

**A PHYLOGENETIC CIRCUMSCRIPTION OF *POLYTRICHASTRUM*
(POLYTRICHACEAE): REASSESSMENT OF SPOROPHYTE
MORPHOLOGY SUPPORTS MOLECULAR PHYLOGENY¹**

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Mosses arguably possess the most structurally complex sporangia of any extant land plants, a consequence of being the monosporangiophyte lineage most strongly adapted to terrestrial environments. Morphological and functional variation in the mechanisms that regulate spore release in one of the major classes of mosses, the Polytrichopsida, is largely unexplored, while recent research indicates that the most distinctive structure, the peristome, has evolved independently in the Polytrichopsida and in other mosses. The genus *Polytrichastrum* was separated from *Polytrichum* on the basis of such sporangial characters, although the critical features had until recently only been examined using light microscopy, and strong evidence from molecular data indicated that *Polytrichastrum* as currently circumscribed is polyphyletic. Here we use Bayesian ancestral character state reconstruction in conjunction with extensive scanning electron micrographic studies to elucidate probable morphology at ancestral nodes and define natural taxa. As well as clarifying the structure, evolution, and aspects of development of the peristome–epiphragm complex in this highly prominent group of mosses, the results provide a basis for a revised phylogenetic taxonomy in which the species of *Polytrichastrum* sect. *Aporotheca* are recognized once more within *Polytrichum*.

Key words: ancestral character state reconstruction; Bayesian phylogeny; epiphragm; peristome; Polytrichaceae; *Polytrichastrum*; Polytrichopsida; *Polytrichum*; scanning electron microscopy; sporophyte; taxonomy.

Mosses arguably possess the most elaborate sporangia of any extant land plants, a consequence of being the monosporangiophyte lineage most strongly adapted to terrestrial environments. (We use the term monosporangiophyte, implicitly defined by the polysporangiophyte concept sensu Kendrick and Crane [1997], to signify any fossil or extant embryophyte in which the sporophyte has only a single sporangium). This structural complexity is apparent even under the traditional interpretation that the spore capsule alone corresponds to the sporangium in mosses, although Kato and Akiyama (2005) hypothesize that the entire sporophyte in all three “bryophyte” lineages is best regarded as a sporangium for purposes of homology with polysporangiophytes. Under either interpretation, because the sporophyte in mosses consists only of a capsule, a stalk (seta), and a foot specialized for nutrient exchange with the vegetative gametophyte, the sporangium itself must regulate the release of its own spores. By contrast, in extant polysporangiophytes, the sporophyte is a highly complex vegetative assemblage bearing multiple sporangia, and extrasporangial structures (e.g., sporo-

phylls, strobili) usually play a role in regulating the release of spores or pollen.

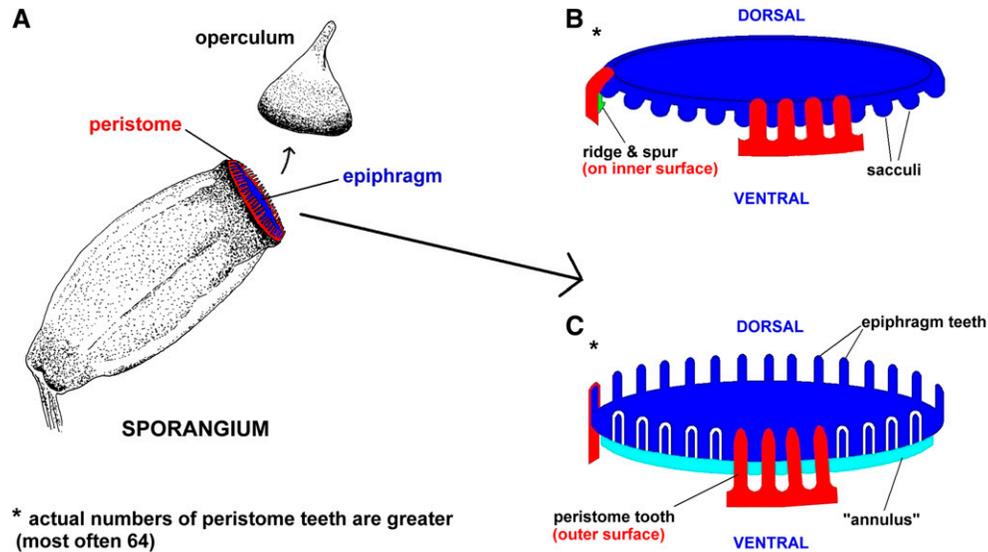
Most moss sporangia dehisce by detachment of a small cap, the operculum (Fig. 1), to expose the peristome, a ring of tooth-like processes responsible for regulating spore release. Recent research (Bell and Hyvönen, 2008) strongly suggests that the peristome has evolved independently in the largest group of mosses (the class Bryopsida or arthroodontous mosses) and in the class Polytrichopsida. While the arthroodontous peristome has been much studied as a source of phylogenetic information (e.g., Vitt, 1981, 1984; Edwards, 1984; Shaw et al., 1987, 1989a, b; Shaw and Rohrer, 1984; Vitt et al., 1998; Goffinet et al., 1999), structural diversity in the very different polytrichopsidan peristome, and its unique associated structure, the epiphragm, remains to be adequately explored. The Polytrichopsida may thus be the most poorly known of all embryophyte classes sporophytically. Here we combine a detailed study of the peristome–epiphragm complex in the genera *Polytrichum* and *Polytrichastrum* with Bayesian ancestral state reconstruction of critical characters to identify patterns of phenotype evolution in the sporophyte. The Bayesian framework offers a method of quantifying probabilities of character states at nodes of interest that integrates multiple uncertainties in the context of a simple model of morphological character evolution. As such, it can provide statistical support for hypotheses of ancestral phenotypes that increases confidence in their use for taxon circumscription, particularly where reversals of definitive apomorphies in derived taxa partially obscure natural groupings.

Polytrichum (Fig. 2) and *Polytrichastrum* are among the most familiar of all mosses, especially in boreal and temperate regions, due to their abundance, distinctiveness, and large size. They are of considerable ecological significance in some communities and are often used as model organisms in physiological

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* actual numbers of peristome teeth are greater (most often 64)

Fig. 1. Diagrammatic representation of the sporangial dehiscence and spore release apparatus in *Polytrichum* and *Polytrichastrum*. (A) Representative spore capsule (*Polytrichastrum ohioense*, Jamieson & Zales 4641, DUKE) showing dehiscence mechanism, with the operculum detaching to expose the peristome and the epiphragm. The peristome (red) is derived from the inner layers of the amphithecium, the tissue surrounding the spore sac, while the epiphragm (blue) derives from the expanded apex of the columella, formed from the endothelial layer that also gives rise to the sporogenic tissue. (B) General morphology of the peristome and epiphragm as found in *Polytrichum* sensu Smith, showing the ridge and spur structures on the inner sides of the peristome teeth that alternate with sacculi on the edge and lower surface of the mature epiphragm. The margin of the true dorsal surface is shown as a black line. (C) Typical morphology as found in most species of *Polytrichastrum* sect. *Polytrichastrum*, showing epiphragm teeth projecting above dorsal epiphragm surface, and "annulus" on ventral surface of mature structure.

studies. The Web of Science (<http://apps.isiknowledge.com/>) contains more references to *Polytrichum commune* than to *Ilex aquifolium*, the European holly. All but one of the species currently placed in *Polytrichastrum* were recognized in *Polytrichum* prior to Smith's (1971) segregation. The status and circumscription of the two genera have been controversial ever since, partially because some of the characters used to separate them are unfamiliar to many bryologists and not always easy to observe. Additionally, the generic placement of *Polytrichastrum alpinum* has always been uncertain; many North American workers treated it in *Pogonatum* prior to Smith's (1971) conspectus, with subsequent tentative molecular results based on limited taxon sampling implying that it might not be closely related to either *Polytrichum* or to the other *Polytrichastrum* species (Derda et al., 1999; Hyvönen et al., 2004). While *Polytrichastrum* has gained acceptance, some bryologists remain uncomfortable with the generic concept, especially as studies based on electrophoretic data (Derda and Wyatt, 2000; van der Velde and Bijlsma, 2001) suggest that some species may be allopolyploids produced by hybridization between *Polytrichum* and *Polytrichastrum*.

With these controversies in mind, we sampled widely from *Polytrichastrum* during our recent large-scale phylogenetic study of the Polytrichopsida (Bell and Hyvönen, 2010). The results, which are based on sampling of all species including several exemplars of *P. alpinum* sensu lato, indicate that the genus is polyphyletic and suggest a more natural circumscription. However, as this entails a novel arrangement of common species, it is necessary to securely place it within a morphological context.

