Arctoa (2018) **27**: *1–17*
doi: 10.15298/arctoa.27.01

PERISTOME DEVELOPMENT PATTERN IN *ENCALYPTA* POSES A PROBLEM: WHAT IS THE PRIMARY PERISTOMIAL LAYER IN MOSSES?

ОСОБЕННОСТИ РАЗВИТИЯ ПЕРИСТОМА У *ENCALYPTA* ПОДНИМАЮТ ВОПРОС О ТОМ, ЧТО ЖЕ ТАКОЕ ПЕРВИЧНЫЙ ПЕРИСТОМНЫЙ СЛОЙ У МХОВ?

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Abstract

Peristome development was studied in mosses of the subclass Funariidae: *Encalypta procera, E. longicolla, E. rhaptocarpa, E. vulgaris*, *Timmia bavarica* and *Funaria arctica*. Peristome of Encalyptaceae is characterized by thick inner peristomial layer (IPL), so up to the late stages of its development its cells do not divide, maintaining basic peristomial formula 4:2:2 (omitting preperistomial layers). No regular offsetting is seen, and cells in all amphithecial layers are aligned by their anticlinal cell walls. Further development to the stage of 4:2:4 may proceed very late, so the formula 4:2:2 may remain in mature peristomes. Moreover, occasional anticlinal divisions occur in primary peristomial layer (PPL), so one IPL cell sometimes adjoins to two cells in the PPL. Such anticlinal divisions in PPL are also observed in *Timmia*, though we failed to find them in *Funaria*. The revealed additional divisions in PPL pose a question on its definition. Original criteria for PPL recognition given by Blomquist & Robertson (1941) are discussed and found contradicting the pattern in *Encalypta* in a number of important aspects. In all studied species of *Encalypta* the IPL seems to keep a development regulatory functions, which are a characteristics of PPL in peristomes of most arthrodontous mosses. This developmental pattern of peristome in *Encalypta* may explain an extraordinal diversity of peristomes in this genus, varying from 5-layered to simple and then to totally reduced. Neglected aspects of the peristome development in *Funaria* are also discussed.

Резюме

Изучено развитие перистома у мхов подкласса Funariidae: *Encalypta procera, E. longicolla, E. rhaptocarpa, E. vulgaris*, *Timmia bavarica* и *Funaria arctica*. Перистомы Encalyptaceae характеризуются сильным утолщением клеток внутреннего перистомного слоя (ВПС), и в процессе их развития долгое время расположение клеток в перистомных слоях соответствует базовой перистомной формуле 4:2:2 (не принимая во внимание предперистомные слои). Смещения клеток в перистомных слоях не наблюдается, и периклинальные стенки всех перистомных слоев остаются выровненными относительно друг друга. Переход к следующей стадии развития, которой соответствует перистомная формула 4:2:4, может происходить очень поздно, или формула 4:2:2 может сохраняться в зрелых перистомах. Более того, в клетках первичного перистомного слоя (ВПС) иногда происходят антиклинальные деления, и в таких случаях к одной клетке ВПС будут примыкать две клетки первичного перистомного слоя (ППС). Такие антиклинальные деления в ППС также происходят у *Timmia*, однако мы не нашли их у *Funaria*. Выявленные дополнительные деления в ППС ставят вопрос о том, что такое первичный перистомный слой. Обсуждаются критерии, изначально предложенные Бломквистом и Робертсоном (Blomquist & Robertson, 1941) для различения ППС; показано, что многие важные признаки ППС отсутствуют у перистомов *Encalypta*. По-видимому, у всех изученных видов *Encalypta* ВПС играет основную роль в регуляции развития перистома, которая обычно приписывается ППС у большинства мхов с артродонтным перистомом. Эта особенность развития перистома у *Encalypta* может служить объяснением экстраординарного разнообразия перистомов у видов этого рода, которые варьируют от пятислойных до полностью редуцированных. Обсуждаются также некоторые аспекты развития перистома *Funaria,* которым ранее не уделялось внимание.

KEYWORDS: bryophytes, evolution, haplolepideous, arthrodontous, sporophyte

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INTRODUCTION

Peristome is a structure for controlling spore discharging in mosses, and therefore it is of crucial importance for their life. In most groups of mosses, peristome is composed of either one circle of 16 elements, called teeth, or of two circles of 16 elements each. These elements are usually developed from three concentric layers of cells, which are largely decomposing, excepting tangental cell walls adjoining in a specific ways, so the inner cell wall of the outer peristomial layer adjoins to the outer cell wall of the middle cells layer, while the inner cell wall of this middle layer adjoins to the outer cell wall of inner peristomial layer (Edwards, 1979, 1984; Shaw & Robinson, 1984; Shaw & Renzaglia, 2004; Shaw *et al.*, 2011).

An outstanding regularity in cell divisions in the developing capsule has been already observed by Kienitz-Gerloff (1878); then Evans and Hooker (1913) counted number of cells in the developing peristome and found a fixed number of cells in two concentric layers which produce peristome in *Ceratodon*.

Homology and terminology of peristomial layers were developed by Blomquist & Robertson (1941) and Kreulen (1972), and Edwards (1979) introduced peristomial formulae, which present the proportion of cells in three (or more) peristomial layers. Subsequent studies revealed not only usefulness of the peristomial formulae, which spread over handbooks, but also it was found that they nicely correlate with the main phylogenetic moss lineages (Newton *et al.*, 2000; Shaw *et al.*, 2011; Frey & Stech, 2009; Goffinet *et al.*, 2009). In Fig. 1 this principal scheme is summarized in accordance with current knowledge. This general rule of peristome evolution provided a possibility to evaluate specific exceptional cases, such as *Catoscopium* (Ignatov *et al.*, 2015) and *Pseudoditrichum* (Fedosov *et al.*, 2016).

Representatives of most moss orders were already studied for the peristome development, although the Encalyptales were not specially addressed in this respect up to now. At the same time, peristomes of Encalyptaceae attracted attention in the first bryological studies in 18th century (see review of Horton, 1982). The fact that such small genus has so diverse peristome structure was noted already by Nees *et al.* (1827), and a rather modern species grouping in the genus is dated by the second edition of Synopsis Muscorum Europaeorum' (Schimper, 1876). The important step forward was done by Philibert (1889, revised and abridged in English by Taylor, 1962), who noticed the similarity of peristomes of *E. longicollis* and *E. brevicollis* with *Polytrichum, Buxbaumia,* and *Tetraphis*, and concluded that other species of *Encalypta* may be cosidered as a transition from *E. longicollis* to the normal arthrodontous mosses by means of reduction of the peristomial layers, through structures like in *E. affinis*, *E. procera*, *E. streptocarpa*, and *E. ciliata*. Philibert correctly concluded that the haplolepideous peristome of *E. ciliata* is homologous to the endostome of *E. procera*, which makes the genus *Encalypta* of important phylogenetic significance, maybe comprising an early stages of transition to the haplolepideous mosses.

Consequently the discussion on the peristome of *Encalypta* by Edwards (1979, 1984) and Horton (1982) contributed much to understanding of the Encalyptaceae morphology and evolution. However, the developmental aspects were still scarcely observed, so we decided to perform this study for better understanging of peristome structure in the Encalyptaceae and reasons for its great variation.

MATERIAL AND METHODS

Sampling. Although the molecular phylogenetic studies never adressed *Encalypta* specifically, a rather broad selection of its species was represented in a number of broaded phylogenies (Tsubota *et al*., 2004; Ignatov *et al.*, 2016), which confirms the general system of the genus worked out already by Philibert (1889), Brotherus (1924), and Horton (1982, 1983). Four species of *Encalypta* were selected for this study in order to include both basal and teminal representatives of the genus: *E. procera,* one of the basalmost species in the molecular phylogenetic trees (Tsubota *et al*., 2004); *E. longicollis*, another species from the basal grade, with the "secondary nematodontous" peristome formed by 4-5 cell layers; *E. rhaptocarpa*, a representative of the terminal clade, with a well developed endostome and exostome only a little shorter and adhaerent to endostome; and *E. vulgaris*, a species closely related to the previous one, but eperistomate. Two other mosses of the subclass Funariidae, or 'diplolepideous opposite', *Funaria arctica* and *Timmia bavarica* were taken for comparison, in order to have comparable images using the same methods and based on the material collected at similar stages of development.

The material was collected when setae reached one third of their full length, but the capsule itself did not start to become broader than the seta. As sporophyte develompent proceeds acropetally, the earliest stages of peristome development were seen in its distal parts, while below the later stages were available. As we mostly did not trace all stages for each individual species, the structure of IPL (where divisions are commonly delayed in *Enclaypta*) was observed under SEM, by observations on peristome surface from inside.

