Amer. J. Bot. 75(7): 1019–1032. 1988.

PERISTOME DEVELOPMENT IN MOSSES IN RELATION TO SYSTEMATICS AND EVOLUTION. II. TETRAPHIS PELLUCIDA (TETRAPHIDACEAE)[1]

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ABSTRACT

The Tetraphidae is a small subclass of mosses with a nematodontous peristome that has frequently been interpreted as primitive among the true mosses. The developmental cell sequence leading to the formation of the four peristome teeth of *Tetraphis pellucida* is described for the first time. Comparisons are made with sequences known for other nematodontous and arthrodontous mosses. Peristome development in *T. pellucida* is more like that described previously for arthrodontous peristomes than to published developmental sequences for nematodontous peristomes of species in the Polytrichaceae. On the other hand, our observations confirm a basic uniformity of the earliest developmental stages in all mosses studied thus far, regardless of their systematic position.

THE TETRAPHIDAE is the smallest subclass of mosses, consisting of a single family, the Tetraphidaceae Schimp., and two genera, *Tetraphis* Hedw. and *Tetrodontium* Schwaegr. *Tetraphis* is comprised of two species, *T. pellucida* Hedw. and *T. geniculata* Girg. *ex* Milde, and *Tetrodontium* is a monotypic genus erected for *T. brownianum* (Dicks.) Schwaegr. All three species of the Tetraphidae are north-temperate in distribution.

Although there are no serious taxonomic problems at the specific level within the Tetraphidae, the phylogenetic and taxonomic status of the subclass has been disputed through the years. Many bryologists have expressed the view that the Tetraphidae retain a significant number of features that are primitive in the true mosses. The protonema of species in the Tetraphidaceae has thalloid "flaps" that are often compared to the thalloid protonema of *Sphagnum* L. and *Andreaea* Hedw. (e.g., Campbell, 1905; Goebel, 1905; Schofield, 1985). Although homology between the thalloid protonema in these groups has not been explicitly hypothesized, the suggestion, or at least the possibility, of homology is implicit in the frequent comparisons that have been made. It is, however, still an open question as to whether the thalloid protonema of the Tetraphidaceae represents a primitive trait retained from an ancestor in common among the Tetraphidae, Sphagnidae, and Andreaeidae.

It is the structure of the peristome that has been most frequently cited as evidence for a primitive phylogenetic position for the Tetraphidae. The peristome in both *Tetraphis* and *Tetrodontium* consists of four massive teeth that fill the opercular region of the mature capsule. The teeth are nematodontous in that they are made up of whole, thick-walled cells, rather than just remnants of cell walls, as in arthrodontous peristomes.

Moss peristomes can be classified as arthrodontous or nematodontous on the basis of comparative morphology without necessarily making hypotheses about the phylogenetic relationship between the two types of peristomes. When Philibert (1884) distinguished arthrodontous and nematodontous peristomes, he compared them in terms of cellular structure and the patterns of lines on their inner and outer surfaces. The morphological distinction is a phenetic and not a phylogenetic one, although Philibert and many other bryologists since have attached phylogenetic significance to the differences in morphology.

Fundamental similarities in the basic ground plan among superficially divergent arthrodontous peristomes have led many bryologists to the interpretation that mosses having arthrodontous peristomes are monophyletic. That is, basic similarities suggest to many that the arthrodontous peristome has evolved only once in the mosses. These fundamental features include the formation of teeth from adjacent periclinal walls rather than whole cells, the articulated appearance resulting from the pat-

[1] Received for publication 3 June 1987; revision accepted 1 October 1987.

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This research was supported by NSF Grant No. BSR-8506992. We thank B. D. Mishler for assistance with field work and for commenting on an earlier manuscript draft. We also thank M. Turner for his able technical assistance.

terns of lines on the inner and outer surfaces, and the uniform number of cells in the outer and primary peristomial layers throughout arthrodontous mosses. Anatomical studies have shown that almost all arthrodontous mosses have 32 cells in the outer peristomial layer (OPL) and 16 in the primary peristomial layer (PPL) (Edwards, 1984; Shaw and Robinson, 1984). Moreover, developmental studies, although rather few, indicate a remarkable uniformity of development among arthrodontous peristomes (Evans and Hooker, 1913; Blomquist and Robertson, 1941; Saito and Shimoze, 1954; Saito, 1956).

Edwards (1979) devised a convention, which he termed the peristomial formula, to make comparisons of the number of cells in the peristomial layers of mosses easier. The formula lists the number of cells in each of the three layers, from the outside inward, in only one eighth of the capsule's circumference. The peristomial formula of almost all arthrodontous mosses ranges from 4:2:2 to 4:2:14 (Edwards, 1984; Shaw and Robinson, 1984). That is, the OPL (outer peristomial layer) uniformly consists of 32 cells and the PPL (primary peristomial layer) of 16 cells, but the inner peristomial layer (IPL) ranges from 16 to 112 cells in circumference. Most of this variation in the number of cells in the IPL occurs between species; within a given species the number of cells in all three layers is relatively constant.

A monophyletic origin for groups having nematodontous peristomes is less clear. If nematodontous peristomes are primitive with respect to arthrodontous peristomes, as many bryologists assert (e.g., Cavers, 1911; Dixon, 1924; Edwards, 1984; Schofield, 1985), then the nematodontous structure itself cannot be evoked as evidence for monophyly. It would simply be a feature that is retained from a common ancestor. Furthermore, the two subclasses of mosses which are characterized by nematodontous peristome structure (Polytrichidae and Tetraphidae) have peristomes that are extremely different in cellular ground plan, in spite of sharing the feature of being formed from whole, persistent cells. Gametophytic structure in the two subclasses also suggests, because of extreme differences, that the Polytrichidae and Tetraphidae may not be closely related (e.g., Crum and Anderson, 1981).

In this paper we describe the sequence of cell divisions in the peristomial region of developing sporophytes of *Tetraphis pellucida*. This is the first developmental study of peristome structure in any species of the Tetraphidae, and we will compare the sequence of divisions preceding deposition of the peristome teeth in this species to published sequences for species in other subclasses of mosses. In particular, we have sought to answer the following questions during the course of our study: 1) Is there a pattern of development (i.e., a sequence of cell divisions) that is unique to and shared by the nematodontous peristomes of species in the Polytrichidae and Tetraphidae? 2) At what stage of development do *Polytrichum*- and *Tetraphis*-type peristomes diverge? 3) At what stage of development do *Tetraphis*-type and arthrodontous peristomes diverge? In order to make comparisons between peristome development in *Tetraphis* and that in other mosses, we will rely on published reports by others, as well as on our own published (Shaw, Anderson, and Mishler, 1987) and unpublished observations. A detailed review of peristome structure and development, intended to serve as a background and rationale for this and our future contributions, was provided in Shaw et al. (1987).

MATERIALS AND METHODS—*Tetraphis pellucida* is widespread across the boreal and north-temperate regions of the Northern Hemisphere. In North America, it extends northward into the tundra of Alaska and the Yukon Territory, and southward in the East to South Carolina and Alabama and in the West to Arizona and California, where it occurs in the mountains (Crum and Anderson, 1981). Populations sampled for this study originated in the Appalachian Mountains of North Carolina, where *T. pellucida* is common on rotten tree stumps, logs, and on soil or rocks near streams. Collection data for the populations we sampled are as follows: Mitchell Co.: Roan Mt., *Anderson* and *Mishler 24,533*; *Anderson* and *O'Toole 24,711*. Voucher specimens are deposited in DUKE. Plants were collected during autumn and midwinter when very small sporophytes (<4 mm long) were visible among the perichaetial leaves. Sporophytes at this stage were fixed immediately in formalin-acetic-alcohol, and the remaining plants with young sporophytes were allowed to mature in the laboratory. Plants were kept slightly moist in covered dishes and were sampled periodically for sporophytes of different developmental stages.

