American Journal of Botany 94(3): 460–467. 2007.

DEVELOPMENT OF THE ENIGMATIC PERISTOME OF *TIMMIA MEGAPOLITANA* (TIMMIACEAE; BRYOPHYTA)[1]

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The Timmiaceae (Bryophyta) have been traditionally classified within the Bryales based on peristome architecture. Phylogenetic analyses of nucleotide sequences have revealed relationships that are incongruent with this hypothesis and have implicated an origin for this lineage early in the radiation of arthrodontous mosses (Bryopsida). This unexpected phylogenetic placement raises important questions about the evolutionary significance of the *Timmia* peristome, which differs from all other mosses by 64 isomorphic filaments topping the endostomial membrane. A developmental study of the peristome in *Timmia megapolitana* was undertaken to examine alignments of anticlinal cell walls in the inner peristomial layer (IPL) with those of the primary peristomial layer (PPL), a character that has been used to define major arthrodontous lineages. Criteria were established for assessing longitudinally homologous regions that contribute to the peristome-forming region. Young sporophytes were examined using histological techniques, and the alignment of the cell wall divisions was quantified. Critical divisions in the IPL of *T. megapolitana* were determined to be symmetrical, similar to patterns in the Funariales. This research provides novel developmental observations for a putative ancestral lineage of arthrodontous mosses, reevaluates criteria used to compare developmental studies of different lineages and discusses the phylogenetic implications of these observations.

Key words: arthrodontous mosses; Bryophyta; development; Funariales; peristome; sporophyte ontogeny; Timmiaceae; *Timmia megapolitana*.

Bryophytes s.l. are a basal grade of embryophytes consisting of mosses (Bryophyta), hornworts (Anthocerophyta), and liverworts (Marchantiophyta) (Kenrick and Crane, 1997). With approximately 12 500 species (Crosby et al., 1999), mosses have been categorized as the second-most diverse lineage of land plants after the angiosperms. Despite their small stature, over 300 million yr of evolution have produced substantial morphological innovations and diversification. One such innovation that arose early in the evolution of mosses is the peristome, a ring of teeth that line the mouth of the sporangium. The peristome can regulate the opening of the sporangium and thereby facilitate spore dispersal (Mueller and Neumann, 1988).

Features of the peristome have been used as the foundation for moss classification for the past 200 yr (Hedwig, 1782; Palisot de Beauvois, 1805; Mitten, 1859; Philibert, 1884–1902, abridged translation in Taylor, 1962). These classifications have divided peristomate mosses into two major groups based on the structure of the mature teeth: nematodontous mosses have teeth composed of whole cells, whereas arthrodontous mosses, which compose approximately 90% of moss species diversity (Goffinet and Buck, 2004), have teeth built only from cell wall remnants (Mitten, 1859).

Peristome characteristics distinguish three main lineages of arthrodontous mosses, Bryales (plus Hypnales), Funariales, and Dicranales (Buck and Goffinet, 2000, Fig. 1C–E). These features include the arrangement of the outer ring of teeth (exostome) in relation to the inner ring of segments (endostome), whether both rings or only the endostome are present, and the presence of cilia. In addition to external morphological characteristics, cellular patterns that form the peristome teeth also distinguish these orders. Within the moss sporophyte, two major tissues are established early during development: the endothecium, which forms the columella and typically also the spore mass, and the amphithecium, which produces external structures, such as the peristome and capsule wall. Three concentric amphithecial cell layers form the two rings of peristome teeth in arthrodontous mosses (Blomquist and Robertson, 1941). Thickened periclinal cell walls from the inner and primary peristome layers (IPL and PPL) form the endostome, whereas walls from the PPL and outer peristome layer (OPL) contribute to the exostome (Fig. 1A, B). The intervening cell walls and cellular contents degrade (Fig. 1C–F; dotted lines). Arthrodontous mosses have a standard number of cells in both the OPL and PPL, 32 and 16 cells, respectively, but the IPL can vary across lineages: Bryales 32–96 cells, Dicranales 24 cells, and Funariales 32 cells (Edwards, 1979).

Although peristome types diagnose major lineages of mosses, peristome characteristics alone are insufficient to reconstruct relationships among these orders (Vitt et al., 1998). Phylogenetic analyses based on sequence data have supported a majority of the historical groupings originally based on peristome characteristics. These analyses not only resolved relationships among the major lineages (Buck and Goffinet, 2000; Goffinet and Buck, 2004) but also revealed unanticipated shifts in the phylogenetic positions of several smaller lineages.

The Timmiaceae are a small family of mosses with peristomes composed of both an endo- and exostome, a characteristic that is shared with the Bryales and Funariales. However, the endostome is unique to this family because it is composed of a basal membrane topped by 64 identical

[1] Manuscript received 25 July 2006; revision accepted 16 January 2007.

The authors thank J. Romanow for SEM assistance; L. Lewis and two anonymous reviewers for helpful comments on earlier versions of this manuscript; the Eastern New York Chapter of The Nature Conservancy for permission to collect specimens from the Limestone Rise Nature Preserve; and J. Kekes for assistance in locating collection sites. Funding was provided by the R. Bamford Endowment from the Department of Ecology and Evolutionary Biology (EEB) and the Connecticut State Museum of Natural History at the University of Connecticut. This paper represents a portion of the master's thesis of J.M.B. at the University of Connecticut.