Preparation and Microscopy. All material was collected in the field, fixed shortly after collecting in 2.5% glutaraldehyde in 0.05M PBS. Further steps vere done after several weeks or few months. Specimens were post-fixed with 1% osmium tetroxide in PBS, pH 6.8, for 6 hours. Then material was dehydrated through an ascending ethanol-acetone series to 100% acetone. After that samples were embedded in araldite 6005 medium, according to the manufacturer's protocol. Sections were cut 2 μm thick with glass knives, put on glass slides without mounting medium, stained with 0.01% berberine or its combination with DAPI and scanned under LSCM Olympus FV-1000 based on Olympus BX61, using 473 nm or

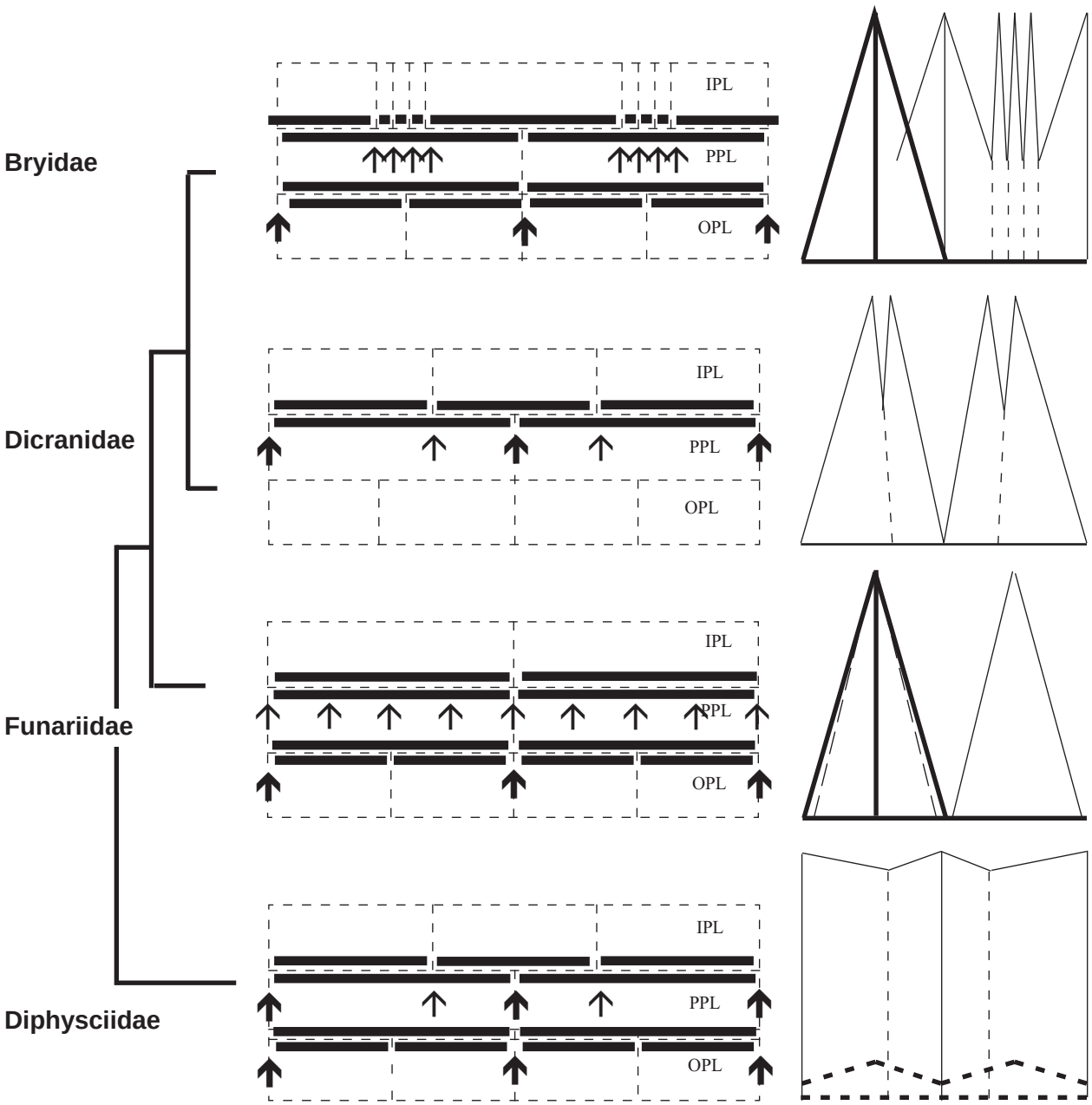

Fig. 1. Scheme of the main peristome types in anthrodontous mosses of the subclasses Diphysciidae, Funariidae, Dicranidae, and Bryidae. Note that Diphysciidae, the basalmost lineage, has clear 4:2:3 peristomial formula, although the peristome in this group is highly specific, due to absence of splitting into separate teeth. The 4:2:3 peristomial formula seems to be basic for Dicranidae and appeares at early stages of development in the Bryiidae, but it does not form in the ontogenesis in some lineages, especially in the Funariidae.

combination of 405 and 473 nm lasers. Z-stacks of several scans were usually obtained and presented here.

For transmission electron microscopy (TEM) observations of the fully developed peristome, mature and recently opened capsules of *Encalypta longicollis* were used; this species has an especially complicated peristome structure. Material intended for TEM obsertvation was taken from the the same araldite embeddings. Search of appropriate position was made by the the same 2 μm thick cuts, which were also studied under the light microscope and LSCM. Then sectioning (50 nm thick) was done with a Leica-5 ultratome. Sections were examined under JEM-1011 TEM (Jeol, Japan) at 80 kV and a CCD ORIUS SC1000W under control of GATAN Digital Micrograph in the Laboratory of electron microscopy at the Faculty of Biology of Lomonosov Moscow State University.

Scanning electron microscope (SEM) observations of peristome structure were done with the SEM Jeol 6380 for specimens coated by gold without additional preparation.

Note on terminology. Names of peristomial layers are applied here in the traditional sense: the innermost amphithecial layer will be called IPL (Inner Peristomial Layer), the second one PPL (Primary Peristomial Layer), and the third OPL (Outer Peristomial Layer). Cells of OPL may continue to form additional peristomial layers outwards, resulting in OPL1 (closest to PPL) and consequent OPL2, OPL3, OPL4, and occasionally even OPL5. Exceptional cases occur occasionally at a level of teeth base, where the IPL cells may undergo periclinal divisions, resulting in IPL1 (closer to PPL) and IPL2 (closer to endothecium) (Fig. 10).

RESULTS

Encalypta

The complete series of 2 μm sections show the principally similar pattern in peristome development in four species of *Encalypta*. Selected photographs from these series were obtained for three samples of *E. procera* from two populations, two samples of *E. longicollis* from one population, four samples of *E. rhaptocarpa* from two localities and one sample of *E. vulgaris*; they are given in Figs. 2, 3, 5.

The developmental stages show in these figures start either from the "fundamential cross" or from eight-cells pattern where four endothecial and four amphithecial cells are differentiated. Downwards to juvenile sporophyte, the successive series present patterns characterized by peristomial formulae: 1, 2:1, 2:2:2, 4:2:2, with some deviations.

Longitudinal sections available for some species indicate the position of respective transverse sections, calculated as the difference between given section and by first section where endothecium is seen, and multiplied by 2 μm (the thickness of sections).

As the series might not cover the latest stages of peristomial development occurred, e.g., during sporogenesis time, we supplemented the series of transverse sections with SEM observations of inner (ventral) surface of basal parts of teeth. The occurrence of anticlinal divisions, albeit partly fairly irregular, indicates the delayed divisons in IPL cells of proximal part of teeth in all species of *Encalypta*. At the same time, middle, and moreover, distal parts of inner surface of teeth indicate that the formula 4:2:4 is performed only in proximal part of peristome, while more distally the formula remains to be 4:2:2.

Species-specific characteristics are listed briefly for individual species, based on these few studied specimens.

Encalypta procera (Fig. 2)

The studied sequence of divisions results in a pattern characterised by the following formulae: 2:1 — 2:2:2 — 4:2:2 — 8-4:4:2:2. The IPL:PPL cells thickness ratio is ca. 2.5:1. Few anticlinal divisions occur at the later stages of development in both IPL and PPL (Fig. 2: H, I, J).

Encalypta longicollis (Figs. 3, 4)

This species is known as having the greatest number of layers among arthrodontous mosses. Some of them are obviously developed at the later stages during sporogenesis, as at the earlier stages used in the present analysis sequence of divisions results in pattern characterised by the following formulae: 2:1 — 2:2:1— 4:4:2:2 — 4:4:4:2:2 — 8:4:4:2:2. The IPL:PPL cells thickness ratio is 1.4–2:1. Anticlinal divisions occur at the later stages in few PPL cells (Fig. 3G). In some sectors the peristomial formula comprises 4:2:3 pattern (Fig. 3H), although this case was observed in only one series of sections. Various irregularities, including, e.g., anticlinal divisions in some PPL cells, are more numerous comapred to *E. procera*. Longitudinal sections (Figs. 3 A, B) illustrate less regular cell arrangement in *E. longicollis* compared to *E. procera* (Fig. 2 C).

Multilayered peristome at its mature stage is shown in Fig. 4. It is formed of five layers with decomposed cell content only in outermost and innermost layers. Content of cells corresponding to PPL, OPL1 and OPL2 transforms into the fibrillose substance that fills them, without any tendency for sedimentation along cell walls (Figs. 4 D, F). Berberin staining of cell walls indicates the position of cellulose only at cell walls (Fig. 4E), thus the substance filling the main volume of cells remains unknown.

Encalypta rhaptocarpa (Fig. 5: A–I).

The studied sequence of divisions results in a pattern characterised by the following formulae: 2:1 — 2:2:1— 4:2:2 — 8:4:2:2 — 8:4:4:2. The IPL:PPL cells thickness ratio varies from 3:1 to 1.2:1 in different series and at different stages. Anticlinal divisions are numerous at later stages of development in both IPL and PPL (Fig. 5 H, I), and occasionally periclinal divisions occur in IPL at the level of proximal part of teeth. In later (lower in the series) stages, cell walls from different layers are quite perfectly aligned, while in a medium stage, 4:2:2, the aligning is only moderately perfect, and in one sector 4:2:3 pattern is seen. Being solitary, it could be considered as an exception, however in another sector the cell wall is also quite offset (Fig. 5E, arrowed).