Sporophytic tissue was embedded in paraffin and sectioned on a rotary microtome according to the standard techniques (Johansen, 1940). For additional details regarding our methods of fixation, embedding, and sectioning, see Shaw et al. (1987).

RESULTS—The sporophyte of *T. pellucida* grows in length as a result of divisions of a two-

sided apical cell, as in other mosses. However, as also seems to be the case in other mosses which we presently have under study, the apical cell of *T. pellucida* ceases dividing at a very early stage of sporophytic development. We found no evidence of a functioning apical cell in sporophytes longer than approximately 3–5 mm, a developmental stage at which the young spear was barely exerted from the perichaetial leaves. After the apical cell ceases dividing, the apex of the sporophyte becomes broadly rounded and divisions occur in the youngest merophytes just below the tip (Fig. 1). These observations suggest that most elongation of the sporophyte of *T. pellucida* occurs as a result of enlargement and division of cells below the apex, but we did not determine the manner in which the setae develop. Since the mature sporophyte of *T. pellucida* is often 15–20 mm long, only about one-third of this length is the direct result of divisions of the apical cell.

While the apical cell is still functioning, the distal portion of the sporophyte is relatively sharply pointed (Fig. 2). The first transverse section closest to the apex of such a young sporophyte sometimes shows a single cell which is the apical cell itself (Fig. 3). At a slightly lower level (Fig. 4), a transverse view shows two cells, one of which is probably the apical cell and the other is the first merophyte. Slightly lower still (Fig. 5), three cells are visible; the apical cell is in the center, flanked on either side by the two most recently formed and still undivided merophytes. Sections of any given sporophyte do not always show each of these arrangements of cells. Sometimes, for example, the most distal section obtained from the young sporophyte is below the level at which the apical cell alone is visible in transverse view. In other cases, the orientation of the walls of the first two merophytes is such that both derivatives plus the apical cell cannot be viewed in a single transverse section like that shown in Fig. 5. In many cases, the arrangement of cells typically seen in the first transverse section in a series from apex to base is as in Fig. 4.

Each of the most recently formed merophytes first undergoes an anticlinal division resulting in four cells in tranverse view (Fig. 6). Shortly thereafter, the first stage in the differentiation of the two embryonic layers, endothecium and amphithecium, commences with the formation of a series of curved anticlinal walls (Fig. 7). One wall is formed in each cell shown in the previous quadrant (Fig. 6), producing a transient stage in which the sporophyte consists of eight cells in transverse view (Fig. 7). Almost immediately, curved periclinal walls are formed, resulting in an outer layer of eight cells surrounding four inner cells (Fig. 7, 8). The eight outer cells are the amphithecium and the four inner cells, the so-called fundamental square, are the endothecium.

The sequence of cell divisions described up to this point represents serial views of the developing sporophyte from the apex downward through progressively older portions. The remaining descriptions that follow, however, describe the proliferation and differentiation of the embryonic cell layers at the level of dehiscence of the operculum (i.e., the "annular" level—*Tetraphis* has no specialized annulus per se). Development as seen from transverse sections will be emphasized since major peristome types in mosses are distinguished mainly on the basis of the numbers and arrangement of cells in the peristome-forming layers.

Soon after the endothecium and amphithecium have differentiated, divisions begin in the amphithecium (Fig. 8). Anticlinal divisions occur in each of the eight amphithecial cells, and, more or less synchronously, periclinal divisions occur, initiating the formation of a two-layered amphithecium (Fig. 8). In general, the periclinal divisions occur first, resulting in two amphithecial layers, each of which is composed of eight cells. Then anticlinal divisions occur in the outer of these two layers, resulting in an outer layer of 16 cells and an inner layer of eight cells. However, the sequence of divisions is not always so regular, nor are all of the anticlinal or periclinal divisions in each layer absolutely synchronous. Figure 8, in fact, shows several amphithecial cells dividing anticlinally before they have divided periclinally. In this figure, two other cells have divided periclinally before any anticlinal divisions have occurred; the latter represents the typical order of divisions.

As a result of these anticlinal and periclinal divisions in the first and second amphithecial layers, whether the divisions are absolutely synchronous or not, eventually two layers are formed (Fig. 9). At this stage there are almost invariably 16 and 8 cells in the outer and inner layers, respectively. The inner of these two layers is the inner peristomial layer, or IPL. Next, periclinal divisions occur in the outer layer, producing a three-layered amphithecium (Fig. 10). The middle layer, with 16 cells at this stage, is the primary peristomial layer, or PPL. The peristomial formula at this stage is 0:2:1; the divisions that form the OPL have not yet occurred.

Almost as soon as the amphithecium is three-layered, cells in the outermost layer undergo anticlinal divisions (Fig. 10—many of the cells in the right half of the section have divided;

