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Aboral (pallial) epithelium in bryozoan larvae: a comparative morpho-functional analysis

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ABSTRACT: The outer epithelium of free-swimming larvae of marine invertebrates is composed of several cell types and performs a variety of vital functions. In bryozoan larvae, early anatomical studies revealed a rather complex organization of this epithelium. The present contribution re-examines the available morphological descriptions, as well as investigates anatomically and ultrastructurally the epispheral (aboral) part of the outer pallial epithelium in contrasting larval types of six marine bryozoans from three orders — Cheilostomatida, Ctenostomatida, and Cyclostomatida. A total of 11 cell types were identified — three of them are typical for most of the studied larvae, the rest are unique to specific larval types. Based on the data obtained, we conducted a comparative analysis and assigned potential functions to particular types of cells during larval life. We proposed the hypothesis that immersion/invagination of the larval pallial epithelium (which was accompanied by cell enlargement in ciliary locomotory corona) occurred independently in different bryozoan lineages and was associated with acquisition of incubation. We also consider the presence of the cuticle above the episphere and the absence of cilia in most principal pallial epithelial cells as plesiomorphic traits of bryozoan larvae.
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Аборальный (паллиальный) эпителий личинок мшанок: сравнительный морфо-функциональный анализ

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РЕЗЮМЕ: Наружный эпителий плавающих личинок морских беспозвоночных состоит из разных типов клеток и осуществляет множество важных функций. У личинок мшанок он имеет достаточно сложную организацию, что было продемонстрировано уже на ранних этапах изучения их строения. В данной работе мы исследовали микроанатомию и ультраструктуру наружного (паллиального) эпителия аборальной полусферы (эписферы) (а также провели ревизию ранее опубликованных морфологических описаний) у разных типов личинок шести видов морских мшанок из трех отрядов Cheilostomatida, Ctenostomatida и Cyclostomatida. В общей сложности мы описали 11 типов клеток — три из них типичны для большинства изученных личинок, остальные уникальны для определенных видов. На основании полученных данных мы провели сравнительный анализ и сделали предположения о потенциальных функциях клеток. Мы выдвинули гипотезу о том, что в связи с возникновениям вынашивания независимо в разных линиях мшанок происходило погружение паллиального эпителия, сопровождающееся увеличением клеток локомоторного ресничного кольца (короны). Мы также предположили, что наличие кутикулы над эписферой и отсутствие ресничек у основных клеток паллиального эпителия можно рассматривать в качестве анцестральных признаков личинок мшанок.
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КЛЮЧЕВЫЕ СЛОВА: личиночный эпителий, ультраструктура, вынашивание эмбрионов, морские беспозвоночные, эволюция.

Introduction

The outer epithelium (integument) of Eumetazoa is a derivative of the ectodermal germ layer, which is the first to differentiate in the course of embryonic development. Its main functions are the protection of the internal milieu and interaction with the environment (thus being involved in maintaining homeostasis), including barrier and sensory functions, gas exchange, and excretion of metabolites (Bereiter-Hahn *et al*., 1984; Schmidt-Rhaesa, 2007). The outer epithelium is usually associated with the external and internal extracellular matrix. The external matrix often shows the ability to harden (chitinize or biomineralize), forming various skeletal structures (needles, plates, and shells), thus providing support and protection. Moreover, epithelial cells can serve as an anchor for muscles and can accumulate metabolic products and store nutrients (Beklemishev, 1970; Schmidt-Rhaesa, 2007). In various tiny organisms (including larvae), the outer epithelium can absorb dissolved organic matter from the environment via endocytosis (Boidron-Métairon, 1995; Boucaud-Camou, Roper, 1995; Johnson, Wendt, 2007).

The integument undergoes active changes in the course of both ontogenetic and evolutionary transformations (Bereiter-Hahn *et al*., 1984), whereby animals with larval development are of particular interest. In invertebrates, during metamorphosis, the integumentary cells often change their cytological and functional properties or are even replaced by other cell types (e.g., Ivanova-Kazas, Krichinskaja, 1988; Reed, 1991; Hiebert *et al*., 2010; Martín-Durán, Egger, 2012). Similar changes occur during shifts from long-lived exotrophic larvae developing in the external environment to short-lived endotrophic ones, which is often associated with the emergence of incubation, and sometimes with the appearance of extraembryonic nutrition (reviewed in Ostrovsky *et al*., 2016). Changes in the habitat can also alter the integument of both larvae and adult forms (Reed, Cloney, 1982b).