Analyses of all data sets in Bell and Hyvönen (2010) found that the species of *Polytrichastrum* sect. *Aporotheca* formed a well-supported clade with *Polytrichum* and (more distantly) all

or some species of *Pogonatum*, while the other *Polytrichastrum* species, including the type, *P. alpinum*, were outside of this group. Most of the latter formed a clade together with *Meiotrichum lyallii*, while the positions of *P. sexangulare* and *P. sphaerothecium* were ambiguous. Further significant findings were that *P. sexangulare* and *P. sphaerothecium* appear to be mutually distinct, as do *P. alpinum* sensu stricto and an entity approximately corresponding to some concepts of *P. norwegicum* and/or *P. alpinum* var. *septentrionale*, which also included *P. altaicum*.

Smith's concepts of *Polytrichum* and *Polytrichastrum* (Smith, 1971, 1974) were based loosely on Lindberg's (1868) *Polytrichum* subgenera *Pterygodon* and *Leiodon*, distinguished on the basis of peristome and epiphragm characters. Smith (1971) used these features (Fig. 1) in the description of *Polytrichastrum*, while later noting (Smith, 1974) that spore morphology also supported the distinction. The most important characters are the presence in *Polytrichum* sensu stricto of vertical ridges on the insides of the peristome teeth, often associated with spurs on the lower parts of the teeth (Figs. 1B, 3A), and distinct lobes (sacculi) on the ventral and lateral margin of the epiphragm (Figs. 1B, 3B). The sacculi alternate with the peristome teeth such that the ridges project between them. After dehiscence, they partially retract to further open the gaps between the teeth, and it appears that hydration of the dehiscence peristome causes the gaps to be at least partially closed again from expansion of the sacculi combined with subtle changes in the overall shape of the epiphragm and peristome. In *Polytrichastrum* and all other peristomate Polytrichopsida, these ridges and sacculi are lacking, and the lateral surface of the epiphragm (folded onto the ventral surface when mature and dry) is more or less continuous (Figs. 1C, 3C). In some taxa, this is clearly visible as an "annulus" (Fig. 3C, not to be confused with the



Fig. 2. *Polytrichum commune*, showing green leafy gametophytes and a mature sporophyte (center).

annulus associated with operculum dehiscence), while in others (apparently including some *Polytrichastrum* species) the dry ventral surface is more or less unornamented.

Another character used by Smith in separating *Polytrichastrum* from *Polytrichum*, the presence of “epiphragm teeth”, occurs only in some *Polytrichastrum* species. In taxa such as *Polytrichastrum alpinum*, linear, tooth-like processes project above the dorsal surface of the epiphragm and are the sites of attachment of the peristome teeth when the peristome is mature (Figs. 1C, 3D, 3E). In some other species of *Polytrichastrum* (and in *Polytrichum*), these teeth are lacking, and the peristome appears to attach either to the lateral side of the epiphragm or to the outer edge of the dorsal surface (Figs. 1B, 3F). As Smith (1974) noted for *Polytrichastrum formosum*, the apparent attachment of the peristome to the true dorsal epiphragm surface is an artifact (see Results).

A number of other sporophyte characters are variable within or between *Polytrichum* and *Polytrichastrum*. *Polytrichum* species have a prominent basal constriction defining the apophysis, within which the stomata are found, and pitted exothecial cells. *Polytrichastrum* species lack a discrete basal constriction and may or may not have thin spots on the exothecial cells, but cells are not pitted (Smith, 1971). The presence and number of capsule angles is well known to vary within *Polytrichastrum*. Although recent treatments (e.g., Merrill, 1992) have tended to view these characters as taxonomically uninformative, the distribution of states among terminals in our recent results (Bell and Hyvönen, 2010) suggested otherwise. The presence or absence of epiphragm teeth also appeared to correspond well (al-

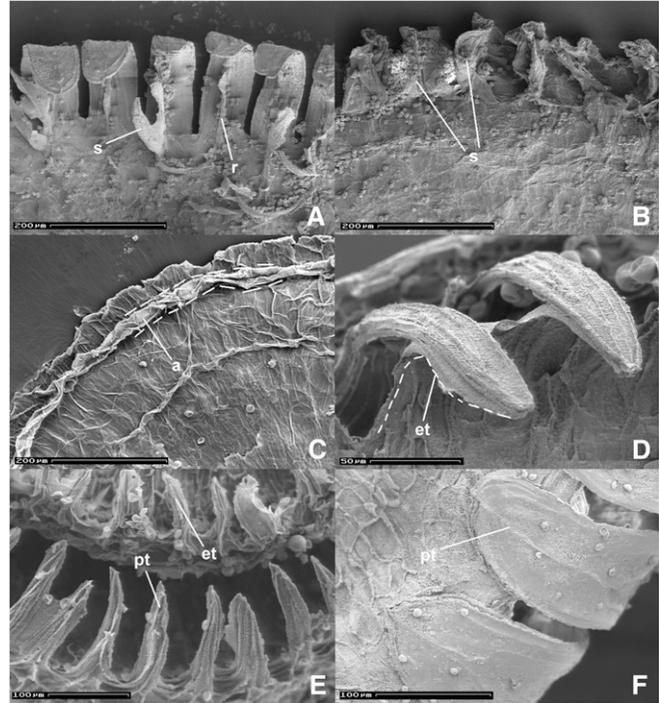


Fig. 3. Scanning electron micrographs illustrating some characters used in separating *Polytrichastrum* from *Polytrichum* sensu Smith (1971, 1974). All specimens were prepared in the dry state, i.e., without critical point drying. Scale bars indicate magnification. (A) *Polytrichum commune*, inner surface of peristome showing ridges (r) and spurs (s). (B) *Polytrichum commune*, ventral surface of detached epiphragm with sacculi (s). (C) *Polytrichastrum ohioense*, ventral surface of epiphragm with annulus (a). (D) *Polytrichastrum alpinum*, detail of attachment of peristome to epiphragm, showing epiphragm teeth (et). (E) Part of peristome and epiphragm of *Polytrichastrum sexangulare* in which peristome teeth (pt) have become detached from epiphragm teeth (et). (F) *Polytrichum commune*, detail of peristome–epiphragm attachment with peristome teeth indicated (pt).

though not precisely) to clades. Because our studies of polytrichopsidan sporophytes using scanning electron microscopy (SEM) offered insights into possible developmental homologues and analogues, we decided to investigate the nature and possible evolutionary trajectories of some key characters relevant to the results of Bell and Hyvönen (2010). An improved understanding of these features provides the context for a revised circumscription of *Polytrichum* and *Polytrichastrum*.

MATERIALS AND METHODS

Scanning electron microscopy—As part of an ongoing study of the peristome–epiphragm complex in Polytrichopsida we have examined multiple specimens using SEM, including species of *Polytrichum* and *Polytrichastrum*. Vouchers for taxa referred to in this study are provided in the Appendix. Most specimens were examined in the dry state, while some were subjected to critical point drying to mimic the hydrated state. Whole capsules were cut transversely, and the upper portions were mounted on aluminum stubs with adhesive tape. Some capsules were dissected to allow individual parts (epiphragms, parts of peristomes) to be mounted separately. Specimens subjected to critical point drying were initially thoroughly hydrated, sometimes with the help of KOH, and then dehydrated in an ethanol series prior to critical point drying in a CPD 030 unit (Bal-Tec, Balzers, Liechtenstein). All specimens were platinum coated before being examined using a DSM 962 scanning electron microscope (Carl Zeiss AG, Oberkochen, Germany).

Sampling, phylogeny, and ancestral character state reconstruction—According to Bell and Hyvönen (2010), all species of *Polytrichum* and *Polytrichastrum* occur within a large “northern hemisphere” clade, together with *Psilopilum*, *Steereobryon*, *Atrichum*, *Pogonatum*, and a group of *Oligotrichum* species (excluding a distantly related southern hemisphere segregate). To obtain a phylogenetically appropriate sample of trees for Bayesian ancestral character state reconstruction and without unnecessary duplication of terminals, we reanalyzed a subset of the combined chloroplast and mitochondrial matrix used in that study. The regions sampled were the *trnL-F* region (including the group I intron and the *trnL-F* spacer), the *rbcL* and *rps4* genes, the *rps4-trnS* spacer (all from the chloroplast), and the mitochondrial *nad5* gene (also including a group I intron). In Bell and Hyvönen (2010), the nuclear 18S region was also sampled but found to be locally incongruent with the chloroplast and mitochondrial data, most notably for the positions of the basal *Pogonatum* species. We initially considered using results from an analysis also including the 18S data to integrate uncertainty deriving from dataset incongruence in the ancestral character state estimations. Because the source of the incongruence is uncertain, however, and the 18S topology may be artifactual (Bell and Hyvönen, 2010), we here base our phylogeny on the chloroplast and mitochondrial data alone. The focus of this study is *Polytrichastrum*, and while our sampling of known species from this genus is complete, sampling from *Polytrichum* sensu stricto is representative, including several species from both sections. Morphology is very consistent within *Polytrichum* sensu stricto, however, and to the extent that the sporophyte characters of interest vary at all within the genus, this variability has been represented in our sampling. All known species of *Polytrichum* would be scored identically for the characters being reconstructed.