Encalypta vulgaris (Fig. 5: J–L)

Available material for this species was imperfect. However, it is interesting, showing a great variation with numerous additional anticlinal divisions in both IPL and PPL, so the formulae 2:1, 2:2, and 4:4:4 were observed. The IPL:PPL cells thickness ratio varies even more than in *E. rhaptocarpa*, from 4:1 to 1:1 at different stages.

* * *

Structure of the IPL cells formed at the latest stages of development is seen in the SEM images of the lowermost parts of teeth from inside (Fig. 6), where each tooth has two cells on its surface. This allow conclusion that IPL cell divisions are delayed in *Enclaypta*, thus mostly not seen in series of transverse section (Figs. 2, 3, 5), however obviously happens later, thus the number of IPL cells in peristome formulae of *Encalypta* should be 4, not 2.

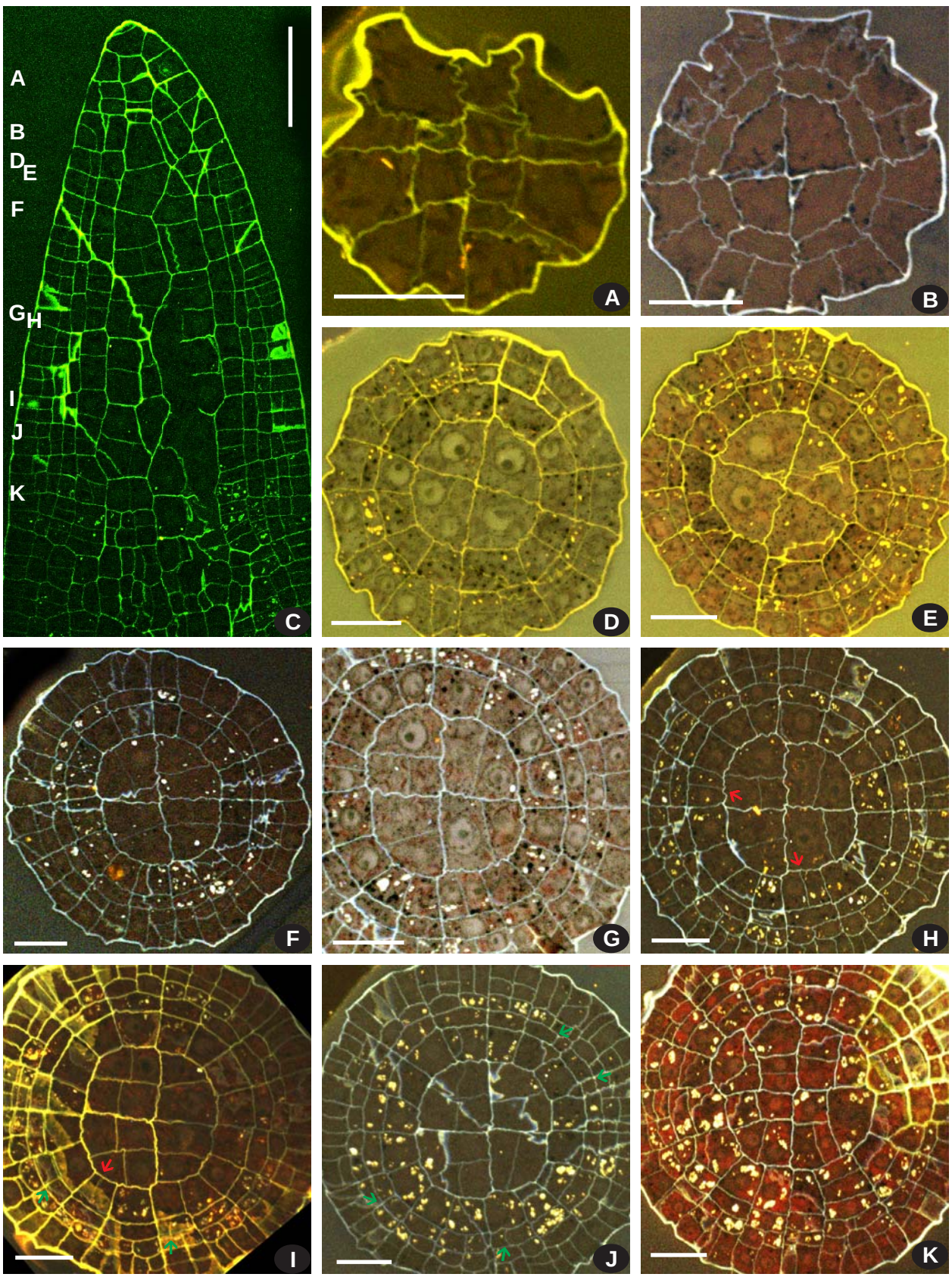

Fig. 2. *Encalypta procera* (from Yakutia, Ignatov & Ignatova 16-353, MHA): longitudinal (C) and transverse (A–B, D–K) sections of sporophyte, showing succesive stages of the peristome develompent, corresponding to peristomial formulae [in general, some sectors have exceptions]: 1 (A), 2:1 (B), 2:2:1–2 (D), 4:2:2 (E–F), (4–8):4:2:2 (G–K). Red arrows point anticlinal divisions in IPL cells, and green one in PPL cells at later stages of development. Note that the latter are sometimes more numerous (I–J). Levels of transverse sections are shown in C. Scale bars: 50 μm for C, 20 μm for all others.

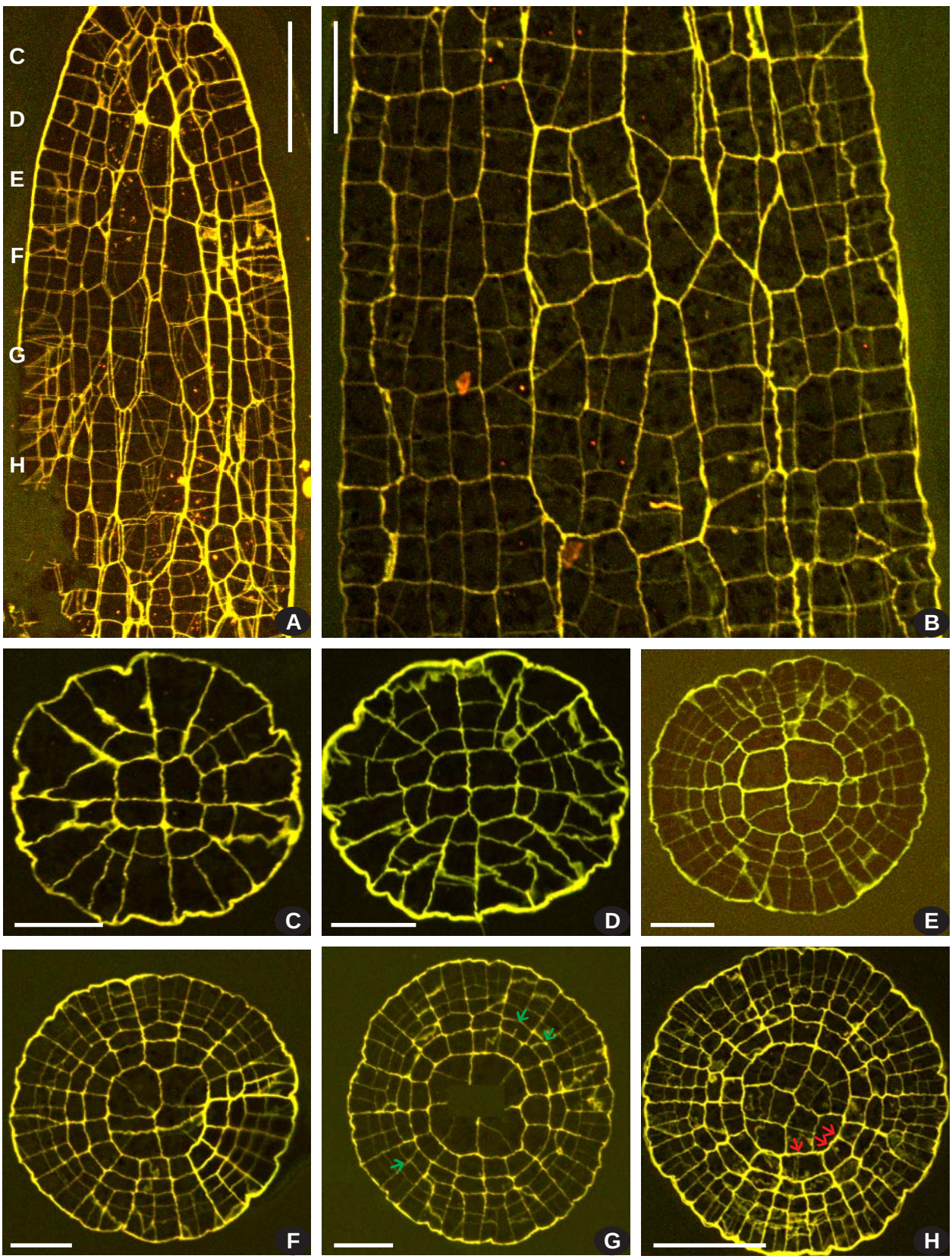

Fig. 3. *Encalypta longicollis* (from Yakutia, Ignatov & Ignatova 17-675, MHA): Longitudinal (A–B) and transverse (C–H) sections of sporophyte, showing successive stages of the peristome develompent, corresponding peristomial formulae [in general, some sectors have exceptions]: 2:1 (C), 2:2:1 (D), 4:4:2:2 (E, F, G); 8:4:4:2: (H). Red arrows point anticlinal divisions in IPL cells, and green one in PPL cells at later stages of development. Levels of transverse sections are shown in A. Scale bars: 50 μm for A, 20 μm for all others.