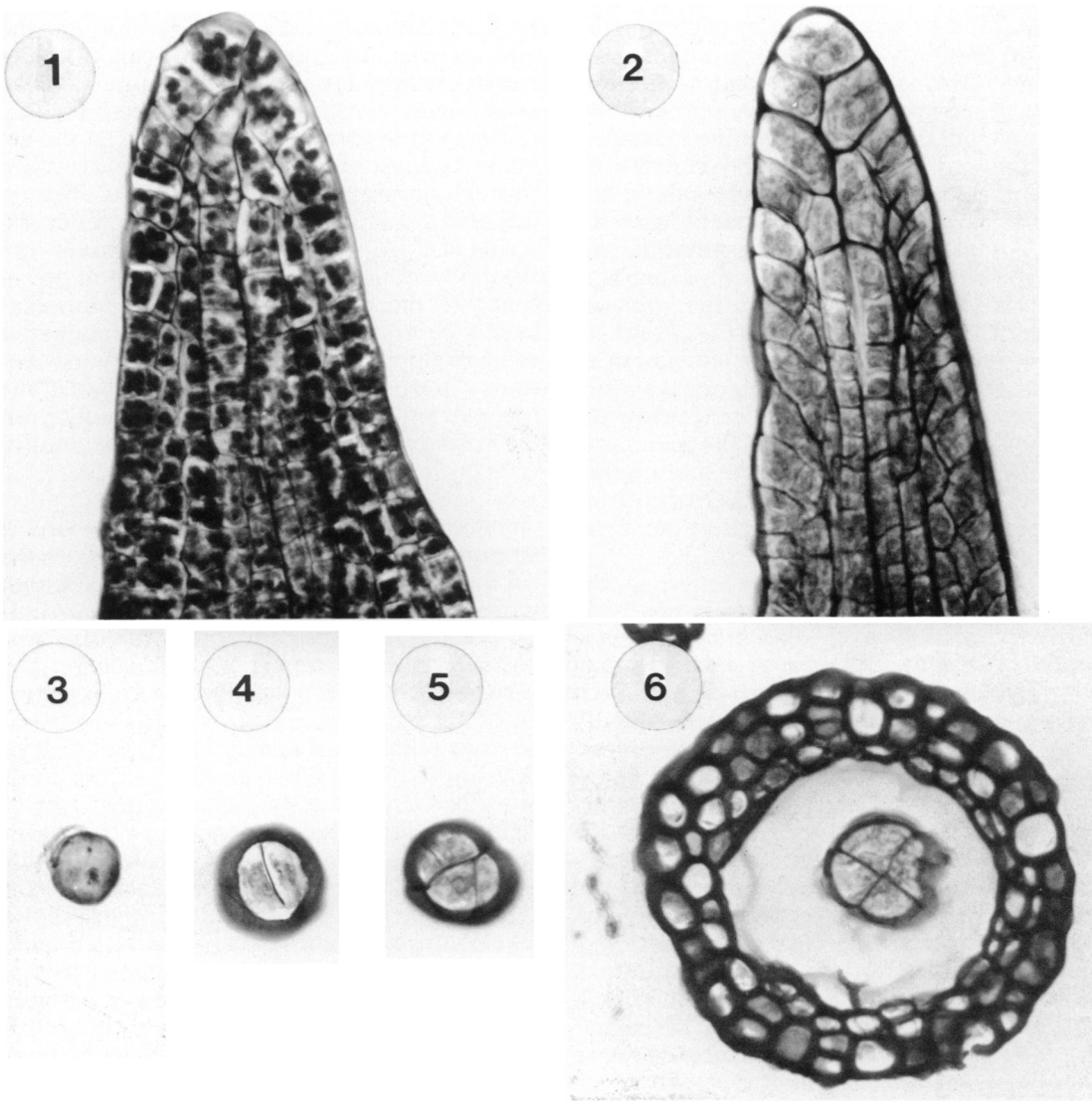

Fig. 1–6. Transverse and longitudinal sections through young sporophytes of *Tetraphis pellucida*. (Fig. 1, 2 ×400; 3–6 ×648). **1.** Longitudinal section of a sporophyte in which the apical cell has ceased dividing. **2.** Longitudinal section of an embryonic sporophyte in which the apical cell is still dividing. The most recently formed merophyte is on the left; the second merophyte is on the right. **3.** Transverse section through the apical cell. **4.** Transverse section from just below the apex of a sporophyte as in Fig. 2, showing two cells. **5.** Transverse section from just below the apex of a sporophyte as in Fig. 2. The apical cell is in the center, flanked by the first and second merophytes. **6.** Transverse section of a young sporophyte slightly lower than Fig. 4, 5. Each merophyte has divided anticlinally, producing four cells. The calyptra surrounds the sporophyte.

those in the left half have not). When these anticlinal divisions have been completed, the outer of the three amphithecial layers consists of 32 cells (Fig. 11).

At about the same time that anticlinal divisions in the outer layer have increased the number of cells in this layer to 32, and sometimes slightly before the divisions have been completed, anticlinal divisions in the IPL commence (Fig. 11). Each IPL cell divides roughly in half, increasing the number of cells in this layer to 16 (Fig. 11–13). Each new anticlinal wall in the IPL is offset slightly with regard to corresponding anticlinal walls in the PPL, this resulting in one of the IPL cells being slightly larger than the other of each pair. The degree

to which the new walls are not aligned with walls in the PPL is somewhat variable, but the positions seen in Fig. 11 are typical.

Divisions in the four endothecial cells also commence at this stage (Fig. 11). The early pattern of divisions in the endothecium is almost exactly like the sequence of divisions leading to the original differentiation of endothecium and amphithecium. Each of the four endothecial cells first undergoes an anticlinal, or tangential, division (Fig. 12), followed by a periclinal division. The result is an inner square of four cells, surrounded by an outer layer in which additional anticlinal divisions occur almost immediately so that this layer consists of 12 cells (Fig. 13). This outer endothecial layer gives rise to the sporogenous tissue in the capsule urn. Few additional divisions occur in the endothecium in the opercular region of the capsule and even at a relatively mature stage the operculum contains relatively few, conspicuously large, cells (Fig. 22).

At approximately the same time that anticlinal divisions occur in the IPL, periclinal divisions begin in the outer layer, producing a four-layered amphithecium. When the fourth amphithecial layer has been completely formed, the three peristomial layers, OPL, PPL, and IPL, are differentiated, and the peristomial formula is 4:2:2 (Fig. 12, 13).

Up to this point in development, the arrangement of cells in the amphithecial layers is highly organized, and anticlinal walls in the three layers are for the most part aligned with one another. However, as development progresses, involving increases in both the number and size of cells, transverse sections reveal a decreasing degree of orderliness in the arrangement of cells (e.g., Fig. 13–16).

Periclinal divisions occur in the outer amphithecial layer so that the 4-layered amphithecium becomes 5-layered (Fig. 13, 14). The periclinal divisions in the outer layer are even less synchronous than the earlier divisions in the amphithecial layers, and typically any given section shows some, but not all, cells of the outer layer divided (e.g., Fig. 14). Also at this stage, cells in the IPL, and to a lesser extent, the PPL, begin to enlarge (Fig. 14). The IPL cells then undergo periclinal divisions, again in a somewhat disorganized and asynchronous fashion, to produce an additional amphithecial layer adjacent to the endothecium (Fig. 15). It is noteworthy that all previous increases in the number of amphithecial layers had been the result of periclinal divisions in cells of the outermost layer. In order to maintain a consistent application of the terms, IPL, PPL, and OPL, we will refer to this most recently derived innermost amphithecial layer as the IPL-2. The alternative, calling it the IPL, would effectly result in a displacement of the three peristomial layers inward, and would make it virtually impossible to make interpretations about homologies between the peristomial layers in *Tetraphis* with those in other mosses.

At about the same time that cells in the IPL are enlarging, anticlinal divisions occur in the PPL (Fig. 14). Such divisions do not occur in every cell of the PPL, and, in fact, they occur in roughly every other PPL cell. As a result, where there were previously two PPL cells adjacent to four OPL cells in each eighth of the capsule's circumference, the number of PPL cells increases to three. The resulting peristomial formula at this stage is thus 4:3:2 (Fig. 14).

Because of the differentiation of the IPL-2, along with additional periclinal divisions in the outermost amphithecial layer, the amphithecium becomes six to seven layers in thickness (Fig. 15, 16). Also, anticlinal walls in the amphithecium become noticeably less aligned in the concentric layers than they were in earlier developmental stages, and as a result, it becomes more difficult to specify a peristomial formula. Additional anticlinal divisions occur in the PPL so that the formula becomes 4:4:2, at least in some areas in the circumference of the sporophyte (Fig. 16). Divisions in the IPL also increase the number of cells in this layer and result in a formula of roughly 4:3–4:3–4 (Fig. 16). Over 20 sporophytes of *T. pellucida* were sectioned in this study; variation in the number of primary and inner peristomial layer cells in one eighth of the capsule's circumference occurred both within and between sporophytes. However, the formula was consistently 4:3–4:3–4. That is, the number of PPL and IPL cells range from 24 to 32, but do not seem to vary beyond these limits.

While cells in the amphithecial layers are undergoing anticlinal and periclinal divisions, as described above, the cells in each layer are roughly isodiametric to short-rectangular as seen in longitudinal view (Fig. 17). At this stage six or seven or occasionally fewer layers of cells make up the amphithecium at the level of dehiscence of the operculum, which, in Fig. 17, is toward the base of the photograph. The spore layer, which is the outermost layer of the endothecium, is clearly recognizable in the latter figure. Its cells stand out because they are more heavily stained and have thicker walls than the adjacent cells. The spore layer can thus be used as a marker to distinguish amphithecium and

endothecium. It should be noted that the open space in the lower right of the section in Fig. 17 is a tear and is not an air space. The capsules of *Tetraphis* lack an air space.