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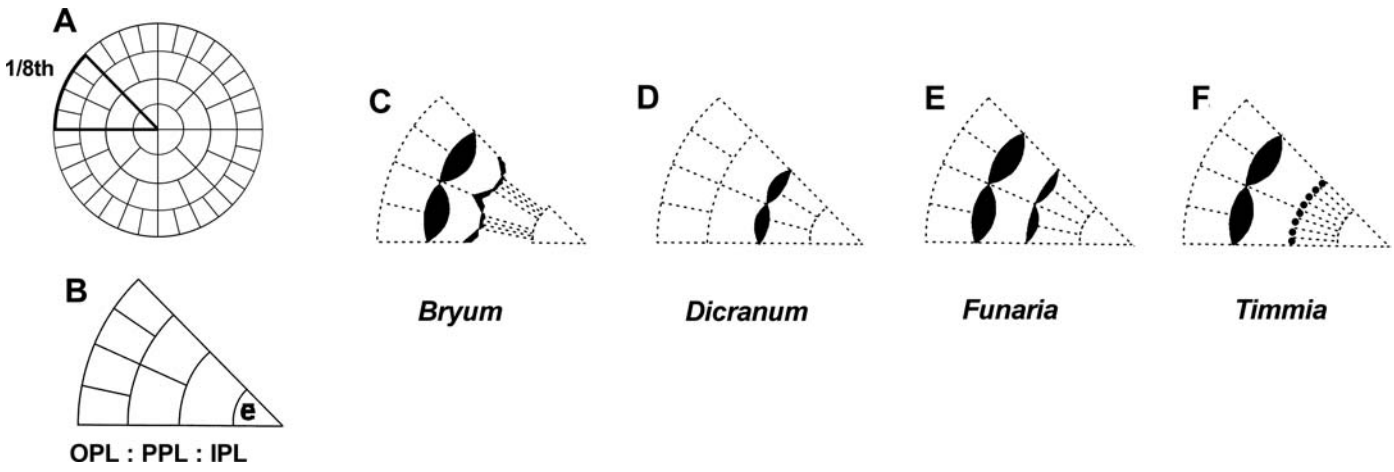


Fig. 1. Diagrams of cross sections through the peristome region of moss sporophytes. (A) Cross section through the peristome-forming region, showing only the innermost layers. (B–F) Diagrams of one-eighth of the cross-sectional area. (B) The three innermost layers of the amphithecium. e, endothecium; IPL, inner peristome layer; OPL, outer peristome layer; PPL; primary peristome layer. (C–E) Mature peristomes of major lineages of arthrodontous mosses. Modified from Vitt (1981; Fig. 13). Darkened regions indicate areas of cell wall thickening that form the teeth. Dotted lines indicate cell walls that are degraded at maturity. (C) *Bryum*-type peristome, representative of the Bryales. (D) *Dicranum*-type peristome, representative of the Dicranales. (E) *Funaria*-type peristome, representative of the Funariales. (F) Mature peristome of *Timmia.*

filaments (Fig. 2; Murphy, 1988). These filamentous appendages are reminiscent of cilia of the *Bryum*-type peristome and account for the historical placement of the Timmiaceae within the Bryales (e.g., Vitt, 1984). Subsequently, inferences from molecular analyses have placed the Timmiaceae near the base of the evolution of arthrodontous mosses (e.g., Newton et al., 2000; Goffinet et al., 2001; Cox et al., 2004) although the precise affinities of the Timmiaceae within this basal radiation are ambiguous: sister to the Funariales or sister to a clade containing the Bryales and Dicranales (Cox et al., 2004). Understanding the structure and development of the Timmiaceae peristome will expand our view of peristome diversity present in early-evolving moss lineages, and with data from additional basal lineages, may shed light on the polarity of transformations in peristomial architectures.

Studies of peristome development in approximately 25 arthrodontous mosses have shown lineage-specific patterns of cell wall alignments at critical developmental stages (e.g., Shaw et al., 1987, 1989a, b; Shaw and Anderson, 1988; Schwartz, 1994; Goffinet et al., 1999). (See Shaw et al. [1987] for a review of earlier peristome developmental studies.) Among all arthrodontous mosses, the very early patterns of cell divisions are conserved (Goffinet et al., 1999). The first stage at which developmental patterns differ is when the IPL divides from eight to 16 cells (Goffinet et al., 1999). At this stage, the degree of cell wall alignment between cells dividing in the IPL and those in the PPL varies among lineages. Specifically, in the Funariales the anticlinal cell walls in the IPL are perfectly aligned with the adjacent PPL walls as a result of a symmetric division, whereas the cell walls are weakly or conspicuously unaligned in the Bryales and Dicranales due to slightly asymmetrical or strongly asymmetrical divisions, respectively (Shaw et al., 1989a, b; Goffinet et al., 1999). Later stages of peristome development in *Timmia megapolitana* Hedw. were presented by Murphy (1988), who noted that at maturity, cell walls of the IPL were clearly unaligned with those of the PPL, which suggests a developmental pattern similar to the Bryales. Whether the observed lack of alignment resulted from originally asymmetric divisions or from symmetric divisions and subsequent uneven cell expansion remains uncertain. Thus, the main goal of this study is to carry out a complete analysis of peristome development for *T. megapolitana* as a representative of the Timmiaceae. Examining early developmental stages will resolve whether the initial alignments of cell walls of the IPL are symmetric as in the Funariales or asymmetric as in the Bryales.