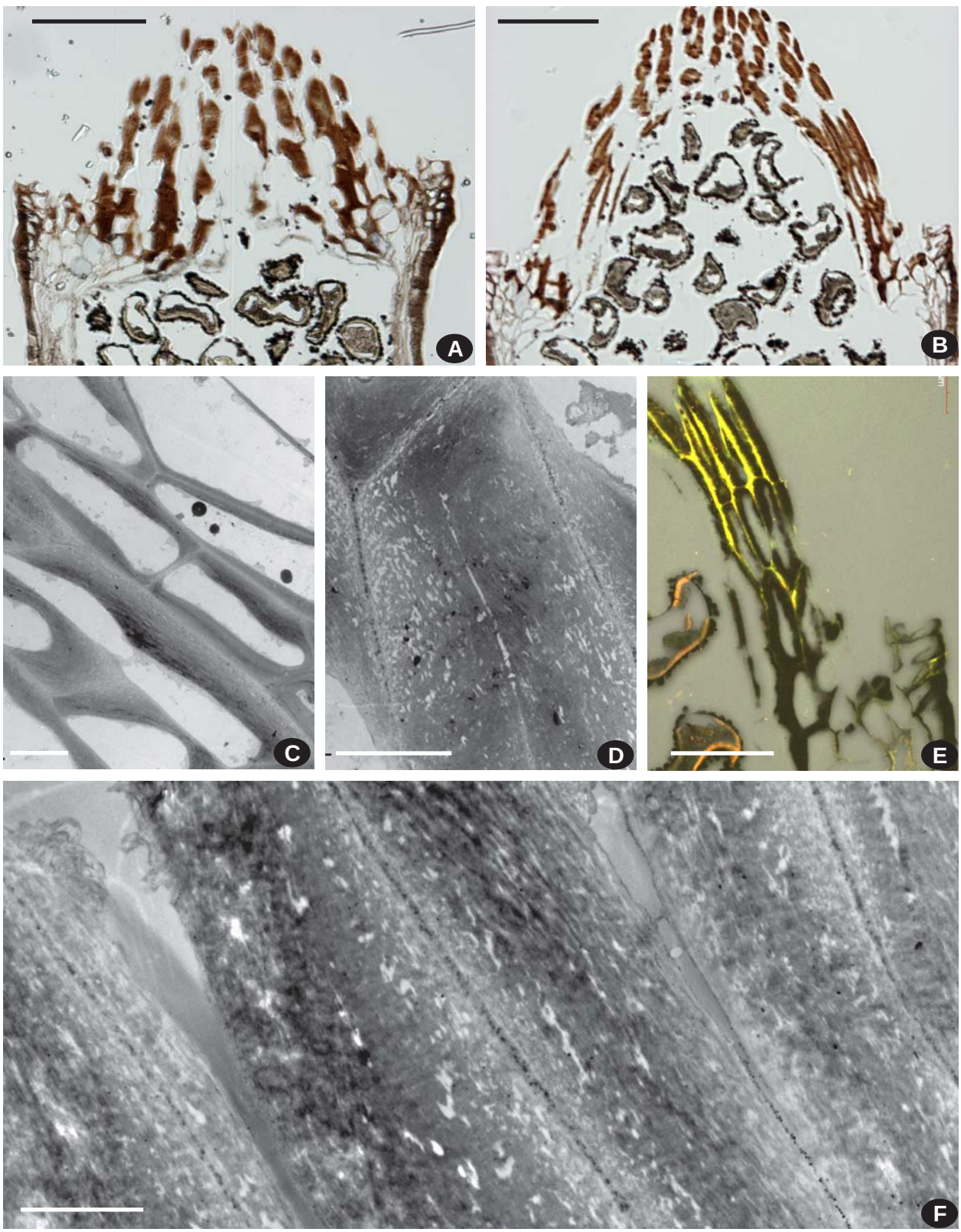

Fig. 4. *Encalypta longicollis* (from Yakutia, Ignatov & Ignatova 17-675, MHA): mature peristome structure (A, B: light microscope; C, D, F: TEM; E: LCSM). A: section tangential to teeth; B, E: same section, longitudinal as related to capsule, slightly acentric, showing longitudinal section of teeth in proximal 2/3 and subtransverse-tangential in distal part, closer to point of teeth fusion by their apices. Dark osmeophilous fibrillose material is filling the main cell volume, while close to cell walls cells material is otherwise lighter. LCSM image shows cellulose (in yellow) along cell walls. Cell content is partly resorbed in cells closer the teeth base (C), while above fibrillose material fills them totally (F), or leaves a transparent part only as a narrow slit (D). Scale bars: 100 µm for A–B; 50 µm for E; 10 µm for C; 5 µm for D–F.

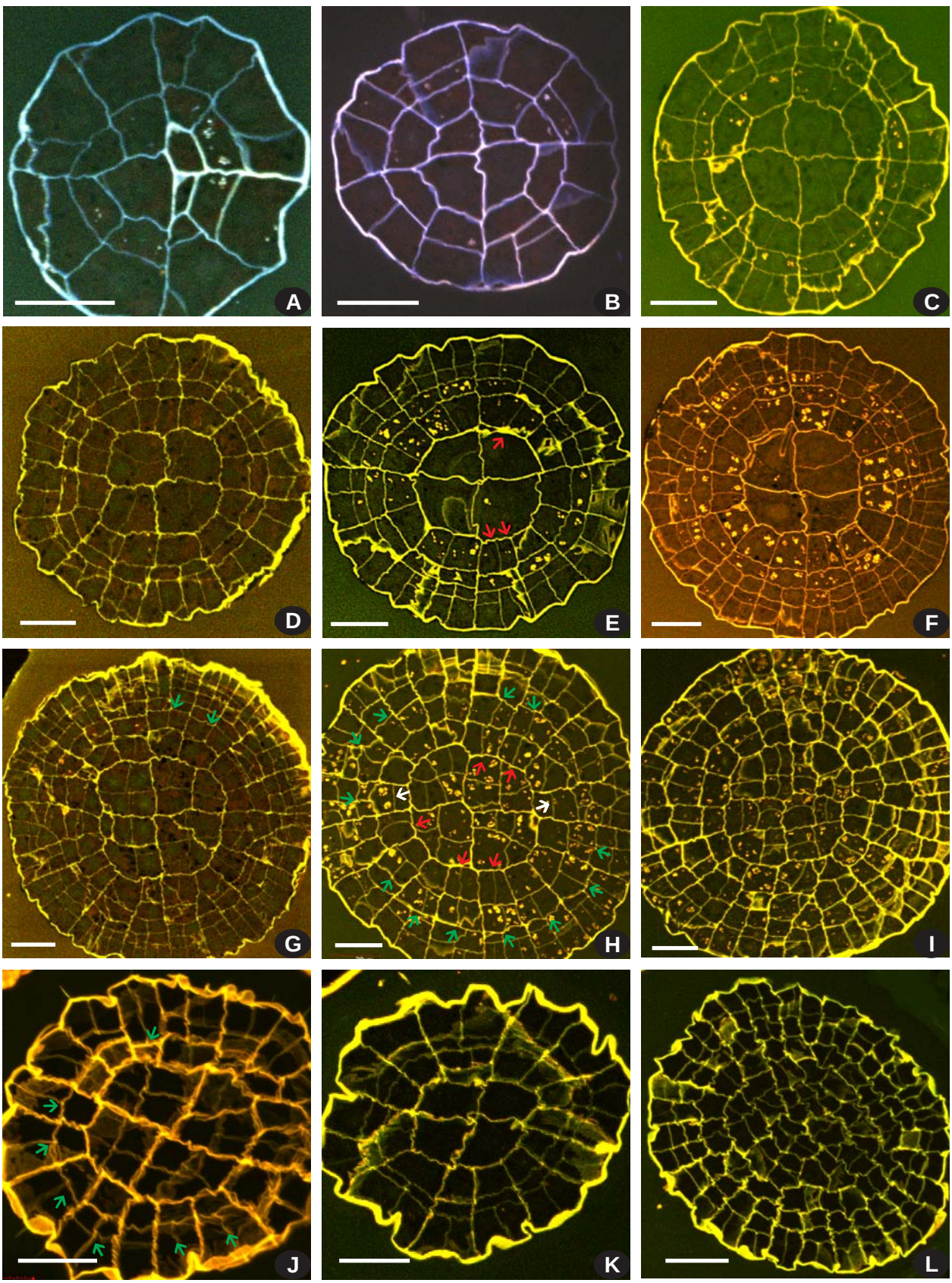

Fig. 5: A–I: *Encalypta rhaptocarpa* (from Yakutia, Ignatov & Ignatova 16-803,MHA) and J–L: *E. vulgaris* (from Moscow Province, Ignatov 18-3001, MW): transverse sections showing succesive stages of the peristome develompent, corresponging to peristomial formulae: 1:1 (A), 2:1 (B, H), 2:2:1 (C, D, I), 4:2:2 (E), 4:4:2:2 (F, G), 8:4:4:2 (F, G). White arrows indicate the places where periclinal division occurs in IPL cells, red arrows point anticlinal divisions in IPL cells, and green one in PPL cells (numerous in H–I) at later stages of development. Scale bars: 20 μm for all.

