EXTENDING THE FOSSIL RECORD OF POLYTRICHACEAE (BRYOPHYTA): INSIGHTS FROM THE EARLY CRETACEOUS OF VANCOUVER ISLAND, CANADA

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A Thesis Presented to

The Faculty of Humboldt State University

In Partial Fulfillment of the Requirements for the Degree

Master of Science in Biology

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May 2018

ABSTRACT

EXTENDING THE FOSSIL RECORD OF POLYTRICHACEAE (BRYOPHYTA): INSIGHTS FROM THE EARLY CRETACEOUS OF VANCOUVER ISLAND, CANADA

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Diverse in modern ecosystems, mosses are dramatically underrepresented in the fossil record. Furthermore, most pre-Cenozoic mosses are known only from compression fossils, which lack detailed anatomical information. Lower Cretaceous deposits at Apple Bay (Vancouver Island, British Columbia, Canada) contain a diverse anatomically preserved flora that includes numerous bryophytes, many of which have yet to be characterized. Among them is a polytrichaceous moss that is described here as *Meantoinea alophosioides* gen. et sp. nov. *Meantoinea alophosioides* represents the first occurrence of gemma cups in a fossil moss and is the oldest unequivocal record of Polytrichaceae, providing a hard minimum age for the group of 136 Ma (Valanginian). In order to assess the phylogenetic relationships of fossil Polytrichaceae (including *Meantoinea*) and compare hypotheses of relationships recovered using molecular vs morphological methods, I conducted a comprehensive morphology-based phylogenetic study of the family. This phylogenetic study used a dataset of 100 morphological characters scored for 44 species of acrocarpous mosses, and parsimony as the optimality criterion. Results of the phylogenetic analysis suggest that morphology is useful in

resolving phylogenetic relationships in the Polytrichaceae and that both fossil Polytrichaceae have stable phylogenetic relationships. However, rooting experiments demonstrate that there is no superior way to root analyses and indicate that relationships within the family are best evaluated using unrooted networks without outgroup taxa. These rooting problems suggest that additional information is needed to understand the phylogenetic relationships of Polytrichaceae. Such additional information could come from fossils of stem group polytrichaceous mosses, which await discovery.

ACKNOWLEDGEMENTS

I am grateful to Glenn Shelton (Humboldt State University) and Adolfina Savoretti (Instituto de Botánica Darwinion) for their insightful discussions regarding fossil bryophytes. I also thank Marie Antoine and Steve Sillett (Humboldt State University), for making a specimen of *Dendroligotrichum dendroides* available for examination, the Willi Hennig Society for making TNT version 1.5 free to use, and to and Russell Bryan for help rendering the phylogenetic matrix. This work was supported by the Paleontological Society G. Arthur Cooper Award, a Botanical Society of America undergraduate student research award, a Botanical Society of America Graduate Research Award, a Humboldt State University Master's Student Research Award, the Alistair and Judith McCrone Graduate Fellowship (Humboldt State University), and a National Science Foundation Graduate Research Fellowship to A.C.B, as well as by a NSF grant [IIA-1322504] to A.M.F.T. Finally, this research would not have been possible without the support and encouragement of numerous friends, family members, and teachers that have helped me along the way.

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CHAPTER ONE

EXTENDING THE FOSSIL RECORD OF POLYTRICHACEAE: EARLY CRETACEOUS *MEANTOINEA ALOPHOSIOIDES* GEN. ET SP. NOV., PERMINERALIZED GAMETOPHYTES WITH GEMMA CUPS FROM VANCOUVER ISLAND

[published in the *American Journal of Botany*]

1.1 INTRODUCTION

The Polytrichaceae, sole family of the class Polytrichopsida, is a diverse and evolutionarily distinct group of mosses characterized by a high level of sporophyte and gametophyte complexity (Smith 1971; Schofield, 1985; Smith Merrill, 2007). Polytrichaceous moss gametophytes are easily recognizable by their complex leaves which typically bear adaxial photosynthetic lamellae and have sophisticated conducting tissues (Smith, 1971; Hébant, 1977). Polytrichaceous sporophytes are equally distinctive, since most species have many non-hygroscopic, nematodontous, peristome teeth, which are considered non-homologous to those of other moss lineages (Smith Merrill, 2007; Bell et al., 2008). While these features are generally conserved within the family, polytrichaceous mosses have a broad range of growth habits, including the largest and most anatomically complex moss gametophytes (e. g. *Dawsonia superba* Greville; Zanten, 1973), as well as mosses with extremely small ephemeral shoots and long-lived protonemata (e.g. *Pogonatum camusii* Tuow; Hyvönen, 1989).

While it is clear that the Polytrichaceae occupy a basal phylogenetic position among peristomate mosses, the family has no close living relatives, and, therefore, it is not clear how distinguishing characters, e. g. photosynthetic lamellae, distinctive peristome, and complex conducting tissues, evolved (Renzaglia et al., 2007; Chang and Graham 2011, 2014). Given this

absence of close living relatives, fossils are crucial for understanding the early evolution of the Polytrichaceae. Fortunately, compared to other mosses, the Polytrichaceae have a rich Cenozoic fossil record (i.e., younger than 66 Ma), with ten species described from Europe, North America, and Asia (Göppert, 1853; Knowlton, 1926; Yasui, 1928; Frahm, 2004, 2010). Unfortunately, very few moss fossils reported from older sediments are available to throw light on earlier stages in the evolution of this basal moss lineage.

Here we describe an anatomically preserved polytrichaceous moss of Early Cretaceous age (Valanginian, ca. 136 Ma), based on five permineralized gametophyte shoots from the Apple Bay locality on Vancouver Island (British Columbia, Canada). This moss is described as a new genus and species, characterized by terminal gemma cups bearing lenticular gemmae, leaves with short photosynthetic lamellae restricted to the costa, and a bistratose lamina with an adaxial layer of mamillose cells. This is one of the most completely known pre-Cenozoic fossil mosses to date and represents the oldest unequivocal record of the Polytrichaceae and the first report of gemma cups in a fossil moss. This discovery offers a crucial look at pre-Cenozoic polytrichaceous moss diversity, which is necessary for understanding evolution in this distinctive and diverse bryophyte lineage.

1.2 MATERIALS AND METHODS

The focus of this study is the most completely known unicostate moss gametophyte from Apple Bay. Five gametophytes of this moss are preserved by cellular permineralization in four carbonate concretions, as part of an allochthonous fossil assemblage deposited in nearshore marine sediments (e.g., Stockey and Rothwell, 2009). The concretions were collected from sandstone (greywacke) beds exposed on the northern shore of Apple Bay, Quatsino Sound, on

the west side of Vancouver Island, British Columbia, Canada (50° 36’ 21” N, 127° 39’ 25” W; UTM 9U WG 951068) (Stockey and Rothwell, 2009). The concretion-bearing layers are regarded as Longarm Formation equivalents and have been dated by oxygen isotope analyses to the Valanginian (Early Cretaceous, ca. 136 Ma) (Stockey et al., 2006; D. Grocke, personal communication, 2013).

This diverse Early Cretaceous flora includes lycophytes, equisetophytes, at least 10 families of ferns (Smith et al., 2003; Hernandez-Castillo et al., 2006; Little et al., 2006a, b; Rothwell and Stockey, 2006; Stockey et al., 2006; Vavrek et al., 2006; Rothwell et al., 2014) and numerous gymnosperms (Stockey and Wiebe, 2008; Stockey and Rothwell, 2009; Klymiuk and Stockey, 2012; Rothwell and Stockey, 2013; 2016; Rothwell et al., 2014; Atkinson et al., 2014a, b; Ray et al., 2014; Klymiuk et al., 2015), as well as fungi (Smith et al., 2004; Bronson et al., 2013) and a lichen whose thallus shows modern heteromerous organization (Matsunaga et al., 2013). The Apple Bay flora is also emerging as the most diverse pre-Cenozoic assemblage of fossil bryophytes (Shelton et al., 2015; Tomescu, 2016), with liverworts and many distinct moss morphotypes. Recently, a new family of tricostate mosses was erected and subsequently expanded based on permineralized material from Apple Bay (Shelton et al., 2015, 2016). These mosses, including *Tricosta plicata* Shelton, Stockey, Rothwell, et Tomescu (as the most completely known moss gametophyte in the fossil record) and *Krassiloviella limbelloides*, also provide the earliest hard evidence for hypnanaean pleurocarpous mosses.

Fossil-containing concretions were sliced into slabs and sectioned using the cellulose acetate peel technique (Joy et al., 1956). Slides were prepared using Eukitt, xylene-soluble mounting medium (O. Kindler GmbH, Freiburg, Germany). Micrographs were taken using a Nikon Coolpix E8800 digital camera on a Nikon Eclipse E400 compound microscope. Images

were processed using Photoshop (Adobe, San Jose, California, USA). All specimens and preparations are housed in the University of Alberta Paleobotanical Collections (UAPC-ALTA), Edmonton, Alberta, Canada.

1.3 RESULTS

1.3.1 SYSTEMATICS

Class Polytrichopsida Doweld

Order Polytrichales Fleisher

Family Polytrichaceae Schwägrichen

Genus *Meantoinea* Bippus, Stockey, Rothwell et Tomescu, gen. nov.

Generic diagnosis Gametophytes unbranched. Leaves strongly costate with distinct sheathing base and free blade; costa with stereids and central arc of deuters. Photosynthetic lamellae restricted to costa of leaf blade. Leaf blade with bistratose lamina; adaxial cells mamillose, abaxial cells bulging. Leaf margins bearing unicellular teeth. Terminal gemma cups comprised of densely packed leaves.

Etymology *Meantoinea* is named in recognition Marie E. Antoine's (Humboldt State University) key contribution to the bryological training of many students of the Apple Bay bryoflora.

Type species *Meantoinea alophosioides* Bippus, Stockey, Rothwell et Tomescu, sp. nov.

Specific diagnosis Gametophyte shoots unbranched, at least 4 mm tall. Stem diameter ca. 0.3 mm. Stem cross sections with epidermis of small cells; central strand ca. 0.1 mm thick. Leaves densely packed along stem, 2/5 phyllotaxis. Leaves at least 2.64 mm long. Leaf base with unistratose lamina, 540-960 µm wide, clasping stem along ca. 0.6 mm. Leaf blade much narrower than sheathing bases, ca. 150-500 µm wide; linear, with bistratose lamina. Costa

strong, abaxially convex, up to 290 µm wide in leaf base, up to 153 µm wide in leaf blade. Costal anatomy complex; deuters forming central arc. Stereid band thick, adaxial to deuters; abaxial epidermis with isodiametric cells; smaller conducting parenchyma adaxial to deuters; overlain by adaxial layer of intermixed parenchyma and stereids. Adaxial cells of bistratose leaf blade lamina thick-walled, mamillose; abaxial cells of lamina smaller, bulging in distal leaf region. Leaf margins with thick-walled unicellular teeth. Adaxial lamellae in 4-10 rows restricted to costa of leaf blade. Lamellae 2-3 cells (31-40 µm) tall with mamillose marginal cells and smaller isodiametric regular cells. Terminal gemma cups of densely packed leaves, containing ca. 6 stalked gemmae. Gemma cups ca. 2.6 mm wide, 1.2 mm deep. Gemmae lenticular, ca. 100 x 100 x 50 µm; gemma stalk short, rhizoid-like.

Etymology *alophosioides* refers to the close similarity between this species and *Alophosia azorica* (Renauld et Cardot) Cardot.

Holotype hic designatus Gemmiferous gametophyte shoot in rock slab UAPC-ALTA P15393 B (slides B bot series a).

Paratypes UAPC-ALTA P13158 Cbot; P15800 Cbot.

Locality Apple Bay, Quatsino Sound, northern Vancouver Island, British Columbia (50° 36’ 21” N, 127° 39’ 25” W; UTM 9U WG 951068).

Stratigraphic position and age Longarm Formation equivalent; Valanginian, ca. 136 Ma (Early Cretaceous).

1.3.2 DESCRIPTION

Habit and stem anatomy Gametophyte shoots, traced for up to 4 mm of length have stems 192-346 µm in diameter (mean = 272.9 µm; n = 9). None of the specimens exhibits branching. Anatomically, stems are composed of three distinct layers (Fig. 1B, 1C): an outermost epidermal

layer, one to several cells thick, consisting of small isodiametric cells 6-10 µm in diameter (mean = 7.5 µm; n = 12); a cortex composed of larger isodiametric cells 10-24 µm in diameter (mean = 15.4 µm; n = 10); and a central conducting strand ca. 100 µm in diameter, preserved in only one of the specimens and consisting of narrow and taphonomically compressed cells (Fig. 1B, 1C).

Leaf morphology and anatomy The shoots have 2/5 phylotaxis (Fig. 3). In the apical portion of a shoot that terminates in a gemma cup (Figs. 3, 4), leaves at successive nodes are spaced 60-90 µm apart. The leaves have a broad base that sheathes the stem and a much narrower blade that is adaxially concave and diverges at a wide angle (Figs. 1A, 1F, 2C-2G). The transition from leaf base to leaf blade, observed in serial transverse sections, is associated with a sharp decrease in width (Figs. 1A, 2C-2G). Leaf tips are sometimes recurved, and are incompletely preserved (Figs. 2E-2G; 6). Leaves have a strong costa that runs the entire preserved length of leaves and comprises 25-30% of leaf width. The costa protrudes abaxially and is broader and thinner in the leaf base (Fig. 1D), becoming narrower and thicker, with a semi-circular profile distally (Figs. 1F, 2C-2G). The leaf blades bear adaxial photosynthetic lamellae covering the entire width of the costa (Figs. 1F-1G; 2C-2G; 2I) and the entire preserved length of the leaf.

Leaf bases are 540-960 µm wide (mean = 694.3 µm; n = 17), sheathing the stem for ca. 0.6 mm before the leaf blade curves away from the stem. The linear blade, at least 2 mm long and 75% of overall leaf length, is much narrower than the base, 142-482 µm (mean = 308 µm; n = 21) (Fig. 6). Leaf length was reconstructed based on three series of sections that represent partial lengths of three distinct leaves; the three leaves have closely similar sizes (in cross section) and the three series are partially overlapping longitudinally. Leaf bases have a broad unistratose lamina consisting of square-isodiametric cells 7.2-15.6 µm (mean = 10.7 µm; n = 9), with the marginal cells significantly smaller than other laminal cells. Leaf blades have a much

narrower bistratose lamina. The adaxial cells of the lamina are thick-walled, mamillose, and 8.4-13.0 µm wide x 12.0-16.8 µm tall x 12.0-16.8 µm long. The abaxial cells of the lamina are isodiametric in cross section, with an abaxial bulge, 7.2-12.0 µm wide x 8.4-13.2 µm tall x 14.4-15.6 µm long; in the transition zone between leaf base and leaf blade, cells of the abaxial lamina lack an abaxial bulge and are not as tall as those further up the blade. Leaf margins bear thick-walled unicellular teeth 27 µm long x 10.8-23.0 µm wide (mean = 16.2; n = 2).

The costa is 120-288 µm wide (mean = 185.5 µm; n = 23) and 40.8-92.4 µm thick (mean = 62.9 µm; n = 14) in the sheathing leaf bases. In the leaf blade, the costa is up to 153 µm wide and 90-104 µm thick basally, tapering toward the leaf tip. In cross section, the costa shows several distinct layers. The central region is occupied by an arc of large (9.6-16.8 µm; mean = 13.5 µm; n = 8), elongated, thick-walled deuter cells with circular cross-sectional outline, comparable to those of extant Polytrichaceae (e.g. Smith, 1971; H*é*bant, 1977); "deuter" is a term used for specialized cells of the leaf costa that are though to conduct photosynthates (see H*é*bant, 1977). Abaxial to the deuters, is a 3-5 cell thick zone of small-diameter (3.6-6µm; mean = 4.1µm; n = 13), thick-walled stereids. Abaxial to this band of stereids, the epidermis consists of small, 6.0-7.2 µm (mean = 6.6 µm; n = 8) diameter cells; the arrangement of these cells gives the costa an abaxially grooved surface (Figs. 1F, 2G). Adaxial to the deuters, is a layer of smaller-diameter circular cells (8.4-12.0 µm; mean = 10.3 µm; n = 10) comparable to the conducting parenchyma described. in the costa of some polytrichaceous mosses (Hébant, 1977; Scheirer, 198). Primarily adaxial to, but also intergrading with, the conducting parenchyma is a second thin band of stereids similar to the abaxial ones.

The leaf blades bear for their entire preserved length 4-10 photosynthetic lamellae restricted to the adaxial surface of the costa (Figs. 1F-1G; 2C-2G, 2I; 6). The lamellae are 2-3

cells tall (ca. 30-40 µm overall height; mean =36.1 µm; n = 8) and consist of small, isodiametric cells (8.4-14.4 µm; mean = 11.8 µm; n = 7). The marginal cells of lamellae are thick-walled and mamillose in a similar way to the adaxial lamina cells. These marginal cells are 12-14.4 µm wide (mean = 13.4 µm; n = 7) and 15.6-19.2 (mean = 17.3 µm; n = 7) µm tall.

Asexual reproductive structures One of the five gametophyte shoots terminates in a gemma cup formed by densely packed leaves, similar to the gemma cups of *Tetraphis pellucida* Hedwig (Crum, 2001) and *Alophosia azorica* (Renault and Cardot) Cardot (Smith, 1971). The cup is 2.6 mm in diameter and 1.2 mm deep, and contains six laterally-flattened gemmae (Fig 4A, C; 5A). The gemmae measure ca. 100 x 100 x 50 µm and are borne on short stalks ca. 8.5 µm in diameter. In cross sections of the cup, each gemma displays up to 10 relatively large, thin-walled isodiametric cells (13.2-24 µm; mean = 18.; n = 10). These lenticular gemmae have a unistratose margin and are closely similar to those of *Tetraphis pellucida* (Fig 4D; 5B) and *Alophosia azorica* (Crum, 2001; Smith, 1971).

1.4 DISCUSSION

1.4.1 TAXONOMIC PLACEMENT OF *MEANTOINEA ALOPHOSIOIDES* GEN. ET SP. NOV.

A diagnostic suite of characters unequivocally place *Meantoinea* in the moss family Polytrichaceae. First, the leaves have a complex costal anatomy with deuters, stereids, and conducting parenchyma typical of polytrichaceous mosses (Smith, 1971; Hébant, 1977; Scheirer et al., 1983). Second, the leaves are differentiated into a broad sheathing leaf base with a unistratose lamina and a narrower leaf blade, a morphology found in many polytrichaceous mosses (Smith, 1971; Schofield, 1985). Third, the leaf blade has a bistratose lamina with an adaxial layer of mamillose cells, a feature of basal Polytrichaceae (e.g., *Alophosia* Cardot,

Lyellia Brown, and *Bartramiopsis* (James) Kindberg; Smith, 1971; Bell and Hyvonen, 2010). Fourth, the stem has a robust conducting strand, which is found in almost all polytrichaceous mosses (Smith, 1971). Fifth, the leaves of *M. alophosioides* bear short adaxial, unbranched photosynthetic lamellae with mamillose marginal cells. Finally, *M. alophosioides* produces stalked lenticular gemmae that are extremely similar to those produced by the polytrichaceous moss *Alophosia azorica* (Smith, 1971). . Several moss lineages combine some features from this list, but the Polytrichaceae is the only group in which all the above features co-occur.