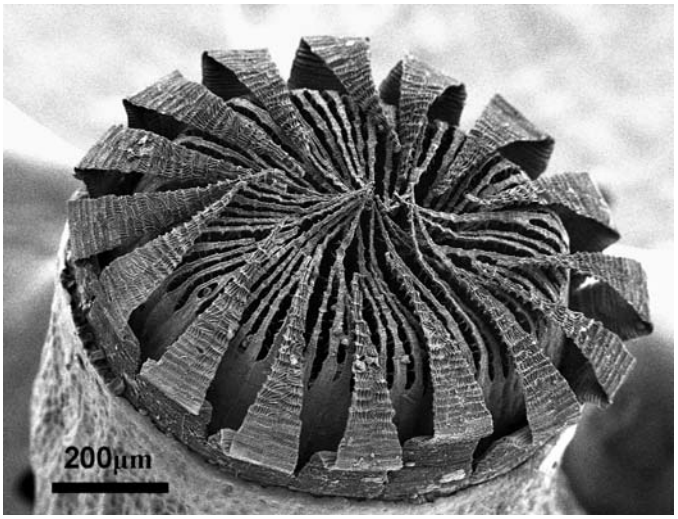


Fig. 2. Scanning electron micrograph of the mature peristome teeth of *Timmia megapolitana*.

Authors of previous developmental studies described the alignment of cells walls of the IPL as strongly asymmetrical, slightly asymmetrical, or symmetrical (e.g., Shaw et al., 1987, 1989a, b; Shaw and Anderson, 1988; Schwartz, 1994; Goffinet et al., 1999). However, careful examination of published images upon which these classifications were based showed variation in cell wall alignment both in single species and even within a histological section. This variation has been noted

previously (Shaw et al., 1989a) yet never formally quantified. Our second goal in this study is to establish a system for quantifying variation in cell wall symmetry in order to compare alignments observed in our investigation of *T. megapolitana* with previous developmental studies.

Variation in cell wall alignments could arise if histological sections are compared that were not taken from longitudinally homologous regions of the inner amphithecium layer (Fig. 1A), i.e., regions that would not contribute to the peristome at maturity. Because the critical divisions are occurring at very early stages when the sporophyte is a small spear, there are no external markers indicating the peristome-forming region. Thus our third goal is to develop criteria for assessing longitudinally homologous regions of peristome formation, based on sporophyte meristem activity, the degree of attachment of the calyptra, and the histological observations of Wenderoth (1931).

MATERIALS AND METHODS

Timmia megapolitana is distributed across temperate areas of the northern hemisphere, with outliers that occur along major northern rivers (Brassard, 1984). This species is most commonly found on calcareous substrates and is the only member of the Timmiaceae that occurs in manmade habitats. One population was sampled from Limestone Rise Nature Preserve, Knox County, New York, USA, which belongs to the Eastern New York Chapter of the Nature Conservancy. A voucher specimen (*Budke 101*) was deposited in the herbarium of the University of Connecticut (CONN).

In *T. megapolitana*, fertilization occurs in June with sporophytes developing to maturity the following spring (Murphy, 1988; J. Budke personal observations). Gametophytes with developing sporophytes attached were collected in August 2004. Sporophytes were fixed in a solution of formaldehyde, alcohol, and acetic acid (FAA) for at least 72 h (Johansen, 1940). These samples were dehydrated through a tertiary butyl alcohol series (Ruzin, 1999) and embedded in Peel-A-Way paraffin (Polysciences, Warrington, Pennsylvania, USA). Twenty-nine sporophytes ranging from 0.45 to 8.48 mm in height were sectioned at 5 μm in a transverse plane and stained with safranin O and fast green FCF (Ruzin, 1999). Eight sporophytes in this range were embedded in JB4 plastic (Polysciences) and 3-μm thick cross sections were cut with a glass microtome knife. Sections were floated on water droplets on slides and dried on a slide warmer at 50°C. These sections were stained using toluidine blue O in a 0.5% benzoate buffer (O'Brien et al., 1964). Mature peristomes of *T. megapolitana* were also examined using scanning electron microscopy. Dry capsules were mounted onto stubs with adhesive tape, covered with gold palladium (60% Au, 40% Pd) using a E5100 SEM coating system (Polaron, Hertfordshire, UK) and then examined using a DSM982 Gemini field emission scanning electron microscope (FESEM) (Zeiss, Thornwood, New York, USA).

Criteria for quantifying cell wall alignments—Critical anticlinal divisions in the IPL are those that increase the number of cells in this layer from eight to 16. All divisions in cross sections that could be examined were classified into one of three symmetry categories: symmetrical, with complete alignment between cell walls of the IPL and adjacent PPL (0%); slightly asymmetrical, with IPL cell walls from one to 33% offset from alignment with those of the PPL; and strongly asymmetrical, with IPL cell walls 34 to 66% offset. Only cell walls in regions where the IPL was actively dividing from eight to 16 cells were scored to avoid measuring cell walls that had later shifted due to cell expansion (Shaw et al., 1989a).