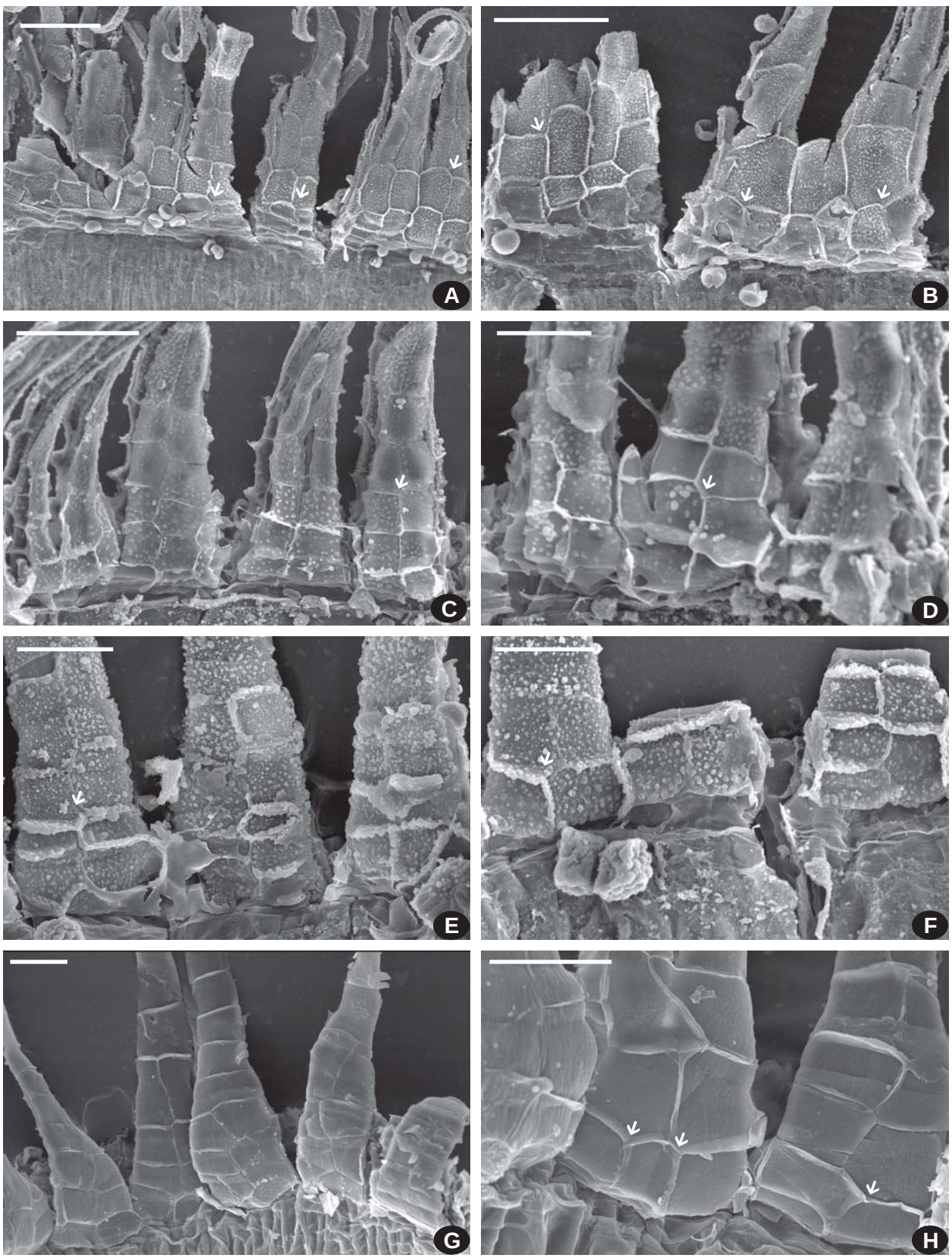

Fig. 6. Inner surfaces of peristomes of *Encalypta* species at their bases, allowing count the number of cell in IPL by remnants of anticlinal cell walls (arrowed). Note that such remnants more distally are absent, which correspond to the peristomial formula 4:2:2, whereas at peristome base the 4:2:4 pattern occurs. Anticlinal divisions are mostly unseen in transverse sections in Figs. 2, 3, 5, which allow conclusion that IPL cell divisions are delayed in *Enclaypta*, but usually occur later, and sometimes quite irregularly. A–B: *E. procera* (from Yakutia, Ignatov & Ignatova 16-353, MHA); C–D: *E. longicollis* (from Yakutia, Ignatov & Ignatova 17-675, MHA); E–F: *E. rhaptocarpa* (from Yakutia, Ignatov & Ignatova 16-803, MHA); G–H: *E. ciliata* (from Altai, Ignatov 34/35, MHA). Scale bars: 100 μm for A–B; 50 μm for C–H.

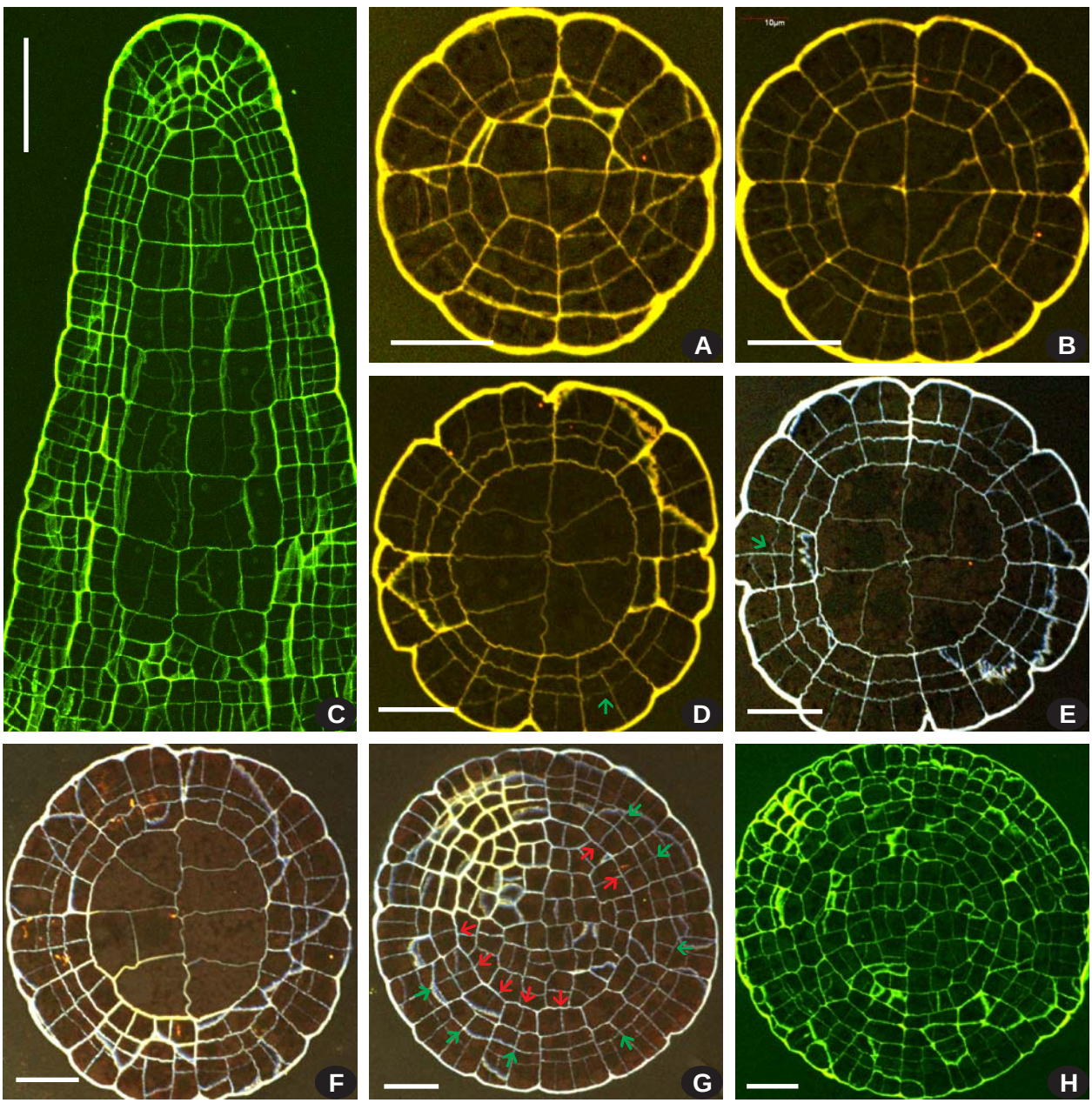

Fig. 7. Longitudinal (C) and transverse (A–B, D–H) sections, showing succesive stages of the *Timmia bavarica* (from Yakutia, Ignatov & Ignatova 16-337, MHA) peristome development: 2:2:1 (A), 4:2:2 (B, D), 8:4:4:4 (G). These formulae are not fully identical in the whole circumference. Section H illustrates irregular cell arrangement at the short distance below the base of peristome teeth. Scale bars: 50 µm for C, 20 µm for all others.

Timmia bavarica (Fig. 7)

The studied sequence of divisions results in pattern characterised by the following formulae: 2:1(not shown) — 2:2:1 — 4:2:2 — 8:4:4:4 (opposite). The IPL:PPL cells thickness ratio is 1.5–2:1. Repeated anticlinal divisions occur at later stages of development in both IPL and PPL (Fig. 7D, E, G). Cells of peristomial layers, as seen in longitudinal sections (Fig. 7C), are more regularly arranged compared to *Encalypta* (Figs. 2C, 3A).

Funaria arctica (Figs. 8, 9)

As the genus *Funaria* has been already a subject of the peristome development studies, we display here mostly the later stages. Fig. 8A shows the stage where some IPL cells are already underwent anticlinal divisions (corresponding to 4:2:4 formula), while some did not, thus fitting 4:2:2 formula, which is characteristic for earlier stage of development. IPL cells have divided periclinally at the level reached by sporogeneous tissue: darker cell content in the outermost endothecial layer is not yet discernible in Fig. 8D, but becomes more or less apparent 10 µm below, at Fig. 8E, F, and clearly seen further below, at Figs. 8G, H. Note that the regular pattern of peristomial layers is still distinct at the level of urn, where sporogeneous tissue is apparent (Figs. 8, 9).