A few genera in the Pottiaceae (*Pterygoneurum* Juratzka, *Aloina* Kindberg, *Aloinella* Cardot, and *Crossidium* Juratzka) have adaxial outgrowths on leaves, a central strand in the stem, and complex costal anatomy (Delgadillo, 1975; Zhao et al., 2008; Zander, 2007). However, only in *Pterygoneurum* are the adaxial outgrowths organized into longitudinal files forming lamellae; adaxial leaf outgrowths in the other three pottiaceous genera are just irregularly arranged filaments (Zander, 2007; Zhao et al., 2008). Species of *Pterygoneurum* differ from *Meantoinea* in several characters: taller lamellae (ca. 12 cells tall), occasional branching of stems, absence of mamillose marginal cells, unistratose lamina, leaves not differentiated into a broad sheathing base and narrow blade, and absence of gemma cups. *Aligrimmia peruviana* Williams (Grimmiaceae) also has complex costal anatomy, adaxial photosynthetic lamellae, and a central strand, but is significantly different from *Meantoinea* in its taller lamellae (6-7 cells tall) with undifferentiated marginal cells, a unistratose lamina, and lack of gemma cups (Murray, 1984).

1.4.1.1 Justification for a new genus

Compared to extant members of the Polytrichaceae, *Meantoinea* is most similar to three genera: *Alophosia*, *Lyellia*, and *Bartramiopsis* (Table 1). In addition to polytrichaceous characters more broadly shared within the family, *Meantoinea* shares with these three genera the bistratose

lamina with an adaxial layer of mamillose cells and leaves differentiated into a broad sheathing base and a narrow blade, as well as comparable costal anatomy. Despite these similarities, the gametophytes of *Meantoinea* are different from each of the three genera in a number of ways (Table 1). First, *Alophosia*, *Lyellia*, and *Bartramiopsis* all have multicellular teeth at their leaf margins, whereas *Meantoinea* has unicellular teeth. Second, in contrast to *Meantoinea*, *Bartramiopsis* and *Lyellia* have taller lamellae with round marginal cells and do not produce gemma cups. Third, unlike *Meantoinea*, *Lyellia* produces double teeth and short lamellae abaxially on leaves (Ivanova and Ignatov 2007). Fourth, although *Alophosia* produces gemmae in gemmacups quite similar to those of *Meantoinea* and has costal anatomy closely similar to the latter, that genus differs significantly from the fossil by completely lacking photosynthetic lamellae.

Meantoinea is also substantially different from both of the known genera of extinct Polytrichaceae: *Polytrichites* Britton and *Eopolytrichum* Konopka, Herendeen, Smith Merrill et Crane (Mägdefrau, 1957; Frahm, 1999, 2010; Konopka et al., 1997). *Eopolytrichum antiquum* Konopka, Herendeen, Smith Merrill et Crane is based on charcoalified sporophyte capsules from the Campanian (Late Cretaceous) of Georgia, USA, but several charcoalified gametophytes with polytrichaceous features have been described in association (but not physical connection) with the type material and may represent the same species (Konopka et al., 1997). *Meantoinea* is notably different from the gametophytes associated with *E. antiquum* in two ways. First, *Eopolytrichum* bears lamellae on both the costa and lamina of the leaf blade, whereas *Meantoinea* bears lamellae only on the costa. Second, *Meantoinea* has a bistratose leaf blade lamina with mamillose cells, whereas *Eopolytrichum* has a unistratose lamina.

The genus *Polytrichites* is a form genus for polytrichaceous fossils that do not preserve enough diagnostic information to be placed in any of the other known genera (Frahm, 2010). This genus includes two species known from Eocene Baltic amber (Frahm, 2010), one species known from compressions in the Miocene of Washington, USA (Knowlton, 1926), and an anatomically-preserved shoot fragment from the Upper Miocene of Japan (Yasui, 1928; Yamada et al., 2015). Given the disparity between the level of preservation of *Meantoinea*, which provides tremendous detail on the morphology and anatomy of this moss, and the much less completely characterized fossils included in genus *Polytrichites*, the latter is not an appropriate placement for the Apple Bay moss.

The genera of living Polytrichaceae are differentiated primarily based on sporophyte characters (Smith, 1971; Konopka, 1997). In some genera, gametophyte characters can vary substantially between species (e.g. *Pogonatum* P. Beauv.; Smith, 1971; Hyvönen, 1989). Because of this, gametophyte characters have been considered less reliable taxonomically, in general, for the Polytrichaceae. In this context the taxonomic placement of *Meantoinea*, a polytrichacean known only from gametophytes, has to be considered carefully. On the one hand, the differences between *Meantoinea* and other polytrichaceous genera are not greater than the intrageneric gametophyte variation seen in the most heterogeneous polytrichaceous genera. This would suggest that separation of *Meantoinea* as a distinct genus may not be warranted. On the other hand, most polytrichaceous genera do not show nearly as much variation in gametophyte morphology. Moreover, gametophyte characters are a significant component of most generic concepts in the family. In this context, separation of *Meantoinea* as a distinct genus is justified not only by the significant differences between the gametophytes of this moss and those of all

known Polytrichaceae, but also by the fact that *Meantoinea* combines features of several polytrichaceous genera (Table 1).

1.4.2 POLYTRICHACEOUS MOSSES IN THE FOSSIL RECORD

Family Polytrichaceae has a surprisingly rich fossil record, with three genera described from Cenozoic and Cretaceous records (Table 2). *Eopolytrichum antiquum* is based on charcoalified sporophyte material from the Campanian (Late Cretaceous) of Georgia (Konopka et al., 1997). If the gametophyte material in the same assemblage, which exhibits polytrichaceous features, represents the same species, then *E. antiquum* is the most completely known fossil polytrichacean. *Eopolytrichum* combines features of derived peristomate Polytrichaceae (*Polytrichum* Hedwig sect. *Polytrichum* and sect. *Juniperifolia*) with features of a basal eperistomate grade (*Alophosia*, *Lyellia*, and *Bartramiopsis*) (Konopka et al., 1997; Hyvönen et al. 2004; Bell and Hyvönen, 2008, 2010). The most recent phylogenetic study of the Polytrichaceae to include *E. antiquum* (Hyvönen et al., 2004) recovered the fossil in a clade with *Polytrichum*, suggesting that *Eopolytrichum* secondarily lost its peristome and convergently evolved similarities with the basal eperistomates. However, support for the *Eopolytrichum*+ *Polytrichum* clade is low (Hyvönen et al., 2004; Bell et al., 2015).

All other fossil Polytrichaceae are known only from gametophytes. Most of these fossils are preserved in Middle Eocene Baltic Amber (Wolfe et al., 2016). The amber fossils include several species of the extant genus *Atrichum* P. Beauv., as well as two species of the polytrichaceous form genus *Polytrichites* (Table 2). Except for *A. mamillosum* Frahm, which exhibits mamillose cells in the lamina and lamellae, unlike any extant *Atrichum*, the fossil *Atrichum* species described from amber are very similar to extant species (Frahm, 2004). Three

Polytrichum species listed by Göppert (1853) have no descriptions and are considered invalid (Tropicos.org – Missouri Botanical Garden, 10 Jul 2016, http://www.tropicos.org).

Two other species of *Polytrichites* have been described from Tertiary rocks. *Polytrichites spokanensis* Britton, a compression reported from the Miocene Latah Formation of Washington State, cannot be reliably assigned to the Polytrichaceae (or any other group of acrocarpous mosses) because of insufficient taxonomically diagnostic characters (Knowlton, 1926). *Polytrichites aichiensis* Yasui is an anatomically preserved stem fragment from the Upper Tertiary of Japan (Yasui, 1928). This fossil has a central strand with both hydroids and leptoids, a feature unique to the Polytrichaceae, but lacks any other taxonomically informative characters (Smith, 1971; Hébant, 1977).

A fossil exhibiting some similarity with the Polytrichaceae, *Livingstonites gabrielae* Vera, is known from permineralized moss gametophytes discovered in the Aptian of Antarctica (Vera, 2011). *Livingstonites* is described as an *incertae sedis* member of the basal acrocarp grade. The moss has a strong costa with complex anatomy including a band of deuters and at least one abaxial stereid band, which are found in several moss lineages, including the Polytrichaceae. Additionally, a leaf cross section (Figs. 4 and 5 in Vera, 2011) of *Livingstonites* may show short lamellae typical of polytrichaceous leaves at the intersection between leaf blade and sheathing leaf base. Further detailed examination of these structures in *Livingstonites* is necessary to determine if this moss is indeed a polytrichacean.

Meantoinea alophosioides is ca. 50 Ma older than any other unequivocal Polytrichaceae, thus providing a hard minimum age of 136 Ma for the family. This species is only the second report of pre-Cenozoic Polytrichaceae and documents the best characterized fossil polytrichaceous gametophytes, with details of internal anatomy, leaf morphology, and asexual

reproduction. The exquisite preservation of *M. alophosioides* supports the ideas that bryophytes have better preservation potential than previously thought, and that the scarcity of pre-Cenozoic bryophyte fossils reflects primarily a lack of bryological expertise in the paleobotanical community rather than paucity of fossils (Tomescu, 2016). *Meantoinea alophosioides* also expands the diversity of thoroughly characterized bryophytes in the Apple Bay flora to two families: the Tricostaceae (a family of extinct pleurocarpous mosses; Shelton et al., 2015) and the Polytrichaceae. A diverse array of permineralized fossil mosses from Apple Bay, including more species of Polytrichaceae, awaits further description (Tomescu, 2016).

1.4.3 GEMMAE IN THE FOSSIL RECORD

Naiadita lanceolata Brodie provides the oldest unequivocal evidence of gemmae in the fossil record. *Naiadita* is a leafy liverwort abundant in the Rhaetian (Late Triassic) of England and produces terminal gemma cups on gemmiferous shoots that are ubiquitous in the fossil layers (Harris, 1939). The gemma cups are composed of numerous leaves and contain sessile gemmae ca. 400 µm in diameter. The gemmae are lenticular with an oval outline and four cells across; they have unistratose margin and are 2 cells thick at the center.

Marchantites huolinhensis Li et Sun is a complex thalloid liverwort preserved as compressions with cuticular preservation from the Lower Cretaceous (Valanginian-Hauterivian) of China (Li et al., 2014). *Marchantites huolinhensis* bears gemma cups with an elliptical to circular outline, 1.1-2.5 mm in diameter. The content of the cups is incompletely preserved and gemmae could not be identified unequivocally. The gemma cups of *M. huolinhensis* are nevertheless compelling, and this fossil represents the oldest record of such structures in a thalloid liverwort. Anatomically preserved discoid gemmae from mid-Cretaceous deposits in Australia have been described as *Marchantites marguerita* Dettman et Clifford (2000).

Preserved as dispersed fossils, these uni- to bistratose gemmae are 160-440 µm across and are borne on short unicellular stalks 60 µm in diameter. Additionally, the gemmae bear a meristematic peripheral notch on either side. The small size, discoid shape, unicellular stalk, and paired peripheral meristems are features shared with extant *Marchantia* L. and *Lunularia* Adanson gemmae, indicating that these fossils are the gemmae of a complex thalloid liverwort.

Another liverwort occurrence, from the Lower Cretaceous (Aptian-Albian) of Spain, has been reported to include specimens bearing gemma cups, as well as dispersed gemmae (Diéguez et al., 2007). However, in our opinion additional, better preserved specimens are required to provide unequivocal evidence for the two types of structures and hence, for the inferred marchantiacean affinities of these fossils.

For mosses, the only unequivocal reports of fossil gemmae come from three species of *Calymperes* Swartz (Calymperaceae) described from Early-Middle Miocene Dominican Amber (Vintent and Macphee, 1996). Specimens referred to the extant species *Calymperes palisotii* Schwägrichen were the first moss fossils discovered with gemmae (Frahm and Reese, 1998). This moss, like all extant species of *Calymperes*, bears a terminal cluster of fusiform to clavate gemmae adaxially on leaf apices (Reese, 2007). Gemmiferous specimens belonging to two additional extant species of *Calymperes* (*C. levyanum* Bescherelle and *C. smithii* Bescherelle) have subsequently been reported from Dominican Amber (Frahm and Newton, 2005).

Cup-like structures have been reported in *Palaeocampylopus buragoae* Ignatov et Shcherbakov, a *Campylopus*-like moss from the Early Permian of Russia (Ignatov and Shcherbakov, 2009). Similarities with the perigonia of *Campylopus* Brid. have led the authors to describe these structures as putative perigonia, but they could alternatively represent terminal gemma cups. However, because *Palaeocampylopus* are preserved as compression fossils that do

not show anatomical detail, the nature of the cup-like structures cannot be determined with certainty.

Meantoinea alophosioides provides the first unequivocal fossil record of gemma cups containing gemmae, in mosses. These also represent the only record of such moss structures for the Mesozoic. The gemmae of *Meantoinea* are remarkably similar in anatomy and morphology to those of the extant species *Tetraphis pellucida* (Fig. 4). This demonstrates that gemma cups formed from modified leaves, as well as gemmae very similar to those of extant species, had evolved in mosses by the Early Cretaceous. While *Naiadita* demonstrates that the leafy liverworts had evolved gemma cups by the Triassic, no extant leafy liverworts have similar gemma cups formed from modified leaves. The complex thalloid liverworts from China and Australia (*Marchantites huolinhensis* and *M. marguerite*; Li et al., 2014; Dettman and Clifford, 2000) indicate that gemma cups anatomically and morphologically similar to those of extant species had evolved in the group by the mid-Cretaceous. In light of this fossil record, it is possible that gemma cups evolved independently in both liverworts and mosses by the Mesozoic, and have persisted, virtually unchanged, to the present.

1.4.4 MOLECULAR CLOCK CALIBRATIONS AND THE FOSSIL RECORD OF POLYTRICHACEAE

As pointed out by Wilf and Escapa (2015, 2016), in a phylogenetic context, fossils have dual utility in studies addressing the age of lineages. On the one hand, new fossil discoveries serve as independent direct tests for existing divergence age estimates. On the other hand, when incorporated as calibration points, these fossils can be used to improve the precision of such estimates.

Most fossil Polytrichaceae are known from Eocene Baltic amber and are, thus, not useful in attempts to date basal phylogenetic divergences in a group whose evolutionary history extends

far beyond the Eocene, into the Mesozoic (Bell et al., 2015). The only fossils that can provide critical calibration points in the Mesozoic are *Eopolytrichum antiquum* and *Meantoinea alophosioides*. Unfortunately, the phylogenetic position of *E. antiquum* as part of a clade with *Polytrichum* is weakly supported (Hyvönen et al., 2004; Bell et al., 2015); therefore, its usefulness as a calibration point for that clade is limited. Additionally, if *Eopolytrichum* is a close relative of *Polytrichum*, then, as a highly- derived member of the Polytrichaceae, it cannot provide a reliable divergence age estimate for the family as a whole. On the other hand, *M. alophosioides* is closely similar to basal members of the Polytrichaceae (*Alophosia*, *Lyellia*, *Bartramiopsis*). This suggests that *Meantoinea* may occupy a more basal position in the family and, therefore, provides a more useful calibration point for basal divergences within the Polytrichaceae.

If *Eopolytrichum*, a Campanian (ca. 83 Ma) moss, is a derived member of the Polytrichaceae, and *Meantoinea*, a Valanginian (ca. 136 Ma) moss, occupies a position close to the base of the Polytrichaceae, then it is possible that significant evolutionary radiation leading to the emergence of many extant genera occurred during the Cretaceous. A recent study addressing the tempo of evolution across all of bryophyte phylogeny has proposed that the Polytrichopsida (i.e. the class whose sole family is Polytrichaceae) emerged between 297 and 471 Ma (Laenen et al., 2014, supplementary information, p. 118). As the oldest unequivocal Polytrichaceae, *Meantoinea* only provides a *minimum* hard age for the group, which certainly arose prior to the Valaginian, 140 Ma ago. However, the age ranges proposed for the Polytrichaceae by Laenen et al. (2014) are two to three times older than the base of the Cretaceous. Such extreme age ranges are probably a result of the choice of the Early Permian fossil *Palaeocampylopus buragoae* (273 Ma) as the calibration point for stem group Polytrichaceae. This choice is intriguing, given that

P. buragoae lacks features that allow unambiguous assignment to the Polytrichaceae and is considered to show closer affinities with the Dicranaceae (Ignatov and Shcherbakov, 2009). We suggest that the use of *Meantoinea* as a calibration point for basal Polytrichaceae may provide a better constraint for estimating clade age in future studies of the tempo of bryophyte evolution.

1.5 CONCLUSIONS

Anatomically preserved mosses are exceptionally rare in pre-Cenozoic rocks (Smoot and Taylor, 1986; Konopka et al., 1997, 1998; H*ü*bers and Kerp, 2012; Heden*ä*s et al., 2014; Tomescu, 2016). Given this meager record, fossils are generally not considered to be very valuable for understanding moss evolution. In this context, *Meantoinea alophosioides* provides an important addition to a growing body of evidence suggesting that fossils are crucial for understanding moss phylogeny. We emphasize that a diverse anatomically preserved fossil bryoflora is preserved in the Early Cretaceous Apple Bay locality of Vancouver Island, Canada (Tomescu, 2016). *Meantoinea* is the third moss described in detail from this locality, broadening the taxonomic diversity of the Apple Bay bryoflora to include the Polytrichaceae in addition to the extinct hypnanaean family Tricostaceae (Shelton et al., 2015, 2016). *Meantoinea* also marks the oldest unequivocal record of Polytrichaceae, providing a hard minimum age of ca. 136 Ma (Valanginian) for the family and the first record of fossil moss gemma cups.

Meantoinea preserves a high level of anatomical and morphological detail, based on multiple anatomically preserved gametophyte shoots. This level of detailed information is known from only a handful of other pre-Cenozoic moss fossils (Smoot and Taylor, 1986; Konopka et al., 1997, 1998; Shelton et al., 2015, 2016). Detailed anatomical and morphological information on fossil mosses serves two important purposes. First, it allows accurate taxonomic

placement, contributing to understanding of pre-Cenozoic moss diversity. Second, it is a prerequisite for any attempt to address the deep phylogeny of mosses circumventing the taxon sampling issues that plague 'extant-only' phylogenies (e.g. Rothwell and Nixon 2006; Rothwell et al., 2009). Study of fossil species that preserve high levels of morphological and anatomical detail broadens the range of taxon sampling by adding novel combinations of characters whose existence could not have been foreseen from studies based exclusively on extant plants. Every time phylogenetic studies have sampled systematically the fossil record, their results have provided new perspectives (e.g., Rothwell, 1999; Rothwell and Nixon, 2006; Hilton and Bateman, 2006). This second purpose is particularly relevant, since fossil information is crucial for understanding evolution of a group such as the Polytrichaceae, which lack close living relatives. When incorporated in phylogenetic studies, *Meantoinea* may provide some of the information needed to confidently resolve the phylogenetic position of Polytrichaceae with respect to other moss lineages (Chang and Graham, 2014).