All the above changes in the external epithelium and their causes can be studied using Bryozoa, a phylum of aquatic colonial invertebrates. These suspension feeders have a long evolutionary history being among the dominating fouling groups in many benthic marine, brackish and freshwater communities since Cambrian (Taylor, 2020; Schwaha, 2021; Zhang *et al*., 2021). Their life cycle includes the larval phase that undergoes catastrophic metamorphosis to form a primary zooid(s) — ancestrula or ancestrular complex (Reed, 1991; d'Hondt, 1994). The colony then grows by iterative zooidal budding.

Bryozoa comprises two major clades — Phylactolaemata and Myolaemata (Schwaha *et al*., 2020). Phylactolaemata are exclusively freshwater, non-skeletal bryozoans with placental matrotrophy and highly modified sexual and asexual development (Bibermair *et al*., 2021). This class is not considered in this study. Myolaemata is divided into Stenolaemata with one recent order Cyclostomatida, and Gymnolaemata with two orders Cheilostomatida and Ctenostomatida.

Monophyletic Cyclostomatida are exclusively marine calcified bryozoans with viviparous matrotrophic incubation, polyembryony, and characteristic short-lived, so-called 'cyclostome larvae' (Harmer, 1893; Borg, 1926; Nielsen, 1970; Nekliudova *et al*., 2021). Cheilostomatida comprises a monophyletic group of mostly marine calcified bryozoans, whereas Ctenostomatida is paraphyletic, including marine, brackish- and freshwater unmineralized forms. Most gymnolaemates are brooders having short-lived endotrophic larvae, either lecithotrophic or placentally nourished (Woollacott, Zimmer, 1975; Reed, 1991; Ostrovsky *et al*., 2009; Moosbrugger *et al*., 2012; Ostrovsky, 2013a, b; Schwaha *et al*., 2019; Nekliudova *et al*., 2019a). Such larvae have an expanded ciliary ring (corona) and are termed coronate larvae. A few gymnolaemates species (both cteno- and cheilostomes) are zygote-spawners having a relatively long-lived planktotrophic larva (cyphonautes) with a characteristic triangular two-valved shell and functioning gut. In addition, morphologically 'intermediate' larval forms (pseudocyphonautes, paracyphonautes, etc.) with vestigial gut and, sometimes, a shell, were described in a few gymnolaemates (Nielsen, 1971; Zimmer, Woollacott, 1977a; d'Hondt, 1977a, 1997, 2012). Cyphonautes is considered by most researchers as being similar to an ancient larval type in Bryozoa (Reed, 1991), whereas all other types are evolutionary modified (reviewed in Ostrovsky, 2013a).

Bryozoan larvae have a high structural diversity and complexity, consisting of several dozen or even hundreds cells of various types (Zimmer, Woollacott, 1977a; Gruhl, 2021). Despite their variety, myolaemate larvae share common axial properties and zonality of epithelia (d'Hondt, 1975, 1977a; Zimmer, Woollacott, 1977a). The outer surface is represented by the upper (aboral, or epispheral) and the lower (oral, or hypospheral) epithelium with the locomotory coronal cell ring between them. In cyclostome larvae, the corona consists of many cell rows (d'Hondt, 1977b; Zimmer, Woollacott, 1977b), while in other myolaemates, it is a single- or double-cell ring. The aboral epithelium of the bryozoan larvae most likely corresponds to the epispheral epithelium of the trochophore larvae of other coelomate spiralians, since it originates from the same blastomere descendants (Vellutini *et al*., 2017). In bryozoans, it is usually called 'pallial' epithelium, by analogy with mollusks, in which pallium (or mantle) secretes a shell, as it is in cyphonautes and some pseudo- and paracyphonautes.