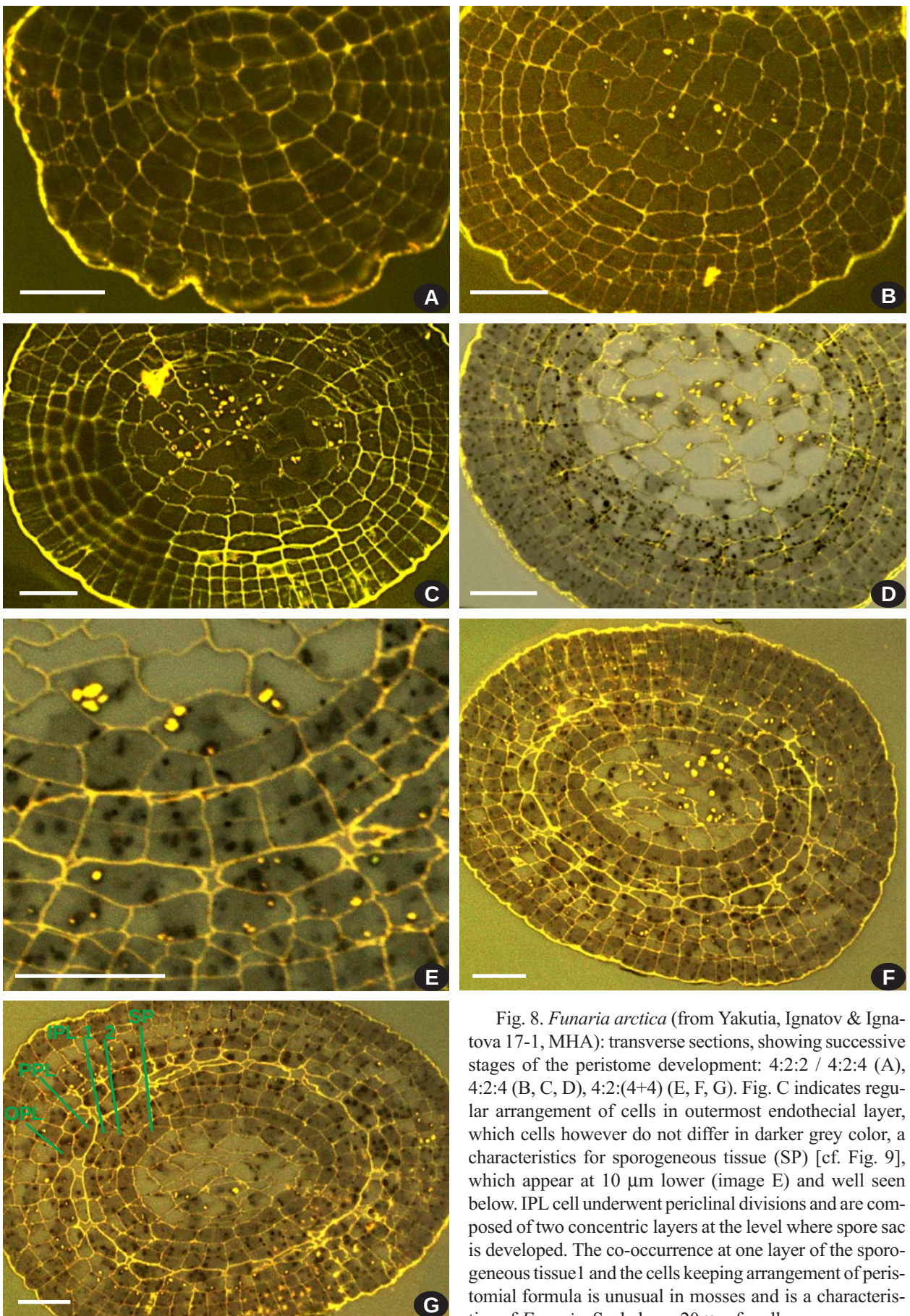

Fig. 8. *Funaria arctica* (from Yakutia, Ignatov & Ignatova 17-1, MHA): transverse sections, showing successive stages of the peristome development: 4:2:2 / 4:2:4 (A), 4:2:4 (B, C, D), 4:2:(4+4) (E, F, G). Fig. C indicates regular arrangement of cells in outermost endothecial layer, which cells however do not differ in darker grey color, a characteristics for sporogeneous tissue (SP) [cf. Fig. 9], which appear at 10 μm lower (image E) and well seen below. IPL cell underwent periclinal divisions and are composed of two concentric layers at the level where spore sac is developed. The co-occurrence at one layer of the sporogeneous tissue1 and the cells keeping arrangement of peristomial formula is unusual in mosses and is a characteristics of *Funaria*. Scale bars: 20 μm for all.

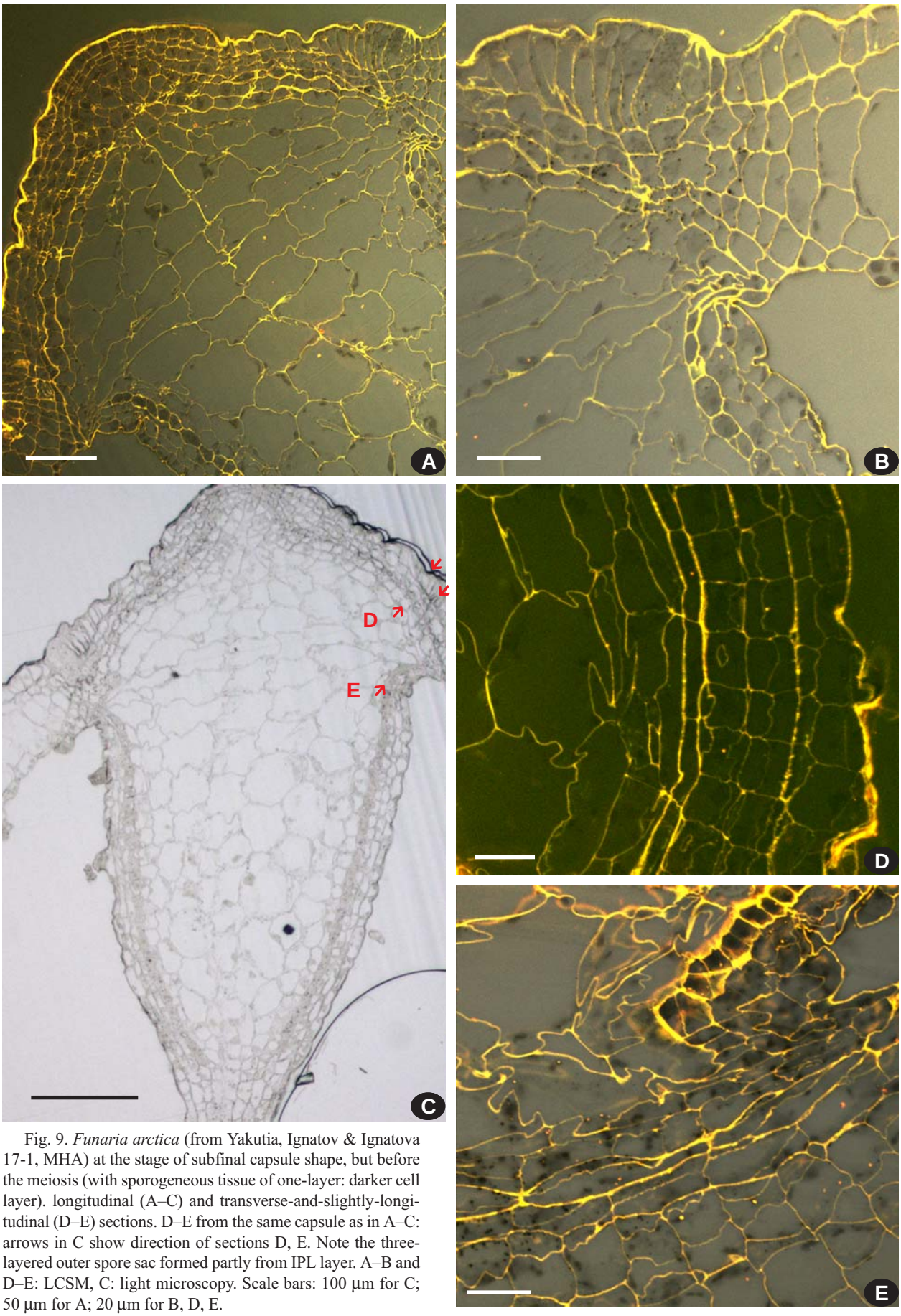

Fig. 9. *Funaria arctica* (from Yakutia, Ignatov & Ignatova 17-1, MHA) at the stage of subfinal capsule shape, but before the meiosis (with sporogeneous tissue of one-layer: darker cell layer). longitudinal (A–C) and transverse-and-slightly-longitudinal (D–E) sections. D–E from the same capsule as in A–C: arrows in C show direction of sections D, E. Note the three-layered outer spore sac formed partly from IPL layer. A–B and D–E: LCSM, C: light microscopy. Scale bars: 100 μm for C; 50 μm for A; 20 μm for B, D, E.

