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# AN ELECTRON MICROSCOPIC STUDY OF THE ANNULUS IN SPLACHNACEAE (MUSCI)

# ЭЛЕКТРОННО-МИКРОСКОПИЧЕСКОЕ ИЗУЧЕНИЕ КОЛЕЧКА СПЛАХНОВЫХ МХОВ

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Abstract

The annulus of *Tetraplodon angustatus* B. S. G. has been studied with transmission electron microscopy and of *Tayloria tenuis* (With.) Schimp. with scanning electron microscopy. Both species have an annulus which serves as a dehiscence mechanism of the capsule. Dehiscence takes place through cell wall separation at the middle lamella. The annulus remains attached to the operculum. Longitudinal section of the capsule of *T. angustatus* shows an annulus consisting of three cells in two layers (outer with two cells, inner with one cell). Ultrastructural peculiarities of the annulus cells are: 1) alternating electron-dense and more electron-translucent areas in the cell walls, representing a system of radial canals in the cell wall; 2) dense cytoplasm lacking vacuoles and occupying only a part of cell volume; 3) extraplasmic space and cell wall canals filled with a substance that appears to be mucilage; 4) outer parts of outer cell walls especially electron-dense and stratified. The annulus cells are considered to produce mucilage and hydrolytic enzymes in the course of their development.

Резюме

Строение колечка изучали у двух видов сплахновых – *Tayloria tenuis* (с помощью сканирующей электронной микроскопии) и *Tetraplodon angustatus* (с помощью трансмиссионной электронной микроскопии). Вскрывание коробочки происходит путем расхождения смежных оболочек клеток колечка и края урночки в области срединной пластинки. Колечко остается на крышечке. На продольном разрезе коробочки *T. angustatus* колечко состоит из трех клеток (в наружном слое две клетки, во внутреннем - одна). Ультраструктурными особенностями клеток колечка являются: 1) чередование в их оболочках электронно-плотных участков с более электронно-прозрачными, образующими систему радиальных канальцев оболочки; 2) густая цитоплазма без вакуолей, занимающая лишь часть объема клетки; 3) заполненность экстраплазматического пространства и канальцев оболочки слизью; 4) образующие поверхность коробочки участки стенок клеток колечка имеют особо высокую электронную плотность и слоистую структуру. Вероятно, в ходе своего развития клетки колечка производят слизь, накапливающуюся в экстраплазматическом пространстве, и гидролитические ферменты, вызывающие в конце развития клеток избирательный гидролиз их оболочек. Слизь абсорбирует воду из цитоплазмы клеток колечка, а также и из соседних клеток, что приводит к одновременному увеличению объема клеток колечка и уменьшению объема соседних клеток и, как следствие, к вскрыванию коробочки.

## INTRODUCTION

Most mosses of subclass Bryidae are stegocarpous (operculate), i.e. their capsules are opened by removal of an operculum. The operculum separates from the urn by means of an annulus, formed of one to several concentric rows of hygroscopic exothecial cells at the operculum-urn boundary.

The structure of annulus is very variable in mosses. In most species the annulus remains attached to the operculum after the capsule dehiscence; in others either it remains attached to the urn or it separates from both urn and operculum (Dihm 1894; Goebel 1930; Maier 1967, 1973a, 1973b, 1973c; Mueller 1973). Some taxonomists use the term annulus only for the type that separates from both urn and operculum (cf. Buck, 1980). Others (cf. Vitt, 1981) con-

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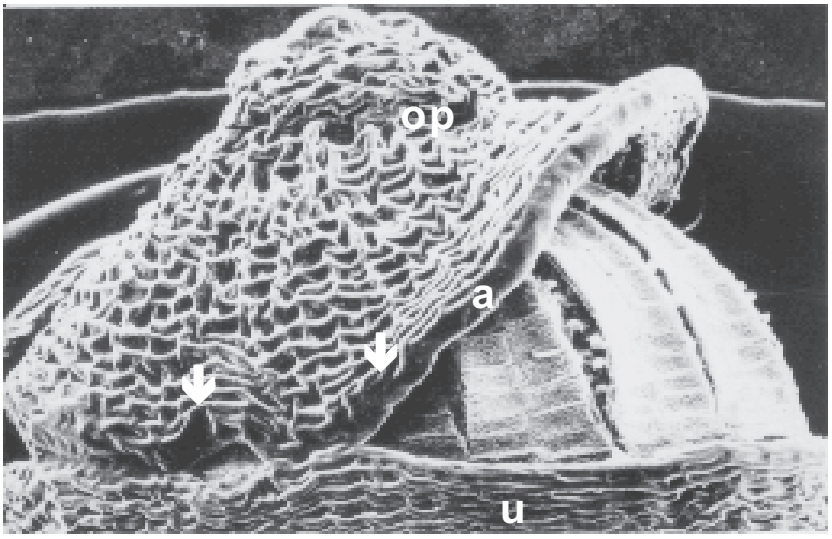


Fig. 1. *Tayloria tenuis* (With.) Schimp. Upper part of capsule showing two areas of dehiscence (arrows); a – annulus; op – operculum; u – urn. SEM, 325×.

sider that all mosses with operculate capsules have annulus, and in mosses without an annulus (cleistocarpous) capsules dehisce by breakage of individual exothecial cells.