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FIGURES

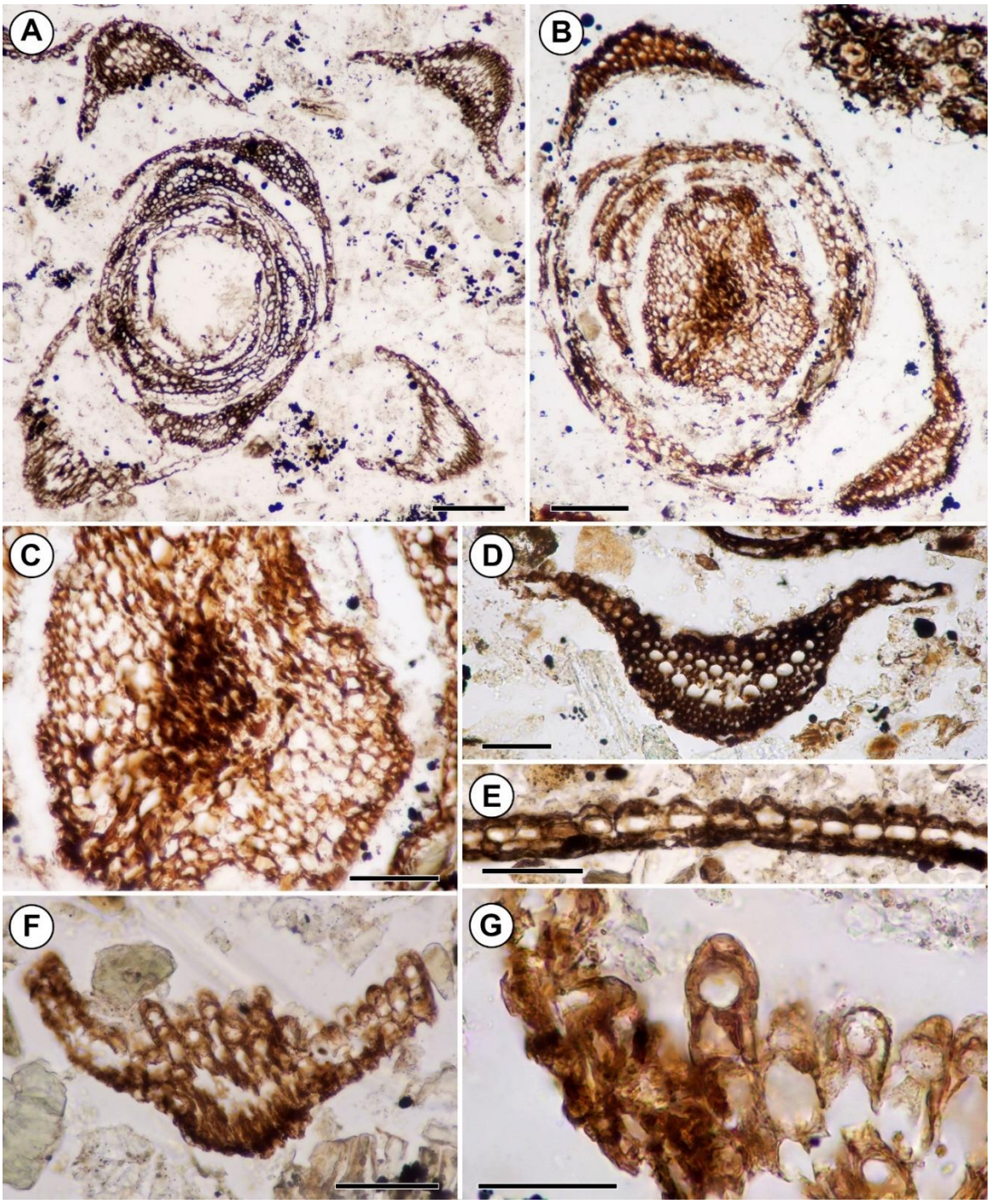

Figure 1 Stem and leaf anatomy of Meantoinea alophosioides *gen. et sp. nov. (A) Cross section of shoot showing several sheathing leaf bases tightly wrapped around stem and four leaves sectioned in transitional region between leaf base and leaf blade; scale bar = 100; P15393 Bbot #7b. (B) Cross section of shoot with stem anatomy fully preserved; scale bar = 100 µm; P13158 Cbot #6c. (C) Detail of stem anatomy in B showing peripheral layer of small-diameter cells, cortex with larger cells, and central conducting strand; scale bar = 50 µm; P13158 Cbot #6c. (D) Cross section of costa in leaf base, showing deuters (layer of large cells), putative conducting parenchyma (layer of smaller cells adaxial to deuters), and small stereids on adaxial and abaxial side of costa; scale bar = 50 µm; P15393 Bbot #13b. (E) Longitudinal section of lamina close to base of leaf blade, showing bistratose lamina with adaxial mamilose cells; scale bar = 50 µm; P15393 Bbot #1b. (F) Cross section of leaf blade with several photosynthetic lamellae adaxial on costa; scale bar = 50 µm; P15393 Bbot #10b. (G) Detail of photosynthetic lamellae in Fig 2D; note mamillose distal-most cell of lamella; Scale bar = 25 µm; P15393 Bbot #16b*

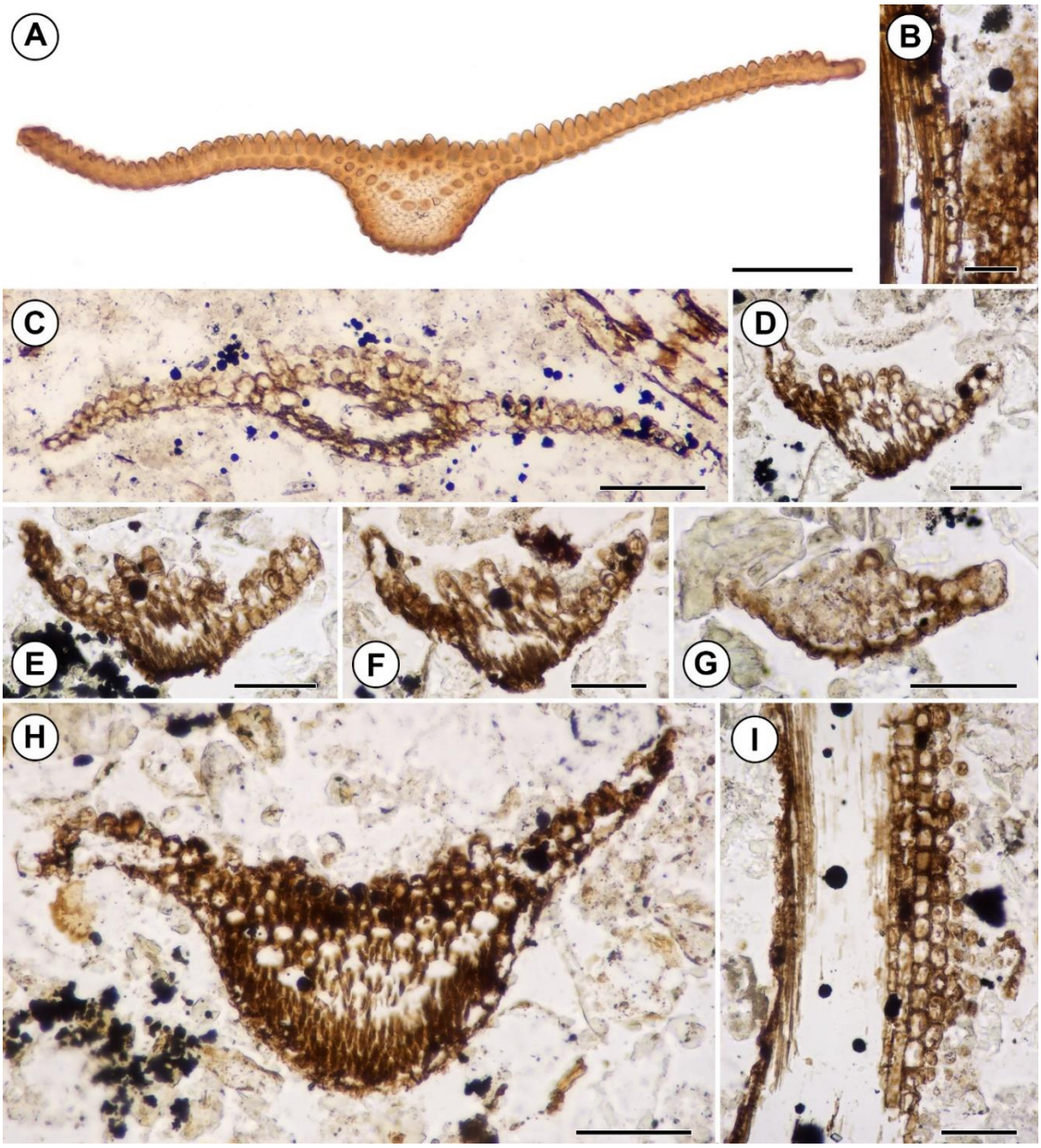

Figure 2 Leaf anatomy and morphology of Meantoinea alophosioides gen. et. sp. nov. and comparison with Alophosia azorica. (A) Cross section of basal portion of Alophosia azorica leaf blade, showing similar anatomy to M. alophosiodes (costa anatomy and bistratose with adaxial mamillose cells); photo courtesy of J. Hyvönen and N. E. Bell; scale bar = 100 µm. (B) Paradermal section of M. alophosioides *leaf blade with unicellular teeth at margin (arrowheads); scale bar = 50 µm; P15393 Bbot #2b. (C) Cross section of basal portion of* M. alophosioides *leaf blade, showing bistratose lamina and adaxial photosynthetic lamellae on costa; note similarities with 2A; scale bar = 100 µm; P15800 Cbot #4c. (D) Cross section of apical portion of* M. alophosioides *leaf blade, showing prominent adaxial photosynthetic lamellae with mamillose distal-most cells; scale bar = 50 µm; P15393 Bbot #16b. (E-G) Cross sections of same* M. alophosioides *leaf blade illustrating morphological change along proximal-distal axis; note persistent costa, reduced lamina and lamellae close to leaf tip (G); scale bars = 50 µm; P15393 Bbot #12b (E), #15b (F), #20b (G). (H) Cross section of* M. alophosioides *leaf at transition from sheathing leaf base to leaf blade; note costal anatomy with deuters (large), conducting parenchyma, and two bands of stereids (arrowheads); scale bar = 100; P15393 Bbot #7b. (I) Paradermal section of leaf blade; note parallel vertical rows of adaxial mamillose lamina cells (round, at right), abaxial lamina cells (rectangular, at right); cells (deuters) in middle of the costa (center) incompletely preserved; scale bar = 50 µm; P15393 Bbot #1b.*

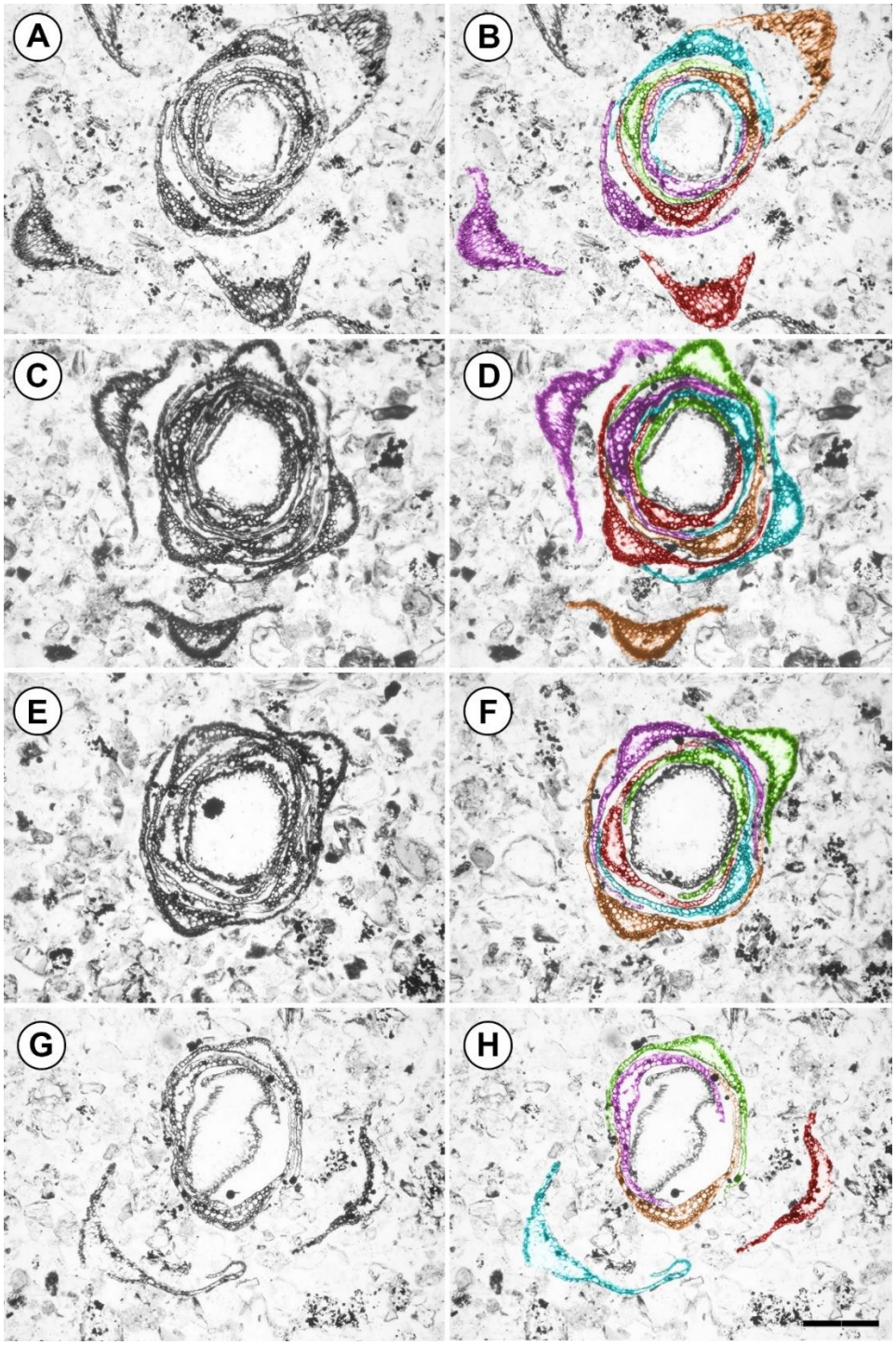

Figure 3 Phyllotaxis and gemma cup morphology of Meantoinea alophosioides *gen. et. sp. nov. Apical (A, B) to basal (G, H) series of cross sections of same shoot showing leaves with 3/5 phyllotaxis and five orthostichies colored purple, orange, green, red, and blue (in B, D, F, H); note increasing leaf density from basal part (G, H) toward apical gemmae cup and reflexed leaf blades around margin of gemmae cup (compare C, D and A, B). P15393 Bbot #7b (A) Cross section of distal portion of gemmiferous shoot*

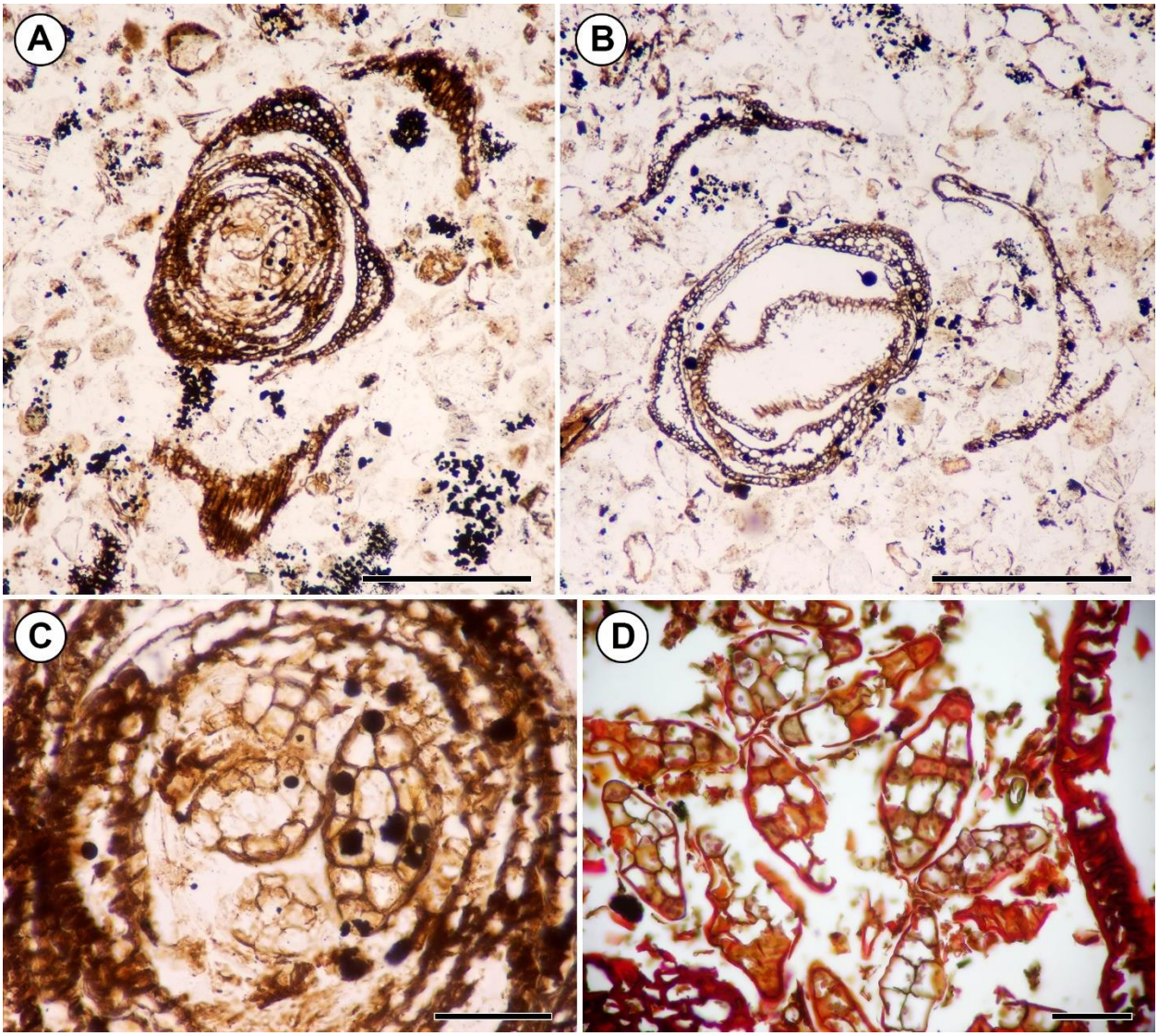

Figure 4 Gemmae and gemma cup of Meantoinea alophosioides gen. et. sp. nov. and comparison with gemmae of Tetraphis pellucida. (A) Cross section of M. alophosioides gemma cup with gemmae inside; scale bar = 250 µm; P15393 Bbot # 1b. (B) Cross section of vegetative region of same shoot, showing far fewer leaves than in specialized part of shoot that comprises gemmae cup; scale bar = 250 µm; P15393 Bbot # 41b. (C) Detail of A, showing four gemmae (large arrowheads); one gemma attached to rhizoid-like stalk (small arrowhead); scale bar = 50 µm; P15393 Bbot # 1b. (D) Cross section of Tetraphis pellucida *gemmae cup, showing details of gemma anatomy; scale bar = 50 µm.*

