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FALSE COLUMELLA AND SPORE RELEASE IN *TETRAPLODON ANGUSTATUS* (HEDW.) BRUCH et SCHIMP. in B.S.G. AND *T. MNIOIDES* (HEDW.) BRUCH et SCHIMP. in B.S.G. (MUSCI: SPLACHNACEAE)

ЛОЖНАЯ КОЛОНКА И ВЫСВОБОЖДЕНИЕ СПОР У *TETRAPLODON ANGUSTATUS* (HEDW.) BRUCH et SCHIMP. in B.S.G. И *T. MNIOIDES* (HEDW.) BRUCH et SCHIMP. in B.S.G. (MUSCI: SPLACHNACEAE)

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Abstract

The capsule development of *Tetraplodon angustatus* and *T. mnioides* was studied by placing emphasis on spore releasing mechanism. The same developmental pattern is observed in both species. Inside the capsule with multi-layered archesporium, a false columella (the axial tissue cylinder below the true columella) begins to develop. This structure consists of small cells. When the capsule matures and opens, the true columella degenerates while the false columella develops further by cell elongation. Spores are pushed out from urn subsequently. A lysigenous cavity often develops in the middle of apophysis, containing probably aromatic substances. The one to two-layered annulus consisting of two to three rows of small cells with irregularly thickened walls is a specialized mechanism for operculum removal.

Резюме

Развитие коробочек *Tetraplodon angustatus* и *T. mnioides* изучали, обращая особое внимание на механизм высвобождения спор. Коробочка у обоих видов развивается сходным образом. В коробочке, имеющей многослойный археспорий, начинает формироваться структура, названная здесь ложной колонкой. Ложная колонка, цилиндр осевой ткани, расположена под настоящей колонкой и до вскрывания коробочки состоит из очень мелких клеток. К моменту вскрывания коробочки, после отмирания настоящей колонки, ложная колонка начинает расти благодаря растяжению клеток, в результате чего споры постепенно выталкиваются из урночки. В центре апофизы ко времени вскрывания коробочки часто развивается лизигенная полость, содержащая, вероятно, пахучие вещества. Одно-двухслойное двух-трехрядное колечко, состоящее из мелких клеток с неравномерно утолщенными стенками, является специализированным механизмом для удаления крышечки.

INTRODUCTION

Splachnaceae is one bryophyte family characterized by zoochory (entomochory). Mosses of this family grow predominantly on substrates of animal origin such as dung, stomach pellets of predatory birds, etc., and attract species of Dipteran flies to visit mature capsules.

There is a whole suite of adaptive characters for entomochory, which includes morphology, chemistry and ecology (Koponen 1990). The chemical adaptation consists of specific attractant for flies, such as odor produced by sporophyte apophysis. The ecological adaptation is the tolerance for high concentration of osmogens in the substrate.

Among bryophyte's sporophytes, there are in these taxa several original and unusual morphological adaptations, i.e., brightly-colored seta (especially in its upper part), large and brightly colored apophysis (or hypophysis), sticky spores, etc. An interesting sporophytic character of *Tetraplodon* was reported by Rieth (1957) who observed that in *T. mnioides* spores are pushing

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out of the capsule by means of an extension of tissue stalk under the spore sac.

In the present paper we describe the development of capsules of *T. angustatus* and *T. mnioides*, with special reference to their spore releasing mechanism.

MATERIALS AND METHODS

Capsules of *T. angustatus* and *T. mnioides* were collected at various developmental stages from July to August of 1986 from different stations throughout Kindo Peninsula (Kandalaksha Bay, Beloye Sea shore at the North Polar Circle latitude, North Karelia, Russia) and adjacent areas. Pure and mixed patches of these two species were found growing predominantly on pellets of predatory birds in moss-lichen ground cover under lichen-pine forests. Capsules were fixed in formalin-acetic acid-alcohol (FAA) solution, dehydrated through an ethanol/xylol series, and then embedded in paraffin block according to standard method. Sections of 15 μm thick were obtained from a microtome, stained with hematoxylin, and examined under light microscope.

RESULTS

A significant phenological displacement between populations of *T. angustatus* and *T. mnioides* was observed. According to our observation, the time of capsule opening in *T. mnioides* is the first half of August, but capsules of *T. angustatus* remain green and not opened up to September. Probably the spore dispersal takes place in early to middle June: in early July we found brown, opened capsules of *T. angustatus* with about a quarter of spore amount.