Unlike cyclostome larvae, those of Gymnolaemata have specialized sensory areas of the outer epithelia — an apical organ on the epispheral (aboral) part of the larva, and the pyriform organ with ciliary tuft and groove at the anterior end of the hypospheral (oral) part. In all myolaemate larvae, the oral epithelium forms an invaginated adhesive organ (internal sac) posteriorly. In cyphonautes and several other gymnolaemate larvae, the hypospheral part of the oral epithelium is screwed under the episphere, forming an atrium. The latter can be divided by ciliary ridges into the inhalant and exhalant chambers. In such larvae, the adhesive organ is displaced upwards and lies in the upper part of the exhalant chamber.

Although several cell types were described within the pallial epithelium of myolaemate larvae (e.g., suprapallial, principal pallial, infra- and subpallial and supracoronal cells, see d'Hondt, 1975), its structure is still poorly understood. Due to the small larval size (which can be less than 100 μm) and difficulties in cultivation, physiological and molecular studies on bryozoan larval cells and their functions are rare (e.g., Okano *et al*., 1996; Fuchs *et al*., 2011). Routine histology and electron microscopy (currently supplemented by CLSM) are still the main research tools in this field. Detailed ultrastructural descriptions are limited to seven myolaemate genera (Supplementary File 1). Data on the larvae of many taxa from different phylogenetic lineages are still completely lacking, which strongly hampers comparative and evolutionary analysis (d'Hondt, 1974, 1997, 2015, 2016).

In this study, we re-examined and studied anew the aboral pallial epithelium (except apical organ) in six species of myolaemate bryozoans: two ctenostomes, three cheilostomes, and one cyclostome (Supplementary File 2). We chose larvae with profound differences in structure and biology, namely: long-lived non-brooded planktotrophic cyphonautes vs short-lived non-feeding (lecithotrophic and placentotrophic) larvae of contrasting morphology developing in incubation chambers of various types. Based on our own and published data, we conducted a comparative morphological analysis of the aboral epithelium in an attempt to determine the functions of various cells and to detect possible changes during the transition between larval types in the bryozoan evolution. We also compared the aboral epithelium in bryozoan larvae with that in the larvae of other marine invertebrates.

Material and methods

Plankotrophic cyphonautes larvae of *Electra pilosa* (Linnaeus, 1767) were collected from a boat using a plankton net (200 μm mesh) at 5 m depth near the Educational and Research Station "Belomorskaia", Saint Petersburg State University (Chupa Inlet, Kandalaksha Bay, White Sea, 66°18′30.6″N, 33°55′ 03.1″E). Non-feeding incubated larvae of the ctenostomes *Flustrellidra hispida* (Fabricius, 1780) and *Alcyonidium hirsutum* (Fleming, 1828), cheilostome *Juxtacribrilina annulata* (Fabricius, 1780) (former *Cribrilina*), and cyclostome *Patinella verrucaria* (Linnaeus, 1758) were obtained after their release from reproducing colonies collected in the same locality at the White Sea by boat dredging and SCUBA-diving from various algal substrates at 1–8 m depth. Non-feeding larvae of *Tendra zostericola* de Nordmann, 1839 were obtained after their release from reproducing colonies growing on *Zostera* sp. collected by snorkeling at the Black Sea at 1–2 m depth (Kazachja Bay, 44°34′02.4″N, 33°24′45.5″E). All incubated larvae were processed immediately after their release.

Larvae were anesthetized with 5% magnesium chloride solution mixed with filtered seawater (1:1)

for about 10–40 min until signs of contraction disappeared, and then fixed. For light and transmission electron microscopy (TEM), fixation was performed at 4°C for 12 h-2 weeks in 2.5% glutaraldehyde in 0.1 M Na-cacodylate buffer, pH 7.4 (containing 0.14% NaCl (750 mOsm) for larvae from the White Sea). After fixation, specimens were rinsed three times in the same buffer and post-fixed for 1 h at 4°C in a buffered 1% solution of OsO_4. After three rinses (for 5 min each) in the same buffer, larvae were dehydrated in a graded alcohol series (30 to 96% at steps of 10%), further in alcohol-acetone mixtures (3:1 – 1:1 – 1:3) and in pure acetone, then embedded in acetone-resin mixtures (3:1 – 1:1 – 1:3), and finally in epoxy resin (Epon 812 kit, Sigma-Aldrich, USA).