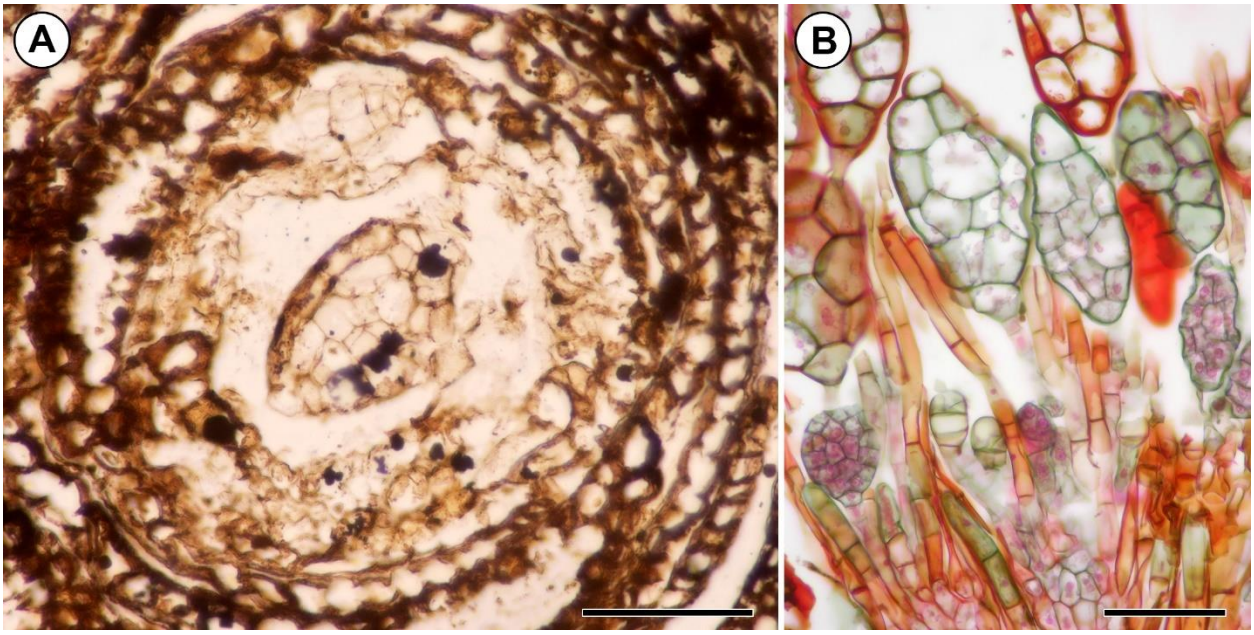

Figure 5 Gemmae of Meantoinea alophosioides *gen. et. sp. nov. and comparison with gemmae of* Tetraphis pellucida. *(A) Cross section of median region of* M. alophosioides *gemma; scale bar = 50 µm; P15393 Bbot # 3b. (B) Longitudinal section of* T. pellucida *gemma cup showing rhizoid-like stalks that bear gemmae and numerous immature gemmae; scale bar = 50 µm.*

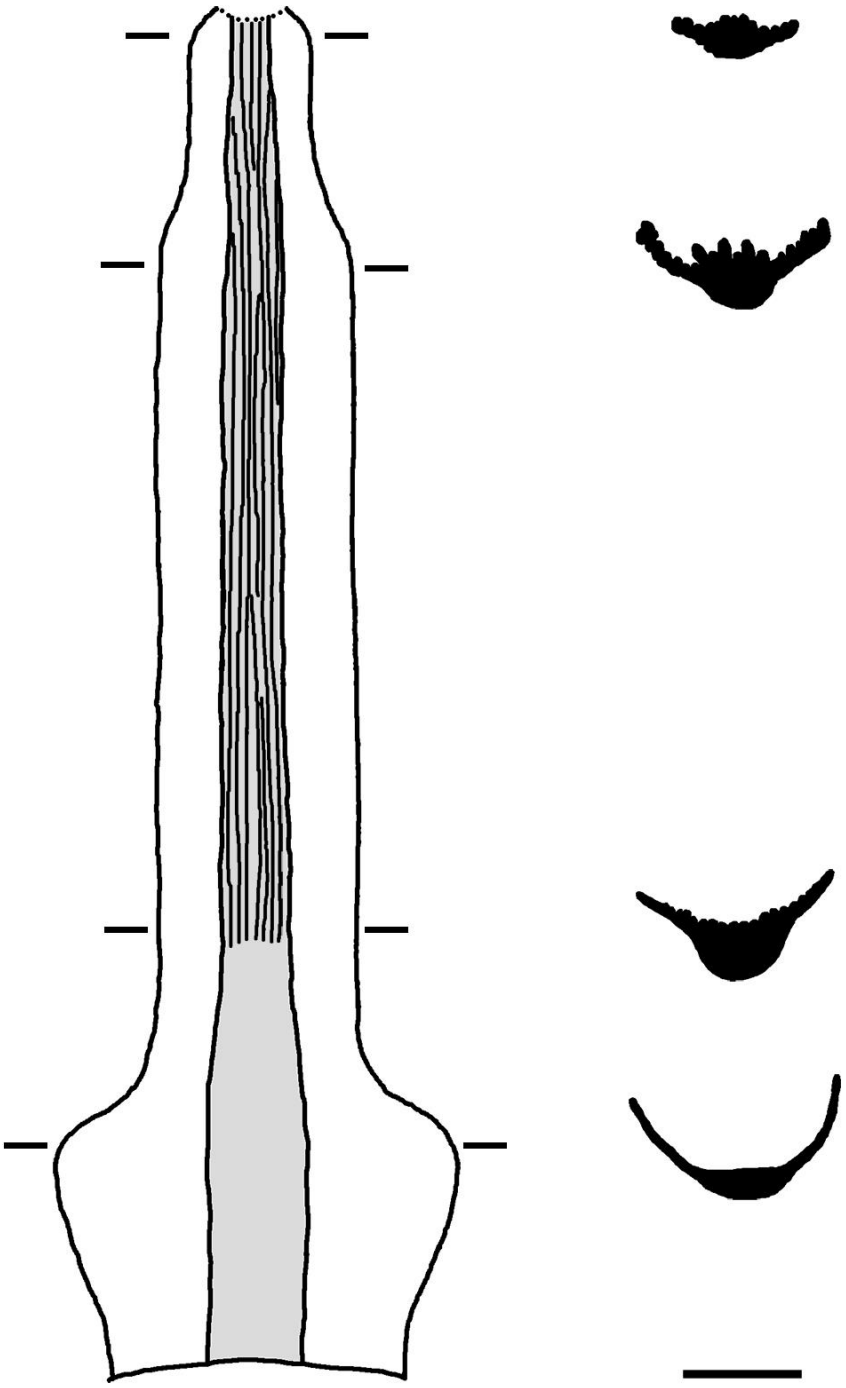

Figure 6 Meantoinea alophosioides *gen. et. sp. nov. leaf model. (A) Overall leaf morphology. Note short, broad sheathing base; narrower, long leaf blade. Photosynthetic lamellae on adaxial surface of costa (gray), along leaf blade. Leaf apex incompletely preserved. (B) Series of leaf cross sections at four levels along leaf; from base to tip, sections correspond to specimens illustrated in Fig 1A, 2H, 1F, and 2G. Scale bar = 250 µm.*

TABLES

Table 1 Meantoinea compared to polytrichaceous mosses with similar features.

	Meantoinea	***Alophosia***	***Bartramiopsis***	***Lyellia***	**Gametophytes associated with *Eopolytrichum***
Abaxial photosynthetic lamellae	present	absent	present	present	present
Lamellae distribution	costa	-	costa	costa	costa + lamina
Lamellae height	2-3 cells	-	4-8 cells	6-8 cells	3-4 cells
Lamella marginal cell	mamillose	-	undifferentiated	mamillose	undifferentiated to mamillose
Gemmae	present	present	absent	absent	absent
Bistratose lamina	present	present	present	present	absent
Mamillose laminar cells	present	present	present	present	absent
Leaf margin	single unicellular teeth	single multicellular teeth	single multicellular teeth	double multicellular teeth	single unicellular teeth

Table 2 Polytrichaceous moss fossils

Species	**Age**	**Stratigraphy**	**Location**	**Preservation**	**Assignment to Polytrichaceae**	**References**
Polytrichites aichiensis	Upper Cenozoic	?	Aichi, Japan	permineralized	unequivocal	Yasui, 1928
Polytrichites spokanensis	Miocene	Latah Formation	Washington, USA	compression	equivocal; branched shoot, no obvious photosynthetic lamellae	Knowlton, 1926
Atrichum subrhystophyllum	Eocene	Baltic Amber	-	amber	unequivocal	Frahm, 2004, 2010
Atrichum groehnii	Eocene	Baltic Amber	-	amber	unequivocal	Frahm, 2004, 2010
Atrichum mamillosum	Eocene	Baltic Amber	-	amber	unequivocal	Frahm, 2004, 2010
Polytrichites pogonatoides	Eocene	Baltic Amber	-	amber	unequivocal	Frahm, 1999, 2010
Polytrichites convolutus	Eocene	Baltic Amber	-	amber	equivocal; photosynthetic lamellae not visible	Mägdefrau, 1957; Frahm, 2010
Eopolytrichum antiquum and associated gametophytes	Late Cretaceous (Campanian)	Gaillard Formation	Georgia, USA	charcoalified	unequivocal	Konopka et al., 1997
Meantoina alophosioides	Early Cretaceous (Valanginian)	Longarm Formation equivalent	Vancouver Island, Canada	permineralized	unequivocal	this study

CHAPTER TWO

WANTED DEAD OR ALIVE (PROBABLY DEAD): STEM GROUP POLYTRICHACEAE

[In press in the *American Journal of Botany*]

2.1 INTRODUCTION

The moss family Polytrichaceae (the sole family of class Polytrichopsida and order Polytrichales) is a diverse and distinctive group of mosses in terms of morphology, anatomy, and ecology. Within mosses, the greatest degree of both gametophyte and sporophyte complexity is achieved in this lineage (Smith, 1971; Schofield, 1985; Smith Merrill, 2007). Gametophytes are typically large, and anatomically complex, with sophisticated conducting tissues (Hébant, 1977) and photosynthetic lamellae on the leaves. Lamellae allow these plants to efficiently photosynthesize in high light environments, an unusual attribute among mosses (Proctor, 2005). Sporophytes have similarly complex conducting tissues (Hébant, 1977) and, in most species, they have distinctive nematodontous peristome teeth (Smith Merrill, 2007) that are regarded as not homologous to those of other peristomate mosses (Smith Merrill, 2007; Bell and Hyvönen, 2008).

Because of the distinctive morphology and anatomy of the Polytrichaceae, and the lack of morphologically similar living relatives, the evolutionary relationships of the family among acrocarpous mosses have long been a subject of interest (Smith, 1971). The Polytrichaceae have been included in almost all broad phylogenetic studies of mosses (e.g. Chang and Graham, 2011, 2014) and phylogenetic relationships within the family have been the focus of many studies (Hyvönen, 1989; Forrest, 1995; Hyvönen et al., 1998, 2004; Bell and Hyvönen, 2008; Bell and Hyvönen 2010a; 2010b; Bell and Hyvönen, 2012; Bell and Hyvönen, 2015). Early morphology-based (Hyvönen, 1989; Forrest, 1995) and combined molecular and morphological (Hyvönen et

al., 1998, 2004) phylogenetic studies suggested that morphology may not be phylogenetically informative for resolving either relationships with other mosses or within the family. However, these studies used small morphological data sets (≤50 characters). In contrast, molecular phylogenetic studies have yielded hypotheses of relationships within the family with high support values for most nodes (e.g. Bell and Hyvönen, 2010a). Nevertheless, phylogenetic relationships of the Polytrichaceae with other acrocarpous moss lineages, as inferred from molecular data are more equivocal (Chang and Graham, 2014). Because the family represents the tip of a very long branch with no close living relatives, rooting phylogenetic analyses of the Polytrichaceae (both molecular and morphological) has proved difficult and several rooting strategies have been employed in different studies. The most recent analyses have been rooted with *Alophosia azorica* Card., a member of the family that is regarded as sister to all other Polytrichaceae (Bell and Hyvönen, 2008).

In 2017, Bippus et al. described the oldest fossil polytrichaceous moss, *Meantoinea alophosioides* Bippus, Stockey, G.W. Rothwell et Tomescu, from Early Cretaceous (136 Ma) deposits of Vancouver Island, Canada. This fossil species combines features of several basal eperistomate genera in the Polytrichaceae (*Bartramiopsis* Kindb., *Alophosia* Card., *Lyellia* R. Br.), but its phylogenetic relationships within the family have not been assessed. Here we present the first morphology-based phylogenetic analysis of the Polytrichaceae in more than 20 years. We incorporate a much larger set of characters (100) than previous morphology-based studies. Additionally, we use continuous characters to codify continuously-varying features for the first time in the Polytrichaceae, and for the second time in mosses (Flores et al., 2017b). The aims of this study are to assess the phylogenetic relationships of two well-characterized fossil members of the family (*Meantoinea alophosioides* and *Eopolytrichum antiquum* Konopka,

Herendeen, Smith Merrill et Crane), to evaluate the effects of rooting on relationships within the family, and to compare hypotheses of phylogenetic relationships supported by morphological and molecular data.

2.2 MATERIALS AND METHODS

2.2.1 CHARACTER SELECTION AND SCORING

Relationships of polytrichaceous mosses were evaluated using a dataset consisting of 100 morphological characters scored for 44 species of acrocarpous mosses (Appendices 1 and 2; Appendix S1; see Supplemental Data with this article). Nine of these characters represent sizes of gametophyte and sporophyte features, and two represent ratios describing leaf shape. Characters were scored from the scientific literature using Mesquite 3.2 software (Maddison and Maddison, 2009). When provided in the literature, the variation of a character among individuals within a species was scored as a range. For taxa and characters where such ranges of natural variation have not been reported, we created an artificial range for each character by bracketing the single value reported in the literature by ± 10% (Appendix S1; see Supplemental Data associated with this article). This was done to avoid artificially emphasizing differences between taxa without ranges. The eleven continuously-varying features were treated as continuous characters, using the methods proposed by Goloboff et al. (2006), and the complete range of each character was standardized as the equivalent to one step of a discrete character. To scale each character scoring to the standardized range, we first determined the minimum and maximum value across the entire set of taxa, i.e., the complete range of variation of the character. For each taxon, we then subtracted the overall minimum value for that character from the end values defining the range of variation of the character in that taxon. The resulting values were then divided by a value representing the entire range of variation of that character across all

taxa in the dataset, thus obtaining a scaled range for each taxon. These methods have been shown to add phylogenetically useful information that is not fully recoverable when codified as discrete character states (Escapa and Pol, 2011). However, these characters may also artificially bias results by generating a single most parsimonious tree, while other trees are just fractions of a step longer (Goloboff et al., 2006). Future studies taking measurements from specimens in herbaria would probably enhance the precision of this analysis. For characters that were used in previous phylogenetic studies, we applied the same scorings as in those analyses (Hyvönen, 1989; Forrest, 1995; Hyvönen et al., 1998, 2004; Koskinen and Hyvönen, 2004). Novel characters were scored from the taxonomic literature (Mitten, 1859; Mullen and Frye, 1947; Smith, 1969; Lawton, 1971; Nyholm, 1971; Smith, 1971; van Zanten, 1973; Tuow, 1986; Shaw et al., 1987; Shaw and Anderson, 1988; Hyvonen, 1989; Shaw et al., 1989; Schwartz, 1994; Smith, 1996; Konopka et al., 1997; Budke et al., 2007; Ivanova and Ignatov, 2007; Smith Merrill, 2007; Malcom et al., 2009; Peralta and Yano, 2010) and by examining the *Meantoinea alophosioides* type specimens of Bippus et al. (2017; P15393, P13158, and P15800) housed in the University of Alberta Paleobotanical Collections (UAPC-ALTA; Edmonton, Alberta, Canada), as well as *Pogonatum contortum* Lesq., and *Polytrichum commune* Hedw. specimens collected in the Arcata Community Forest (Arcata, California, USA), 40° 52' 32.3612" N, 124° 4' 25.0702" W, and *Dendroligotrichum dendroides* Broth. specimens collected by M. E. Antoine (Humboldt State University) near Karamea (South Island, New Zealand), housed in the Humboldt State University Bryophyte Teaching Collection.

The scored matrix has 13% missing data. Discrete character scorings are reported in Appendix 2 and continuous character scorings are reported in Appendix S1. The phylogenetic

matrix is publicly available as a TNT file from Morphobank.org (O'Leary and Kaufman, 2012), Project 2810.

2.2.2 INGROUP TAXON SAMPLING

All extant polyrichaceous genera were sampled in our analysis, except the recently described *Delongia* N.E. Bell, Kariyawasam, Hedd. et Hyvönen (Bell et al., 2015). We included species used in previous morphology-based or combined molecular and morphological phylogenetic studies of the family (Hyvonen, 1989; Forrest, 1995; Hyvönen et al., 1998, 2004) because the greatest amount of morphological data were available for these species. We also included the two oldest and best characterized extinct members of the Polytrichaceae, *Eopolytrichum antiquum* and *Meantoinea alophosioides*. We did not include moss fossils from Eocene Baltic amber assignable to Polytrichaceae (*Atrichum* P. Beauv., *Polytrichites* E. Britton; Frahm, 2004, Frahm, 2010) or other members of the form genus *Polytrichites* (Knowlton, 1926; Yasui, 1928), which includes fossils that are identifiable only to the family level, because these fossils are poorly known.

2.2.3 PHYLOGENETIC ANALYSES

Phylogenetic searches were conducted in TNT 1.5 (Goloboff and Catalano 2016) using equally weighted parsimony as the optimality criterion. The parsimony analyses were initiated using the command "*xmult=hits10*". Using this command, the analysis starts from 50 random addition sequences (RAS), which are refined by tree bisection-reconnection. The resulting trees are then submitted to a combination of Ratchet (default settings), Tree Drifting (default settings), and sectorial searches (default settings). Unrooted networks were generated by saving tree files from TNT 1.5 as .tre files, rendering them in Figtree 1.43 software, using the "display as unrooted

network" option. Bootstrap values were generated using the "bootstrap resampling" command in TNT 1.5 with default tree search parameters and 100 replicates.

2.2.4 EVALUATING OUTGROUP SAMPLING EFFECTS

Because the Polytrichaceae are highly distinctive morphologically and lack close living relatives (Smith, 1971; Bell and Hyvönen, 2008), early combined morphological and molecular studies of polytrichaceous phylogeny sampled all major lineages of basal acrocarpous mosses as outgroups (Hyvönen et al. 1998, 2004). Conversely, a much-reduced outgroup, consisting only of *Tetraphis pellucida* Hedw. and *Tetradontium brownianum* Schwägr., was used in the morphology-based analysis by Forrest (1995). However, the results of a phylogenetic study targeted at rooting the Polytrichaceae (Bell and Hyvönen, 2008), suggested that among living Polytrichaceae, *Alophosia azorica* is the most basal species. Accordingly, the most recent phylogenetic studies exploring relationships within the Polytrichaceae (Bell and Hyvönen 2010a, 2012) have treated *Alophosia* as the outgroup. One of the fossils included in this study, *Meantoinea alophosioides* (Bippus et al., 2017) is very similar morphologically to *Alophosia azorica* and is the oldest unequivocal representative of the Polytrichaceae. To assess the phylogenetic position of *Meantoinea* with respect to *Alophosia* outside of constraints imposed by the rooting role of the latter, we used several outgroup sampling regimes.

In a first set of analyses (Analysis 1A, 1B Table 1), we did not use an outgroup and rooted the trees with *Alophosia*. This is consistent with the findings of Bell and Hyvönen (2008) but does not test if *Meantoinea* forms a clade with *Alophosia* in rooted analyses, as we would hypothesize based on their morphological similarities.

In a second set of analyses (Analysis 2A, 2B Table 1), we included seven outgroup species, representing all major lineages of basal acrocarpous mosses (*Sphagnum palustre* L.