The next two layers outside the spore layer represent IPL-1 and IPL-2, which originated from periclinal divisions of the original IPL, as described above (Fig. 15, 16). The next or third layer of cells toward the outside in Fig. 17 is the PPL, beyond which are the OPL-1 and -2. The outermost layer of the amphithecium becomes the capsule wall.

Shortly after the stage shown in Fig. 17, all of the cells within the operculum begin a period of dramatic elongation, enlargement, and differentiation, as a result of which the capsule assumes its mature shape. The cells of the outermost peristomial layers, including the OPL-1 and -2 and the PPL, undergo a striking elongation with little or no increase in width. This results in long and narrow cells that dovetail past each other, forming prosenchymatous-like tissue (Fig. 18–22). The cells of both IPL-1 and -2 and all of the cells of the endothecium become elongated, considerably widened, and ultimately are emptied of their contents (Fig. 18–22). Wall deposition begins in all of the cells of the peristomial layers immediately after the period of elongation (Fig. 18) and continues through maturation. Figure 18 shows a longitudinal section through the upper portion of the urn and the lower part of the operculum in which cell elongation and enlargement are complete, and secondary wall deposition has begun. The line of opercular dehiscence is near the upper end of the photograph. The spore layer is visible at the lower portion of the figure, identifiable by the dark-staining, compact, square to short-rectangular cells which end considerably short of the ring of dehiscence of the operculum. Although there has been some tearing in sectioning in Fig. 18, the individual peristomial layers can be identified by relating them to the spore sac, which marks the outer layer of the endothecium.

Higher magnification of longitudinal sections through the peristomial region after elongation and considerable wall deposition reveals clearly the cellular details of the developing peristome, shown in Fig. 19, 20. The line of opercular dehiscence in Fig. 19 is near the bottom, while in Fig. 20 it is near the top. The line can be recognized by a series of contrasting narrower cells in the opercular wall. The cells of the three outer peristomial layers (OPL-1 and -2 and the PPL) in these longitudinal views are long, narrow, and pointed, while the cells of the IPL-1 and -2 are much wider and are more or less rectangular. Apparently only the outer wall of the IPL-2 receives secondary

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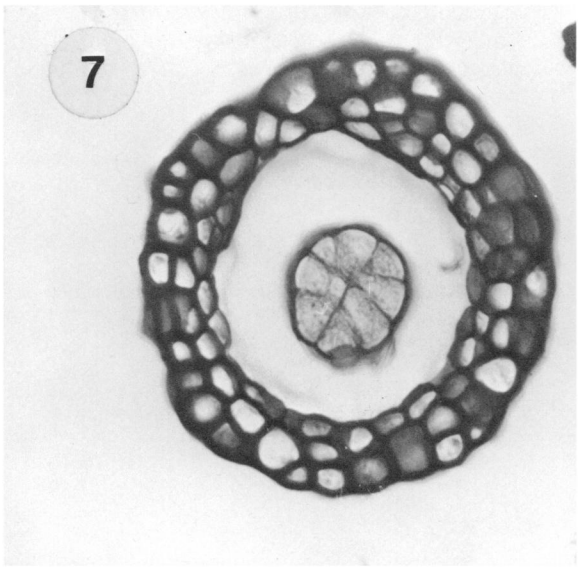
Fig. 7–12. Transverse sections of sporophytes at progressively older developmental stages. (×648). Calyptrae are visible in Fig. 7, 8. **7.** Differentiation of the endothecium and amphithecium by a series of curved anticlinal and periclinal walls. **8.** Four endothecial cells surrounded by the amphithecium in which periclinal and anticlinal divisions are occurring. **9.** Two-layered amphithecium; the inner layer consists of 8 cells, the outer of 16 cells. The four endothecial cells form the so-called fundamental square and have not yet divided. **10.** Three-layered amphithecium surrounding the four endothecial cells. Anticlinal divisions are occurring in the outer amphithecial layer. **11.** Three-layered amphithecium with anticlinal divisions in the outer layer completed; there are now 32 cells in this layer. Anticlinal divisions are occurring in the inner layer. Note that the anticlinal walls of the IPL are not quite aligned with anticlinal walls in the PPL. The endothecial cells are beginning to divide. **12.** Periclinal divisions are occurring in the outer amphithecial layer, producing the OPL. Anticlinal divisions are occurring in the IPL and a second cell of the endothecium has divided.

Fig. 13–18. Transverse and longitudinal sections through sporophytes of *T. pellucida*. (Fig. 13, 14, 17 ×648; 15, 16, 18 ×400). **13.** Four-layered amphithecium surrounding the endothecium which has now undergone repeated divisions. Note that the arrangement of cells in the amphithecial layers is not as organized as in earlier sections. The peristomial formula is 4:2:2. **14.** Periclinal divisions in the outer layer have produced the fifth amphithecial layer. Anticlinal divisions in the PPL have resulted in a 4:3:2 peristomial formula. **15.** Continuing but irregular anticlinal divisions in the outermost amphithecial layer. IPL cells are swelling and undergoing periclinal divisions, producing the IPL-2. **16.** The IPL-2 is completely differentiated; the amphithecium now consists of seven layers. The peristomial formula is still 4:3:2. **17.** Longitudinal section through a young sporophyte. Cells in all the endothecial and amphithecial layers are isodiametric to short-rectangular. **18.** A somewhat older sporophyte in which the cells in the opercular region have elongated.