Semithin (750 nm) and ultrathin sections (60–70 nm) were made using a diamond Diatome Histo Jumbo Knife on a Leica EM UC7 Ultramicrotome. Semithin sections were stained with Richardson's stain (Richardson *et al*., 1960) and examined under a Leica DM2500 optical microscope equipped with a digital camera Leica DFC295. Ultrathin sections were placed on the copper blends with Formwar film, contrasted with uranyl acetate (1–3 min) and lead citrate (1–2 min), and examined using a Jeol JEM-1400 and Jeol JEM-2100HC transmission electron microscopes; photographs were taken with a digital Olympus-SIS Veleta camera and Gatan UltraScan 4000 camera, respectively. The total number of larvae examined by TEM: *E. pilosa* — 4, *F. hispida* — 3, *A. hirsutum* — 4, *T. zostericola* — 6, *J. annulata* — 3, *P. verrucaria* — 2.

For visualization of cilia and cell boundaries using confocal scanning laser microscopy (CSLM), larvae were fixed for 1–6 h in 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS) followed by three rinses (for 5 min each) in the same buffer. Specimens were then transferred to PBS with Triton-X100 (0.1%) (PBT) for 1–2 h, blocked with 1% bovine serum albumin in PBT during 6 h, and incubated with primary anti-acetylated α-tubulin antibodies (mouse monoclonal, Sigma-Aldrich, USA) diluted in blocking solution (1:1000) at 4 °C for 12 h. Before staining, specimens were rinsed three times for 15 min each in the PBS. Incubation in a 1:500 dilution of Goat Anti-Mouse IgG Antibodies labeled with Alexa Fluor 633 (Sigma-Aldrich, USA) took 24 h, followed by 15 min wash in PBS. Incubation in TRITC-labeled phalloidin (Sigma-Aldrich, USA) solution (200 ng/ml) took 24 h, followed by 15 min wash in PBS. Finally, the specimens were mounted in Fluoroshield with Dapi (Sigma Aldrich, USA) and examined under the confocal scanning laser microscope Leica TCS-SP5.

Living and fixed larvae were photographed with a digital Leica DFC295 camera attached to a compound microscope Leica DM 2500. ImageJ v. 1.48e software was used to process data from CSLM. Schemes and reconstructions were done with Adobe Illustrator CS 5.1, and images were modified with Adobe Photoshop CS 5.1.

To describe the cell types in the studied larvae, we used the terminology coined by previous authors, cells were named predominantly by their position relative to the aboral-oral axis (Kupelwieser, 1905; d'Hondt, 1979; Santagata, Zimmer, 2000).

Results

Data presented below are also shortly summarized in Supplementary Files 2 and 3.

Electra pilosa (Figs 1, 2; 8A)

The cyphonautes larva of *E. pilosa* is non-brooded, planktotrophic, living up to one month (Kupelwieser, 1905; reviewed in Shevchenko *et al*., 2020).

The larva has the appearance of a laterally flattened cone with a wide base surrounded by a ciliary corona, and the apical organ on the top (Figs 1A; 2A; 8A) (average height 299 μm (N=18, range 193–389 μm), length along the lower margin 333 μm (N=17, range 225–447 μm), width in the middle 81 μm (N=5, range 69–105 μm)). Laterally the larva is covered by the translucent triangular shell valves that protect most of the aboral surface except the apical organ and elongated areas running between the valves from the apical organ to the corona along the anterior and posterior larval margins. Shell valves are stratified and seemingly slightly mineralized (Figs 1E, G, I; 2E, G). In cross-sections, a thin, flexible cuticular 'film' is visible between the valves (Figs 2C; 8A). It extends around the base of the apical organ, continues in each valve, and basally hangs freely over the corona (Figs 1C; 2F; 8A). When the larva is 'relaxed', the valves lie directly on the pallial epithelium; when it contracts, a narrow cavity appears between the valves and the epithelium, whose cells remain visibly connected to the shell by elongated cytoplasmic processes (Fig. 1B). This cavity is isolated from the external medium by the cuticular 'film' adjoining the apical organ (Fig. 1C) and by peripheral margins of the shell valves abutting the pallial epithelium (Fig. 2D, F).