On basis of the results of Bell and Hyvönen (2010), we used *Polytrichadelphus innovans* as an outgroup. All laboratory protocols, DNA vouchers, and GenBank accession numbers were as detailed in Bell and Hyvönen (2010). Heterogeneous Bayesian analysis was conducted using the program MrBayes 3.1.2. (Ronquist and Huelsenbeck, 2003) with identical partitioning, model choice, and program settings to those of Bell and Hyvönen (2010) and employing the same criteria to assess convergence and adequate sampling of the posterior distribution (four partitions were used, representing the *rps4* gene, the *rbcL* gene, the *nad5* region, and the combined *rps4-trnS* spacer + *trnL-F* region, with the general time reversible model including a proportion of invariable sites and gamma-distributed rate variation across sites (GTR+I+G) applied to each partition independently).

Ancestral character state reconstruction was accomplished using the program BayesTraits v.1.0 (Pagel et al., 2004; available at website <http://www.evolution.rdg.ac.uk>), and 30 000 trees from the post-burnin phase of one of the two MrBayes runs were used with the MultiState option within BayesTraits. Because BayesTraits requires that all trees are rooted with a positive branch leading to the ingroup and the trees produced by MrBayes did not include a bipartition between the outgroup and ingroup, a branch of the minimum permitted length (0.000010) was inserted between the outgroup and the ingroup for all trees (character states were scored as unknown for the outgroup). We explored two characters with particular potential relevance to the circumscription of taxa within *Polytrichum* and *Polytrichastrum*, namely, capsule profile and the presence or absence of epiphragm teeth.

Capsule profile was coded as a four-state character with the following states: (0) terete, (1) with four angles, (2) with five, six, or more angles, (3) ribbed. In our experience, species with more than four angles never consistently have a fixed number of angles (e.g., *Polytrichastrum sexangulare* may have five, six, or occasionally more angles), while many taxa consistently have four angles. BayesTraits allows multiple states to be applied to taxa, enabling them to be assigned any of these states when the evolution of the character is modeled. Hence, taxa such as *Meiotrichum lyallii* and *Polytrichastrum longisetum* were coded as both (1) and (2), because individual sporophytes may have four, five, or occasionally more angles. Some *Oligotrichum* species were coded as (0, 1, 2) because sporophytes may be terete or with weak multiple angles. Although *Polytrichastrum sphaerothecium* generally has terete capsules, it may also have capsules that are weakly five or six angled; hence, this species was coded as (0, 2). While *P. sexangulare* is often described as having either angled or terete capsules, we believe that mature capsules of this species are always angled and that unambiguously terete examples represent *P. “norwegicum”*. The latter species may closely resemble *P. sexangulare* gametophytically in having more or less cucullate leaf apices and ± smooth lamellar apical cells, as well as sometimes growing in the same late snow lie habitats (N. Bell, unpublished data). Taxa coded as ribbed (state 3) were all species of *Pogonatum*. In these plants, the multiple “ribs” are relatively superficial compared with the capsule angles found in other taxa, and it is not clear to what extent, if any, they are structurally and developmentally homologous.

The epiphragm tooth character was coded as present (0) or absent (1). Under SEM magnification, many, perhaps most, taxa have very slight projections of the peristome tooth attachment point beyond the dorsal surface, whether this projection is vertically or horizontally orientated. However, in taxa with true epiphragm teeth, these projections are distinctly elongated. To define an epiphragm tooth, we used the criterion that it should project at least as much as the width of the peristome tooth at its point of attachment, i.e., it should be longer than it is broad. In practice, most epiphragm teeth project considerably more than this. In some taxa, e.g., *Meiotrichum lyallii*, individual epiphragm teeth are occasionally joined together so that the projection in places resembles a continuous fringe. These were coded as having epiphragm teeth, because individual teeth are also present and it seems clear that the structures are developmentally homologous.

We conducted preliminary maximum likelihood analyses for each of the two characters within MultiState to assess the normal range of variation for the likelihood parameter. To obtain posterior distributions of models of evolution for each character, we used the reversible jump MCMC option within MultiState (Pagel and Meade, 2006). An exponential prior was seeded from a uniform hyperprior on the interval 0 to 15 (Pagel et al., 2004). The addmrca command was used to generate posterior distributions of likelihoods for ancestral states at a number of nodes of interest appearing in the majority rule consensus tree. We further used addmrca to produce state probabilities for a hypothetical node uniting *Polytrichastrum sexangulare* and *P. sphaerothecium* with the clade that includes *Meiotrichum lyallii* and the other *Polytrichastrum* species occurring outside the apical clade (MultiState allows the generation of posterior distributions of states for hypothesized most recent common ancestors of any group of terminals, whatever the frequency of that node in the posterior distribution of trees). Analyses were conducted using 2×10^7 iterations, a burnin of 5×10^6 and a sample frequency of 300. The ratedev parameter was set to 1.5 for modeling evolution of the capsule profile character and 0.5 for presence/absence of epiphragm teeth, these values producing optimal ranges for acceptance rates. In many cases, we used the “fossil” command to assess the statistical significance of observed greater probabilities of certain states at nodes using Bayes factors. These analyses were repeated two or three times and used either 2×10^8 , 5×10^8 , or 1×10^9 iterations (burnin = 5×10^7 or 1×10^8), depending on the ambiguity of the preliminary results, to maximize accuracy of estimation of the harmonic mean. Harmonic means from the separate analyses of the states being compared were used to assess significance using the Bayes factor table in Kass and Raftery (1995, p. 777).

RESULTS

Observations of peristome and epiphragm structure: The “annulus”—Smith (1974, p. 143) interpreted the “annulus”, seen on what appears to be the edge of the ventral surface of dry, mature epiphragms of many Polytrichopsida, as “the margin of the ventral surface, contracted and drawn up against the underside of the disk.” Our interpretation is that the annulus represents tissue that would have been part of the lower lateral surface of the cylinder-like immature epiphragm (the apex of the columella) during early development (see fig. 3 in Chopra and Sharma, 1958, for a section of an immature capsule of *Pogonatum*). When the epiphragm is fully mature, it becomes more or less thin and membranous dorsiventrally, but the lateral surface that directly contacts the peristome remains thicker, at least to a later developmental stage. Figure 4A shows the lateral (i.e., “edge on”) surface of a near-mature epiphragm of *Polytrichastrum formosum* that was fully hydrated prior to critical point drying. The “U-shaped” imprints of the base of the peristome can be clearly seen. In this micrograph and in all others, “x” represents the part of the epiphragm that would have been attached to the midpoint of the base of the sulcus between two peristome teeth at some point during development (or in some states of the mature peristome in some species). Figure 4B shows the ventral surface of a mature, dry epiphragm of the same species, while Fig. 4C (close-up in Fig. 4D) is the same view of an epiphragm of *Pogonatum urnigerum*. The images of *P. urnigerum* are included because they illustrate the structures

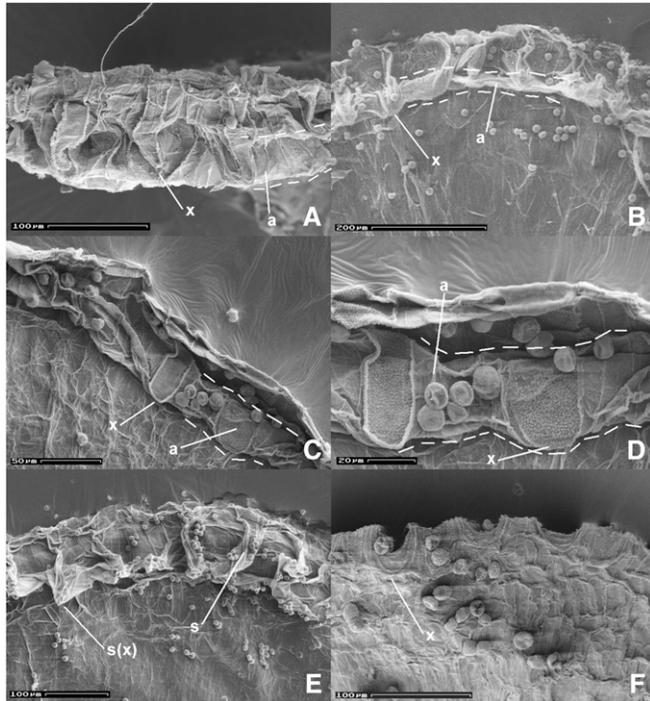


Fig. 4. Scanning electron micrographs illustrating morphology of lateral and ventral epiphragm structures in selected taxa. Scale bars indicate magnification. (A) *Polytrichastrum formosum*, lateral epiphragm surface of specimen subjected to critical point drying to mimic hydrated state. The U-shaped imprints of the base of the peristome can be seen. The lower midpoint of the sulcus between two teeth is indicated by (x) and the part of the lateral surface corresponding to the annulus on the mature, dry ventral surface by (a). (B) *Polytrichastrum formosum*, dry ventral surface of epiphragm showing slightly dissected annulus (a) and imprint of sulcus (x). (C, D) *Pogonatum urnigerum*, dry ventral surface of epiphragm showing annulus (a) and imprint of sulcus (x). (E) *Polytrichum piliferum*, dry ventral surface of epiphragm with morphology intermediate between sacculi and annulus. Sacculi (s) are indicated, also corresponding to base of imprint of sulcus at (x). (F) *Polytrichastrum alpinum*, dry ventral surface of epiphragm. Note lack of distinct annulus or sacculi and imprint of sulcus/base of peristome (x).

particularly clearly, although they are very similar in *Polytrichastrum formosum*. Note that unlike *P. urnigerum*, most *Pogonatum* species lack a clear annulus, presumably because the epiphragm is disassociated from the peristome much earlier in development. In these taxa, the epiphragm is much smaller in relation to the height of the peristome and clearly cannot be involved in blocking the gaps between the peristome teeth at any stage approaching maturity. It can be seen from these figures that the larger part of the lateral surface of the epiphragm eventually forms the structures seen at the edge of the ventral surface of the mature, dry epiphragm, including the annulus. Figure 5B illustrates the hypothesized manner of development of the annulus from lateral epiphragm tissue in a typical member of *Polytrichastrum* sect. *Aporotheca*.