Fig. 19–24. Transverse and longitudinal sections through developing sporophytes. **19, 20.** Longitudinal sections through progressively older sporophytes. Cells in the outer amphithecial layers are narrowly linear and the walls are beginning to thicken. (×648). **21, 22.** Longitudinal sections through immature (Fig. 21 ×100) and nearly mature (Fig. 22 ×160) sporophytes. Note the absence of air spaces in the urn in Fig. 22. **23.** Transverse section of a nearly mature sporophyte at approximately the level of the capsule mouth. (×648). **24.** Transverse section of a nearly mature sporophyte at approximately ⅓ the way up the opercular region. Note wall thickenings in endothecial cells. (×648).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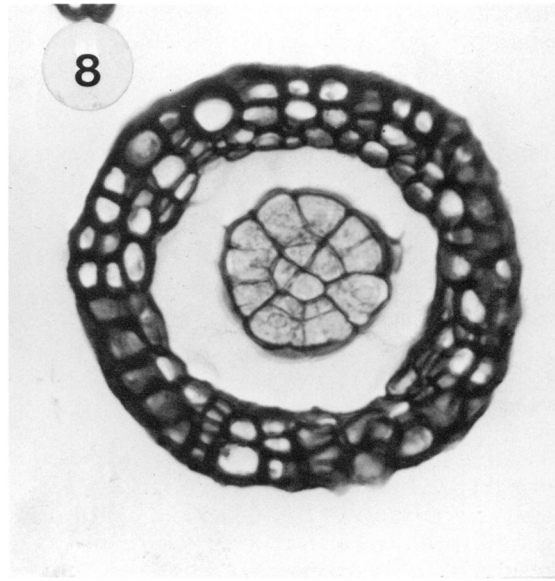
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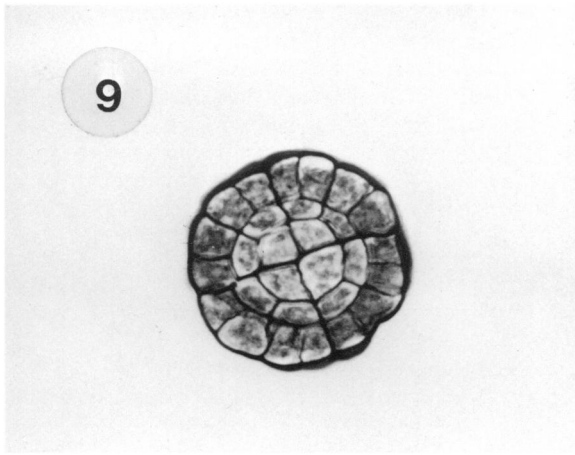
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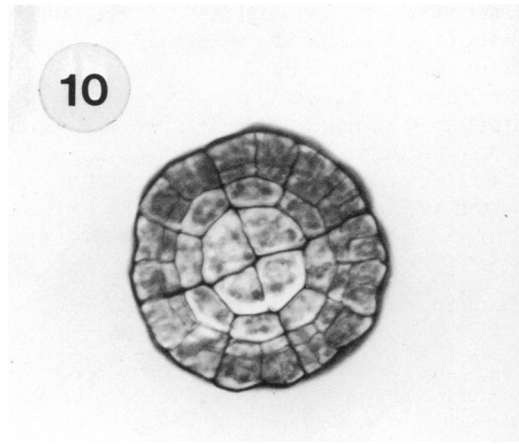
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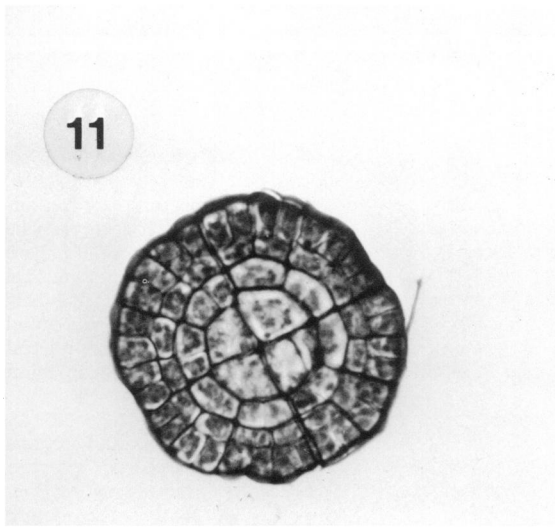
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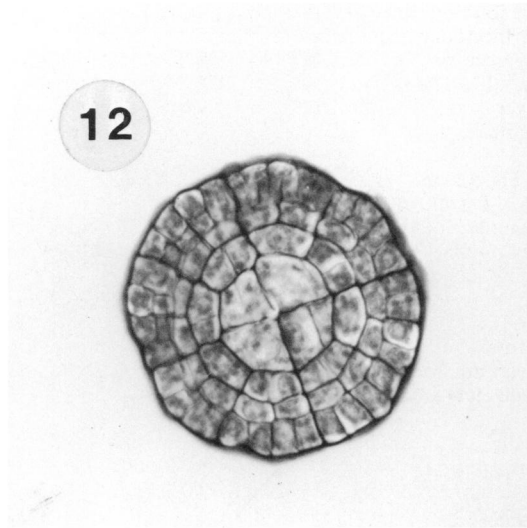