The pallial epithelium comprises seven cell types (Figs 1, 2; 8A): (1) suprapallial secretory cells forming a ring zone beneath the apical organ (Fig. 1C, D), (2) principal pallial cells constituting extensive lateral areas of the pallial epithelium underneath the shell valves (Figs 1E–H; 2E), (3) 'polster' cells (Figs 1H; 2F, G) grouped between the principal pallial cells, (4) ciliated secretory cells forming anterior and posterior marginal zones between the valve edges (Figs 1B; 2A,

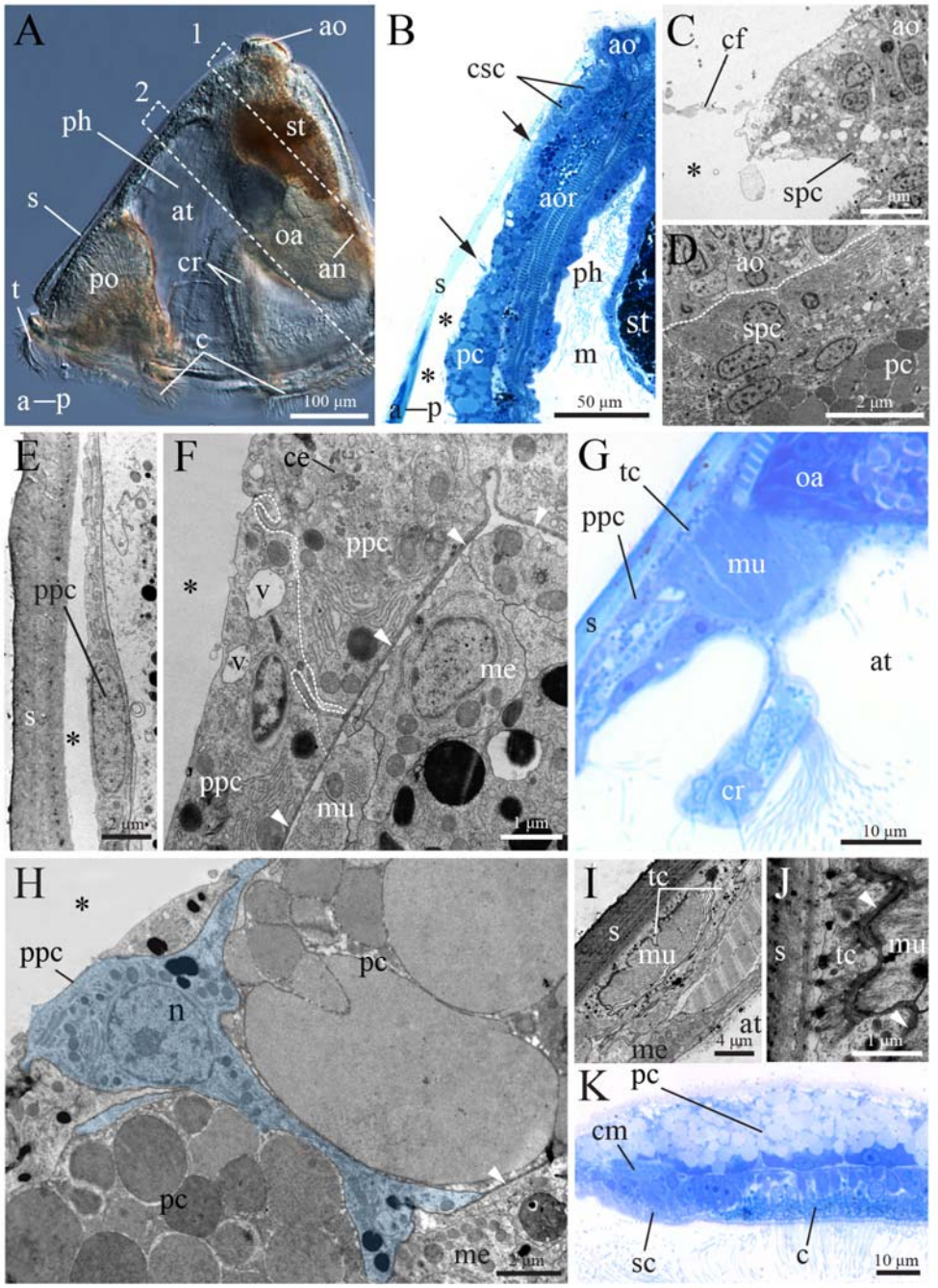

Fig. 1. Anatomy and ultrastructure of cyphonautes of *Electra pilosa* (Gymnolaemata: Cheilostomatida) (unless stated otherwise anterior end is oriented to *left*). A — light microcsopy; B, G, K — stained semithin sections, light microcsopy; C, D, E, F, H–J — transmission electron microscopy. A — lateral view of living mature larva (plane shown by dotted-line 1 corresponds to section plane in E, F; plane shown by dotted-line 2 corresponds to section plane in G, I); B — sagittal section of anterior region of larva to show secretory and 'polster' cells of pallial epithelium under shell; C — sagittal section of apical organ with secretory pallial cells

on periphery (note secreted material forming thin cuticular ‘film’); D — sagittal section through apical organ and suprapallial epitheluim beneath (border shown by dotted line; note numerous interdigitations between cells and cisternae of RER); E — oblique frontal section of shell and squamous principal pallial cells on the level of stomach (section plane 1 as indicated in A); F — oblique frontal section of cuboidal principal pallial cells just above pharynx (section plane 1 as indicated in A; note ‘upper’ cell showing mitotic division, interdigitations between two neighbor cells (marked by dotted line) and thick basal lamina (arrowheads) between principal pallial epithelium and inner mass of muscle and other mesenchymal cells); G — oblique frontal section of tendon cells in place of adductor attachment (section plane 2 as indicated in A); H — sagittal section on