Wenderoth (1931) noted that the peristome-forming region in *Polytrichum juniperinum* Hedw. maintained an endothecium of only four cells in cross section until three layers of the amphithecium had formed via periclinal divisions. Assuming that the observations in *P. juniperinum* can apply to other taxa, we restricted our quantification of cell wall alignment in *T. megapolitana* to histological sections that satisfied two criteria: the endothecium consisted of four cells and the amphithecium had divided to form three cell layers. Divisions were not scored if any cell walls appeared to have shifted during specimen processing. Additionally, the alignments of cell walls that were obscured from view or could not be clearly focused on were not scored. Cell wall alignments were measured as a percentage offset from perfectly aligned using an ocular micrometer on a Leica DMLB compound microscope (Leica Microsystems, Wetzlar, Germany).

In addition to investigating sectioned sporophytes of *T. megapolitana*, other previously published photographs of sporophyte cross sections were examined and quantified in the same manner (Appendix S1, see Supplemental Data accompanying online version of this article). Departure in the position of the IPL cell wall from alignment with the adjacent anticlinal PPL wall were estimated from the published figures using a ruler to the nearest 0.25 mm and scored as a percentage offset from aligned.

RESULTS

The single apical cell (Fig. 3A), first divided into two and, later, four cells (Fig. 3B). An oblique cell wall then formed in each of these cells (Fig. 3C, arrow). A second set of four oblique walls formed, resulting in an eight-celled amphithecium surrounding a four-celled endothecium (Fig. 3D). Once the amphithecium and endothecium were established, periclinal divisions divided the amphithecium into two layers (Fig. 3E, arrow). This was followed by anticlinal divisions that increased the number of cells in the outer layer from eight to 16 cells (Fig. 3F, arrow). Subsequently, periclinal divisions in the outer amphithecial layer formed a three-layered amphithecium (Fig. 3G, arrow). Anticlinal divisions then increased the number of cells around the circumference of the outermost layer from 16 to 32 cells (Fig. 3H, arrow). At these stages, the IPL divided from eight to 16 cells (Fig. 3G, H). The outermost layer then divided via periclinal walls to form a fourth amphithecial layer (Fig. 3I), resulting in the establishment of the three peristome-forming layers (IPL, PPL and OPL; Fig. 1F). Simultaneously, the IPL was dividing anticlinally from 16 to 32 cells. The amphithecium proceeded to divide by way of periclinal divisions to produce five and then six layers, while the IPL continued to divide from 32 to 64 cells (Fig. 3J). Additional anticlinal divisions led to 64 cells composing each of the outer two layers (Fig. 3J).

As the sporophyte progressed through the developmental stages described earlier, the apical meristem actively added new cells to the apex. In very young sporophytes, less than 1.5 mm in height, a zone in which endothecium and amphithecium were not yet delineated extended most of the length of the distal region (Figs. 4A, 5 shown in green, 6A). As the sporophyte increased in height, this region became progressively more restricted toward the apex (Fig. 4B–D). In slightly older sporophytes, the IPL divided from eight to 16 cells in regions in which the amphithecium consisted of only two cell layers, but the endothecium had divided past the four-celled stage (Fig. 4B, hatched zone), i.e., the endothecium was similar to that in Fig. 3F. This region gives rise to the sporangium. For sporophytes that had exceeded a total length of 3 mm (Figs. 4C, 6C), divisions producing a 16-celled IPL were observed only in a narrow longitudinal region (Fig. 4C, gray region), above the zone that would differentiate into the spore mass. In this region, critical divisions in the IPL occurred in conjunction with an amphithecium of three layers and the endothecium at a four-celled stage (Fig. 4C, gray region).

Eight of the 38 sporophytes sectioned had cell divisions occurring in the critical regions defined earlier. Of the 134 cell walls in the IPL that could be measured, a majority (129 cell walls) was determined to be symmetric (Fig. 3K, L). Five divisions were determined to be asymmetric, ranging from 13