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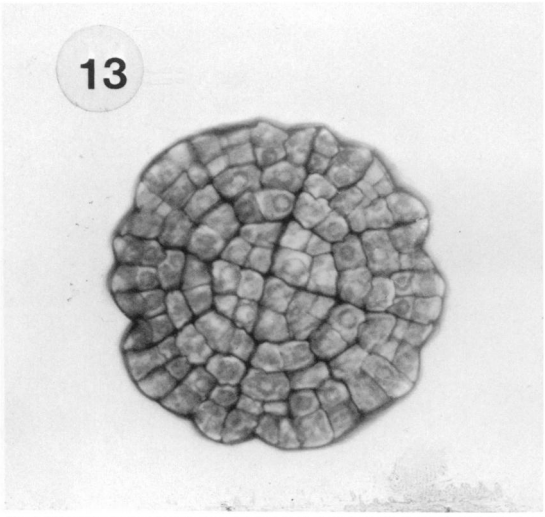
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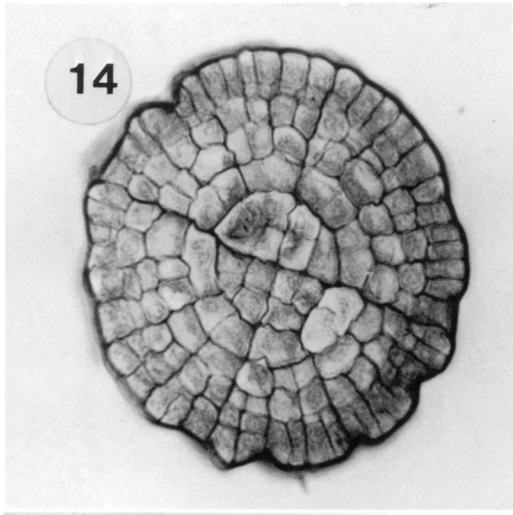
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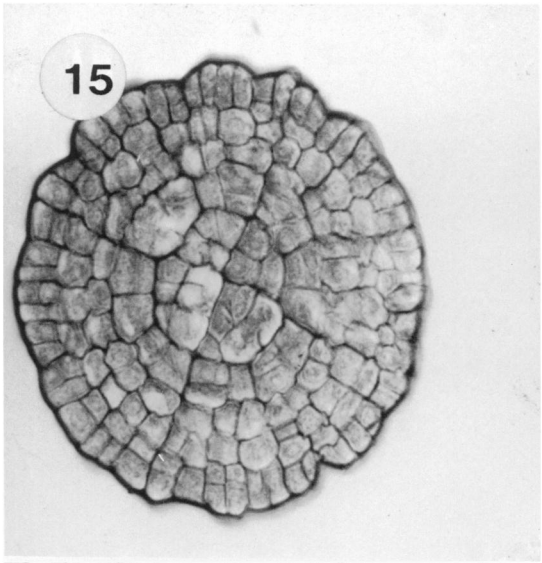
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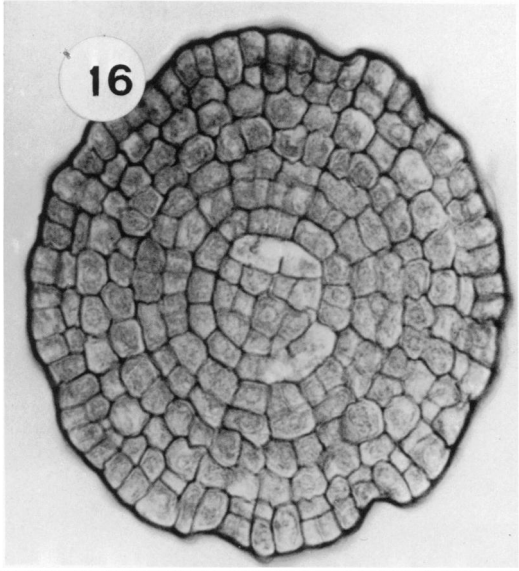
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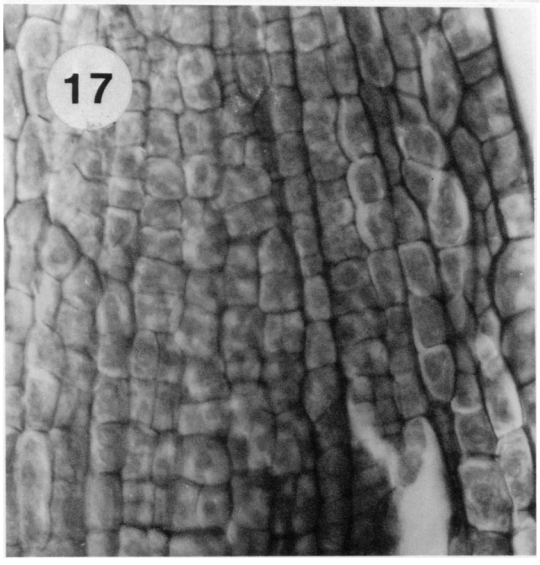
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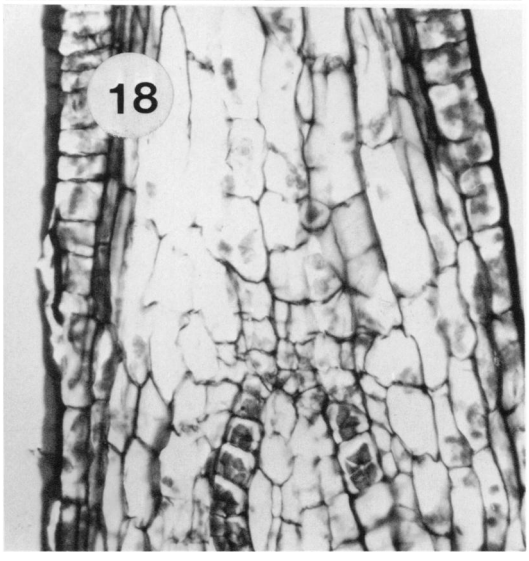
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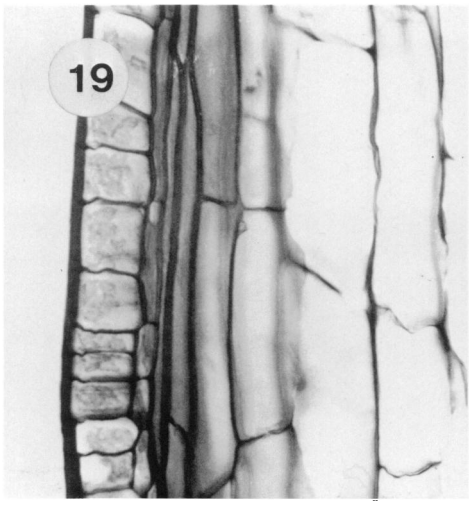
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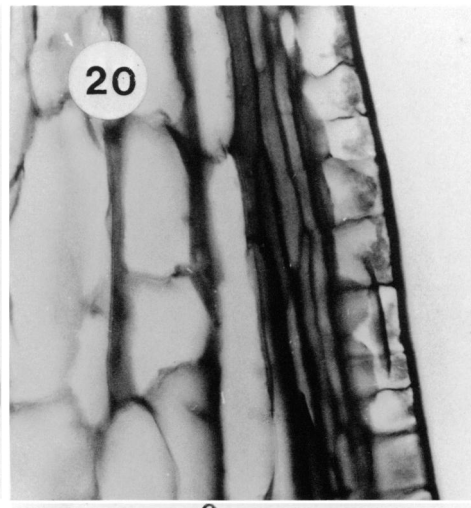
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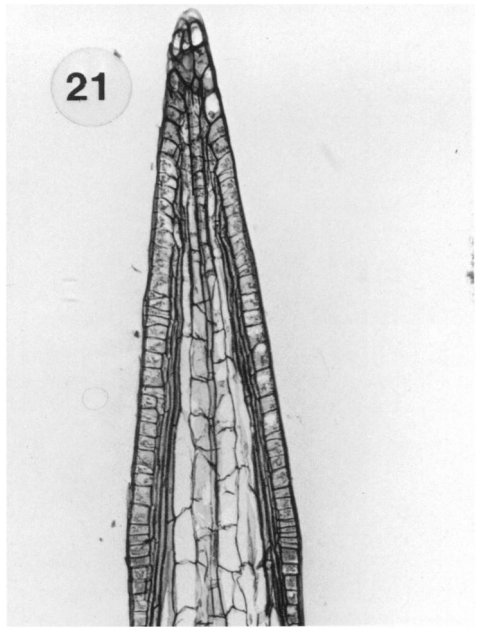
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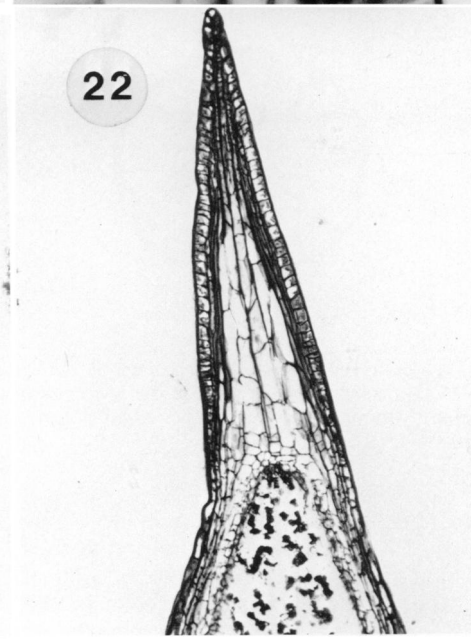
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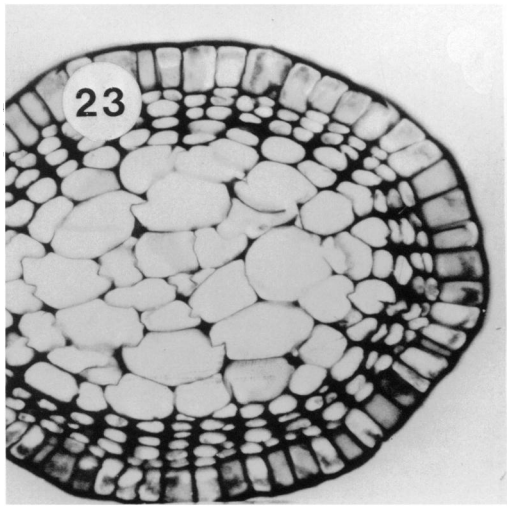
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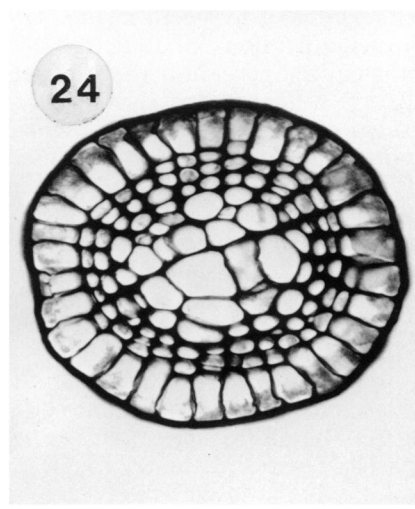
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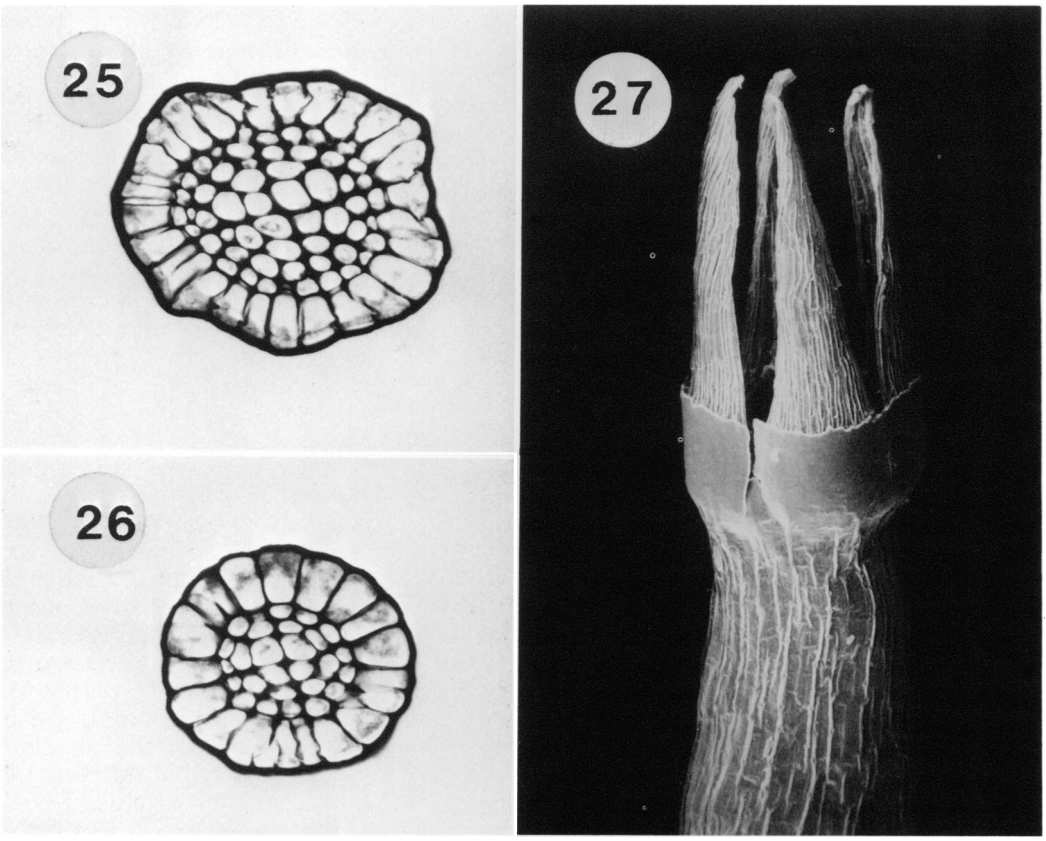