Andreaea rupestris Hedw., *Tetraphis pellucida*, *Oedipodium griffithianum* Shwägr., *Buxbaumia aphylla* Hedw., *Funaria hygrometrica* Hedw., *Diphyscium foliosum* D. Mohr), and rooted trees with *Sphagnum palustre*. This is similar to the approach used in early combined morphological and molecular analyses (Hyvönen et al., 1998, 2004). This taxon sampling regime, expected to introduce higher levels of homoplasy in the analysis, allowed for testing whether *Alophosia* and *Meantoinea* form a clade, and if *Alophosia* is the basal-most member of the family.

Finally, in a third set of analyses (Analysis 3A, 3B Table 1) we used a reduced outgroup, rooting the trees with *Sphagnum palustre* and including the other two outgroup genera most closely related to the Polytrichaceae (*Oedipodium* and *Tetraphis*). This regime, expected to reduce levels of homoplasy as compared to the second sampling regime, still allowed for testing if *Alophosia* and *Meantoinea* form a clade, and if *Alophosia* is the basal-most member of the family.

2.3 RESULTS

2.3.1 ANALYSIS ROOTED WITH *ALOPHOSIA* (ANALYSIS 1A)

Parsimony analysis of the matrix consisting of all 37 species of Polytrichaceae, scored for all characters (discrete + continuous) and rooted with *Alophosia*, resulted in one most parsimonious tree (length 233.85; fractional tree lengths are due to continuous characters in which character changes are < 1) (Fig. 1). *Meantoinea* and three eperistomate Polytrichaceae (*Alophosia*, *Lyellia*, and *Bartramiopsis*) are recovered as a grade at the base of the tree, with *Bartramiopsis* sister to the peristomate Polytrichaceae. Within the latter, a basal divergence separates the *Pogonatum* P. Beauv clade. *Dendroligotrichum* Broth. is sister to all remaining peristomate Polytrichaceae, which form two clades: (1) a clade consisting primarily of mosses with large gametophytes (the

"large gametophyte clade", including *Dawsonia* R. Br., *Polytrichadelphus* Mitt., *Polytrichum* Hedw., *Eopolytrichum, Polytrichastrum* G. L. Sm.); and (2) a clade comprising mosses with smaller gametophytes (*Itatiella* G. L. Sm., *Hebantia* G. L. Merr., *Oligotrichum* D. C., *Steereobryon* G. L. Sm., *Atrichum, Psilopilum* Brid., *Notoligotrichum* G. L. Sm.). Within the first clade, *Dawsonia* is sister to all other large Polytrichaceae, and *Polytrichadelphus* is sister to a clade including *Polytrichum, Eopolytrichum,* and *Polytrichastrum*. In the second clade, *Itatiella Hebantia,* and *Oligotrichum* form a paraphyletic grade basal to the clade consisting of *Steereobryon, Atrichum, Psilopilum,* and *Notoligotrichum*. *Steereobryon* is basal to the divergence of an *Atrichum* clade and a clade consisting of *Psilopilum* and *Notoligotrichum*. Within the later, *Psilopilum* is sister to *Notoligotrichum*. Two major points of incongruence, due to ingroup rooting (see discussion below), between the results of this analysis and all other analyses in our study are (1) the *Pogonatum* clade is sister to all other peristomate Polytrichaceae, and (2) *Alophosia*, *Meantoinea*, *Bartramiopsis*, and *Lyellia* form a grade, instead of a clade. In the unrooted network of this analysis (Fig. 7), these four genera form a clade, just as they do in all of our other analyses.

2.3.2 ANALYSIS ROOTED WITH *SPHAGNUM*, INCLUDING ALL MAJOR LINEAGES OF BASAL ACROCARPS (ANALYSIS 2A)

Parsimony analysis of the matrix consisting of all 37 species of Polytrichaceae and eight species representing all other major lineages of basal acrocarpous mosses, scored for all characters (discrete + continuous), resulted in one most parsimonious tree (length 292.41) (Fig. 2). In this cladogram, *Timmia* Hedw. is sister to all Polytrichaceae. Within the ingroup, most of the differences between Analysis 1A and 2A are due to rooting (Fig. 1), and affect only the basal node of each clade (see discussion below). The eperistomate Polytrichaceae – *Bartramiopsis*,

Meantoinea, *Alophosia*, and *Lyellia* – form a monophyletic group, with *Bartramiopsis* sister to the other three. This eperistomate clade is sister to all peristomate Polytrichaceae, which form two clades: (1) a clade of mosses with small gametophytes (with similar membership to the "small gametophyte clade" recovered in Analysis 1A); and (2) a clade consisting of *Pogonatum*, *Itatiella*, and the "large gametophyte clade" reported in Analysis 1A. The relationships within the "small gametophyte clade" are different, however, in this analysis because of different ingroup rooting (Fig. 1). *Hebantia* and *Oligotrichum* form a clade which is sister to the clade consisting of *Steereobryon*, *Atrichum*, *Psilopilum*, and *Notoligotrichum*. Relationships within this clade are identical to those recovered in Analysis 1A. Within the second clade of peristomate Polytrichaceae, *Pogonatum* and *Itatiella* form a clade which is sister to the "large gametophyte clade". The relationships within *Pogonatum* in this analysis are identical to those recovered in Analysis 1A. Relationships of the "large gametophyte clade" are very similar to those in Analysis 1A, except that *Dendroligotrichum* is sister to this clade, as a result of different ingroup rooting in this analysis (see discussion below). In general, the results of Analysis 2A are similar to those of the morphology-only results of Hyvönen et al. (2004), who used similar outgroups.

2.3.3 ANALYSIS ROOTED WITH *SPHAGNUM*, INCLUDING A REDUCED OUTGROUP (ANALYSIS 3A)

Parsimony analysis of the matrix consisting of all 37 species of Polytrichaceae in addition to an outgroup consisting of *Sphagnum palustre*, *Tetraphis pellucida*, and *Oedipodium griffithianianum*, scored for all characters (discrete + continuous), resulted in two most parsimonious trees (tree length 265.61). The strict consensus of these two trees suggests a hypothesis of relationships that is different from other analyses primarily because of different ingroup rooting (see discussion below). A *Psilopilum* + *Notoligotrichum* clade is recovered as

sister to the rest of the family (Fig. 1, 3) within which a basal dichotomy separates an *Atrichum* clade. In the other member of this dichotomy, *Steereobryon*, *Oligotrichum*, *Hebantia*, and *Itatiella* form a grade below the dichotomy of two clades: (1) the eperistomate members of the Polytrichaceae (*Bartramiopsis*, *Meantoinea*, *Alophosia*, and *Lyellia*); and (2) a clade consisting of *Dendroligotrichum*, *Pogonatum*, and the "large gametophyte clade" recovered in both of the other analyses. The eperistomate clade has the same topology as in Analysis 2A. Within the second clade, *Pogonatum* forms a grade basal to the "large gametophyte clade", which shows the same relationships recovered in Analysis 1A. There are two major points of incongruence between the results of this analysis and the other two, attributable to differences in rooting (see discussion below). First, *Bartramiopsis*, *Meantoinea*, *Alophosia*, and *Lyellia* are not recovered as basal within the family. Second, the "small gametophyte clade" recovered in Analyses 1A and 2A is a paraphyletic group in this analysis. Another major point of incongruence, namely that *Pogonatum* forms a grade in this analysis instead of a clade as it does in all other analyses in this study, is not the result of differences in rooting between analyses. Instead, it is the result of different polarization of continuous characters.

2.3.4 EFFECT OF CONTINUOUS CHARACTERS

As shown by Analyses 1B, 2B, and 3B, which include only discrete characters, resolution is reduced significantly when continuous characters are removed (Figs. 4, 5, 6). In these three analyses similar small clades were recovered, with no resolution regarding their relationships to each other, irrespective of outgroup sampling regime. In Analysis 1B, *Alophosia*, *Meantoinea*, *Lyellia*, and *Bartramiopsis* are paraphyletic to a large polytomy that includes all other Polytrichaceae (Fig. 4). In this polytomy, only four small clades are resolved. These are: (1) a clade consisting of both species of *Dawsonia*; (2) a clade consisting of all species of *Atrichum*

(*A. undulatum* P. Beauv., *A. crispum* Sull., *A. angustatum* Bruch et Schimp.); (3) a clade consisting of several species of *Pogonatum* (*P. volvatum* Paris, *P. phillippinense* Touw, *P. piliferum* Touw, *P. camusii* Touw); (4) a clade consisting of *Polytrichastrum*, *Polytrichum*, and *Eopolytrichum*. Identical relationships are recovered for the ingroup in Analysis 2B, except that *Alophosia*, *Meantoinea*, *Bartramiopsis*, and *Lyellia* form a clade that is part of the same large polytomy as the rest of the Polytrichaceae, and the *Pogonatum*clade is larger, including all members of the genus (Fig. 5). In Analysis 3B, virtually identical relationships were recovered for ingroup taxa as in Analysis 2B, except that here the *Pogonatum* clade is smaller (as in Analysis 1B), and all species of *Polytrichastrum* as well as some species of *Polytrichum* (*P. formosum* Hedw. and *P. longisetum* Sw.) are part of the basal polytomy (Fig. 6). The differences in relationships between Analyses 1B, 2B, and 3B with respect to the relationships of *Alophosia*, *Lyellia*, *Meantoinea*, and *Bartramiopsis* are primarily due to differences in ingroup rooting between the three analyses (see discussion below).

In most studies that employ continuous characters, these characters resolve primarily the distal nodes, whereas internal nodes tend to be resolved primarily by discrete characters (Escapa and Pol, 2011). In contrast to this general pattern, in our study discrete characters bring little resolution to internal nodes, as shown by Analyses 1B, 2B, and 3B. However, when internal nodes are resolved by addition of continuous characters (Analyses 1A, 2A, and 3A), the synapomorphies supporting these nodes are combinations of continuous and discrete characters. This indicates that the phylogenetic signal introduced by continuous characters is congruent with some relationships supported by discrete characters.

2.4 DISCUSSION

2.4.1 COMPARISON WITH RESULTS OF MOLECULAR PHYLOGENIES

A series of iterative studies (Bell and Hyvönen, 2010a, b, 2012; Bell et al., 2015) has painted an image of the phylogenetic relationships within the Polytrichaceae as resolved using molecular data, with support from detailed micromorphological characters (Bell and Hyvönen, 2010b, 2012). All these analyses were rooted with *Alophosia* and they recover *Alophosia* and a *Bartramiopsis* + *Lyellia* clade (eperistomate Polytrichaceae) as a paraphyletic group at the base of the family. *Dawsonia* (which has a distinctive fibrous peristome; Zanten, 1973), is sister to all Polytrichaceae with a polytrichoid peristome. Among the latter, a paraphyletic group including (1) a clade formed by *Itatiella* and *Notoligotrichum* (Bell and Hyvönen, 2012); (2) *Polytrichadelphus*; and (3) a *Dendroligotrichum* + *Hebantia* clade, is basal to a clade within which resolution varies between the different analyses. This clade includes *Polytrichastrum*, a large clade containing *Oligotrichum*, *Psilopilum*, *Steereobryon*, and *Atrichum* (with *Steereobryon* sister to *Atrichum*), and a *Polytrichum* + *Pogonatum* clade.

The relationships recovered by our analyses vary significantly depending on outgroup sampling regime, because each regime forces a different rooting (Fig. 1). The overall topology we obtained in Analysis 3A is incongruent with the results of other phylogenetic studies, as ingroup relationships are rooted between the *Psilopilum* + *Notoligotrichum* clade and the rest of the family. This is because in this analysis, the *Psilopilum* + *Notoligotrichum* clade is most similar morphologically to the outgroup and, as a result, this clade is forced to the base of the ingroup. In turn, this leads to reorganization of relationships among remaining ingroup species: particularly of the basal-most node in each clade and of species recovered closest to the

Psilopilum + *Notoligotrichum* clade in other analyses, whose placement is the most significantly affected.

Overall, the relationships recovered by Analyses 1A and 2A are similar to those of recent molecular analyses. In these analyses, ingroup relationships are rooted by the node between the eperistomate clade (*Alophosia*, *Meantoinea*, *Bartramiopsis*, and *Lyellia*; Analysis 2A), or by only one of its members (*Alophosia*; Analysis 1A) and the rest of the family (Fig. 1). Because these rooting configurations are similar, the two analyses result in hypotheses of relationships that are similar in several ways: (1) *Pogonatum* is monophyletic; (2) there is a large clade consisting of *Dendroligotrichum*, *Dawsonia*, *Polytrichadelphus*, *Polytrichum*, *Eopolytrichum*, and *Polytrichastrum* ("large gametophyte clade"); and (3) *Hebantia*, *Oligotrichum*, *Steereobryon*, *Atrichum*, *Psilopilum*, and *Notoligotrichum* form a clade ("small gametophyte clade"). Within the small gametophyte clade, *Steereobryon* is basal to the divergence of an *Atrichum* clade from a *Psilopilum* + *Notoligotrichum* clade.

Bootstrap values are generally low in all of our analyses, but some nodes have moderate support values (*Pogonatum phillippinense* + *P. piliferum* + *P. camusii* clade, *Atrichum undulatum* + *A. cripsum* + *A. angustatum* calde, *Polytrichum commune* + *P. piliferum* + *Eopolytrichum antiquum* clade) and the *Dawsonia* clade has generally strong support (Figs. 1-3). However, resampling-based support values, such as bootstrap values, only indicate the level of uncertainty of a particular node for a given dataset. Our low bootstrap values are not surprising, since resolution of these analyses is greatly reduced when continuous characters (11% of the dataset) are removed, partially due to moderate levels of missing data (13%). Nevertheless, comparisons with the results of independent analyses including similar taxon sampling provide another way to assess, qualitatively, node stability. As pointed out above, several of our nodes

with low bootstrap values are congruent with the results of molecular analyses, indicating that at least some nodes with low levels of bootstrap support are still informative.

Some of the relationships recovered in Analyses 1A and 2A are, however, incongruent with those indicated by molecular analyses. Recovery of a "large gametophyte clade" in both these analyses is the result of continuous characters related to size [large number of lamellae (character 5) in analysis 1A and large capsule size (character 8) in analyses 2A and 3A] complementing the signal encoded by discrete characters, which vary between analyses. These characters force *Dawsonia*, *Polytrichadelphus*, and *Polytrichastrum* to be part of the *Polytrichum* + *Eopolytrichum* clade, a clade that includes species with large gametophytes and is very stable (i.e., is recovered even when only discrete characters are used). In contrast, in molecular phylogenies, *Dawsonia* is only distantly related to any of the species grouped in this clade, and both *Dendroligotrichum* and *Polytrichadelphus* occupy different positons. Additionally, *Polytrichum longisetum* and *P. formosum* are recovered within the *Polytrichastrum* clade in all of our analyses, while a recent molecular and micro-morphological analysis by Bell and Hyvönen (2010b) recovers these taxa as part of the *Polytrichum* clade. However, the relationships recovered in our analysis are not surprising, because *Polytrichum longisetum* and *P. formosum* were previously placed in *Polytrichastrum* based on morphological similarities and were only recently moved to *Polytrichum* by Bell and Hyvönen (2010b), based on detailed micromorphological studies.

The "small gametophyte clade" of Analyses 1A and 2A roughly corresponds to the *Oligotrichum* + *Psilopilum* + *Steereobryon* + *Atrichum* clade recovered in molecular analyses, except that the morphologically-similar *Hebantia* and *Notoligotrichum* are added to this clade in our analyses and the topology within the clade is different between the molecular and

morphological analyses. Two discrete characters, which are homoplasic [elongate median blade cell shape (character 32) and adaxial lamellae restricted to the costa (character 48)], and one homoplasic continuous character [reduced number of lamellae (character 5)], support the small gametophyte clade in Analysis 1A. Two homoplasic discrete characters [sheathing leaf base absent (character 27) and elongate median blade cell shape (character 32)] support the small gametophyte clade in Analysis 2A.

Itatiella ulei G. L. Sm. is placed either as sister to the *Pogonatum* clade (Analysis 2A) or sister to the "small gametophyte clade" (Analysis 1A). These different positions of *Itatiella* are due to differences in ingroup rooting. These relationships, neither of which is recovered in molecular analyses, are probably due to the highly divergent morphology of *I. ulei*. Nevertheless, *I. ulei* was recovered as sister to the *Pogonatum* clade in the morphology-only analysis of Hyvönen et al. (2004).

2.4.2 PHYLOGENETIC RELATIONSHIPS OF FOSSIL POLYTRICHACEAE

The phylogenetic relationships of both fossils (*Meantoinea alophosioides* and *Eopolytrichum antiquum*) are consistent between all our analyses, irrespective of outgroup sampling regime and exclusion of continuous characters. *Eopolytrichum* is nested in a clade with *Polytrichum commune* and *P. piliferum* Hedw., and is sister to *P. piliferum* (Figs. 1-7). These results are consistent with the results of previous analyses that included this fossil (Hyvönen et al., 1998, 2004). Intriguingly, these results suggest that *Eopolytrichum*, as a member of the *Polytrichum*-clade, evolved an eperistomate sporangium dehiscence mechanism similar to those of distantly related *Alophosia*, *Lyellia*, and *Bartramiopsis* (Konopka et al., 1997).

Meantoinea is closely related to *Alophosia*, *Bartramiopsis*, and *Lyellia* (Figs. 1-7). In the unrooted network of Analysis 1A (Fig. 7), *Alophosia*, *Meantoinea*, *Lyellia*, and *Bartramiopsis*

form a clade, with *Bartramiopsis* basal to the divergence of *Lyellia* from *Meantoinea* + *Alophosia*. *Meantoinea* occupies the same position in all analyses not rooted with *Alophosia* (Analyses 2A, 2B, 3A, 3B) (Figs 2, 3, 5, 6). However, in analyses rooted with *Alophosia* (1A, 1B), the clade that includes *Alophosia* in the other analyses (i.e., those not rooted with *Alophosia*) forms a paraphyletic group, with *Meantoinea* being sister to the rest of the family (Figs. 1, 4). The fact that *Alophosia* and *Meantoinea* form a clade irrespective of outgroup selection or rooting, as long as *Alophosia* itself is not the root, suggests that *Alophosia* and *Meantoinea* are sister taxa. This is not surprising, considering that *Meantoinea* combines features of *Alophosia*, *Lyellia*, and *Bartramiopsis*, but shares the greatest number of features (gemma cups, gemmae, costal anatomy, and size) with *Alophosia* (Bippus et al., 2017). Another implication of this is that *Alophosia* may not be the basal-most member of the family.

2.4.3 IS MORPHOLOGY PHYLOGENETICALLY INFORMATIVE FOR THE POLYTRICHACEAE?

Early morphological analyses of the Polytrichaceae (Hyvönen, 1989; Forrest, 1995) and combined morphological-molecular analyses (Hyvönen et al., 1998, 2004), suggested that morphology is not phylogenetically informative in the Polytrichaceae. The results of these analyses (especially the morphology-only results of Hyvönen et al., 2004) resemble the results we obtained with exclusion of continuous characters (Analyses 1B, 2B, 3B; Figs. 4, 5, 6): only a few clades are recovered and their relationships with each other are unresolved.