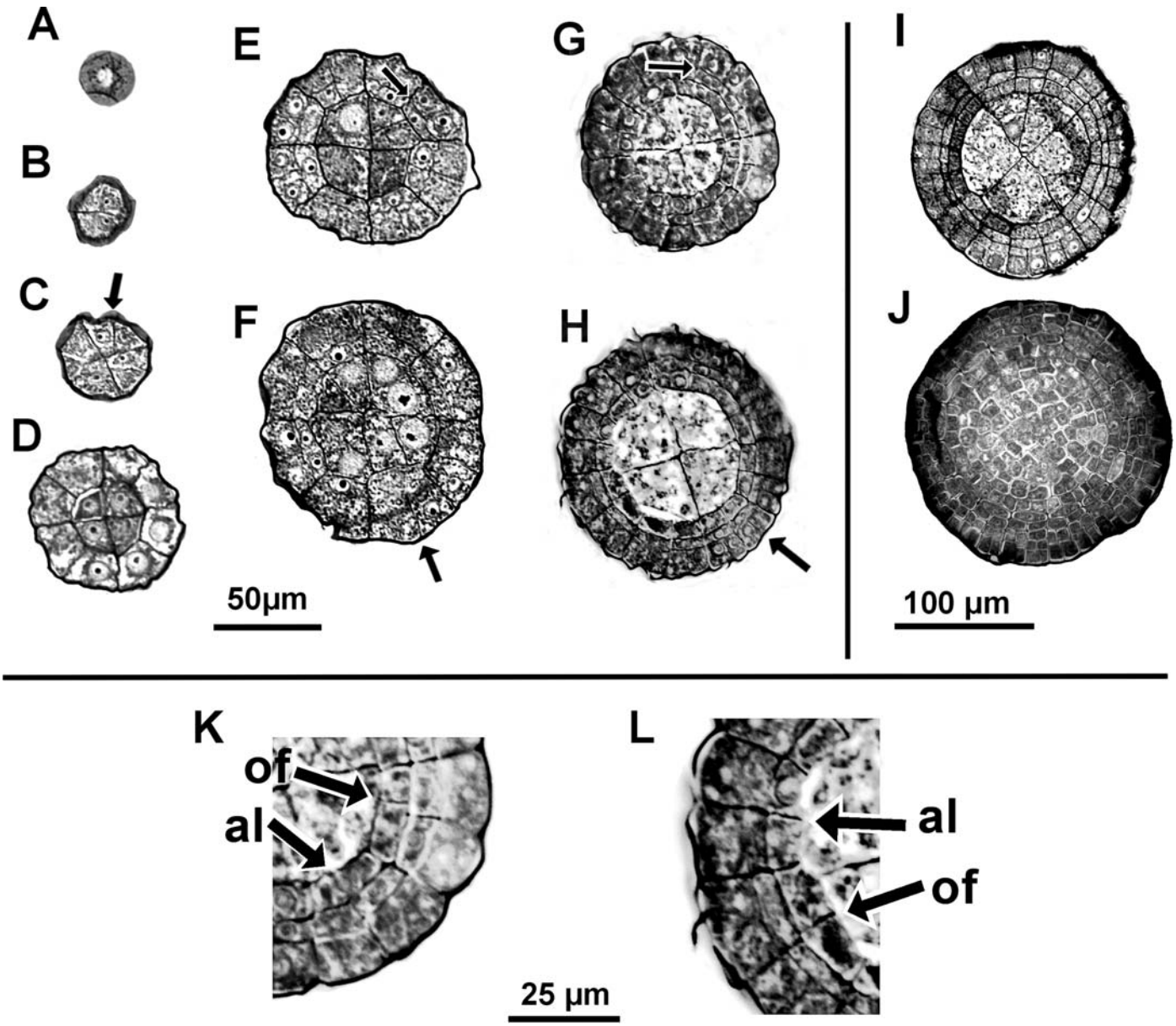
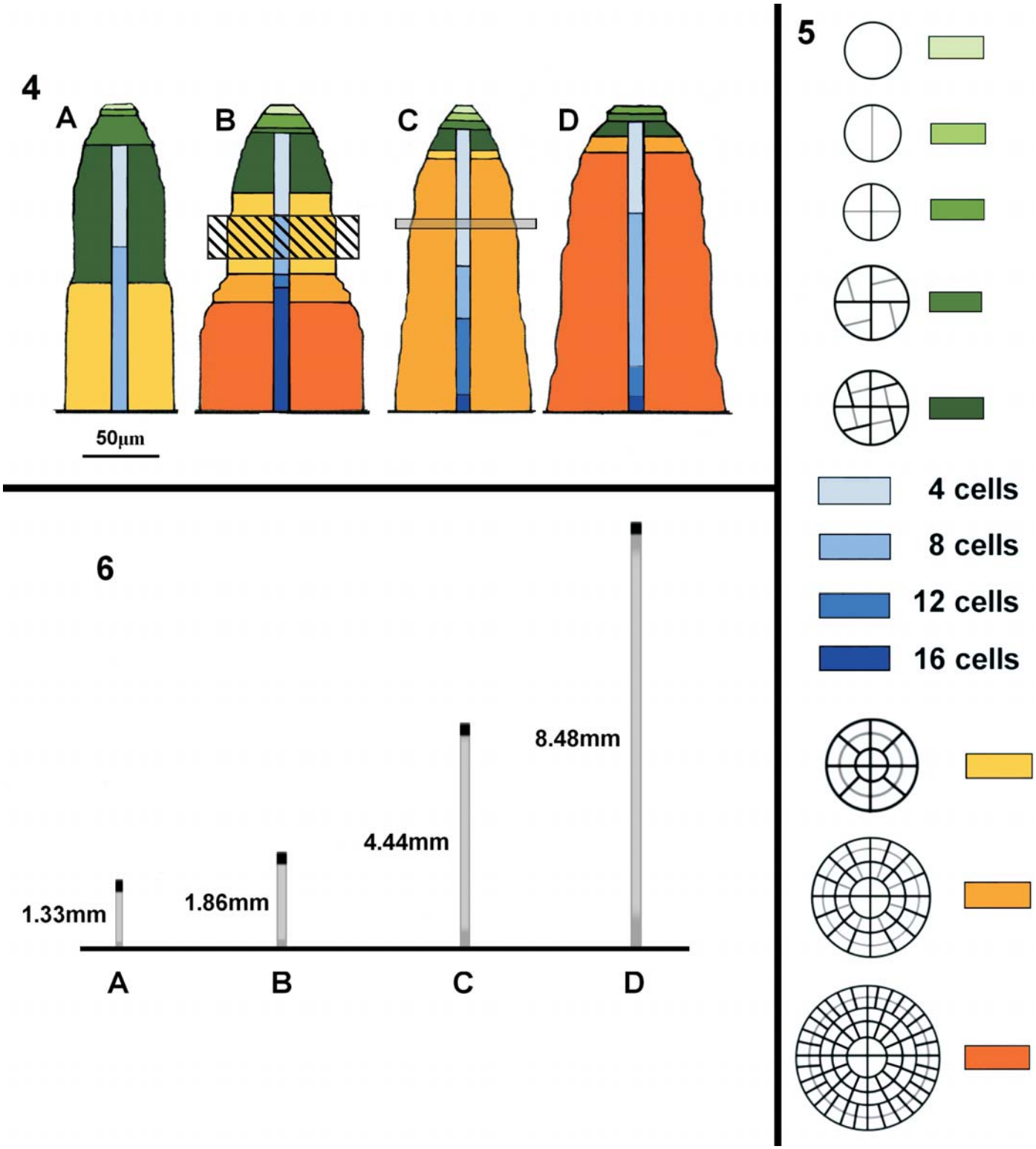


