophyta). *Botanical Journal of the Linnean Society, 195*(4), 545-567.

Bonfim Santos, M., & Stech, M. (2017). Testing hypotheses on suprageneric relationships and morphological evolution in the Leucobryaceae (Bryophyta). *Plant Systematics and Evolution*, *303*(10), 1383–1397.

Bonfim Santos, M., & Stech, M. (2017). Tackling relationships and species circumscriptions of *Octoblepharum*, an enigmatic genus of haplolepideous mosses (Dicranidae, Bryophyta). *Systematics and Biodiversity*, *15*(1), 16–24.

Selected conference abstracts

Bonfim Santos, M., Stech, M., Fedosov, V. (2021). Recent advances in Dicranidae phylogenetics. Symposium State of the art in Dicranidae systematics, BL2021 virtual conference by Université Laval, Quebec, Canada. (talk)

Bonfim Santos, M., Fedosov, V., Hartman, T., Siebel, H. Stech, M. 2017. *Dicranella*, a case study of misunderstood diversity of inconspicuous haplolepideous mosses. XXI Simpósio de Botânica Criptogâmica, Aranjuez, Spain. (talk)

Bonfim Santos, M., Pombo Geertsma, I., Hartman, T., Stech, M. 2016. Phylogeny of haplolepideous mosses – Circumscriptions of families and genera still need our attention. 67° Congresso Nacional de Botânica, Vitória, Brazil. (poster)

Bonfim Santos, M., Stech, M. 2015. Evolution of the leucobryoid morphology in Dicranidae (Bryophyta). Systematics Association Biennial 2015, Oxford, UK. (talk)

Bonfim Santos, M., Gama, R., Lang, A., Schäfer-Verwimp, A., Stech, M. 2014. Disentangling the *Campylopus savannarum* (Müll. Hal.) Mitt. (Bryophyta) species complex. 11th Latin-American Botanical Congress/65th National Botanical Congress, Salvador, Brazil. (poster)

Bonfim Santos, M., Stech, M. 2014. Leucobryaceae Schimp. (Bryophyta, Dicranidae) systematics: Taxonomic history and present challenges. 11th Latin-American Botanical Congress/65th National Botanical Congress, Salvador, Brazil. (poster)

Bonfim Santos, M., Bastos, C.J.P. 2009. O gênero *Campylopus* Brid. (Bryopsida: Leucobryaceae) no Estado da Bahia, Brasil. 60th National Botanical Congress, Feira de Santana, Brazil. (talk)

Theses

Bonfim Santos, M. 2011. Contribuição ao conhecimento do gênero *Campylopus* Brid. (Bryophyta, Leucobryaceae) no Nordeste do Brasil. Dissertação (Mestrado Acadêmico em Botânica), Universidade Estadual de Feira de Santana, Feira de Santana.

Acknowledgements

I thought that following a PhD track meant learning how to do science, while giving a small contribution to the field of research I love and making a few acquaintances on the way. What I did not predict is how much personal growth would come from this adventure, in so many aspects of my life. To live in a new country with such a different language, in contact with people from all over the world, their cultures and personalities, taught me a lot about myself and where I came from. The challenges from this period, science related and not, pushed me further through this journey of self-study. They caused some crises and demanded troubleshooting and re-evaluation of my own ways, but resulted in overdue mental health diagnoses, plenty hard work on their treatment and, finally, in a much happier and healthier Marina with a completed PhD thesis and published articles. I will forever keep in my heart everyone in the crowd who was fundamental for me to get here.

On behalf of my first science colleagues from the Brioflora lab at UFBA, I thank my former supervisor Cid Bastos for his guidance and enthusiasm, and Hermeson Cassiano for literally making me take the first steps to continue my training abroad.

I would like to thank the Brazilian foundation CAPES for the opportunity to pursue my PhD degree at Leiden through its Science without Borders program. I am deeply grateful for public funding to scientific education and research, and the amount of positive change that brings to the world.

I am moreover deeply grateful to my supervisor Michael Stech. Supervising me was not always easy, but he was very understanding and willing to move forward. I had a blast with our discussions and ideas about our shared interest, the phylogeny of haplolepideous mosses. For all that, thank you Michael!

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