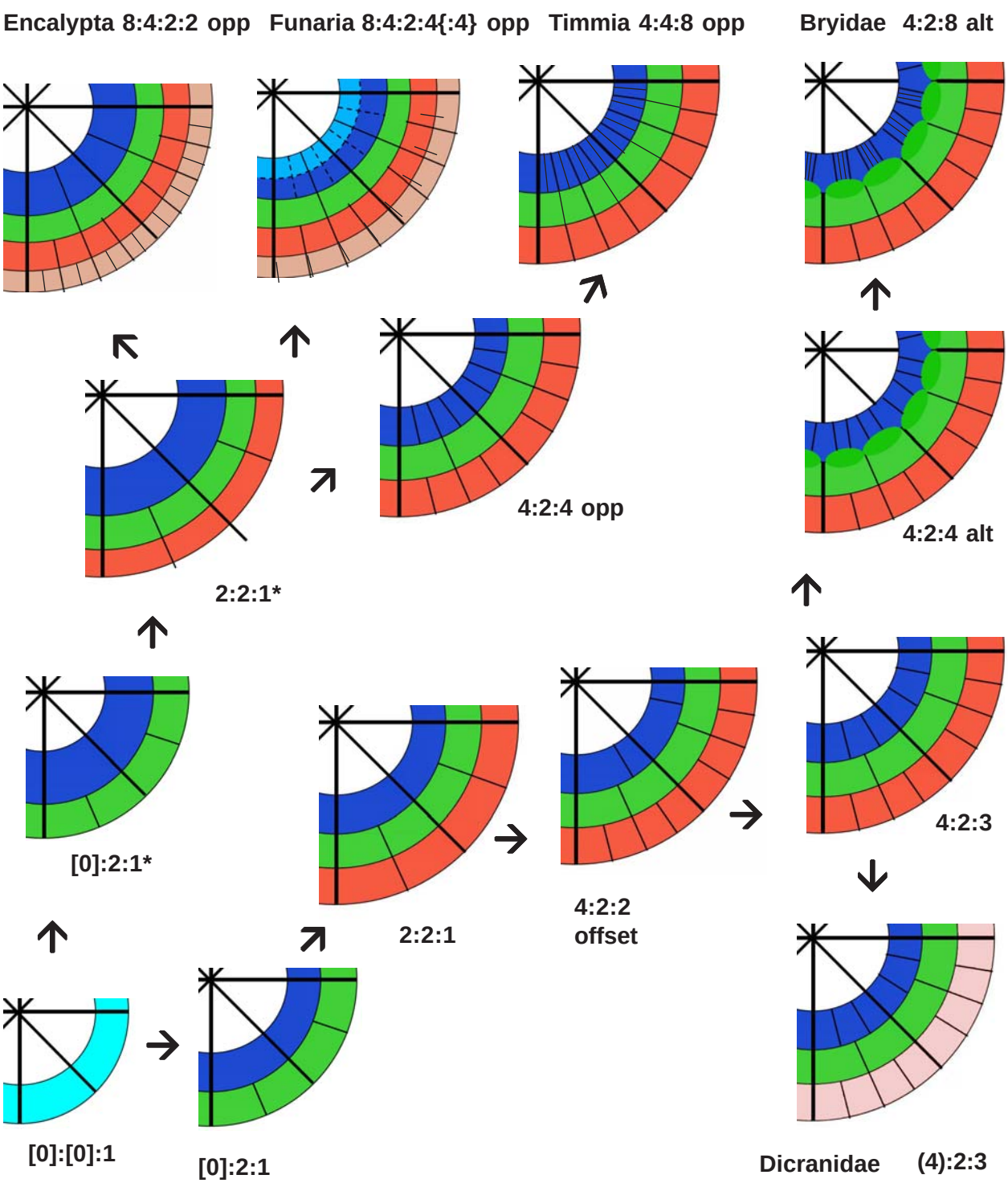

Fig. 10. Scheme of arthrodontous peristome development in three main moss lineages: Bryidae, Dicranidae and Funariidae, shown in quarters of transverse sections of young sporophytes. Funariidae are illustrated here by three studied variants of *Timmia, Funaria*, and *Encalypta*. Note the difference already at the stage [0]:2:1, where in Funariidae lineage the IPL cells are markedly thickened, compared to Bryidae and Dicranidae. At the transition from 2:2:1 to 4:2:2, divisions in IPL are somewhat delayed, whereas in other lineages divisions in IPL occur. In contrast to *Funaria*, IPL cells in *Encalypta* start divisions on a very late stage only (not shown here, but compare with Fig. 6). In Bryidae lineage, the transition from 4:2:3 to 4:2:8 represents putatively one of the variants, while another pathways are characterized by earlier and stronger displacements of IPL anticlinal cell walls against PPL anticlinal cell walls since 4:2:2 stage.

For the present study it is important to note that *Funaria* differs from *Encalypta* and *Timmia* by a relatively better developed PPL, so the IPL:PPL cells thickness ratio is 0.8–1(–1.3):1; the anticlinal divisions in PPL are absent, and cell walls in neighboring peristomial layers are always clearly aligned.

DISCUSSION

The previous observations on the peristome structure of *Encalypta* (Philibert, 1889, revised and abridged by Taylor, 1962; Horton, 1982; Edwards, 1979) are largely supported in the present study: most layers are more or less aligned. There are few exceptions in our observations (Figs. 3H, 5E), where the cell arrangement is approaching to 4:2:3 formula, which is an intriguing pattern, as the basalmost arthodontous moss is *Diphyscium*, with clear 4:2:3 peristomial formula (Shaw *et al*., 1987). However, such cases are so rare in *Encalypta*, so their correspondence with the regular expression of 4:2:3 would be worth to discuss only if further observations will proof their regular presence, while now we treat them as an occasional exceptions.

The series of successive peristome reduction from *Encalypta longicollis* to species with more simple peristome structure were discussed by Philibert (1889); it can be demonstrated in the present series as well. However, its phylogenetic context is different, in a view of the current knowledge of the phylogeny of the genus *Encalypta*. *Encalypta longicollis* has really the most complex structure of peristome in the genus, but its basalmost position in phylogenetic trees is not supported. First, as it was already noticed by Horton (1982) and Nyholm (1998), the second genus of the Encalyptaceae family, *Bryobrittonia* has peristome essentially similar to peristomes of *E. procera* and *E. streptocarpa*. The similarity is so great, that Nyholm (1998) even suggested to submerge *Bryobrittonia* to *Encalypta*, although this suggestion got no wide acceptance and, moreover, it contradicts phylogenetic reconstructions (Ignatov *et al.*, 2016), where *Encalypta* appeared in a sister position to *Bryobrittonia*, but formed a clade on a quite long branch. Phylogenetic analysis of two different sets of sequences, namely *rbc*L only (Tsubota *et al.*, 2004) and concatenated set of *nad*5, *rps*4 and *rbc*L (Ignatov *et al.*, 2016) placed *E. procera* and *E. streptocarpa* in the basal position in the phylogeny of the genus. *Encalypta longicollis* was involved in the latter analysis and it was found in the grade from two mentioned basalmost species to terminal clade of *E. ciliata* and *E. rhaptocarpa*.

Therefore, peristome of *Encalypta longicollis* should be considered as a deviation from the mainstream of the genus evolution. According to our observations, content of cells of peristomial layers seems to be not or only partly dissolved, though light microscopy may provide a misleading picture. On TEM photographs it is seen that space within cells is filled by fibrillose material (Fig. 4D, F), and cell walls are not attracting osmeophilous material as in normally developed peristomes (Mueller, 1973).

Results of the present observations show a pattern common for all four studied species of *Encalypta*. The main distinction of the genus from almost all other mosses studied for peristome development includes an exceptionally thick IPL cell layer, and also scattered to regular additional divisions in the PPL. The latter may be observed especially in the proximal parts of teeth (Figs. 2H, 3G, 5H). A somewhat similar pattern occurs also in *Timmia* (Fig. 7G), where peristome approaches to formula 4:4:4 or 8:4:4 at its final stage.

Divisions in IPL also start late, being observed only in lower parts of the teeth, and are usually fairly irregular: e.g., anticinal in one cell, and periclinal in neighboring cell. It seems that at the latest stages additional anticlinal divisions in IPL occur almost always, but they were not observed at the earlier stages used for transverse sectioning in the present study. However, SEM observations almost always show remnants of anticlinal cell walls on adaxial (ventral) surface of teeth from inside (Fig. 6). A putatively similar case, i.e. additional anticlinal divisions in proximal parts of teeth were observed in haplolepideous peristomes as well (Shaw *et al.*, 1989b).

In *Funaria* peristomial formula 4:2:4 is apparent mostly closer to the base of peristome, whereas at the level of half the length of exostome teeth and higher, endostomial segments usually lack median line on their ventral surface, thus corresponding to formula 4:2:2 (which can be seen at places in Fig. 8A). An interesting structural detail of *Funaria* is the occurrence of regular cell arrangement somewhat below the level of annulus. Loeske (1929) illustrated part of this structure, showing in transverse capsule section that the spore sac is hanging within the expanded air space on 16 trabeculae, i.e. repeating the number of peristome teeth.

Peristome of *Funaria arctica* is very similar to that previously described for *F. hygrometrica* (Shaw *et al*., 1989a; Schwartz, 1994). Its IPL cells are thick at the early stages of development, though not so much as in *Encalypta*. Anticlinal divisions in IPL regularly occur, whereas the PPL cells do not divide further in a way similar to *Encalypta* and *Timmia*, remaining 16 in number, as in other arthrodontous mosses, both haplolepideous and diplolepideous.