Fig. 3. Light micrographs of cross sections through the upper 200 µm of developing sporophytes of *Timmia megapolitana*. The arrows indicate a representative of recently formed cell walls. al, cell walls in the inner peristome layer (IPL) that are aligned (0% offset); of, cell walls in the IPL that are offset (1–33%). (A) Apical cell, 3 µm from the tip. (B) Four cells, 3 µm from the tip. (C) Fundamental square has begun dividing, 12 µm from the tip. (D) Fundamental square divided with the endothecium consisting of four cells and amphithecium of one layer with eight cells, 36 µm from the tip. (E) Amphithecium has divided to form two layers, and the IPL divides from 8 to 16 cells, 81 µm from the tip. The endothecium has begun to divide from four to eight cells. (F) The outer amphithecial layer has 16 cells, and the inner layer (IPL) divides from eight to 16 cells, 81 µm from the tip. Endothecium had divided to an eight-celled stage. (G) Amphithecium divides to produce three layers of cells, 50 µm from the tip. (H) Outermost layer of the amphithecium divides from 16 to 32 cells via periclinal divisions, 90 µm from the tip. (I) Section from sporophyte 9.99 mm tall. Amphithecium consists of four cell layers, and IPL divides from 16 to 32 cells. (J) Section from sporophyte taller than 1 cm. Amphithecium consists of five to six layers, and IPL divides from 32 to 64 cells. (K & L) Close-ups of section G and H showing cell walls that are aligned and offset in the IPL.

to 25% offset. By the time the sporophytes had grown to approximately 6 mm and taller in height (e.g., Figs. 4D, 6D), all the critical divisions had occurred in the IPL, the zone of differentiation for the endothecium and amphithecium had become restricted to a very small region at the apex and the tip of the sporophyte had widened (Figs. 4D, 5 shown in green, 6D).

Published images of histological sections of other species for which the alignment of cell walls in the IPL could be scored ranged from one to four images per species, for a total of 29. In 22 of these images, the endothecium had divided to eight cells or more. Of the 14 species examined, eight were illustrated solely by sections in which the endothecium had divided into eight cells or more. The degree of symmetry of the IPL division varied within all but four taxa (i.e., *Diphyscium*, *Funaria*,



Figs 4–6. Diagrams of sporophytes of *Timmia megapolitana*. Figs. 4–5. Reconstructions of the top 200 μm of sporophytes based on serial cross-sections 3 to 5 μm thick. The most recent cell walls formed are indicated by gray lines. Green regions indicate the cells dividing from the apical cell stage to the establishment of the endothecium and amphithecium. Blue regions, located down the center of the sporophyte (Fig. 4), indicate the number of cells in the endothecium. Yellow and orange regions indicate periclinal divisions that increase the number of amphithecial layers. Anticlinal divisions that increase the number of cells per layer have been omitted for ease of display. **4.** Longitudinal reconstructions. (4A) The amphithecium and endothecium have divided. Where the IPL has formed, it consists of eight cells. (4B) Hashed region indicates where the inner peristome layer (IPL) actively divides from eight to 16 cells. The remainder of the sporophyte below this region has an IPL that has divided past the 16-cell stage. (4C) Gray region indicates where the

Schlotheimia, and *Trematodon*). In the 10 other species examined, the alignment of the IPL cell walls with those of the adjacent PPL, varied by as much as 40% (e.g., *Bryum bicolor*; Appendix S1). Despite this variation, a majority of the divisions occurred within one of our three categories, which resulted in characterizations of symmetric for three taxa, slightly asymmetric (1–33% offset) for seven taxa, and strongly asymmetric (34–66% offset) for four taxa (Appendix S1, see Supplemental Data with online version of this article).

TABLE 1. Symmetry of cell walls in inner peristome layer (IPL) with those established walls in the primary peristome layer (PPL) for eight sporophytes of *Timmia megapolitana*.