The pattern of development is similar in both species. The youngest capsules observed (Figs. 1, 2) had an one to two layered archesporium. At this stage the width of apophysis was the same as the urn. The apophysal cortex began to develop a schizogenous air spaces (Figs. 1a; 2a,c), and air space appeared between the capsule wall and outer spore sac (Figs. 1a,c; 2a). The irregular divisions of inner cells of capsule wall in *T. angustatus* produced an assimilatory ridges, the special structures of two (three) cell rows (Figs. 1c, 3c) on inner surface of the urn wall. These structures were not observed in *T. mnioides*. At this stage of development two to three rows of epidermal cells which were smaller than the neighbouring ones and stained more intensively, are visible at operculum-urn boundary in longitudinal section of young capsule (Figs. 1b, 2b).

Capsules with multi-layered archesporium had an apophysis with the same or slightly smaller in diameter than the urn proper (Figs. 3a, 4a). Apophysal cortex had developed many air spaces (Figs. 3a, d; 4a) and outer epidermal cell walls were thickened. Between the capsule wall and outer spore sac, well-developed air space occurred (Figs. 3a, 4a). By now, the columella consisted of large parenchymatous cells without intercellular spaces and lied closely adjacent to the inner spore sac. Underneath the columella was a short cylinder of tissue consisting of very small, intensively stained cells. The circular air space which developed schizogenously around the basal part of the cylinder became separated from the air space situated between the capsule wall and the archesporium by a thin septum (Figs. 3a, 4a). Small epidermal cells with irregularly thickened walls and inner exothecial cells with thin walls (Figs. 3b, 4b) were observed at the operculum-urn boundary.

By the time of spore ripening (Figs. 5a, 6a), outer periclinal and anticlinal walls of epidermal cells of the capsule became more thickened (Figs. 5b, 6b). The columella and the inner spore sac began to degenerate. Outer spore sac remained and became adjoining to the urn wall (Figs. 5a, 6a). By the moment of opening of the capsule, the elongate axial lysigenous cavity developed frequently inside the central part of the apophysis (Figs. 8a,b).

Just before capsular dehiscence, the true columella usually had completely degenerated. This time, the small-celled axial cylinder underneath the columella began to enlarge and formed a structure which can be called a false columella (Figs. 7a,b, 8a,b). In a few samples of *T. mnioides* was observed a preservation of the true columella, sometimes with one or two upper peristome plates attached to its top (Fig. 8c). In this case, the true columella protruded out of the urn as far as the false columella stretched. Operculum was removed by means of the dehiscence of irregularly thick-walled cells at the operculum-urn boundary (Fig. 6b).

Growth of false columella resulted in the successive dissipation of spore mass from the capsule. At the end of its growth (a few weeks after capsule opening), the tip of false columella nearly reached the level of urn mouth (Fig. 7b), increasing its length more than two times. Simultaneous with this process, the width of the urn narrowed by cell shrinkage. Longitudinal walls of epidermal cells were observed to be 2-3 times thicker than the transverse walls (Fig. 7c) along most of the urn length. Thus, cell shrinkage easily resulted in the decrease of urn diameter.

DISCUSSION

Our phenological data were similar to that of Russian Arctic and Canada (Abramova & al., 1961; Marino, 1988a; Vitt & al., 1988) where *T. angustatus* produced mature sporophytes earlier in the season than *T. mnioides* (in spring-early summer versus middle summer-autumn). However, in Great Britain and Romania *T. mnioides* produces sporogones earlier, than

T. angustatus (Mohan, 1990; Smith, 1978).

In his phenological review of pottiaceous mosses, Zander (1979) indicated that some taxa can show relatively great variation of maturation dates of sporophytes in various geographic regions. The maturation date of a population correlates with sexual condition, latitude, maximum of regional precipitation, habitat and the taxonomic groups, as well as with the geographical distribution and climatic optimal requirement of the species. Phenological variability of *T. angustatus* can be attributed also to the existence of distinct races, or to differential response of the species to the ecological conditions of the regions. The data of Marino (1988b) which showed that *T. angustatus* and *T. mnioides* differed in their north to south distribution in North America (*T. mnioides* is found in both arctic and boreal regions, and *T. angustatus* in boreal region only) permit us to suppose that these species have differences in their climatic requirements. Or probably, these species possess a different evolutionary history.