The close phylogenetic relationship of *Encalypta* and *Funaria* is well proved (Cox *et al*., 2010; Ignatov *et al*., 2016), while the position of *Timmia* remains unstable (Budke *et al.*, 2007). Nevertheless, the developmental pattern of *Timmia* is more similar to *Encalypta*, as the most conspicuous layer in *Timmia* is IPL and the divisions in PPL regularly occur (Fig. 7). At the same time, in Furaniaceae the overall traits of peristome reduction are similar to most mosses with double peristome: the reduction commonly involves endostome (in species of *Enthostodon*) and no one case in Funariaceae is known where endostome would be conspicuously better devel-

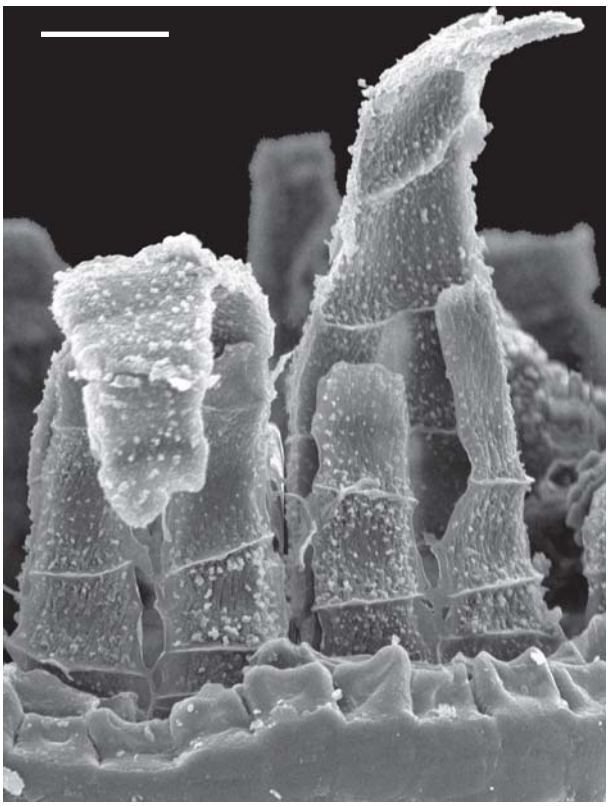

Fig. 11. *Encalypta rhaptocarpa* (from Perm Province, 23.VI.1995, Bezgodov 298, MW), peristome from outside view. Note short exostome element split to the base and more or less attached to well-developed endostome teeth. Scale bar: 100 μm.

oped than exostome (Liu *et al.*, 2012; Medina *et al*., 2018).

Philibert (1889) considered Encalyptaceae as an anomalous group, the heterolepideous, due to inclusion of both haplolepideous and diplolepideous mosses.

Important difference between diplolepideous and haplolepideous mosses in this case is that in the series of gradual reduction along the evolutionary lineages in diplolepideous mosses, it usually starts from inside, involving first of all endostome (e.g., *Orthotrichum, Bartramia, Neckera/Homalia, Fabronia, Anacamptodon, Leucodon, Fontinalis, etc*.), while in haplolepideous mosses reduction starts from outside, and almost in all groups OPL and PPL material is represented only as a poorly developed prostomes. Only two genera with a relatively well-developed exostome are currently known in Dicranidae, the lineage usually considered as haplolepideous. These are recently discovered cases of *Catoscopium* (Ignatov *et al.*, 2015) and *Pseudoditrichum* (Fedosov *et al*., 2016).

The reduction of peristome from outside, starting from OPL and PPL, is a peculiar character of *Encalypta*. Interestingly, in haplolepideous mosses, where the exostome is more severely reduced, IPL is not so much thicker than PPL, compared to *Encalypta,* cf. Figs. 2–6 (Evans & Hooker, 1913; Saito, 1956; Shaw et al., 1989a, b; Schwatrz, 1994).

This peculiarity of the *Encalypta* peristome returns us to the re-evaluation of the role of particular layers in the peristome development. In most artrodontous mosses the outstanding role belongs to the second amphithecial layer, which has been designated by this reason as the primary peristomial layer. This term has been introduced by Blomquist & Robertson (1941) in their study of the peristome development in diplolepideous alternate moss, *Aulacomnium heterostichim*, and their terminology became a standard in all further studies of moss peristomes.

For the sake of present discussion, the original definition of the PPL is cited here in a whole. "The primary peristomial layer is composed of 16 cells which divide **no further**. They **enlarge rapidly and their inside walls soon become distinctly convex** (Figs.16, 17). This is, therefore, the **first peristomial layer to appear conspicuously different** from the other layers. For this reason and because, as will be shown later, the **number of cells of which it is composed determines the number of teeth**, it seems desirable to designate this layer as the primary peristomial layer" (Blomquist & Robertson, 1941, with boldfacing by the authors of the present paper).

Thus, by the original definition, there are four key characters, identifying a layer as the PPL. Their applicability can now be compared one by one with the obtained observation on the peristome development in *Encalypta,* as well as with the general current knowledge of peristome development.

1. The absence of further divisions is not observed in *Encalypta* in any layer, including PPL, albeit they are usually few and irregularly distributed, except near the base they could be performed in more than half cells in both IPL and PPL. Note that the situation in *Timmia* is somewhat parallel, with the difference that anticlinal divisions in IPL start earlier and only later they appear in PPL, reaching formula 4:4:8. We do not know other mosses with arthrodontous peristomes where PPL cells are dividing further (excepting for *Catascopium*, see Ignatov *et al.*, 2015). It is noteworthy that additional divisions in PPL were not observed in *Funaria* either in the present study, or in previous ones (Kienitz-Gerloff, 1878, Shaw *et al.*, 1989b; Schwartz, 1994).

2. Rapid cell enlarging and their inside walls soon becoming distinctly convex. In case of *Encalypta,* rapid enlarging is a characteristic of IPL, rather than PPL, where cells are considerably thinner in radial dimension (Figs. 2, 3, 5). Convex faces are not seen in *Encalypta* and *Timmia*, although in *Finaria* PPL cells are somewhat inflating already at a rather early stage (Figs. 8B, C).

The rapidness of enlargement and distinctness of convexity are criteria which are somewhat difficult to apply. For example, sections of *Ulota* (Goffinet *et al*., 1999) and *Ephemerum* (Schwartz, 1994) would not fit this criterion, and such examples may obviously be multiplied .

3. **First peristomial layer to appear conspicuously different** is no better that previous criterion, at least nothing conspicuous can be noticed in PPL of *Encalypta* and *Timmia*. At the same time, in both haplolepideous and diplolepideous alternate mosses and in *Funaria* this criterium is true (Evans & Hooker, 1913; Blomquist & Robertson, 1941; Shaw *et al.*, 1989a, b; Saito, 1956; Saito & Shimoze, 1955).

4. Number of cells of which it is composed determines the number of teeth. This criterion is interesting by a number of aspects. First, it is a kind of definition of what is 'teeth'. True, that 16 cells in the PPL layer in artrodontous mosses obviously correspond to 16 teeth, although these teeth can be fused into 8 pairs or 4 double-pairs, as in, e.g., some species of *Splachnum* and *Tetraplodon*, or split into 32 "teeth divided to the base" in some species of *Tayloria,* or divided into more numerous filiform parts in Pottiaceae, *etc*. Such cases make this criterion somewhat conditional, although the fact that arthrodontous mosses have mostly 16 PPL cells and teeth are also mostly 16 is doubtless.

In *Encalypta* and *Timmia* this criterion also works, but conditionally. Peristome of *Timmia* has 32 PPL cells (Fig. 7G), but it does not affect normal development of 16 exostome teeth. However, in *E. rhaptocarpa,* where PPL cells are also divided anticlinally (Fig. 5H), appoaching to 32 in number, the exostome elements are usually 32 (Fig. 11). At the same time, the number 16 of the main peristomial elements in *E. rhaprocarpa* is defined by the number of IPL cells.

The above mentioned account may be used in favor of an alternative interpretation of the peristome structure of *Encalypta*. One may explain it as total reduction of the IPL in *Encalypta*, so its innermost amphithecial layer is in fact PPL. In favor of this approach, one may stress on the importance (although rarely clearly formulated) of one more criterion, which appears as a combination of criteria of Blomquist & Robertson (1941). The PPL cells stop their division by an unknown cytostatic factor, while cells next outwards of them continue divisions, forming triads of one PPL and two OPL cells. This criterion could be in fact very practical for searching PPL cells among not clearly arranged cells (*e.g.*, in Fig. 9E). In most cases in both *Encalypta* and *Timmia* this criterion will recognize PPL as a second amphithecial layer, although this pattern is rather scattered within the peristomial layers.

Thus, we are far from such ridiculous re-interpretation, which requires numerous complex re-definitions. We would rather consider the case of *Encalypta* as an exceptional one, where the "cytostatic zone" is to some extent and temporarily shifted from the second amphithecial layer towards the first amphithecial layer, thus IPL at the middle stages of development may partially function as PPL.

Similar conflicts between positional versus functional definition in morphology, the heterotopies, are a well known phenomenon in zoology and to a lesser extend in botany, dated back to the classical works of Haeckel (1866). The centuries-lasting debates on the bird wing digits (*i.e.*, are three bird digits homologous to I–II–III or II–III–IV digits of reptiles) is probably one of the most famous example. Hundred publications with facts from comparative morphology, palaeontology, anatomy, embryology (including experimental transplantations) argue one or another hypothesis (Young *et al.*, 2011; Zhu & Mackem, 2013a,b). Despite the problem is not yet fully solved, it is obvious that the changes in coverage of zones of gene expression plays an important role, making a compromise between two seemingly uncompatible solutions.

A contemporary level of the developmental studies in bryophytes precludes further discussion, as it would be necessarily too speculative. However, the hypothesis that in case of peristome development in *Encalypta* similar shifts occur, changing areas of regulation (expression?) of morphogenetical factors and therefore defining the pathways of cell divisions resulting in different structures. It could be a right direction for further research.

ACKNOWLEDGEMENS

We thank Prof. H. Deguchi and Dr. Y. Inoue for valuable suggestions of the manuscript and RFBR for support of the present study, the project 15-04-00936.

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