Studying the annulus of *Fissidens limbatus* Sull. by means of transmission electron microscopy (TEM), Mueller (1973) observed that the annulus cells are noticeably less vacuolate than other epidermal cells of the capsule, their cytoplasm is dense, and they have large areas which appear to contain a mucilaginous substance. Maier (1973a) found that in *Plagiomnium cuspidatum* (Hedw.) T. Kop. the annulus cells, even after dehiscence of the capsule, have functional chloroplasts with starch grains, and that the outer wall of the annulus cell is incrusted by a specific substance characterized by a reddish-brown color and intensive and rapid staining by metal ions.

Functioning of the annulus in some species was assumed to be related to mucilaginous cell content (Goebel 1930; Lorch 1931; Mueller 1973). Maier (1973c) reported that functioning of the annulus in *Funaria hygrometrica* Hedw. and *Plagiomnium cuspidatum* is related to an increase of its volume. Factors inducing dehiscence of the capsule, in Maier's opinion, are different in these two species. The mature capsule in *Funaria hygrometrica* consists of dead cells (excluding the spores), and dehiscence occurs after a number of wet-dry cycles of the capsule, resulting in breakage along cell walls. In *Plagiomnium cuspidatum*, however, the dehiscing capsule consists of living cells. Cell wall dissolution in the annulus of this species, in Maier's opinion, is a part of the process of capsule ripening and has an enzymatic character (Maier 1973c).

Splachnaceae provide an example of mosses with a non-separating annulus, often described as absent or poorly developed (Brotherus 1924; Lazarenko 1955; Bardunov 1969; Smirnova 1970). Recently light microscopic studies on capsule development in *Tetraplodon angustatus* B. S. G. and *T. mnioides* B. S. G. (Demidova, Filin 1994) have shown an annulus consisting of small, irregularly thick-walled cells. This paper presents observations with TEM on the annulus of *T. angustatus* that allow more precise delimitation of its structure and function. Some scanning electron microscopy (SEM) observations on *Tayloria tenuis* (With.) Schimp. also are included.

## MATERIALS AND METHODS

Nearly mature (urn green to brownish, annulus brown, operculum brownish) unopened capsules of *Tetraplodon angustatus* and *Tayloria tenuis* were collected on Kindo Peninsula (Kandalaksha Bay, Beloye Sea shore at the North Polar Circle latitude, North Karelia, Russia) in July 1993. Pieces of capsules of *Tetraplodon angustatus* were fixed in 5% glutaraldehyde in sodium phosphate buffer at pH 7.2 overnight, postfixed in 2% osmium tetroxide in phosphate buffer overnight, dehydrated in an acetone series and at the grade of 70% acetone block stained in 2% uranyl acetate solution in 70% alcohol overnight,

embedded in epoxy resin, sectioned on LKB Ultratome V, stained with uranyl acetate and lead citrate, and viewed and photographed with a JEOL 100B TEM at 80 kV.

Capsules of *Tayloria tenuis* were fixed in acetic acid-alcohol (1:3), stored in 70% alcohol, air dried; sputter-coated with platinum-palladium, and viewed and photographed with the Hitachi S-405A SEM at 15 kV.

For TEM studies one capsule at the beginning of dehiscence and one nearly mature capsule, but with operculum still tightly attached to the urn, were used.

## Results and discussion

Capsules of *Tayloria tenuis* and *Tetraplodon angustatus* were found partially dehisced (Fig. 1, 2), probably, as a result of dehydration. In *Tayloria* dehiscence of the capsule takes place by cell wall separation in several sites. Along the border of operculum is seen a row of cells that differ from adjacent epidermal cells (both opercular and urnal) in having non-convex surfaces (Fig. 1). These cells are considered to be the annulus.

In *Tetraplodon* dehiscence occurs by cell wall separation along the middle lamella, without breakage of thick-walled cells; the adjacent inner thin-walled cells, however, appear destroyed (Fig. 2).