Although species of *Polytrichastrum* sect. *Aporotheca* lack true sacculi as found in *Polytrichum* sensu stricto (Figs. 1B, 3B, 4E), there is a tendency toward a slight dissection of the annulus into sections corresponding to gaps between peristome teeth in some specimens of some species (Fig. 4B). Further, while all *Polytrichum* sensu stricto species more obviously possess sac-

culi than do any *Polytrichastrum* species, in some members of *Polytrichum* sect. *Juniperifolia* they are considerably less pronounced, and something resembling a dissected annulus may be present. Figure 4E shows the ventral surface of an epiphragm of *Polytrichum piliferum*, which in many respects resembles some specimens of *Polytrichastrum formosum* (Fig. 4B) as much as it does *Polytrichum commune* (Fig. 3B). In species of *Polytrichastrum* sect. *Polytrichastrum* however, a clear annulus is generally lacking (Fig. 4F). In these taxa, the boundary between the lateral and ventral surfaces is much less discrete due to the pronounced concavity of the mature epiphragm (Fig. 3D), and the part of the epiphragm that would have been attached to the base of the peristome (again the U-shaped imprint can be seen at "x" in Fig. 4F) does not become markedly folded. In *Meiotrichum lyallii*, however, a distinct annulus is usually present.

The peristome–epiphragm junction—In *Polytrichum* sensu Smith, as well as a number of other Polytrichopsida such as *Pogonatum* and some species of *Polytrichastrum*, the peristome teeth appear to be joined to the dorsal surface of the mature epiphragm. Some early workers (e.g., Hagen, 1914) believed that the elliptic areas on the epiphragm marking the attachment points of the peristome teeth represented (in the words of Smith, 1971, p. 19) "inflexed marginal tooth-like processes, which are appressed to the upper surface of the epiphragm." Smith (1971) was uncertain, noting that observations of herbarium material were often equivocal. Subsequently, however, Smith (1974, p. 144) noted that in *Polytrichastrum formosum*, the imprints of the peristome teeth on what appears to be the edge of the dorsal epiphragm surface are actually outside the true margin of the dorsal surface and that "the apparent margin of the epiphragm is an artifact: the edge of the epiphragm is creased about midway between the dorsal and ventral margins" (i.e., on the lateral margin).

Some of our initial observations of fully mature specimens of *Polytrichum commune* (Fig. 6A) strongly suggested that inflexed marginal teeth could be present, i.e., that the attachment points of the peristome on the epiphragm are homologous to the epiphragm teeth found in species such as *Polytrichastrum alpinum*, only folded over onto the dorsal surface. Subsequent study of material in the later stages of development prior to dehiscence, however, particularly using critical point drying, revealed a more complex picture (Figs. 6B, 6C). In Fig. 6B, it can be seen that the principal area of attachment is developmentally the extreme uppermost portion of the lateral surface of the epiphragm and that, during the final developmental stage, this area becomes rolled or folded over onto the dorsal surface and subsequently more or less compressed onto it (illustrated diagrammatically in Fig. 5B). A small part of the peristome tooth attachment area (see Fig. 6C) is homologous to an epiphragm tooth, because it projects beyond the dorsal surface, but the majority is not. This small projection, together with the line representing the margin of the dorsal and lateral surfaces once it has become compressed (the process varies slightly from one tooth to the next), may easily create the false impression that the entire semicircular attachment area of the peristome tooth is an inflexed epiphragm tooth. Figures 5B and 6C may be compared with fig. 33 in Wenderoth (1931), showing a section of a *Polytrichum juniperinum* capsule at a similar developmental stage. The small part of the epiphragm to which the peristome tooth is attached in this illustration is the part of the lateral surface that becomes rolled over to form the slightly raised edge of the dorsal surface. In this figure, the remainder of the lateral surface,

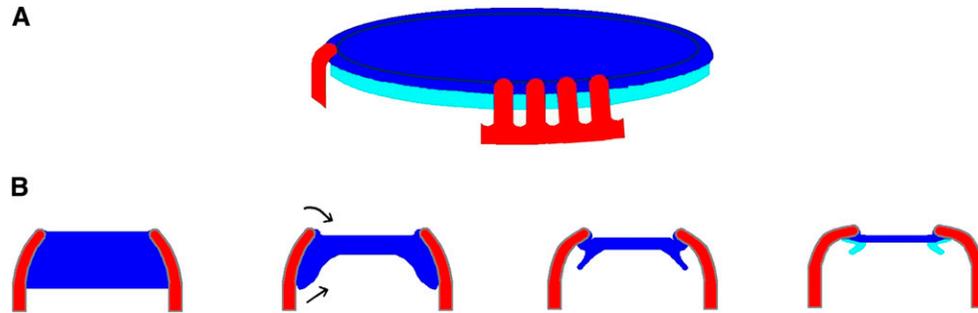


Fig. 5. Diagrammatic representation of hypothesized developmental processes by which selected structures on the mature dorsal and ventral surfaces of some species of *Polytrichum* and *Polytrichastrum* are formed. (A) Generalized peristome and epiphragm structure as found in most species of *Polytrichum* sect. *Aporotheca* (cf. Fig. 1). In these taxa, the peristome teeth apparently attach to the dorsal epiphragm surface as in *Polytrichum* sensu Smith, although an annulus is present rather than sacculi and the peristome teeth are unornamented. (B) Process of formation of the annulus from the lower lateral surface of the partially mature epiphragm, also showing how part of the upper lateral surface develops into what appears to be the edge of the dorsal surface in the fully mature structure.

forming the edge of a sacculus, is already detached from the lower peristome. These observations are consistent with Smith's (1974) comments on *Polytrichastrum formosum* outlined above, although in some specimens of *P. formosum* the rolling or folding over of the upper lateral epiphragm surface is less pronounced, and the teeth may clearly be seen to adhere to the lateral surface even in mature specimens.

In all species of *Polytrichastrum* sect. *Aporotheca* we have observed, with the exception of *P. longisetum*, the peristome–epiphragm junction is more or less the same as is found in *Polytrichum* sensu stricto, as illustrated diagrammatically in Figs. 5A and 1B. Figure 6D shows *P. xanthopilum*; again, the “rolling over” of the upper lateral surface can clearly be seen, creating what often appears as an intermittent raised rim at the edge of the mature dorsal surface. *Polytrichastrum longisetum* appears to represent a genuinely intermediate condition between *Polytrichum*/*Polytrichastrum* sect. *Aporotheca* and the other species of *Polytrichastrum*. Epiphragm teeth, while shorter and broader than those found in other species, are clearly present (Fig. 6E, 6F), and while there is a slight indication of a folding over of the upper lateral surface onto the dorsal surface (Fig. 6F), it is the epiphragm teeth that are the principle attachment points for the peristome.

In *Polytrichastrum* sect. *Polytrichastrum* and in *Meiotrichum*, this situation is considerably more pronounced. In nearly all species, the epiphragm teeth are distinctly elongated, the mature peristome is attached to them, and the upper lateral surface of the epiphragm (excluding the epiphragm teeth) is not significantly rolled over onto the edge of the dorsal surface (Figs. 1C, 3D, 3E, 7A, 7B, 7E, 7F). In immature or recently dehisced specimens, the epiphragm may be attached all the way to the base of the peristome (Fig. 7A), and in some species, the epiphragm teeth and/or a continuous membrane projecting beyond the dorsal surface may be folded toward the dorsal surface (Fig. 7E, 7F), but there is not a folding or rolling over of the true lateral surface onto the dorsal surface. In most of these species, the epiphragm is rapidly deciduous, as the peristome teeth soon “peel off” from the bases of the epiphragm teeth upward, becoming only very weakly attached at their apices (Fig. 7B). Again there is an exception; in *P. emodi*, the epiphragm teeth are very short or absent, and the peristome mostly attaches to the lateral epiphragm surface, which is not, however, significantly folded over (Fig. 7C, 7D). This is similar to the situation observed in most northern hemisphere species of *Oligotrichum*.