	Aligned (0% offset)	Offset 1–33%	Offset 33–66%	Unable to be scored	Not divided	Total
No. cell walls	129	5	0	53	85	272
Scorable cell walls (%)	96.3	3.7	—			

DISCUSSION

Development of the peristome—The sequence of cell divisions in the sporophyte of *T. megapolitana*, from a single apical cell through the stage at which three amphithecial layers have been established (Fig. 3A–I), is identical to those described for other mosses (i.e., Shaw et al., 1987, 1989a, b; Shaw and Anderson, 1988; Schwartz, 1994; Goffinet et al., 1999), except *Archidium* (Snider, 1975). The developmental patterns of distinct peristome types diverge when the IPL undergoes anticlinal divisions that yield a 16-celled layer (Fig. 3G, H, K, L). Additional cell divisions produce a peristome with 32 OPL, 16 PPL, and 64 IPL cells for *T. megapolitana* (Fig. 1F; Murphy, 1988). At maturity, the peristome consists of two rings of teeth, with the endostome composed of a basal membrane topped by 64 filaments (Fig. 2). All species of Timmiaceae share an identical peristome architecture; the peristomes differ in the ornamentation of the filamentous appendages (Brassard, 1979, 1980, 1984). Although *T. megapolitana* is derived within the genus (Budke and Goffinet, 2006), the ontogeny of its peristome is most likely representative of the other taxa.

Of the critical anticlinal cell walls in the actively dividing IPL of *T. megapolitana*, the vast majority (96.3%) are in complete alignment with those of the PPL, with only some (i.e., 3.7%) deviating from perfect alignment (Table 1). This variability in the cell walls is found even within an individual sporophyte of *T. megapolitana* (Fig. 3K, L). Overall, the pattern of cell divisions in the IPL for *T. megapolitana* is similar to that described from other peristomate species of the Funariales (Shaw et al., 1989a; Schwartz, 1994).

Variation in the alignment of the IPL cell walls was observed in both *T. megapolitana* and in other taxa (Table 1; Appendix S1, see Supplemental Data with online version of this article). For example, in *Dicranum scoparium* the cell walls range from 33–46% offset from symmetrical. Because 80% of the divisions are within the category of 34–66% offset, they can be classified overall as strongly asymmetric (Appendix S1). The alignments of the cell walls in species of *Bryum* (*B. bicolor* and *B. pseudocapillare*) are also variable. Although a majority of the cell walls fall into the category of 1–33% asymmetric, some are perfectly aligned and others up to 40% offset (Appendix S1), showing considerable variation within a single species. So far, constancy in the alignment of cell divisions is observed in *Funaria*, wherein all anticlinal cell walls are perfectly aligned with the adjacent PPL walls, *Schlotheimia* where all divisions are slightly asymmetric and in *Diphyscium* and *Trematodon*, both of which have strongly asymmetric divisions (Appendix S1). All other taxa studied (i.e., 10) vary in the alignment of their IPL cell walls across these three categories.

We support continued use of the historical categories (symmetrical, slightly asymmetrical, and strongly asymmetrical) to classify divisions in the IPL overall for a taxon, with the recommendation that this assessment be based on the percentage of cell divisions that fall into the categories of 0%, 1–33%, or 34–66% offset. We acknowledge that this is an artificial system and that difficulties may arise from our attempts to divide a continuous character into discrete categories for use as a systematic character. However, utilizing numerical definitions for these historical categories provides a standard for quantification and comparison across taxa. It also provides a system to examine the variation in this character within a taxon.

Homology assessment—For all moss taxa thus far examined, the endo- and amphithecium differentiate early in sporophyte development (Edwards, 1984; Goffinet et al., 1999). The amphithecium is composed of multiple layers that extend the length of the sporangium. The apical portions of the three innermost layers (IPL, PPL, and OPL) contribute to peristome formation (Fig. 1B; Edwards, 1984). Below the line of dehiscence and thus below the peristome, the amphithecium forms the wall of the spore sac (archesporium). The pattern of cell divisions in the apical region of the amphithecium dictates the architecture of the peristome, whereas in the region below the peristome, no obvious functional constraint influences the alignments of the anticlinal walls. Although these two regions fulfill two distinct functions at maturity, their differentiation is not obvious early in development. If homologous divisions are to be compared in the IPL across lineages of mosses, the cells that will be part of the peristome at maturity must be identified and then distinguished from those that will line the sporogenous tissues.

Longitudinal growth of the sporophyte is accounted for by the activity of two meristems: an apical and an intercalary. The apical meristem actively divides only in the early stages of development. When the apical meristem becomes inactive, the intercalary meristem, located proximal to the tissues that will form the capsule, becomes the sole area of cell division and contributes to seta (stalk) formation (French and Paolillo,

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inner peristome layer (IPL) is actively dividing from eight to 16 cells. The remainder of the sporophyte below this region has an IPL that has divided past the 16-cell stage. (4D) All regions of the sporophyte have an IPL that has more than 16 cells. **5.** Transverse sections showing cell wall arrangements. **6.** Total length of sporophytes is symbolized by the vertical gray bars. Regions representing the upper 200 μm are in black and correspond to the regions drawn in Fig. 4. In the sporophyte in 6D, the calyptra has detached from the base of the archegonium venter, and it covers the upper 5.27 mm.