Odor emission through the apophysal stomata of mature capsules in the Splachnaceae was hypothesized by Wettstein (1921) who observed in inner cells of mature apophysis a great quantity of a liquid which was supposed to contain aromatic substances. Observation on *Splachnum melanocaulon* in Finland (Koponen, 1990) also indicated the existence of a liquid inside the mature capsule. Results of the identification of these volatile compounds in sporophytes and gametophytes of the entomochorous Splachnaceae species (Pyysalo, 1983; Koponen & al., 1990) suggested that some could possibly be produced in the apophysis only. It is possible that the process of lysis of apophysal pith cells is connected with the production of these substances. The lysigenous cavity in apophysal pith (or cells of apophysal pith) appears to be a site of the accumulation of volatiles-containing liquid.

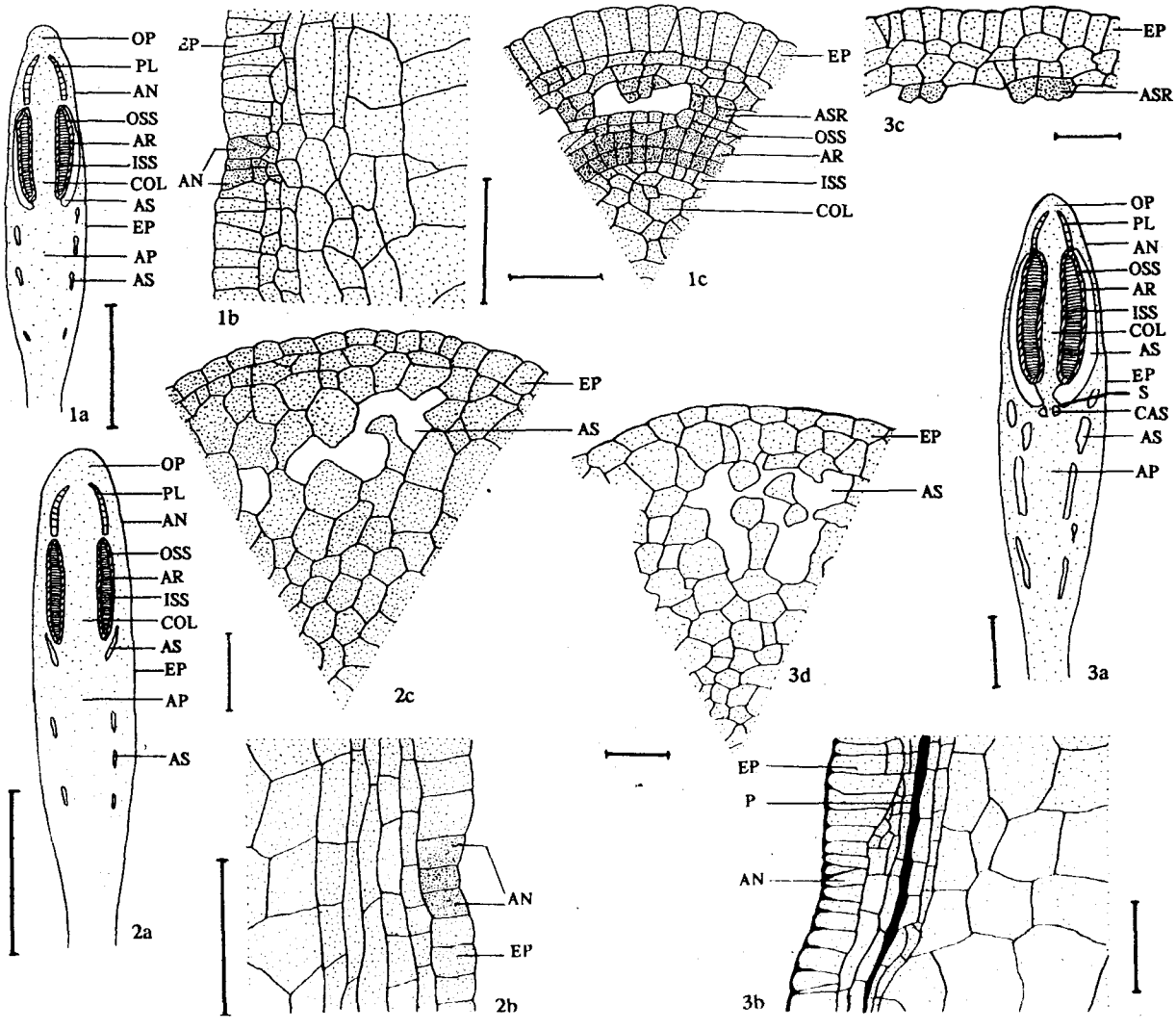
Lysigenous cavities (see Fahn 1979) are observed in different vascular plant families. For some, the lysis of nectariferous tissues in extrafloral nectaries was observed. Ecological role of these lysigenous cavities has been assumed to be for defence and also, as an attraction mechanism. Lysigenous cavity in the apophysis of *Tetraplodon*, as in the Tracheophyta, may take part in attracting of the insects through the accumulation of specific substances at the time of capsule ripening. The existence of lysigenous space in upper part of the seta of *Splachnum luteum* and some other *Splachnum* species was noted by Vaizey (1890). We also have observed apophysal lysigenous cavities extending to the upper part of the seta in the samples of *Splachnum rubrum, S. luteum*, and *S. vasculosum* and in *Tayloria tenuis* (Demidova, Filin, 1993). This character may have a systematic significance.