In our analyses, continuous characters complement the discrete characters and recover significant phylogenetic signal from the morphological data set. When continuous characters are used (Analyses 1A, 2A, 3A), they codify subtle phylogenetic signals that are missing from the discrete character data set, and they fully resolve the relationships (Figs. 1, 2, 3). Similar clades are recovered as in molecular studies, which suggests that the signals codified by continuous

characters are congruent with those of molecular data. Continuous characters complement the signal of discrete morphological characters, in combination with which they can improve phylogenetic resolution. Thus, if a robust set of discrete and continuous characters is used, morphological data do seem to contain phylogenetically informative signals. Continuous characters, especially those related to size have a strong influence in some clades (e.g. the "large gametophyte clade"). Taxa with extremely divergent morphologies as compared to the rest of the family (e.g. *Dawsonia* and *Itatiella ulei*) are especially susceptible to this kind of spurious grouping that is due to similarities in features coded by continuous characters (size, leaf shape) that can introduce homoplasy. For this study, we scored characters from the literature so there is a significant amount of missing data (13%). A more detailed morphological study, including also more comprehensive taxon sampling and scored from specimens, would probably yield more precise results and may circumvent some of the homoplasy introduced when using continuous characters. Use of morphometric characters (such as partial landmarks to describe leaf shape) may also enhance the precision of morphological studies (Goloboff and Catalano, 2016).

According to Bell and Hyvönen (2010a, b), much of the micro-morphology of polytrichaceous sporophytes is unknown as yet but, when studied, it yields taxonomically useful information. For instance, the diversity of sporangial dehiscence mechanisms encompassed by the Polytrichaceae is far broader than in any other moss family (Bell and Hyvönen, 2008). Detailed micro-morphological studies of polytrichaceous sporophytes would undoubtedly uncover useful morphological characters to improve the precision of morphological phylogenetic studies. We expect that such characters would be especially useful for understanding the evolution of unusual sporangial dehiscence mechanisms, such as the fibrous "peristome" of

Dawsonia (Zanten, 1973). A total evidence approach (as used by Bell and Hyvönen, 2010b; Bell et al., 2015) for the whole family, incorporating information from both morphological and molecular data, would a powerful tool for resolving some of the group's problematic phylogenetic relationships.

2.4.4 DO WE KNOW HOW TO ROOT THE POLYTRICHACEAE?

The root of a clade refers to its basal-most node. Accordingly, in this discussion we refer to the basal-most node of a phylogenetic tree as the *root* and to the basal-most node of the ingroup as the *ingroup root*. As pointed out by Graham et al. (2002), incorrectly rooted phylogenetic trees may result in profoundly misleading evolutionary and taxonomic inferences, and this may be a relatively widespread phenomenon in phylogenetic studies. Because rooting has profound effects on tree topology, it is important to consider the ingroup roots recovered by the different outgroup sampling regimes in our analyses. In our study, each outgroup sampling regime resulted in a different ingroup rooting. In turn, this generated the vast majority of incongruence we see between the analyses (Analyses 1A, 2A, 3A). However, some incongruence in distal nodes cannot be attributed to rooting issues (e.g. polyphyletic *Pogonatum* in Analysis 3A; Fig. 3), and is the result of differences in character polarization between analyses.

In Analysis 2A, which uses an outgroup with representatives of all major lineages of acrocarpous mosses, *Timmia* is recovered as sister to the Polytrichaceae (Fig. 2), and the ingroup root is between a clade containing *Alophosia*, *Meantoinea*, *Bartramiopsis*, and *Lyellia*, and the rest of the family (Figs. 1, 2). This is not inconsistent with the basal position of *Alophosia*, *Bartramiopsis*, and *Lyellia* in the family, suggested by all recent phylogenetic studies. However, it is very unlikely that *Timmia* is sister to the Polytrichaceae, given the distinctive bryopsid-type

sporophyte morphology of *Timmia*, and considering the results of numerous phylogenetic studies that place *Timmia* among the Bryopsida (Chang and Graham, 2011, 2014).

Analysis 3A uses a reduced outgroup, consisting of *Sphagnum*, *Tetraphis*, and *Oedipodium*, which recovers a clade comprised of *Tetraphis* and *Oedipodium* as sister to the Polytrichaceae (Fig. 3). Whereas this placement of *Oedipodium* is congruent with the results of recent molecular phylogenetic studies (Chang and Graham, 2014), *Tetraphis* is not recovered as sister to *Oedipodium* in these studies. The ingroup root is between a clade consisting of *Psilopilum* and *Notoligotrichum* and the rest of Polytrichaceae (Figs. 1, 3). This is incongruent with the results of recent phylogenetic studies of the family (Bell and Hyvönen, 2008, 2010a), wherein *Psilopilum* and *Notoligotrichum* are recovered in a derived position, and is not predicted by the morphology and anatomy of these taxa.

Analysis 1A does not include outgroup taxa and generates an unrooted network (Fig. 7) in which *Alophosia*, *Meantoinea*, *Lyellia*, and *Bartramiopsis* form a clade. Rooting this network with *Alophosia*, an ingroup taxon, as advocated by Bell and Hyvönen (2008), leads to a situation wherein the root is also the ingroup root. The position of *Alophosia* at the root forces it outside of the clade recovered in the unrooted network (Fig. 1) and, consequently, the remaining members of the clade (*Meantoinea*, *Lyellia*, and *Bartramiopsis*) are forced into a paraphyletic arrangement at the base of the tree. Although Bell and Hyvönen (2008) present cogent arguments for constraining the position of *Alophosia* as sister to the rest of Polytrichaceae, the relationships of *Alophosia* in the unrooted network (Analysis 1A), and all analyses not rooted with *Alophosia* (Analyses 2A, 2B, 3A, and 3B) indicate that morphological data do not support its placement at the root of Polytrichacesae. Interestingly, Bell and Hyvönen (2010a) also recover *Alophosia*, *Bartramiopsis*, and *Lyellia* as a clade in an unrooted network generated with

molecular data and in absence of an outgroup. Even though sequence alignment issues make inclusion of an outgroup highly problematic with molecular data (Bell and Hyvönen, 2008), these results indicate that an outgroup is necessary for empirical evaluation of phylogenetic relationships within this group, based on rooted phylogenetic trees.

Additionally, rooting the phylogeny of Polytrichaceae with a member of the family (*Alophosia*) does not allow for empirical testing of the phylogenetic relationships of extinct polytrichaceous mosses that may be more basal members of the family (and are much older) than *Alophosia*. Recently, three previously unkown fossil Polytrichaceae that are morphologically similar to basal members of the family (*Alophosia*, *Bartramiopsis*, *Lyellia, Meantoinea*) have been discovered in the Early Cretaceous of Vancouver Island (Canada) and northern California (Tomescu 2016), and in the Middle Jurassic of Argentina (Tomescu et al., 2018). These taxa await description and phylogenetic assessment. If the phylogenetic relationships of these fossils are to be evaluated, we cannot use *Alophosia* to root the tree, since the fossils may be more basal than *Alophosia*. Besides, the results of our study strongly suggest that *Alophosia* forms a clade with the fossil *Meantoinea* and, thus, may not be the basal-most member of the family.

In summary, our experiments with outgroup sampling regimes confirm the results of previous morphological (Smith, 1971; Smith Merrill, 2007) and molecular phylogenetic studies (Bell and Hyvönen, 2008; Chang and Graham, 2014), which indicate that polytrichaceous mosses represent a morphologically unique group with no known close living relatives (long branch). Our experiments also suggest that there is no way to root phylogenetic analyses of the Polytrichaceae that does not distort ingroup relationships. Thus, relationships within the family are best evaluated from unrooted networks without outgroup taxa, for the time being, and more data are needed to reliably root phylogenies of the Polytrichaceae and unambiguously resolve

both the phylogenetic relationships within the family and the position of the family among basal acrocarpous mosses.

2.4.5 DOES THE FOSSIL RECORD HOLD THE KEY?

It is unlikely that increasing the amount of molecular data analyzed will allow us to root the Polytrichaceeae, since the rooting issue stems from the absence of closely related extant lineages, the only ones that could provide sequence data. This rooting problem could be solved by discovery of stem group members of the polytrichaceous lineage (we use "stem group" in its commonly accepted sense – e.g., Wilf and Escapa, 2015 –, with the caveat that the possibility of an extant member of Polytrichaceae that is basal to all currently known living Polytrichaceae, being discovered in the future, cannot be ruled out completely), which would provide better supported hypotheses of character polarity within the family and would facilitate comparisons with other moss lineages. In principle, a representative of the polytrichaceous stem group could be hiding in the modern biota, undiscovered as yet. Although this is unlikely, it is worth considering that the three living presumedly basal Polytrichaceae (*Lyellia*, *Bartramiopsis*, and *Alophosia*) have either limited or highly disjunct geographic ranges, which could represent remnants of broader past distributions. Because our understanding of modern bryophyte diversity is still far from complete, it is not impossible that a basal member of the Polytrichaceae with extremely narrow distribution (like that of *Alophosia*, which is found only in the Açores islands; Smith, 1971) may still await discovery.

Concurrently, detailed surveys of mosses in anatomically preserved plant fossil assemblages highlight the potential of the fossil record to produce a stem group member of the Polytrichaceae. It is encouraging that four anatomically preserved fossil polytrichaceous gametophytes, currently undescribed, have been discovered in Cretaceous rocks from Canada

(136 Ma), USA (125 Ma), and Portugal (125 Ma), and in Middle-Late Jurassic (178-151 Ma) rocks from Argentina (Tomescu et al. 2018). Our initial assessment of these North American and Argentine fossils suggests that they are morphologically similar to *Alophosia* and *Meantoinea*. These fossils are old enough to provide a glimpse of polytrichaceous diversity in deep time, before the last global mass extinction. Additionally, a recent assessment of the taphonomy and fossil record of bryophytes (Tomescu et al. 2018), suggests that the current paucity of bryophytes in the fossil record is not due to a low preservation potential of these plants and may be a temporary artifact of incomplete exploration of the fossil record, combined with the rarity of bryological expertise in the paleontological community. Considering that several Mesozoic fossil Polytrichaceae have been recently discovered, we predict that additional Mesozoic Polytrichaceae await discovery and that some of these fossils are stem group members of the family.

2.5 CONCLUSIONS

Compared to the results of previous morphology-based (Hyvönen, 1989; Forrest, 1995) and combined morphological-molecular phylogenetic analyses of the Polytrichaceae (Hyvönen 1998, 2004), our results suggest that morphology is phylogenetically informative for the family. In analyses employing discrete characters only, resolution was low, like in previous morphology-based studies. Nevertheless, discrete characters provide a backbone for the resolution provided by continuous characters. Continuous characters provide useful information and complement the phylogenetic signal of the discrete characters, improving resolution. These results suggest that studies using a total evidence approach, which incorporate both molecular and morphological data (including continuous characters), may be particularly useful for resolving phylogenetic

relationships within polytrichaceous mosses, similar to the level of resolution obtained by Flores et al. (2017a) with the marchantiidean liverworts.

Our study resolves the phylogenetic positions of the two most completely-known fossil members of the Polytrichaceae. *Meantoinea alophosioides*, as predicted by its morphological and anatomical similarities with *Alophosia*, is closely related to the eperistomate "lower Polytrichaceae" (*Alophosia*, *Bartramiopsis*, *Lyellia*), and forms a clade with *Alophosia* in all analyses not rooted with the latter. We recover *Eopolytrichum antiquum* as a member of the *Polytrichum* clade, which confirms the results of Hyvönen et al. (1998, 2004) and suggests that *Eopolytrichum* illustrates an instance of secondary loss of the polytrichoid peristome.

Using outgroup sampling experiments, we demonstrate that outgroup selection has significant effects on the phylogenetic relationships recovered in the family. These experiments demonstrate that the Polytrichaceae are rooted differently by each combination of outgroups. Currently, there is no reliable way to root phylogenetic analyses of the Polytrichaceae, and relationships are best evaluated from unrooted networks. This is because the Polytrichaceae have no close living relatives that can be used as outgroup taxa. More data are needed to reliably root phylogenies of the Polytrichaceae and unambiguously determine the phylogenetic position of the family among basal acrocarpous mosses. The most expedient source for this type of data would be discovery of a stem group member of the family, either in the modern biota or, more likely, in the fossil record. Recently, several Cretaceous and Jurassic Polytrichaceae have been discovered in Europe, North America, and South America, and await description (Tomescu 2016; Tomescu et al., 2018). Because the current paucity of fossil bryophytes is probably due to incomplete exploration of the fossil record and to the dearth of bryological expertise in the

paleontological community, these recent discoveries predict that there are more Mesozoic fossil Polytrichaceae still undiscovered, some of which are stem group members of the family.

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FIGURES

Figure 1 Most parsimonious tree (tree length 233.85) from Analysis 1A, rooted with *Alophosia* and including continuous characters. The open circles at nodes represent the ingroup root of Analyses 2 and 3, respectively. Numbers at nodes represent bootstrap values. Bootstrap values below 10 are not reported. Extinct taxa are indicated by a † symbol.

Figure 2 Most parsimonious tree (tree length 292.41) from Analysis 2A, rooted using *Sphagnum,* including all lineages of basal acrocarpous mosses in the outgroup, and continuous

characters. Numbers at nodes represent bootstrap values. Bootstrap values below 10 are not reported. Extinct taxa are indicated by a † symbol.

Figure 3 Strict consensus of two most parsimonious trees (tree length 265.61) from Analysis 3A, rooted with *Sphagnum*, using a reduced outgroup (*Oedipodium* and *Tetraphis*) and continuous characters. Numbers at nodes represent bootstrap values. Bootstrap values below 10 are not reported. Extinct taxa are indicated by a † symbol.

Figure 4 Strict consensus of 20 most parsimonious trees (tree length 216) from Analysis 1B, rooted with *Alophosia* and excluding continuous characters. Extinct taxa are indicated by a † symbol.

Figure 5 Strict consensus of 10 most parsimonious trees (tree length 273) from Analysis 2B, rooted using *Sphagnum*, including all lineages of basal acrocarpous mosses in the outgroup, excluding continuous characters. Extinct taxa are indicated by a † symbol.

Figure 6 Strict consensus of 23 most parsimonious trees (tree length 247) from Analysis 3B, rooted with *Sphagnum*, using a reduced outgroup (*Oedipodium* and *Tetraphis*), excluding continuous characters. Extinct taxa are indicated by a † symbol.

Figure 7 Most parsimonious unrooted network (tree length 233.80) from Analysis 1A, including continuous characters. Extinct taxa are indicated by a † symbol.

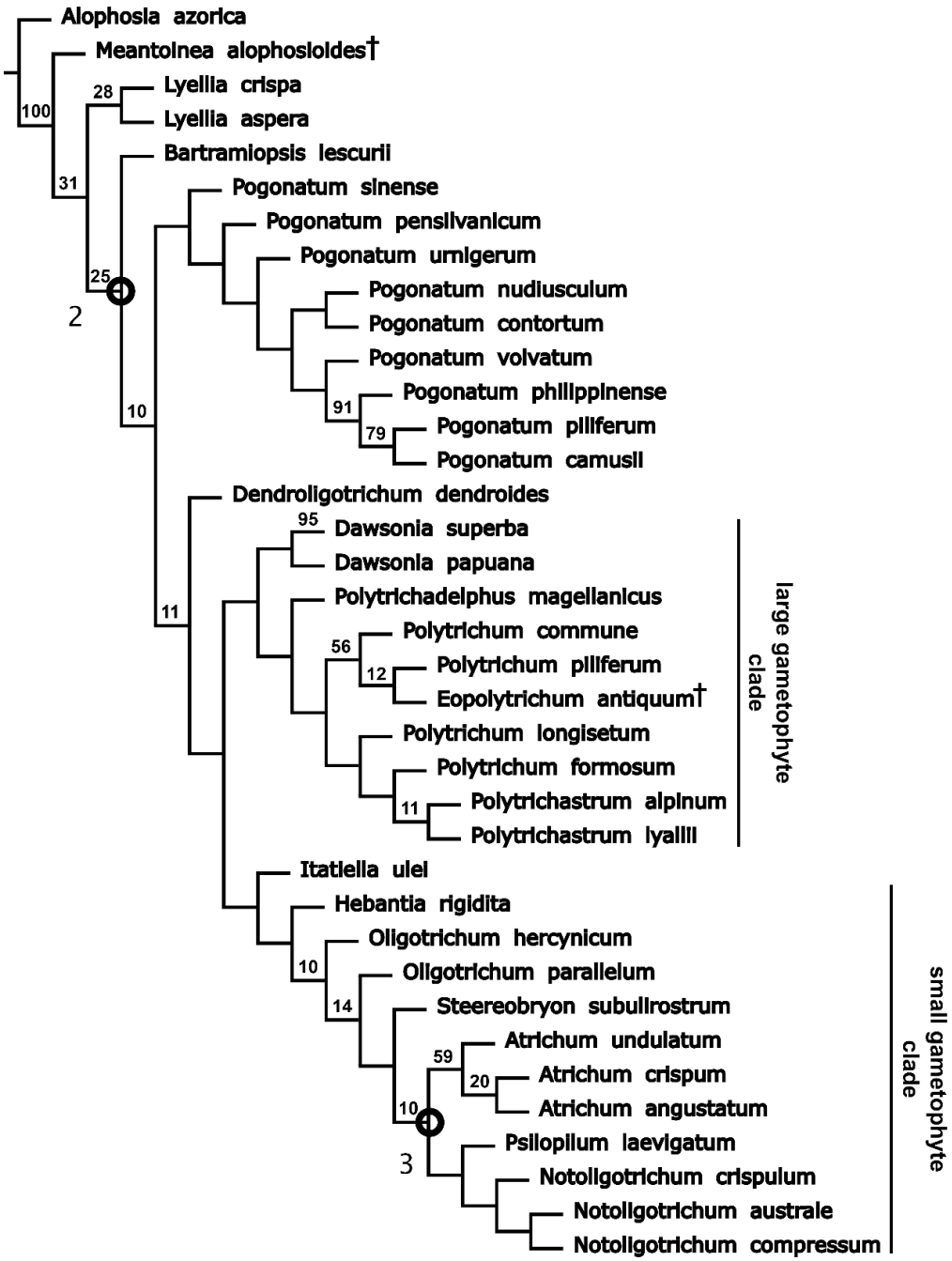

Figure 1 Most parsimonious tree (tree length 233.85) from Analysis 1A, rooted with Alophosia *and including continuous characters. The open circles at nodes represent the ingroup root of Analyses 2 and 3, respectively. Numbers at nodes represent bootstrap values. Bootstrap values below 10 are not reported. Extinct taxa are indicated by a † symbol*