Ancestral character state reconstruction—Figures 8 and 9 show diagrammatical representations of relative posterior probabilities of character states at selected nodes of interest for capsule profile and presence/absence of epiphragm teeth, respectively. Relevant numerical values are provided below.

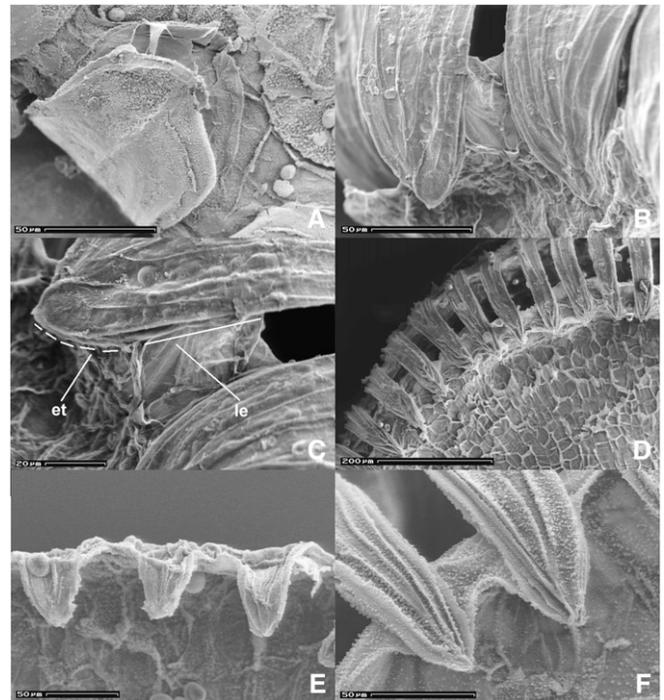


Fig. 6. Scanning electron micrographs illustrating attachment of peristome to epiphragm in selected taxa. Scale bars indicate magnification. (A) *Polytrichum commune*, fully mature specimen in dry state, apparently suggesting presence of epiphragm teeth bonded to dorsal epiphragm surface. (B, C) *Polytrichum commune*, immature specimen subjected to critical point drying, showing attachment of peristome to small section of upper lateral epiphragm surface that is rolled over onto dorsal surface. The small portion corresponding to an epiphragm tooth is indicated by (et), with (le) representing true lateral epiphragm tissue not extending beyond the dorsal surface. (D) *Polytrichastrum xanthopilum*, dorsal epiphragm surface with “rolled over” section of lateral surface as in *Polytrichum* species. (E) *Polytrichastrum longisetum*, epiphragm teeth. (F) *Polytrichastrum longisetum*, attachment of peristome to epiphragm teeth.

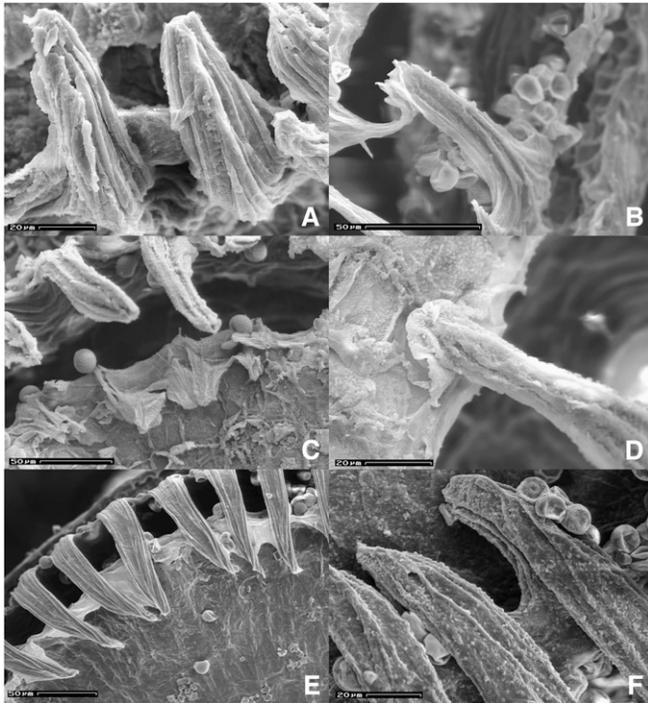


Fig. 7. Scanning electron micrographs illustrating attachment of peristome to epiphragm in selected taxa. All specimens were prepared in the dry state. Scale bars indicate magnification. (A) *Polytrichastrum papillatum*, nearly mature specimen prepared prior to dehiscence. (B) *Polytrichastrum sphaerothecium*. (C) *Polytrichastrum emodi* with peristome detached from epiphragm. (D) *Polytrichastrum emodi*, attached peristome tooth. (E) *Meiotrichum lyallii*, dorsal epiphragm surface showing peristome and epiphragm teeth. (F) *Meiotrichum lyallii*, close-up of peristome and epiphragm teeth.

At the ancestral node for the ingroup, there is a 71% probability of angled capsules with five or more ridges being the ancestral state (given the phylogeny as represented by the sample of trees, the simple model of character evolution implemented within BayesTraits and the assumptions inherent in the character coding). There is a 27% probability of terete capsules and a 2% probability of capsules with four angles, with the Bayes factor test indicating positive evidence in favor of multiangled capsules over terete and strong evidence for multiangled over four angles (BF multiangled/terete = positive, BF multiangled/4 angled = strong). For the most recent common ancestor (MRCA) of the clade spanning *Meiotrichum* and *Polytrichastrum altaicum* (i.e., *Polytrichastrum* sect. *Polytrichastrum* excluding *P. sexangulare* and *P. sphaerothecium* but including *Meiotrichum*), there is a 61% probability of multiangled capsules, a 23% probability of four angled capsules, and a 16% probability of terete capsules (BF multiangled/terete = positive, BF multiangled/four angled = strong). For a hypothetical clade that includes these taxa in addition to *Polytrichastrum sexangulare*

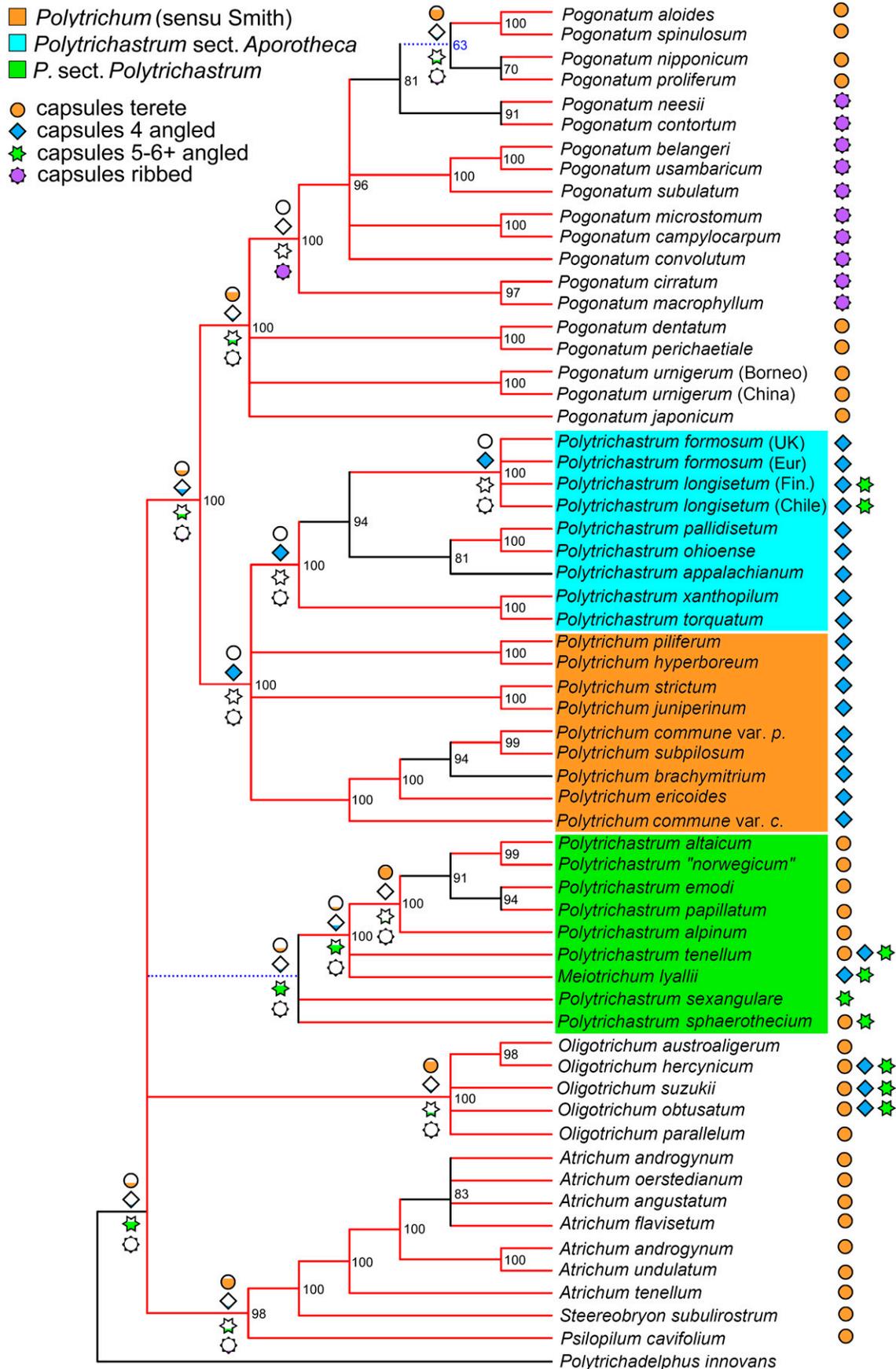
and *P. sphaerothecium*, the probability of multiangled capsules in the MRCA rises to 76%, while four-angled capsules falls to 2%, and terete capsules have a probability of 22% (BF multiangled/terete = positive). At the MRCA node for *P. alpinum*, *P. papillatum*, *P. emodi*, *P. "norwegicum"*, and *P. altaicum*, all of which have exclusively terete capsules, the probability of this state is 97%. (BF terete/multiangled = strong). There is a 100% probability of capsules with four angles for the MRCA of the combined *Polytrichum* sensu stricto/*Polytrichastrum* sect. *Aporotheca* clade, as well as for the MRCA of *P.* sect. *Aporotheca* and also the clade that includes *P. formosum* and *P. longisetum* (the latter is the only species in the section that does not nearly always have capsules with four angles). Results appear ambiguous for the combined *Pogonatum*/*Polytrichum* sensu stricto/*Polytrichastrum* sect. *Aporotheca* clade, with a 32% probability of terete capsules, 37% for four-angled, 28% for multiangled, and 3% for ribbed. However, the Bayes factor test was marginally positive for favoring terete capsules over four angles for this MRCA (despite the apparent slightly higher probability of capsules with four angles). Bayes factors were not significant for other comparisons between the three most probable reconstructions at this node. For the MRCA of *Pogonatum*, there is a 67% probability of terete capsules, 20% for multiangled, 11% for ribbed, and 2% for four angles (BF terete/ribbed = strong, BF multiangled/ribbed = strong, BF terete/multiangled = not significant). At the MRCA of the apical *Pogonatum* clade (see Bell and Hyvönen, 2010), there is a 99% probability of ribbed capsules, while for the MRCA of *Pogonatum aloides*, *P. spinulosum*, *P. nipponicum*, and *P. proliferum*, there is an 80% probability of terete capsules (Bayes factors were not tested for reconstructions at internal nodes within *Pogonatum* because these are peripheral to the focus of the study and multiple analyses using the "fossil" command within MultiState are computationally intensive).