There is a discrepancy in past accounts about the presence of an annulus in Splachnaceae, including *Tetraplodon*. Some bryologists have pointed out the existence of a one-cell-row annulus in *T. mnioides* (Smirnova 1970), or the absence of it (Brotherus, 1909; Bardunov, 1969), or a faint expression of the annulus (Lazarenko, 1955). According to our data, the one to two-layered annulus in *T. angustatus* and *T. mnioides* which consists of 2-3 cell rows begins its differentiation at an early stage of capsule development. The nearly mature, green capsule has the annulus often visible to the unaided eyes as a thin brown strip at the operculum-urn boundary. At this stage, the annular cells dehisced easily (during the slide preparation) with preliminary dehydration. This is due to the considerably irregular cell-wall thickening and the hygroscopic nature of the annulus.

Thus, in *T. angustatus* ans *T. mnioides*, the annulus may be considered a special mechanism for operculum removal. According to Vitt's (1981) classification, it is classified as the second type (annulus of small, thick-walled cells). Operculum removal in *Tetraplodon* may be promoted further by the shrinking of urn diameter and the hygroscopic movement of peristome, which, in the case of *Tetraplodon*, belongs to the group of xerocastique peristomes; more over, by the elongation of false columella.

In sample of *T. mnioides* from China, Rieth (1957) found that the structure of nearly mature, green capsules is the same as that of other mosses. The mature, opened, brown capsules, however, had much enlargered a "der Sporensackstiel" (spore sac stalk). It was so long that spore sac with columella remains had been displaced to reach the level of urn mouth. Rieth pointed out that this stalk reached more than half of the urn length. As a result of the pushing out of spore sac and the drying of capsule, volume of spore sac diminished. He hypothesized that the sticky spores of *T. mnioides* were dispersed by insects rather than by wind, and that spores were pushed out of the capsule and became availabile to insects. Our results confirmed the Rieth's data. We observed that in *T. angustatus* and *T. mnioides* the false columella differentiated during the stage of development of multilayered archesporium. Its growth in the opened capsule was by means of cell elongation, rather than cell divisions.

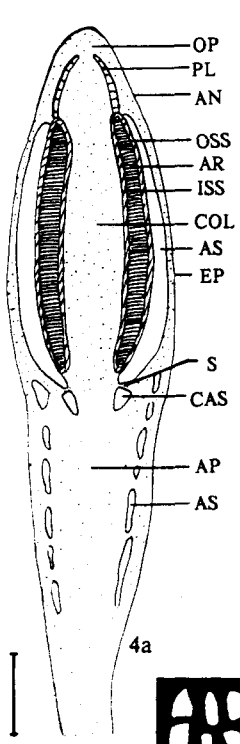
The presence of false columella in both species and their identical mode of differentiation permit us to speculate that this structure may be common in the genus as whole. Since the expression of false columella becomes significant after the capsule is opened, most bryologists may have neglected in the past its development. However, when we examined the capsules of some species of *Splachnum* and *Tayloria tenuis*, the same structure was not observed. Thus, this character may have a certain phylogenetic and systematic significance in the family. It is also probable that