Fig. 25–27. Transverse sections through nearly mature capsules in the opercular region and an SEM of the mature peristome. **25.** Transverse section through the opercular region in the upper third. (×648). **26.** Transverse section from near the apex of the opercular region. Note wall thickenings in endothecial cells in 25, 26. (×648). **27.** SEM of the mature peristome. (×64).

thickening (Fig. 19, 20), but there is clear evidence from SEM studies of mature peristomes (Fig. 27) that these broad rectangular cells of the IPL-2 become the innermost cells of the peristome teeth. These cells show clearly on the inner surfaces of the teeth in Fig. 27.

Lower magnifications of longitudinal sections of the entire operculum after the peristomial cells are differentiated show clearly the relationships of the various layers whose developmental sequences we have described above. Figure 21 is not quite median, but the approximately five peristomial layers are clearly distinguishable. The outermost of the broad, rectangular, clear cells comprise the IPL-2, while the three columns of cells in the center are endothecial. (The central column is narrow because the section is not median.)

A still lower magnification is shown in Fig. 22, in which the upper portion of the spore sac is apparent. The entire upper portion of the columella has lysed and the sporocytes, now in meiosis, lie free in the relatively large spore cavity. Farther below, not shown in the figure, the columella persists. Final wall deposition in the peristomial cells is not complete until well past meiosis and the individual spores are freed from the tetrad.

Cross sections through the operculum of nearly mature capsules reveal the cellular structure and organization of the peristome of *Tetraphis* (Fig. 23–26). The cross section shown in Fig. 23 is near the dehiscence line of the operculum. The large, empty, thin-walled cells in the center of the section are mostly endothecial cells. The outermost layer of larger cells is presumed to be the IPL-2, judging from its position in longitudinal sections. Mostly, only the outer walls of this layer are thickened. In contrast to the precision and regularity found in the peristomial layers of arthrodontous peristomes, the peristome of *Tetraphis* shows considerable variability. The alignment of cells in the various layers in Fig. 23 is not precise, indicating that there have been anomalous periclinal and anticlinal divisions. It is not al-

ways possible to distinguish between endothecium and amphithecium.

Figures 24 to 26 represent cross sections selected from points progressively toward the apex of the operculum and reflect the conical nature of the operculum. There is a progressive reduction in the number of endothecial cells and it is likely that toward the tip of the operculum OPL-2 and IPL-2 layers are lacking. In Fig. 25, 26 all of the opercular cells are thick-walled and become the terminal portion of the peristome teeth. It is difficult in these positions in the operculum to distinguish between amphithecium and endothecium with certainty, but at least the four central cells in Fig. 26 and 27, which are clearly a part of the peristome teeth, are endothecial in origin. These sections thus demonstrate that in the upper opercular region, endothecial cells become thickened at the time peristomial wall material is deposited in the amphithecial layers, and close to the apex become a portion of the peristome.

At maturity, the tissue forming the opercular region of the capsule becomes divided into four massive teeth. Each tooth is composed of the thickened, elongated, amphithecial cells (Fig. 27). The central thin-walled endothecial cells in the opercular region break down just prior to dehiscence so that the teeth are not triangular in transverse view. Rather, both the inner and outer faces of each tooth are convex outward (Fig. 27). The linear shape of the outer cells of the peristome teeth can be discerned in the mature peristome; they range from approximately 40–100 μm in length. The teeth are approximately 700 μm in total length.

DISCUSSION—Considering that much, if not most, of the basis for modern classifications of the true mosses is based on peristome structure, it is remarkable how little is known about the development of peristome teeth. There have been a few thorough developmental studies of arthrodontous peristomes (Evans and Hooker, 1913; Blomquist and Robertson, 1941; Saito and Shimoze, 1954; Saito, 1956; Stone, 1961; Shaw et al., 1987) and even fewer of nematodontous peristomes (van der Wijk, 1929; Wenderoth, 1931; Chopra and Sharma, 1958, 1959; Chopra and Bhandari, 1959). However, one prominent feature of the developmental observations presently available is the striking uniformity of the earliest sequences of cell divisions. Up to at least the point where the amphithecium has become 3-layered (approximately Fig. 11), the pattern of development we have described for *Tetraphis* is virtually indistinguishable from the patterns reported for *Diphyscium foliosum* (Hedw.) Mohr, *Ceratodon purpureus* (Hedw.) Brid., *Aulacomnium heterosticum* (Hedw.) B.S.G., *Bartramia crispata* Schimp., and *Dicranum japonicum* Mitt. (op. cit.). Our unpublished observations on approximately eight additional arthrodontous species support the impression of developmental uniformity gained from the literature. This uniformity is all the more impressive considering that the developmental observations have been obtained from a broad range of both haplolepideous and diplolepideous species, and now also from *Tetraphis pellucida,* a nematodontous species. Data presently available indicate that development of the peristomial layers in these species also shares significant features in common with those of members of the Polytrichaceae, yet some noteworthy differences exist as well. These differences will be discussed below.

A remarkable feature of peristome development in all mosses that have been studied is the regularity of the early cell divisions, both spatially and temporally. The peristomial layers are clearly defined concentric rings of cells, owing to the fact that periclinal walls of divided cells in any given ring are almost perfectly aligned around the circumference of the capsule and occur in every cell of the ring. Moreover, both anticlinal and periclinal divisions in any given ring occur more or less synchronously. In *T. pellucida,* this synchronization decreases as peristome development progresses, whereas in arthrodontous peristomes the divisions are remarkably synchronous throughout development. Although Blomquist and Robertson (1941) provided a somewhat exaggerated impression when they described the divisions in *Aulacomnium heterostichum* as almost absolutely synchronous, it appears that even the degree of regularity we have described in *T. pellucida* is highly unusual, if not unique, in plant development.