Cells at the lower border of operculum are the most peculiar, as in *Tayloria*. Longitudinal section of the capsule (Fig. 2) shows three cells at operculum edge (arranged in 2 rows, the lower row of two cells, upper of one cell) differ greatly from epidermal and subepidermal cells of the urn and the operculum. These cells are considered to be the annulus. They have dense cytoplasm without vacuoles, and visible extraplasmic space. Cytoplasm occupies only part of the cell volume and is displaced toward the center of the cell.

Extraplasmic space in two cells of the annulus ("ac" in Fig.2) is filled with a relatively loose network of electron-dense fibrils embedded in an electron-translucent matrix (Figs. 2, 3, 4). In the third cell ("acd" in Fig. 2) the cytoplasm has a degenerate appearance and extraplasmic space is electron-translucent. Cytoplasm of the two former cells contains chloroplasts with starch grains and plastoglobuli, mitochondria, elements of endoplasmic reticulum, dictyosomes, ribosomes, and inclusions.

An important ultrastructural peculiarity of the annulus cells is the alternation of electron-dense and more electron-translucent areas in their walls (Figs. 2, 3, 4). Nothing similar has been seen either in the adjacent cells, or in cells of the mouth region at an earlier stage of development (in an undehisced capsule). A thin electron-translucent layer is often discernible at outer surface of annulus cell walls except those facing outside (Figs. 3, 4). This outer layer ("ol" in Figs. 2, 3, 4) is especially well-seen at the upper wall of upper cell. It consists of fibrillar material embedded in electron-translucent matrix (Fig. 4).

The electron-translucent areas in cell walls seem to form a system of radial canals. Content of these canals is very similar to that of extraplasmic space; these canals, extraplasmic space and the outer electron-translucent layer are in communication (Figs. 2, 4).

Outer parts of outer walls of the annulus cells are especially electron-dense and stratified ("opw" in Fig. 3). These regions of cell walls have a reddish-brown color visible under a light microscope in unstained sections; this results in a thin brown strip on a ripening capsule.

A substance occurring in the wall canals of the annulus cells and in the extraplasmic space of the studied species seems to be mucilage. Mucilage appears on electron micrographs as an electron-translucent matrix containing electron-dense fibrils and granuli (Vassiljev 1977). In mucilage-producing cells of some species, mucilage accumulates between the plasmalemma and the cell wall (in extraplasmic space), resulting in displacement of the cytoplasm to the center of the cell. This process can be accompanied by hydrolytic changes in cell walls (Vassiljev 1977).

A mechanism for the functioning of the annulus can be hypothesized as follows. In the course of development, annulus cells produce hydrolytic enzymes, which in inactive form are deposited in cell wall, and mucilage, which accumulates in the extraplasmic space. At the end of development, activation of enzymes takes place and, as a consequence, cell wall canals are formed through selective hydrolysis of annulus cell walls. Mucilage, a very hygroscopic substance, fills wall canals and extraplasmic space