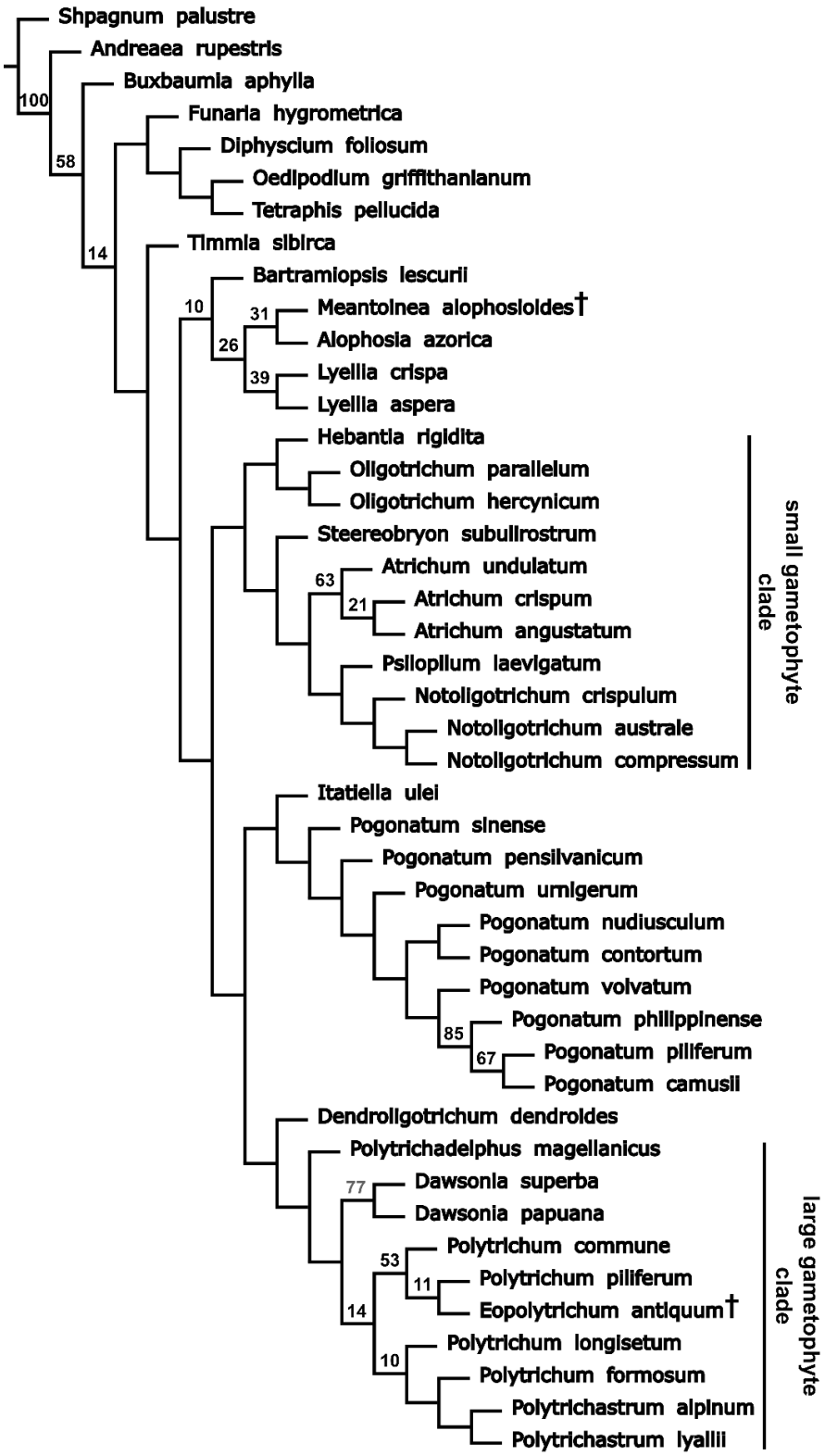

Figure 2 Most parsimonious tree (tree length 292.41) from Analysis 2A, rooted using Sphagnum, *including all lineages of basal acrocarpous mosses in the outgroup, and continuous characters. Numbers at nodes represent bootstrap values. Bootstrap values below 10 are not reported. Extinct taxa are indicated by a † symbol.*

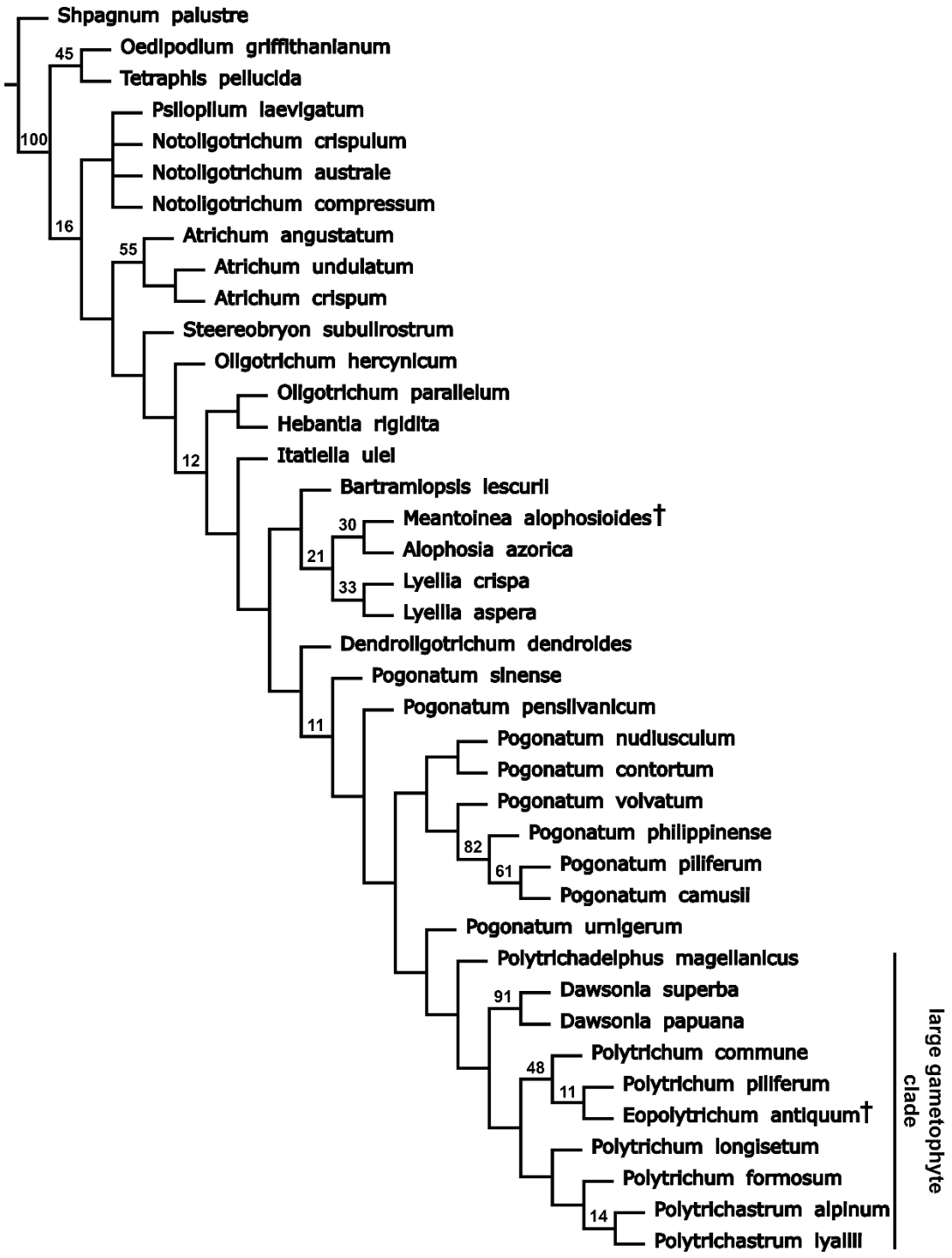

Figure 3 Strict consensus of two most parsimonious trees (tree length 265.61) from Analysis 3A, rooted with Sphagnum*, using a reduced outgroup (*Oedipodium *and* Tetraphis*) and continuous characters. Numbers at nodes represent bootstrap values. Bootstrap values below 10 are not reported. Extinct taxa are indicated by a † symbol.*

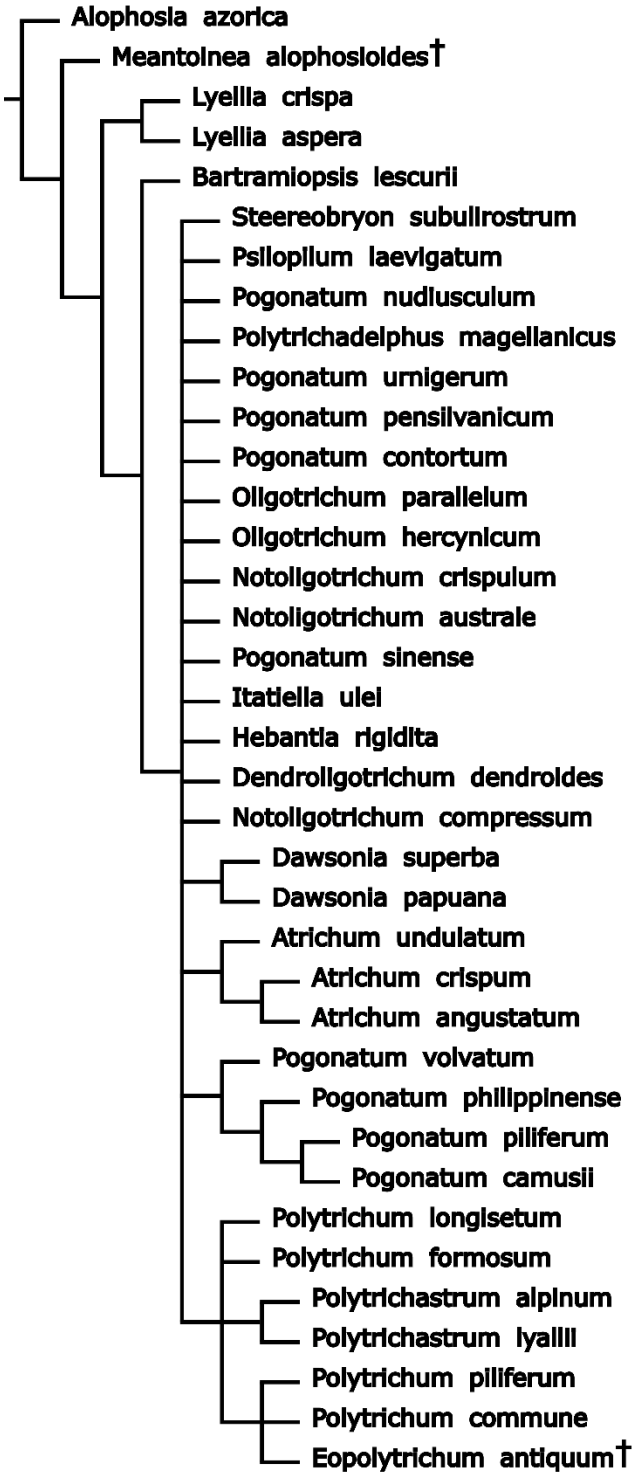

Figure 4 Strict consensus of 20 most parsimonious trees (tree length 216) from Analysis 1B, rooted with Alophosia and excluding continuous characters. Extinct taxa are indicated by a † symbol.

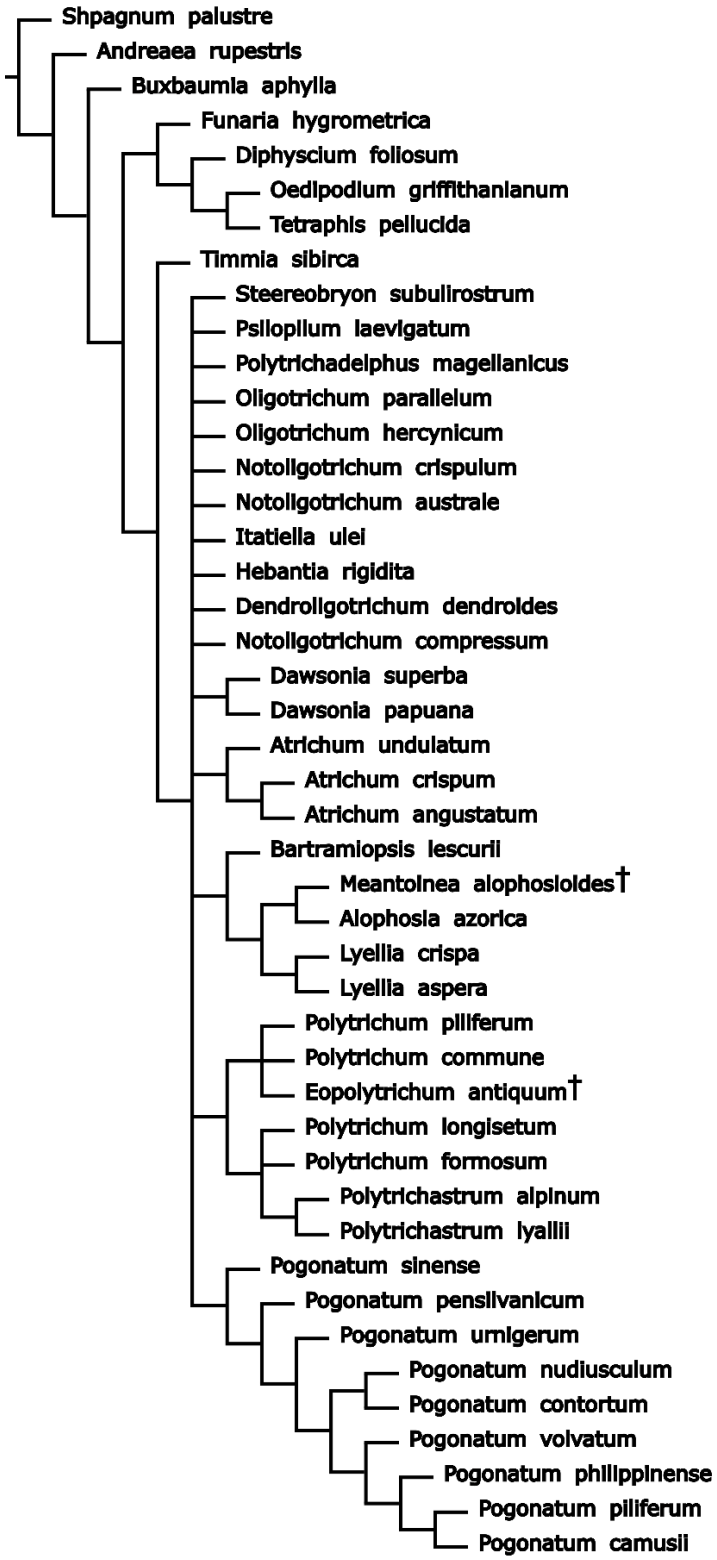

Figure 5 Strict consensus of 10 most parsimonious trees (tree length 273) from Analysis 2B, rooted using Sphagnum, including all lineages of basal acrocarpous mosses in the outgroup, excluding continuous characters. Extinct taxa are indicated by a † symbol

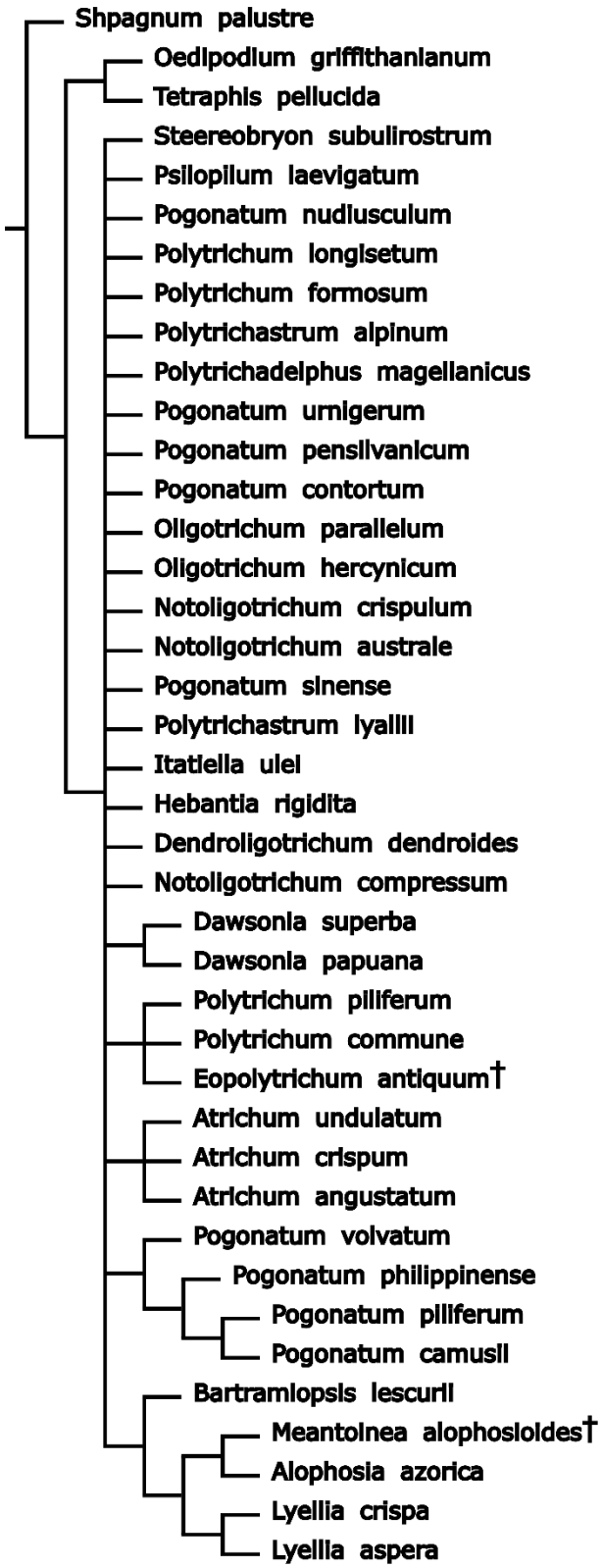

Figure 6 Strict consensus of 23 most parsimonious trees (tree length 247) from Analysis 3B, rooted with Sphagnum, using a reduced outgroup (Oedipodium and Tetraphis), excluding continuous characters. Extinct taxa are indicated by a † symbol.

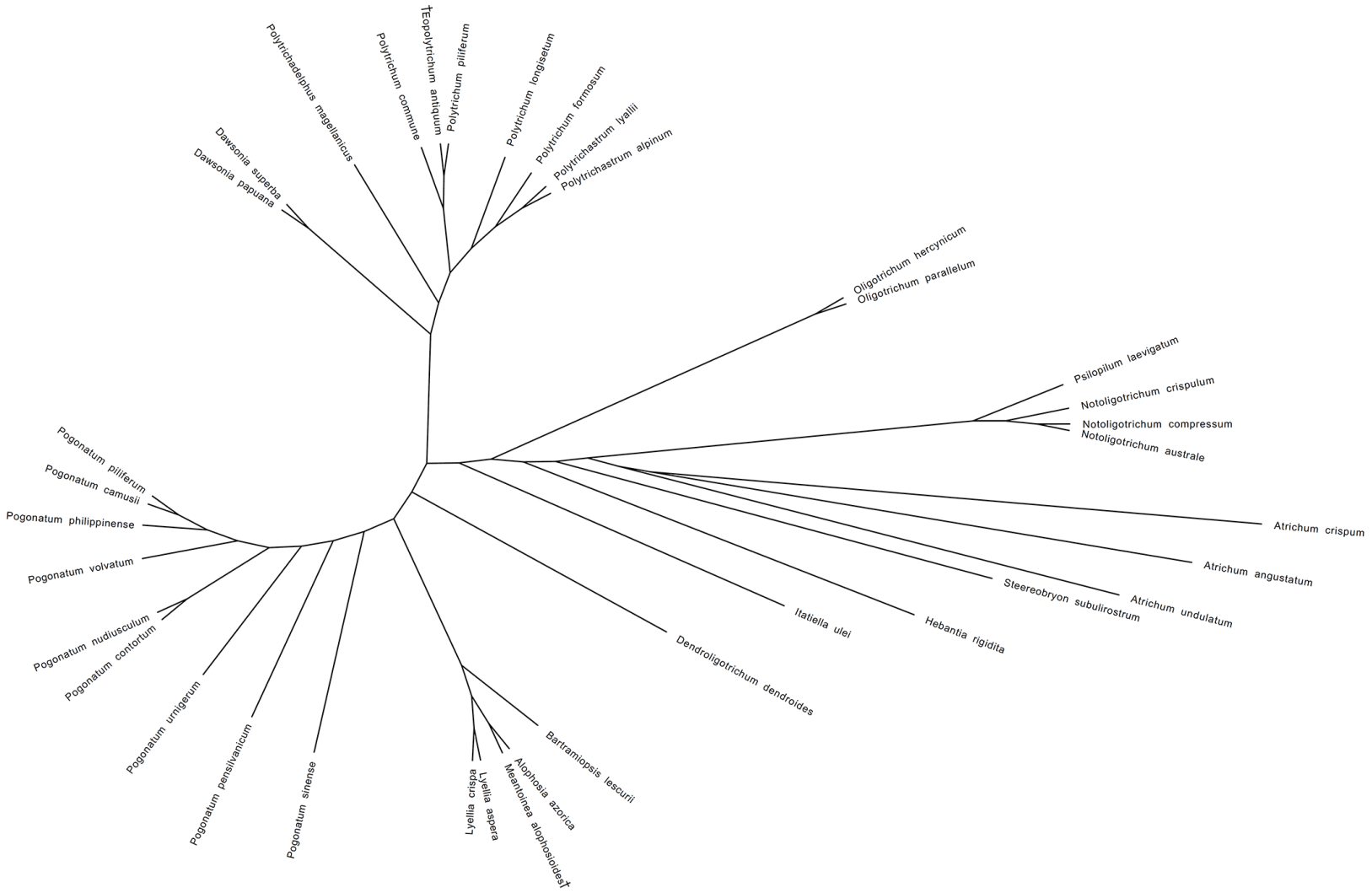

Figure 7Most parsimonious unrooted network (tree length 233.80) from Analysis 1A, including continuous characters. Extinct taxa are indicated by a † symbol

TABLES

Table 1 Analysis Parameters

Analysis	Figures	Root	Outgroup taxa	Continuous characters
1A	1, 7	*Alophosia*	None	Yes
1B	4	*Alophosia*	None	No
2A	2	*Sphagnum*	*Andrea*, *Buxbaumia*, *Diphyscium*, *Funaria*, *Oedipodium*, *Tetraphis*, *Timmia*	Yes
2B	5	*Sphagnum*	*Andrea*, *Buxbaumia*, *Diphyscium*, *Funaria*, *Oedipodium*, *Tetraphis*, *Timmia*	No
3A	3	*Sphagnum*	*Oedipodium*, *Tetraphis*	Yes
3B	6	*Sphagnum*	*Oedipodiu*m, *Tetraphis*	No

APPENDICES

APPENDIX 1. TAXA AND CHARACTERS USED IN THE ANALYSIS.