The probability of epiphragm teeth being plesiomorphic for the ingroup is 87% (BF = positive). For the hypothetical MRCA for all members of *Polytrichastrum* sect. *Polytrichastrum* plus *Meiotrichum lyallii*, there is a 97% probability of epiphragm teeth (BF = positive), rising to 100% for the clade spanning *Meiotrichum* and *Polytrichastrum altaicum* as well as the clade spanning *P. alpinum* and *P. altaicum* (BF = strong and very strong respectively). States of this character at the ancestral nodes of the combined *Pogonatum*/*Polytrichum* sensu stricto/*Polytrichastrum* sect. *Aporotheca* clade, the *Polytrichum* sensu stricto/*Polytrichastrum* sect. *Aporotheca* clade, and the *Polytrichastrum* sect. *Aporotheca* clade are all ambiguous (51%, 49%, and 49% respectively, BF = not significant in all cases). For the clade including *P. formosum* and *P. longisetum*, there is a 64% probability of epiphragm teeth in the MRCA, but again the Bayes factor test is not significant.

DISCUSSION

Among land plants, the Polytrichopsida have a unique apparatus for regulating spore dispersal that evolved within the class

Fig. 8. Diagrammatic representation of reconstructed state probabilities for capsule profile at selected nodes on Bayesian 70% majority consensus tree derived from analysis of major northern hemisphere clade of Polytrichopsida. Branches supported at $\geq 95\%$ posterior probability are in red. Selected branches not supported at 70% posterior probability or else representing hypothesized clades based on morphology are in dashed blue. Relative state probabilities are indicated by proportional shading of state symbols as indicated in key. Numbers at nodes are posterior probabilities for clades. Species of *Polytrichum* sensu Smith are highlighted in orange, *Polytrichastrum* sect. *Aporotheca* in blue, *Polytrichastrum* sect. *Polytrichastrum* in green.



and is now thought to resemble arthrodontous peristomes only through superficial convergence (Bell and Hyvönen, 2008, 2010). The earliest Polytrichopsida almost certainly lacked peristomes and instead developed a primitive epiphragm to control spore release (see micrographs of *Lyellia* in Bell and Hyvönen, 2010). The polytrichopsidan peristome, although derived from the same cell layers as the arthrodontous one, is fundamentally a different structure and evolved in plants that already possessed epiphragms (Bell and Hyvönen, 2008). Failure to fully appreciate that the polytrichopsidan peristome is not simply a type of peristome, but rather a class of peristome types, has been responsible for an over-emphasis on gametophytic characters in previous classifications and generic circumscriptions in particular (Bell and Hyvönen, 2010). Although Smith's (1971) description of *Polytrichastrum* was an admirable exception in this respect, full resolution of natural entities awaited more detailed morphological studies combined with information from molecular data.

It is clear from the results of the SEM studies that in immature stages of development of the epiphragm in *Polytrichum* and *Polytrichastrum*, the lateral surface is more prominent than in the mature sporophyte and is attached to the base of the peristome as well as to the apex. The eventual contraction and folding of this lateral surface and its separation from the base of the peristome results in various structures of what subsequently appear to be the edges of the ventral and sometimes also the dorsal surfaces. These structures vary considerably among taxa due to (1) the stage of development at which the lateral surface of the epiphragm contracts and becomes partially detached from the peristome, (2) differences in the way in which the epiphragm becomes altered dorsiventrally during the final stages of development, particularly how the lateral surface folds and compresses, and (3) earlier stages of development influencing the general shape of the epiphragm.

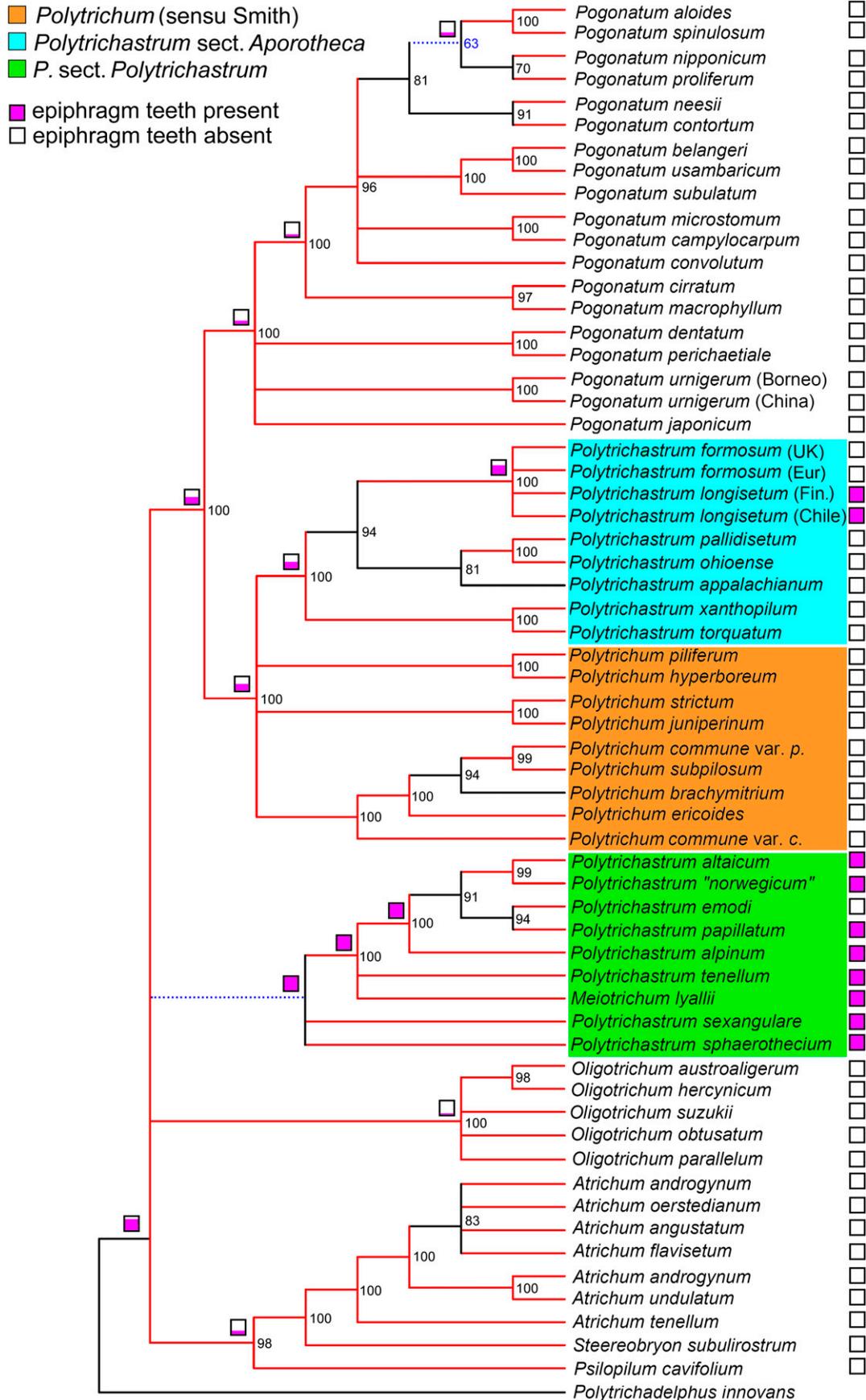
We hypothesize that in *Polytrichum* and *Polytrichastrum*, as well as in most other peristomate Polytrichopsida, the peristome is attached to what is developmentally best viewed as the lateral epiphragm surface, the epiphragm at this stage being a cylinder or lens that is concave or recessed on its dorsal and/or ventral surfaces (see Chopra and Sharma, 1958, fig. 53). In taxa with prominent epiphragm teeth such as most members of *Polytrichastrum* sect. *Polytrichastrum*, the dorsal surface is recessed from the apex of the peristome (its development constrained by the shape of the abscission zone with the operculum), while a membranous extension of the lateral surface extends to the apex and is highly dissected into projecting teeth when mature. The boundary between the lower lateral surface and the ventral surface is usually indistinct, such that the peristome may superficially appear to be attached to the ventral side of the epiphragm, and the annulus is not prominent (cf. *Meiотrichum lyallii*, however). In *Polytrichum* sensu stricto as well as in nearly all species of *Polytrichastrum* sect. *Aporotheca*, epiphragm teeth are lacking or highly reduced, and the upper part of the lateral epiphragm surface (where it is attached to the tips of the peristome teeth) becomes rolled or folded over to form a