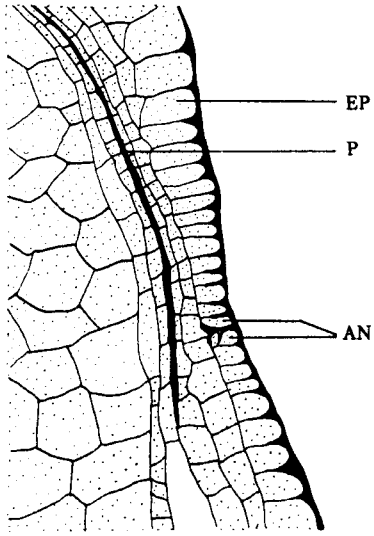
Figs. 1-8. Capsules of *Tetraplodon angustatus* and *T. mnioides* at different stages of development. **1**. *T. angustatus* capsule at two-layered archesporium stage (**a** - longitudinal section of capsule; **b** - longitudinal section at the annular level; **c** - transversal section at the archesporium level). **2**. *T. mnioides* capsule at one-layered archesporium stage (**a** - longitudinal section of capsule; **b** - longitudinal section at the annular level; **c** - transversal section through the apophysis). **3**. *T. angustatus* capsule at multi-layered archesporium stage (**a** - longitudinal section of capsule; **b** - longitudinal section at the annular level; **c** - transversal section of the urn wall; **d** - transversal section through the apophysis). **4**. *T. mnioides* capsule at multi-layered archesporium stage (**a** - longitudinal section of capsule; **b** - longitudinal section at the annular level). **5**. *T. angustatus* capsule at the end of true columella degeneration (**a** - longitudinal section of capsule; **b** - transversal section through the apophysis). **6**. *T. mnioides* capsule with degenerating columella (**a** - longitudinal section of the capsule; **b** - longitudinal section at the annular level). **7**. Opened *T. angustatus* capsule (**a** - longitudinal section of capsule shortly after operculum removal; **b** - the same at the end of false columella expansion; **c** - surface view of the urn epidermis of fresh capsule). **8**. Opened *T. mnioides* capsule (**a** - longitudinal section of the capsule shortly after operculum removal; **b** - the same at somewhat later stage; **c** - ventral view of expanded false columella with retained true columella).

ABBREVIATIONS USED: AN - annulus; AP - apophysis; AR - archesporium; AS - air space; ASR - assimilatory ridge; CAS - circular air space; COL - columella; EP - epidermis; FC - false columella; ISS - inner spore sac; LC - lysigenous cavity; OP - operculum; OSS - outer spore sac; P - peristome; PL - peristomial layers; PT - peristome tips; S - septum; SM - spore mass; UW - urn wall.

Scale bars: for 1a, 2a, 3a, 4a, 5a, 6a, 7a,b, 8a,b,c - 0.5 mm; for others - 50 μm.



4a



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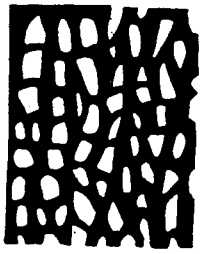
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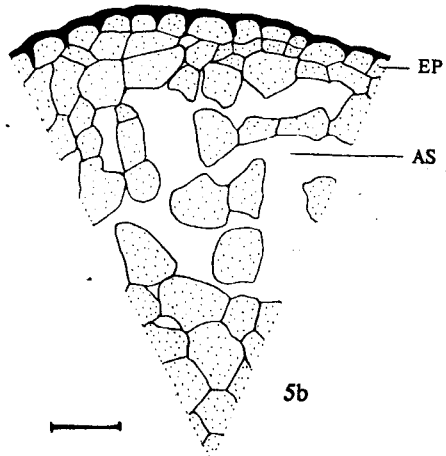