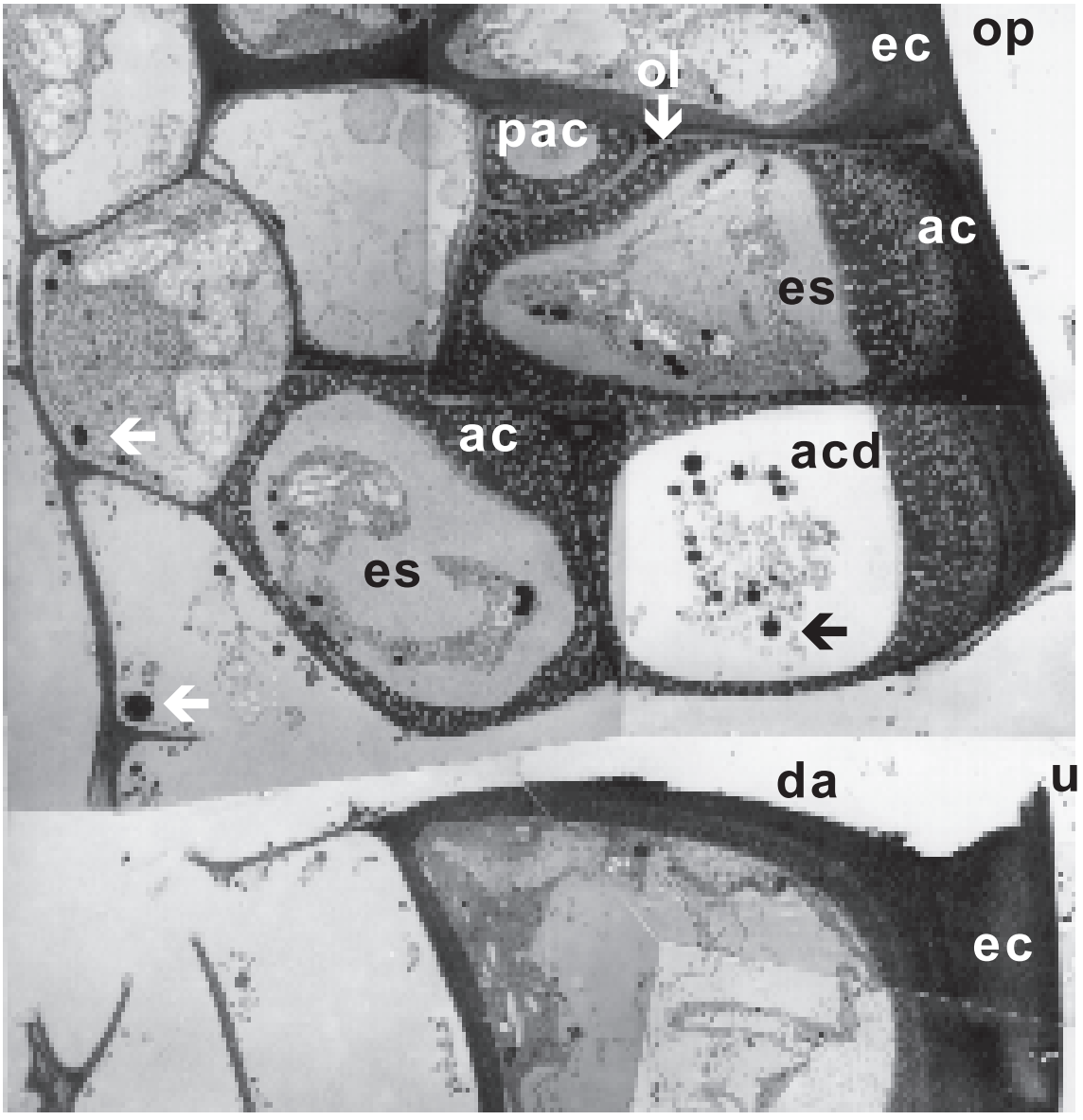


Fig. 2. *Tetraplodon angustatus* B. S.G. Longitudinal section through the annulus of partly dehisced capsule. Four annulus cells (ac) is visible, one of which has degenerated cytoplasm (acd) and one is visible only by its small part (pac). Intensely electron-dense granular deposits in cytoplasm (unlabelled arrows) are likely an artifact; da – dehiscence area; ec – epidermal cell; es – extraplasmic space; ol – outer electron-translucent layer of annulus cell wall; op – operculum; u – urn. The dash-line follows the outline of urn at the dehiscence area. TEM, 4300×.

and absorbs water from the cytoplasm of annulus cells and from adjacent cells. This make the cytoplasm of annulus cells dense. As a result the volume of the annulus cells increases and that of adjacent cells decreases simultaneously, thus tensions arise which lead to capsule dehiscence.

Outer parts of outer walls of annulus cells (especially electron-dense and stratified, "opw" in Fig. 3) seem water-proof and may prevent uptake of water by the annulus cells from outside.

Cytochemical electron microscopic studies on development of the annulus are needed to understand more precisely the mechanism of functioning of the annulus in the Splachnaceae.