One point of variation among the published sequences of peristome development is the manner in which the amphithecium and endothecium are differentiated. Several studies indicate that a single periclinal wall is formed in each cell of a quadrant such as that shown in our Fig. 6. These periclinal divisions differentiate four inner cells, the endothecium, from four outer cells, the amphithecium. Anticlinal divisions in the four amphithecial cells then result in an inner "fundamental square" of four endothecial cells surrounded by a ring of eight amphithecial cells. A sequence like this differentiating the endothecium and amphithecium has been reported for *Funaria hygrometrica* Hedw. (Kienitz-Gerloff, 1878), *Dicranum japonicum* (Saito, 1956), *Bartramia*

crispata (Saito and Shimoze, 1954), and *Polytrichum juniperinum* (Wenderoth, 1931).

Most investigators, however, have observed that the endothecium and amphithecium are differentiated by a series of curved anticlinal and periclinal walls resulting in a pattern similar to that shown in our Fig. 7. A pattern of differentiation like this has been reported for *Aulacomnium heterostichum* (Blomquist and Robertson, 1941), *Diphyscium foliosum* (Shaw et al., 1987), *Polytrichum formosum* (Vaizey, 1888), and *Lorentziella imbricata* (Rushing and Snider, 1980).

Chopra and Sharma (1958) reported both patterns of amphithecial differentiation described above in individual sporophytes. Moreover, these authors found that variation in the manner of endothecial and amphithecial differentiation can exist between adjacent merophytes in the young sporophyte. Stone (1961) also found such variation in the manner in which the amphithecium and endothecium are differentiated in *Mittenia plumula.* Although we have found the pattern described above for *Tetraphis* to be the most common in the mosses we have under study, we too have observed both patterns in *Ditrichum pallidum* (Hedw.) Hampe (unpublished data).

Snider (1975) reported a very different pattern of endothecial and amphithecial differentiation in *Archidium* Brid. This unique pattern of development is sometimes cited as evidence suggesting an isolated phylogenetic position for the Archidiaceae (Snider, 1975; Crum and Anderson, 1981). The two patterns described for other nematodontous and arthrodontous species may also have phylogenetic significance, although present data indicate that the patterns cut across basic and undoubtedly natural systematic groupings defined by many other morphological characters.

The development of the peristomial layers in *Tetraphis* is virtually identical to the patterns described for arthrodontous peristomes at least up to the point at which the eight IPL cells undergo anticlinal divisions (Fig. 12). In *Ceratodon purpureus, Dicranum japonicum,* and *Diphyscium foliosum,* which are haplolepideous species, the first anticlinal divisions in the IPL are extremely asymetric, producing a larger and a smaller cell (Evans and Hooker, 1913; Saito, 1956; Shaw et al., 1987). The next anticlinal divisions in the IPL occur in the larger of these two cells. The result is three IPL cells derived from each of the eight original cells, producing a peristomial formula of 4:2:3. This cellular pattern, or some slight modification of it, is considered to be characteristic of haplolepideous peristomes at maturity (Edwards, 1979, 1984).

In *Bartramia crispata* (Saito and Shimoze, 1954), a diplolepideous species, the first anticlinal division in each of the eight IPL cells is nearly symmetrical, resulting in a 4:2:2 cell arrangement. Each IPL cell then undergoes another anticlinal division, resulting in a 4:2:4 arrangement. The pattern in *Tetraphis* is much like this. Each IPL cell undergoes one nearly symmetrical anticlinal division, followed by anticlinal divisions in each of the derivatives. In *Tetraphis,* however, unlike *Bartramia crispata,* anticlinal divisions also occur in the PPL, resulting in a 4:3–4:3–4 arrangement of cells in the three peristomial layers.

The pattern of development described for nematodontous peristomes of species in the Polytrichaceae differs from that of both *Tetraphis* and the arthrodontous species (van der Wijk, 1929; Wenderoth, 1931; Chopra and Sharma, 1958, 1959; Chopra and Bhandari, 1959). The earliest stages of development are similar to those of the arthrodontous species and *Tetraphis,* to approximately the point at which the amphithecium is 2-layered. However, a greater number of anticlinal divisions occurs in the amphithecial layers of species in the Polytrichaceae than in arthrodontous mosses studied to date. In terms of the sequence of cell divisions preceding deposition of the peristomial wall material, *Tetraphis* is considerably more similar to those arthrodontous species that have been studied than it is to species in the Polytrichaceae. With the latter, *Tetraphis* shares the nematodontous structure of the mature peristome teeth, i.e., the persistence of whole thick-walled cells rather than only portions of cell walls. *Tetraphis* eventually has a cellular pattern of approximately 4:3–4:3–4, and, therefore, has more PPL cells than is typical of arthrodontous mosses, but fewer PPL cells than generally occur in species in the Polytrichaceae. Moreover, the increase in the number of PPL cells occurs in a much later developmental stage in *Tetraphis* than in species of the Polytrichaceae. Evidently, grouping mosses in accordance with the morphology of mature teeth (nematodontous vs. arthrodontous) is not congruent with grouping them according to similar developmental sequences of cell divisions. Our bias is that developmental sequences provide a better estimate of homologies and phylogenetic relationships than do comparisons of mature structure alone. Several bryologists have previously remarked that the Polytrichaceae and Tetraphidaceae do not appear closely related in spite of sharing nematodontous peristome structure (e.g., Limpricht, 1895; Crum and Anderson, 1981; Edwards, 1984).

Many bryologists have expressed the view

that the *Tetraphis*-type peristome is primitive as compared to arthrodontous peristomes (Campbell, 1905; Cavers, 1911; Dixon, 1924; Grout, 1936; Crosby, 1980; Schofield, 1985). However, except for the unique and peculiar structure of the peristome, consisting of four massive teeth, there seems to us to be little objective evidence that the *Tetraphis*-type peristome is, in fact, primitive. Most authors who have suggested a primitive interpretation for the *Tetraphis*-type peristome, and for the Tetraphidaceae, give no explicit reason for the conclusion. Cavers (1911), for example, stated only that "The peculiar and probably primitive peristome of *Tetraphis* may in some respects be compared with the elaterophore of *Aneura* and *Metzgeria*. . . ." Schofield (1985), in his excellent new textbook of bryology, observed that "This subclass [the Tetraphidae] is often considered to be the most generalized of the true mosses!" The precise meaning of the unfortunate term, "generalized," and its phylogenetic significance is not, however, at all clear. Philibert (1889) suggested that the *Tetraphis*-type peristome is a relic, primitive type, and that the *Polytrichum*-type represents a modification of it. However, none of these authors provides any substantive evidence to support the assumption of primitiveness for the *Tetraphis*-type peristome, or the Tetraphidae.

The idea that the Tetraphidae are actually more closely related to the arthrodontous mosses is also not new. Braithwaite (1887) noted that the calyptra of *Tetraphis* is reminiscent of those found in species of the Orthotrichaceae, that the leaf areolation is mnioid, but that the peristome is unique. Edwards (1984) discussed the possibility that the *Tetraphis*-type peristome is secondarily derived rather than primitive. This view is supported by the similarity in gametophytic structure between *Tetraphis* and a number of species in the diplolepideous order, Bryales. Although much additional information is needed on peristome development in other arthrodontous and nematodontous species, our observations strengthen the plausibility of a closer phylogenetic relationship between the Tetraphidae and arthrodontous groups than between either group and the nematodontous Polytrichidae.

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