Taxa used in the analysis

Alophozia azorica Cardot

Andreaea rupsetris Lindb.

Atrichum angustatum Bruch et Schimp.

Atrichum crispum Sull.

Atrichum undulatum P. Beauv.

Bartramiopsis lescurii Kind.

Buxbaumia aphylla Hedw.

Dawsonia papuana F. Muell.

Dawsonia superba Grev.

Dendroligotrichum dendroides Broth.

Diphyscium foliosum D. Mohr.

Eopolytrichum antiquum Konopka, Herend. G. L. Merr., et P. Crane

Funaria hygrometrica Hedw.

Hebantia rigidita G. L. Merr.

Itatiella ulei G. L. Sm.

Lyellia aspera Frye

Lyellia crispa R. Br.

Meantoinea alophosioides Bippus, Stockey, G. W. Rothwell, et Tomescu

Notoligotrichum australe G. L. Sm.

Notoligotrichum compressum N. E. Bell et Hyvönen

Notoligotrichum crispulum G. L. Sm.

Oedipodium griffithanianum Schwägr.

Oligotrichum hercynicum Lam. et DC

Oligotrichum parallelum Kind.

Pogonatum contortum Lesq.

Pogonatum nudiusculum Mitt.

Pogonatum pensilvanicum P. Beauv.

Pogonatum phillippinense Tuow

Pogonatum sinense Hyvönen et P. C. Wu

Pogonatum urnigerum P. Beauv.

Pogonatum volvatum Paris

Polytrichadelphus magellanicus Mitt.

Polytrichastrum alpinum G. L. Sm.

Polytrichastrum lyallii G. L. Sm.

Polytrichum commune Hedw.

Polytrichum formosum Hedw.

Polytrichum longisetum Hook.

Polytrichum piliferum Hedw.

Psilopilum laevigatum Lindb.

Sphagnum palustre L.

Steereobryon subulirostrum G. L. Sm.

Tetraphis pellucida Hedw.

Timmia sibirica Lindb. et Arnell

Characters used in the analysis

Continuous characters

0. Stem max length (mm)

1. Leaf length (mm)

2. Leaf maximum width (mm)

3. Sheath:Blade ratio

Most polytrichaceous mosses have leaves differentiated into a sheathing leaf base and a free leaf blade. Using line drawings of leaves, we measured the ratio of leaf sheath length to leaf blade length.

4. Constriction ratio

Most polytrichaceous mosses have a sharp decrease in leaf width at the transition from sheathing leaf base to free leaf blade. Using line drawings of leaves, we measured the ratio of leaf width in the sheathing leaf base to leaf width at the base of the free leaf blade.

5. Number of lamellae

6. Lamellae height (cells)

7. Seta length (mm)

8. Capsule length (mm)

9. Peristome tooth length (μm)

10. Spore size (μm)

Discrete characters

11. Protonema persistence (from Forrest, 1995)

0=persistent; 1=ephemeral

12. Branching

0=not branched; 1=branched

13. Branching architecture (from Hyvönen et al., 1998)

0=dendroid; 1=branches in fascicles

14. Conducting strand in gametophyte axis

    0=present; 1=absent

15. Conducting strand with or without leptoids

    0= hydroids and leptoids; 1= without well differentiated hydroids and leptoids

    The gametophyte stems of polytrichaceous mosses are unusual in having a conducting strand with well-developed leptoids and hydroids. The gametophyte stems of most other mosses lack leptoids and some also lack hydroids (Hébant, 1977).

16. Dry leaf crisping (from Forrest, 1995)

    0=crisped; 1=not crisped

17. Costa

    0=present; 1=absent

18. Strength of costa (from Forrest, 1995)

    0=well defined; 1=weak

19. Costa ending (from Forrest, 1995)

    0=subpercurrent; 1=precurrent; 2=excurrent

20. Toothing on abaxial side of costa (from Forrest, 1995)

    0=absent; 1=present

21. Deuters in costa

    0=present; 1=absent

    Most polytrichaceous mosses have large, circular, photosynthate-conducting cells called deuters. Several outgroup taxa and species of *Pogonatum* with poorly-developed costa lack these cells (Smith, 1971).

22. Stereids in costa

0=absent; 1=present

All polytrichaceous mosses have thick-walled cells called stereids in their costa. Some outgroup taxa (e.g. *Sphagnum*) lack these cells.

23. Number of stereid bands

0=1; 1=2

In polytrichaceous mosses, there are frequently two bands of stereids: one abaxial to a layer of deuters, and one adaxial to the deuter layer. Some taxa have a reduced costa with only one stereid band (Smith, 1971).

24. Extent of dorsal stereid band (from Koskinen and Hyvönen, 2004)

0=almost as wide as blade (as in *Polytrichum juniperinum*); 1=narrowed (as in *Pogonatum aloides*); 2=present only in costa that is restricted to central portion of leaf (as in *Pogonatum contortum*)

25. Extent of ventral stereid band

0=Ventral stereid band well developed; 1=ventral stereid band reduced

26. Outer walls of dorsal cells of costa (from Koskinen and Hyvönen, 2004)

0=incrassate, thicker than transverse cell walls (as in *Polytrichum commune*, *P. Juniperinum*);1=thin to firm, similar to transverse cell walls (*Oligotrichum parallelum*)

27. Sheathing leaf base (from Forrest, 1995)

0=present; 1=absent

28. Hyaline sheath margin (from Hyvönen et al., 1998, 2004)

0=present; 1=absent

29. Sheath/leaf base margin (From Hyvönen et al., 1998, 2004)

0=entire; 1=ciliate; 2=serrate

30. Hinge tissue (from Hyvönen et al., 1998, 2004; Koskinen and Hyvönen, 2004)

    0=present; 1=absent

31. Median blade cell shape (from Forrest, 1995)

    0=subquadrate to hexagonal; 1=elongate

32. Median blade cell wall thickness (from Forrest, 1995)

    0=incrassate (thick walled); 1=thin

33. Thickness of lamellae-free lamina (from Hyvönen et al., 1998, 2004)

    0=unistratose; 1=bistratose

34. Bistratose lamina with adaxial layer of mamillose cells

    0=adaxial layer not mamillose; 1=adaxial layer mamillose

    Alophosia, *Lyellia*, and *Bartramiopsis* have a bistratose lamina with an adaxial layer of mammillose cells. Other polytrichaceous mosses (e.g., *Dendroligotrichum*) have a bistratose lamina, but lack an adaxial layer of mammillose cells.

35. Serrate leaf margin

    0=absent; 1=serrate

36. Extent of serration (modified from Koskinen and Hyvönen, 2004)

    0=essentially on whole blade; 1=serrate portion less than 1/3 blade length or entire

    This character (and character 16) is modified from character 16 in Koskinen and Hyvönen (2004). The character originally addressed extent of both dentation and serration. We chose to split it into two separate characters, for serration and dentation, respectively, because these features are non-homologous.

37. Toothed leaf margin

    0=present; 1=absent

38. Extent of dentation (modified from Koskinen and Hyvönen, 2004)

0=essentially on whole blade; 1=dentate portion less than 1/3 blade length or entire

This character (and character 14) is modified from character 16 in Koskinen and Hyvönen (2004). The character originally addressed extent of both dentation and serration. We chose to split it into two separate characters, for serration and dentation, respectively, because these features are non-homologous.

39. Thickness of leaf blade margin (from Hyvönen 1998, 2004; Koskinen and Hyvönen, 2004)

0=unistratose; 1=two or more-stratose

40. Differentiated leaf blade border

0=absent; 1=present

Some polytrichaceous mosses (e.g., *Atrichum*) have a conspicuously differentiated leaf border (Smith, 1971).

41. Leaf blade border cell shape (modified from Forrest, 1995)

0=linear; 1=rectangular; 2=irregular

This character is modified from character 12 of Forrest (1995). Originally, "absent" was also a character state. We decided to make a separate character for leaf border presence, because presence/absence of a feature and the different forms of that feature are non-homologous (e.g., Hawkins et al. 1997).

42. Leaf blade border cell ornamentation (from Forrest, 1995)

0=very papillose; 1=almost smooth

43.Hyaline lamina margin (from Koskinen and Hyvönen, 2004)

0=absent; 1=present

44. Lamina back toothing (from Forrest, 1995)

0=smooth; 1=toothed

45. Outer wall of dorsal cells of blade (From Hyvönen, 1989)

0=cell walls thick; 1=cell walls thin

46. Abaxial lamellae (from Forrest, 1995)

0=absent; 1=present

47. Adaxial lamellae (from Hyvönen et al.,1998, 2004)

0=present; 1=absent

48. Extent of adaxial lamellae (from Hyvönen 1998, 2004)

0=numerous, occupying full width of the lamina; 1=restricted to costa

49. Number of lamella marginal cells (from Forrest, 1995)

0=single; 1=gemminate

50. Cross-sectional shape of lamella marginal cells (from Forrest, 1995)

0=isodiametric (similar to other cells in lamellae); 1=mammillose; 2=notched (retuse); 3=pyrifrom

51. Size of lamella marginal cells (from Forrest, 1995)

0=smaller than other lamella cells; 1=same size as other lamella cells; 2=Larger than other lamella cells

52. Lamella cuticle (from Forrest, 1995)

0=smooth; 1=papillose; 2=longitudinally pitted

53. Thickness of lamellae marginal cell walls (Hyvönen, 1989)

0=evenly thin; 1=incrassate

54. Apex of leaf blade (from Hyvönen, 1989)

0= acute; 1= wide; 1= cucullate (resembling a hood)

55. Rhizoids on leaves (from Koskinen and Hyvönen, 2004)

0=present; 1=absent

56. Paraphyses (from Hyvönen et al., 1998, 2004)

0=present; 1=absent

57. Distribution of gametangia (from Forrest, 1995)

0=monoecious; 1=dioecious

58. Perigonia shape (from Koskinen and Hyvönen, 2004)

0=elongated; 1=short and ellipsoid

59. Brood bodies (gemmae) (from Hyvönen et al., 1998, 2004)

0=absent; 1=present

60. Calyptra (from Hyvönen et al., 1998, 2004)

0=present; 1=absent

61. Calyptra shape (from Forrest, 1995)

0=mitricate; 1=cucullate

62. Calyptra hair

0=present; 1=absent

Many Polytrichaceae (e.g., *Polytrichum*) have a hairy calyptra. However, most other mosses (including the basal acrocarpous lineages used in analyses 2A, 2B, 3A, and 3B) do not have a hairy calyptra (Schofield, 1985).

63. Calyptra hair density (modified from Forrest 1995)

0=sparse; 1=dense

This character is modified from character 45 of Forrest (1995). Originally, “naked” was also a character state. We decided to make a separate character for calyptra hair

presence, because presence/absence of a feature and the different forms of that feature are non-homologous (e.g., Hawkins et al. 1997).

64. Calyptra hair structure (modified from Hyvönen et al., 1998, 2004)

0=uniseriate; 1=multiseriate

This character was modified from character 16 of Hyvönen et al. (1998) and character 19 of Hyvönen et al. (2004). Originally, "sparse or none" was also a character state. We decided to make a separate character for calyptra hair presence, because presence/absence of a feature and the different forms of that feature are non-homologous (e.g., Hawkins et al. 1997).

65. Pseudopodium (from Hyvönen et al., 1998, 2004)

0=absent; 1=present

66. Seta (from Hyvönen et al., 1998, 2004)

0=present; 1=absent

67. Number of setae per perichaetium (from Forrest, 1995)

0=unisetous; 1=polysetous

In some polytrichaceous mosses, more than one fertilized archegonium of a female gametophyte may develop a mature sporophyte. If this has been reported for a species, it is scored as "polysetous". This data is unavailable for most species.

68. Seta surface (from Hyvönen et al., 1998, 2004)

0=smooth; 1=papillose

69. Capsule attitude

0=erect; 1=nodding

70. Nodding compressed capsule - transverse section symmetry (modified from Hyvönen et al., 2004)

    0=dorsiventrally compressed; 1=laterally compressed

    This character was modified from character 23 of Hyvönen et al., 2004. "Symmetrical" was originally a character state. We decided to make a separate character for calyptra hair presence, because presence/absence of a feature and the different forms of that feature are non-homologous (e.g., Hawkins et al. 1997)

71. Capsule cross-sectional outline

    0=circular; 1=compressed; 2=angular

72. Number of capsule angles (modified from Hyvönen et al., 2004)

    0=two; 1=(4-6); 2=(6-8)

    This character was modified from character 24 of Hyvönen et al., 2004. "None" was originally a character state. We decided to make a separate character for calyptra hair presence, because presence/absence of a feature and the different forms of that feature are non-homologous (e.g., Hawkins et al. 1997).

73. Capsule angles (from Hyvönen et al., 2004)

    0=blunt; 1=sharp, knife edged; 2=ribbed

74. Capsule dehiscence (from Hyvönen et al.,1998, 2004)

    0=longitudinal slits; 1=operculum

75. Capsule mouth (from Forrest, 1995)

    0=wide; 1=narrow

76. Capsule rim disk (from Hyvönen et al., 1998, 2004)

    0=disk absent; 1=disk present

77. Exothecium (from Hyvönen et al., 1998, 2004)

0=smooth; 1=mammillose; 2=papillose

78. Exothecial pitting (from Hyvönen et al., 1998, 2004)

0=none; 1=thin spots; 2=pitted

79. Apophysis (from Hyvönen et al., 1998, 2004)

0=tapering; 1=contracted; 2=discoid

80. Pseudostomata

0=pseudostomata present; 1=pseudostomata absent

Sphagnum has pseudostomata, which are not considered homologous to stomata of other mosses.

81. Stomata (from Hyvönen et al., 1998, 2004)

0=present; 1=absent

82. Stomata type (from Hyvönen et al., 1998, 2004)

0=superficial; 1=cryptopore

83. Position of stomata (from Hyvönen et al., 1998, 2004)

0=restricted to base of sporangium; 1=dispersed

84. Peristome teeth arthrodontous/nematodontous

0=teeth made from whole cells; 1=teeth made from cell walls

Peristome teeth may either be made from whole cells, or from cell walls. These two types of peristome are considered non-homologous. However, because they develop from the amphithecium (Shaw and Anderson, 1988), we chose to include them as states of the same character. The two types are called nematodontous, if teeth are made from

whole cells, and arthrodontous, if they are made from fragments of cell walls. All peristomate polytrichaceous mosses and *Tetraphis* have nematodontous peristome teeth.

85. Number of rings of peristome teeth

0=peristome with endostome and exostome; 1=peristome with only one ring of teeth

In some mosses, there is only one ring of peristome teeth. However, some derived members of the Bryopsida have two rings of teeth: the endostome and the exostome (Schofield, 1985).

86. Peristome tooth number (from Hyvönen et al., 1998, 2004)

0=32; 1=64; 2=4; 3=16

87. Nematodontous peristome tooth shape (from Forrest, 1995)

0=short,blunt; 1=long, filamentous; 2=long, large

88. Polytrichoid tooth structure (from Hyvönen et al., 1998, 2004)

0=simple; 1=compound, sinus broad; 2=compound, sinus narrow

89. Polytrichoid peristome pigmentation (from Forrest, 1995; Hyvönen et al., 1998, 2004)

0=pale; 1=intensively colored

90. Epiphragm (from Forrest, 1995)

0=absent; 1=present

91. Epiphragm type (from Hyvönen et al., 1998, 2004)

0=discoid; 1=stopper; 2=rod

92. Epiphragm teeth

0=absent; 1=present

Species of *Polytrichastrum* have epiphragm teeth, which are projections of the epiphragm (Bell and Hyvonen, 2010b).

93. Peristome teeth attached to epiphragm

0=attached to epiphragm; 1=not attached to epiphragm

In polytrichaceous mosses, peristome teeth are attached to an expanded columella (epiphragm). In other peristomate moses (including *Tetraphis*), peristome teeth are not attached to an epiphragm (Schofield, 1985).

94. Number of amphithecium cells

0=amphithecium cells 2X; 1=amphithecium cells 1X

The amphithecium is the outer layer of cells that surrounds the sporogenous tissue in a moss sporangium. Various layers of the amphithecium produce peristome teeth, in different lineages of mosses. In the polytrichaceous mosses there are roughly double the number of amphithecial cells (2X) that are present in *Tetraphis* and arthrodontous mosses (X) (Shaw and Anderson, 1988)

95. Amphithecial layers that produce peristome teeth

0=all layers

1=innermost 4-8 layers; 2=3 innermost 3 layers

96. Lysigenous abscission layer

0=present; 1=absent

In polytrichaceous mosses, there is a lysigenous abscission layer in peristome development. This layer is absent in *Tetraphis* (Shaw and Anderson, 1988).

97. Spore origin (from Hyvönen et al., 1998, 2004)

0=endothecium; 1=exothecium

98. Spore sac (from Hyvönen et al., 1998, 2004)

0=overarching collumella; 1=cylindric

99. Spore ornamentation (from Hyvönen et al., 1998, 2004)

0=papillose; 1=echinulate; 2=*Bartramiopsis*-type; 3=*Oedopodium*-typ

APPENDIX 2. SCORING OF DISCRETE CHARACTERS

See appendix 2 at http://morphobank.org/permalink/?2810.