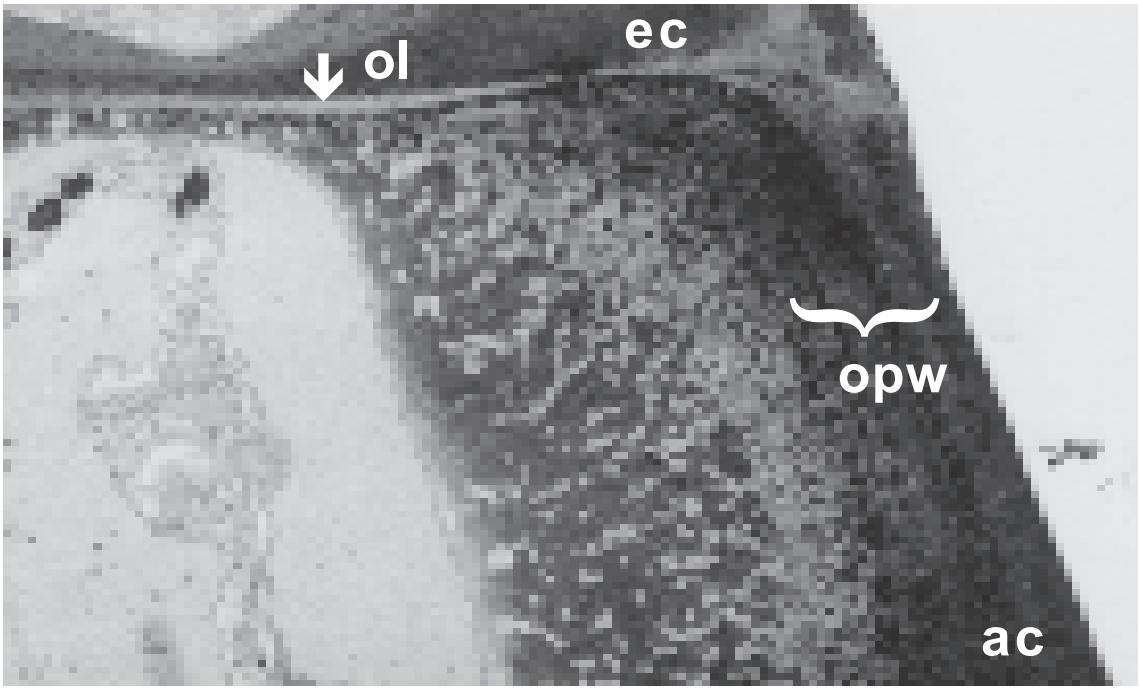


Fig. 3. *Tetraplodon angustatus* B. S. G. Annulus cell (ac) and adjacent epidermal cell (ec); es – extraplasmic space; ol – outer electron-translucent layer of annulus cell wall; opw – outer part of outer wall of annulus cell. TEM, 13700×.

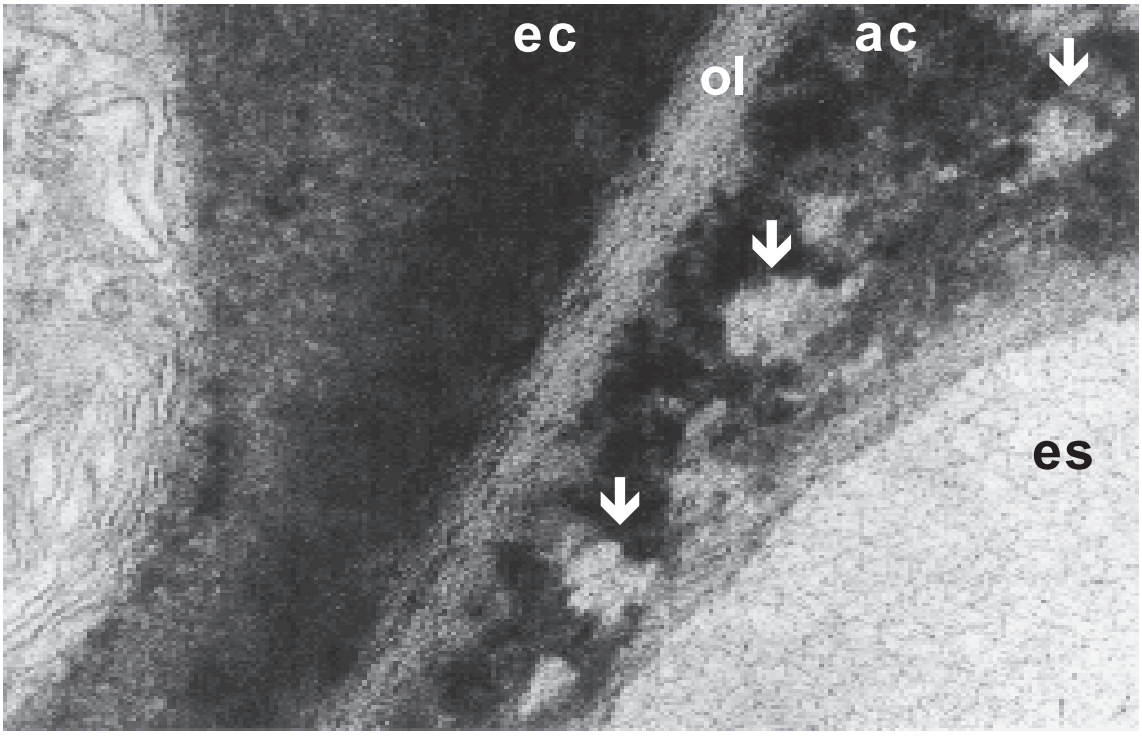


Fig. 4. *Tetraplodon angustatus* B. S. G. Common walls of epidermal (ec) and annulus cells (ac); es – extraplasmic space; ol – outer electron-translucent layer of annulus cell wall; arrows point to canals of annulus cell wall. TEM, 73000×.

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