margin at the edge of the dorsal surface (Fig. 5). The boundary between the lower lateral surface and the ventral surface is distinct, such that when the lateral epiphragm becomes detached from the base of the peristome it appears as a prominent structure or structures at the edge of the ventral surface. In *Polytrichum* sensu stricto, these take the form of sacculi due to dissection of the lower lateral surface by the ridges and spurs on the inside of the peristome, while in *Polytrichum* sect. *Aporotheca* a single, more or less continuous structure (the annulus) is present, although in some species of each group an intermediate condition is often approached. Epiphragm teeth are plesiomorphic in *Polytrichastrum* sect. *Polytrichastrum* (they are secondarily lost in *P. emodi*), while their presence or absence is ambiguous for the MRCA of *Polytrichastrum* sect. *Aporotheca*, as well as the MRCAs of this clade together with *Polytrichum* sensu stricto and the group that includes these taxa together with *Pogonatum*. Nonetheless, only one species in this larger group (*Polytrichastrum longisetum*) has distinct epiphragm teeth.

Contrary to some recent views (e.g., Merrill, 1992), capsule shape seems to be highly phylogenetically informative for *Polytrichum* and *Polytrichastrum*, as well as within *Pogonatum*. It is probable that multiangled capsules (five to six angles or more) is the plesiomorphic condition in the large northern hemisphere clade as a whole and that terete capsules are derived independently in several groups. One of these includes the majority of species in *Polytrichastrum* sect. *Polytrichastrum*, although the plesiomorphic condition in this group (which includes *Meiотrichum*, and in the lack of clear molecular evidence to the contrary can be hypothesized on morphological grounds to include *P. sexangulare* and *P. sphaerothecium*) is five- to six-angled capsules. Terete sporangia seem to be associated with smaller plant size, shorter seta length, and upright capsules. Thin spots on exothecial cells are found exclusively in *Meiотrichum* and in *Polytrichastrum* sect. *Polytrichastrum* (although not in all species), occurring in taxa with both terete and angled capsules.

The possession of distinctly four-angled capsules is a clear synapomorphy for the combined *Polytrichum*/*Polytrichastrum* sect. *Aporotheca* clade, although this is partially reversed in *P. longisetum*. All other species in this group have four-angled capsules (leaving aside relatively unusual aberrations in individual capsules of some specimens), usually with an angle (rather than a flat side) uppermost. Angles are usually considerably sharper in *Polytrichum* sensu stricto than in *Polytrichastrum* sect. *Aporotheca*, and *Polytrichum* sensu stricto additionally has a well-defined basal constriction defining the apophysis along with pitted exothecial cells. In *Polytrichastrum pallidisetum* and some forms of *P. formosum*, the transverse section of the urn is not as distinctly square as in some other taxa; nonetheless, four distinct angles are generally present. Terete capsules appear to be plesiomorphic within *Pogonatum* (although it is not clear whether they are independently derived), with ribbed capsules arising in the MRCA of the large apical clade that includes most species (see Bell and Hyvönen, 2010). A reversal to terete capsules (i.e., nonribbed in this con-

Fig. 9. Diagrammatic representation of reconstructed state probabilities for presence/absence of epiphragm teeth at selected nodes on Bayesian 70% majority consensus tree derived from analysis of major northern hemisphere clade of Polytrichopsida. Branches, numerical values, and highlighting of taxa are as described for Fig. 8. Probability of epiphragm teeth being present in most recent common ancestors of nodes is indicated by proportional shading of boxes.



text) appears to have occurred in the MRCA of a clade that includes *P. aloides*, *P. spinulosum*, *P. nipponicum*, and *P. proliferum*.

It is clear therefore that, while a number of distinctive morphological synapomorphies define *Polytrichum* sensu Smith (1971, 1974), there are also several that define the clade including both *Polytrichum* and *Polytrichastrum* sect. *Aporotheca*. This is consistent with the evidence from molecular data (Bell and Hyvonen, 2010), which provides strong support for the combined *Polytrichum*/*Polytrichastrum* sect. *Aporotheca* clade, indicating that species in the latter are not closely related to those in *Polytrichastrum* sect. *Polytrichastrum*. It is also more or less consistent with the monophyly of *Polytrichum* sensu Smith (although the position of *Polytrichum* sect. *Juniperifolia* is ambiguous based on support indices). In the *Polytrichum*/*Polytrichastrum* sect. *Aporotheca* clade we see what is perhaps the most developed form of the polytrichopsidan peristome, with apparent adaptations toward the control of spore release in response to environmental conditions over extended periods of time. The lower lateral surface of the epiphragm remains attached to the basal peristome until a relatively late developmental stage and, in the form of sacculi or a prominent annulus, appears to play a role in a dynamic response of the peristome–epiphragm complex to subsequent hydration and dehydration, increasing the likelihood of spore release in dry conditions. Correspondingly, the epiphragm remains firmly attached to the peristome, this being associated with a late developmental stage that may facilitate secure attachment of the tips of the peristome teeth to the margin of the epiphragm. These two structures are derived from what are fundamentally different sporophyte tissues during earlier developmental stages. In *Polytrichastrum* sect. *Polytrichastrum*, although the epiphragm may be attached to the base of the peristome at dehiscence, the peristome–epiphragm complex appears to be a more passive structure, with the epiphragm fairly rapidly becoming detached from the peristome from the bases of the teeth upward. Eventually it is shed, and the remaining spores are simply dispersed through the open mouth of the peristome. This is consistent with the generally upright or gently inclined orientation of capsules in this group, and perhaps also with the terete profile in some species. By contrast, in the *Polytrichum*/*Polytrichastrum* sect. *Aporotheca* group, the strongly inclined orientation of the fully mature theca in many species, combined with the angular surface profile, may be further adaptations toward controlled spore release via the persistent peristome–epiphragm complex. We hypothesize that these features may facilitate interactions with environmental variables such as wind and/or precipitation to vibrate the capsule and help dislodge spores at a rate controlled by the peristome and epiphragm. Although certainly most developed in *Polytrichum* sensu stricto, they are also present in *Polytrichastrum* sect. *Aporotheca*.

Taxonomically, there are two plausible solutions to this phylogenetic scenario, either the recognition of *Polytrichastrum* sect. *Aporotheca* at the generic level, or a wider circumscription of *Polytrichum* to include these species. Because the monophyly of *Polytrichum* sensu Smith is not yet firmly established and all species in *Polytrichastrum* sect. *Aporotheca* other than *P. torquatum* have previously been recognized in *Polytrichum*, we prefer to take the latter, more conservative approach at this stage. This follows the principles elucidated by, for example, Wiley et al. (1991) that unnecessary nomenclatural novelties should be avoided. Perhaps more significantly, allopolyploidy

involving hybridization between species of *Polytrichum* and *Polytrichastrum* sect. *Aporotheca*, but not between either of these taxa and *Polytrichastrum* sect. *Polytrichastrum*, appears to have taken place (Derda and Wyatt, 2000; van der Velde and Bijlsma, 2001; see also discussion of these findings in Bell and Hyvonen, 2010). It seems appropriate to recognize section *Aporotheca* under *Polytrichum* (see below). We prefer not to recognize subgeneric taxa within an emended *Polytrichastrum* until the positions of *P. sexangulare* and *P. sphaerothecium* have been clarified.