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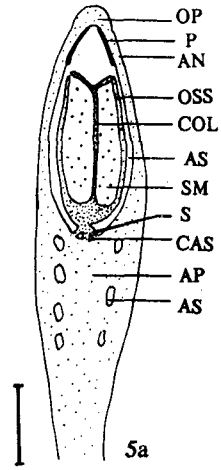

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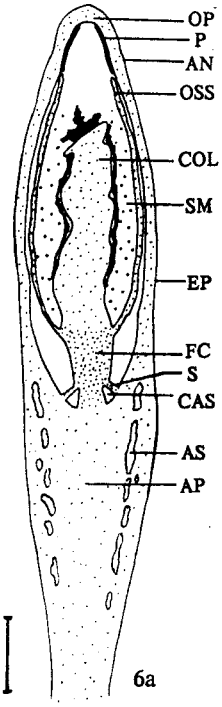
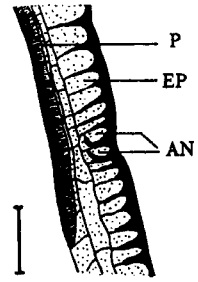


6a



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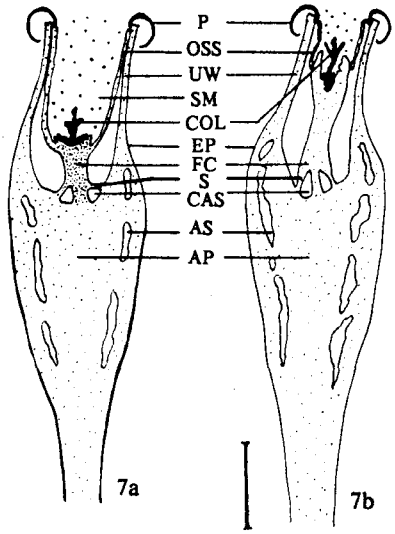
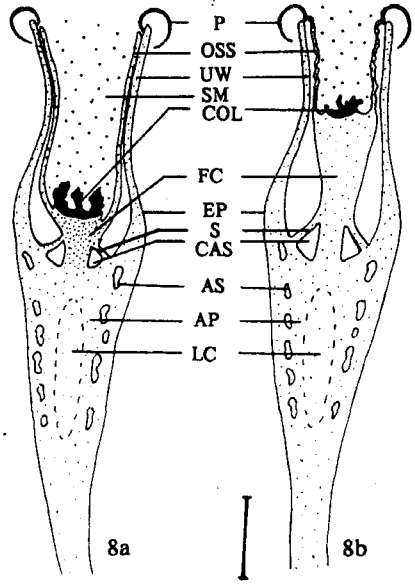


7a 7b



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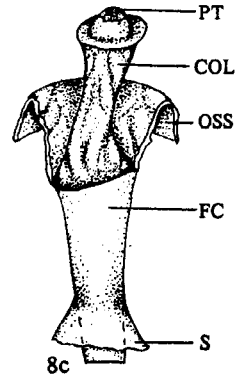

8c

the thin septum around the base of false columella supports the columella preventing it from bending under the weight of spore sac.

Koponen (1978, 1990) attached some important values to the shortening and narrowing of the urn wall during spore releasing in *Tetraplodon*. Our data showed that the pushing of spores by the development of false columella and the shrinking of urn wall occurred simultaneously.

As a result of false columella development, small heap of spores formed at the capsular mouth, making spores available to flies. At the same time, owing to the relatively wide range of suitable substrates colonized by *Tetraplodon* and their sometimes aggregate populations, anemochory can not be excluded from possible ways of spore dispersal.

Our find of some opened capsules of *T. mnioides* possessing the true columella is not unique. The same phenomenon was described and illustrated by Frisvoll (1978) for *T. angustatus*. Presence of broken off upper parts of peristome on top of the persisting true columella in *Tetraplodon* also was observed previously by Brotherus (1909). In all probability, retention of columella is a case of spontaneous reversion of an ancestral character (atavism). However, due to color contrast between green spore mass and yellow columella with brown perisome tips on its top, this phenomenon may also have an adaptive significance. In comparison with a pure patch of plants with only the false columella, a population with such plants may become more attractive to insects. Analogy can be made with the presence of one flower with contrasting colour per umbel in some Apiaceae (Eisikowitch 1980) which plays an important role in insect attraction.

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