1975). Thus all of the cells composing the operculum, peristome, and sporogenous region of the moss sporophyte are produced from divisions of the apical cell and its immediate derivatives very early during development (Shaw et al., 1987). This shift in meristematic activity, from apical to intercalary, has been hypothesized to occur soon after separation of the archegonial venter (the calyptra) from the remainder of the gametophyte and to coincide with a widening of the region surrounding the apical cell (French and Paolillo, 1975; Shaw et al., 1987). In *T. megapolitana*, all sporophytes taller than 6.0 mm had a separation of the archegonial venter and a widening of the apical region, suggesting that the apical cell had ceased dividing by this size (Figs. 4D, 6D). At this stage, all cells of the peristome-forming layers had divided, such that actively dividing peristome cells could only be found in sporophytes smaller than 6.0 mm.

To distinguish the peristome-forming region from the sporogenous region, we considered characteristics of the cross-sectional anatomy. According to Wenderoth's (1931) peristome reconstructions, critical divisions in the IPL occur at a stage when the endothecium consists of four cells and the amphithecium three layers. Therefore, longitudinal regions where three amphithecial layers have not yet formed, or where the endothecium consists of more than four cells, would not be part of the peristome at maturity (Figs. 3E, 3F, 4B, 5). We therefore restricted our assessments of cell alignments in *T. megapolitana* to histological sections in which the endothecium consisted of four cells and the amphithecium had divided to form three cell layers (Figs. 3G, 3H, 4C, 5).

In many previously published studies, sections that were used to represent the alignment of the IPL cell walls had endothecia that consisted of more than four cells (28 with 4–16 cells, one with 48), thus they did not meet our criteria of homology. Some of the variation in alignment we observed (Appendix S1, see Supplemental Data with online version of this article) could be attributed to divisions that would not longitudinally contribute to peristome formation. We assume that previous authors (Appendix S1) used additional material to determine the IPL cell wall alignment for a taxon, such that the symmetry reported may still be representative of homologous peristome regions. Otherwise, the IPL cell wall alignments in these taxa may warrant reexamination in light of our analyses. An alternative is that the proposed criteria, based on Wenderoth's (1931) observations, cannot be broadly applied to other lineages. Even if this is the case, some form of criteria is still needed to ensure that homologous peristome-forming regions are compared across taxa, given the phylogenetic significance attributed to the alignment of cell walls in the IPL (Shaw et al., 1987, 1989a, b; Shaw and Anderson, 1988; Schwartz, 1994; Goffinet et al., 1999).

Contribution to peristome evolution—*Timmia megapolitana* shares symmetrical divisions in the IPL (Table 1) with peristomate taxa of the Funariales (Shaw et al., 1989a). Whether this shared character is strictly homologous or has been acquired independently is not clear. The most recent phylogenetic inferences resolve the Timmiaceae as either sister to the Funariales or as a lineage sister to the Bryales and Dicranales (Fig. 7; Cox et al., 2004). The symmetrical division is most parsimoniously reconstructed on the former topology (Fig. 7A) as a synapomorphy for the clade containing the Timmiaceae and Funariales (i.e., the Funariidae sensu Goffinet and Buck, 2004). Under the alternative phylogenetic hypothesis (Fig. 7B), the symmetric division could have arisen early in the evolution of arthrodontous mosses, and then lost in the ancestor to the Bryales and Dicranales, or have been acquired twice, once in the Timmiaceae and once in the ancestor of the Funariales. With the exception of the Polytrichaceae (van der Wijk, 1930), all other basal lineages of mosses surveyed (e.g., *Diphyscium* and *Tetraphis*, Appendix S1; Shaw et al., 1987; Shaw and Anderson, 1988), have an asymmetrical division in the IPL, suggesting that asymmetrical divisions of the eight IPL cells are the ancestral condition for the arthrodontous mosses.

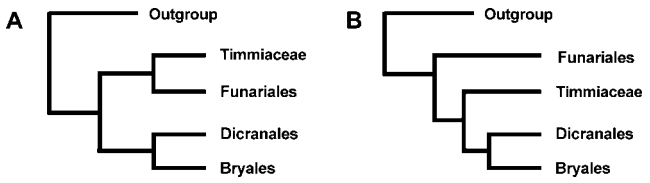

Fig. 7. Pruned phylogenies showing hypothesized relationships between the Timmiaceae and major lineages of arthrodontous mosses. (A) Modified from Fig. 3a in Cox et al. (2004). (B) Modified from Fig. 3b in Cox et al. (2004).

Testing this hypothesis will depend upon a robust phylogeny of major moss lineages and consensus regarding the position of the Timmiaceae. With the exception of the Buxbaumiaceae, peristome development for representatives of all major arthrodontous moss lineages (i.e., Bryopsida sensu Goffinet and Buck, 2004) has been characterized. In contrast, the ontogeny of the sporophyte apex in many lineages that lack a peristome, which form a grade leading to the peristomate mosses (e.g., Takakiaceae, Sphagnaceae, Andreaeaceae, Andreaeobryaceae, and Oedipodiaceae), remains unknown. Further research into moss sporophyte development will aid in the reconstruction of the ancestral peristome architecture and lead to a more thorough understanding of peristome evolution.

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