We further propose that *Meiotrichum lyallii* should be recognized again under *Polytrichastrum*. Although clearly a highly distinct species with a number of features that do not fit easily in that genus, based on the current molecular evidence, it cannot reasonably be recognized at the generic level while *Polytrichastrum tenellum*, *P. sexangulare* and *P. sphaerothecium* are retained within *Polytrichastrum*. The distinguishing characters of *Meiotrichum* appear to be highly plesiomorphic and are perhaps relatively close to those of the common ancestor of the northern hemisphere clade as a whole. In addition to the only very sparsely hairy calyptra, the capsules in some respects resemble those of some austral taxa such as *Notoligotrichum* in being four- to six-angled, gently inclined, sometimes bilaterally compressed with two angles uppermost, and distinctly narrowed toward the mouth. The relatively flat peristome, with narrow, pointed teeth and epiphragm teeth that are sharply creased to lie almost parallel to the dorsal epiphragm surface, is further reminiscent of austral taxa such as *Dendroligotrichum* and *Polytrichadelphus*. Nonetheless, the plant appears in other morphological respects, as well as from the results of the analyses of molecular level data, to be an early-diverging member of *Polytrichastrum*. Many of its features are shared by the closely related *P. tenellum* and to a lesser extent other members of the genus. Notably, the possession of exothecial cells with thin spots unites *Meiotrichum* with hypothesized basal members of *Polytrichastrum*, including *P. sexangulare* and *P. sphaerothecium*.

Only one nomenclatural novelty is required at the species level; we make a new combination for *Polytrichastrum torquatum* to recognize it within *Polytrichum* (see below).

Descriptive key to Polytrichum and Polytrichastrum as newly circumscribed—

1. Capsules with four distinct angles, epiphragm teeth absent or very reduced, peristome usually seeming to attach to margin of upper epiphragm surface, which often appears as a slightly raised rim. Either sacculi or distinct, fleshy annulus present on epiphragm, ridges on back of peristome teeth present or absent. Pitted exothecial cells present or absent, cells never with thin spots.....*Polytrichum*

1. Capsules 5- to 6-angled or terete, or if with 4 angles then not consistently so. Distinct epiphragm teeth present, or if absent then capsules terete. Sacculi and ridges on peristome teeth absent, annulus present or absent. Thin spots on exothecial cells present or absent, cells never pitted.....2

2. Epiphragm teeth short and broad, only slightly longer than wide, vegetative leaves with broad lamina not abruptly narrowed above sheathing base.....*Polytrichum longisetum*

2. Epiphragm teeth elongated (or if absent or very short then capsules terete), vegetative leaves with lamina considerably narrower than sheathing base, often linear and ± channelled.....*Polytrichastrum*

Nomenclature and classification—The following species, all of which have been treated within *Polytrichastrum*, are here treated under *Polytrichastrum* and *Polytrichum* sect. *Aporotheca*. Species of *Polytrichum* sections *Polytrichum* and *Juniperifolia*, none of which have ever been combined under *Polytrichastrum*, are not listed.

Polytrichum Hedw., Species Muscorum Frondosorum 88. 1801.—Type: *Polytrichum commune* Hedw.

Polytrichum sect. *Aporotheca* (Limpr.) N.E.Bell & Hyvönen, comb. nov.—*Polytrichum* subgen. *Aporotheca* Limpr., Die Laubmoose Deutschlands, Oesterreichs und der Schweiz 2: 615. 1893; *Polytrichastrum* sect. *Aporotheca* (Limpr.) G. L. Merr., The Bryologist 95: 271. 1992.

Polytrichum torquatum (Osada and G.L.Sm.) N.E.Bell & Hyvönen, comb. nov.—*Polytrichastrum torquatum* Osada and G.L.Sm., Journal of the Hattori Botanical Laboratory 38: 636. f. 25–36. 1974.

Polytrichum xanthopilum Wilson ex Mitt., Journal of the Proceedings of the Linnean Society, Botany, Supplement 2: 156. 1859.

Polytrichum appalachianum L.E.Anderson, The Bryologist 90: 1. f. 1–7. 1987.

Polytrichum ohioense Renauld & Cardot, Revue Bryologique 12: 11. 1885.

Polytrichum pallidisetum Funck, Cryptogamische Gewächse des Fichtelgebirg's 3: 55. 1802.

Polytrichum formosum Hedw., Species Muscorum Frondosorum 92. pl. 19: f. 1a. 1801.

Polytrichum longisetum Sw. ex Brid., Journal für die Botanik 1800(1): 286. 1801.

Polytrichastrum G.L.Sm., Memoirs of the New York Botanical Garden 21(3): 35. 1971.—Type: *Polytrichastrum alpinum* (Hedw.) G.L.Sm.

Polytrichastrum sexangulare (Flörke ex Brid.) G.L.Sm., Memoirs of the New York Botanical Garden 21(3): 37. 1971.

Polytrichastrum sphaerothecium (Besch.) J.-P. Frahm, Kleine Kryptogamenflora, 6 Auflage 4: 142. 1995.

Polytrichastrum lyallii (Mitt.) G.L.Sm., Memoirs of the New York Botanical Garden 21(3): 40. 1971.

Polytrichastrum tenellum (Müll. Hal.) G.L.Sm., The Bryologist 78: 201. 1975.

Polytrichastrum alpinum (Hedw.) G.L.Sm. Memoirs of the New York Botanical Garden 21(3): 37. 1971.

Polytrichastrum emodi G.L.Sm. Journal of the Hattori Botanical Laboratory 38: 633. f. 1–11. 1974.

Polytrichastrum papillatum G.L.Sm. Journal of the Hattori Botanical Laboratory 38: 633. f. 12–24. 1974.

**Polytrichastrum altaicum* Ignatov & G.L.Merr., Arctoa, a Journal of Bryology 5: 76. f. 29. 1995.

**Polytrichastrum norvegicum* (Hedw.) Schljakov, Novosti Sistematiki Nizaikh Rastenii 19: 210. 1982.

* Note: *Polytrichastrum norvegicum* is probably not the correct name for the entity we recognize in the present study and in Bell and Hyvönen (2010). We believe that there is a morphologically and molecularly distinct species partially corresponding to the usage of this name in the past, but typification is highly confused (see Smith, 1971, notes on p. 37). This entity probably includes *P. altaicum*. These complex nomenclatural issues will be dealt with in a future publication that will also test the distinctness of *P. altaicum* (N. Bell and M. Ignatov, Main Botanical Garden of Russian Academy of Sciences, unpublished data).

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APPENDIX 1. Specimens used for scanning electron micrographs referred to in this study.

Taxon, Voucher, Herbarium (herbarium codes: H = Botanical Museum, Helsinki, Finland; DUKE = Duke University Herbarium, NC, USA), Country of origin.

- Polytrichastrum papillatum*, G. & S. Miehe 6661, H, Pakistan. *Polytrichastrum xanthopilum*, G. & S. Miehe 00-259-15, H, Bhutan. *Polytrichastrum emodi*, G. & S. Miehe 00-381-22, H, Bhutan. *Polytrichastrum sphaerothecium*, B. Jóhannsson s.n. 14.VII.1961, H, Iceland. *Polytrichastrum ohioense*, L. E. Anderson 26394, DUKE, USA. *Meiotrichum lyallii*, R. R. Ireland 8186, H, USA. *Meiotrichum lyallii*, W. A. Weber and T. Grove B-36612, H, USA. *Pogonatum urnigerum*, M. Haapasaari and R. Fagerstén 1040, H, Finland. *Polytrichum piliferum*, K. Heinonen s.n. 24.VII.1969, H, Finland. *Polytrichastrum sexangulare*, Roivainen s.n. 23.VI.1921, H, Finland. *Polytrichastrum longisetum*, M. Brenner s.n. 15.VII.1868, H, Finland. *Polytrichastrum longisetum*, O. Vitikainen 2807, H, Finland. *Polytrichum commune*, S. Hinneri and U. Laine s.n., 16.VIII.1971, H, Finland. *Polytrichum commune*, O. Sundoik s.n., 8.VI.1907, H, Finland. *Polytrichastrum alpinum*, H. Roivainen s.n., 25.VII.1933, H, Finland. *Polytrichastrum alpinum*, E. Therman s.n., 6.VII.1937, H, Finland. *Polytrichastrum formosum*, J. Suominen and T. Koponen 1881, H, Finland. *Polytrichastrum formosum*, J. Hyvönen 6887, H, Finland.