level just above pyriform organ showing two ‘polster’ cells and deformed principal pallial cell (marked by blue color) sandwiched between ‘polster’ cells; I — oblique frontal section of tendon cells beneath the ciliary ridges (section plane 2 as indicated in A); J — close-up of the tendon cell contacting to shell showing hemidesmosomes providing attachment to shell valve, tonofilaments, and thickened basal lamina; K — transversal section on the level of lower ‘polster’ cells, epitheliomuscular cells and biciliated supracoronal cells. Asterisks indicate cavity between shell and pallial epithelium; white arrowheads show basal lamina; black arrows show cytoplasmic processes of principal pallial cells.

Abbreviations: a — anterior region; an — anus; ao — apical organ; aor — apical organ retractors; at — atrium; c — corona; ce — centriole; cf — cuticular ‘film’; cm — cross-striated epitheliomuscular cell(s); cr — ciliated ridge(s); csc — ciliary secretory cell(s) of anterior and posterior marginal areas between valves; m — mouth opening; me — mesenchymal cells; mu — muscle cells; n — nucleus; oa — adhesive organ (internal sac); p — posterior region; pc — ‘polster’ cell(s); ph — pharynx; po — pyriform organ; ppc — principal pallial cell(s); s — shell; sc — supracoronal cell(s); spc — suprapallial secretory cell(s); st — stomach; t — ciliary taft of pyriform organ; tc — tendon cell(s); v — vacuole.

Рис. 1. Анатомия и ультраструктура цифонаута *Electra pilosa* (Gymnolaemata: Cheilostomatida) (если не указано иное, передний конец ориентирован влево). A — световая микроскопия; B, G, K — окрашенные полутонкие срезы, световая микроскопия; C, D, E, F, H-J — трансмиссионная электронная микроскопия. A — прижизненное фото личинки, вид сбоку (плоскость, показанная пунктирной линией 1, соответствует плоскости срезов на E, F; плоскость, показанная пунктирной линией 2, соответствует плоскости срезов G, I); B — полутонкий сагиттальный срез передней области личинки с секреторными и “подушковидными” клетками паллиального эпителия под раковиной; C — сагиттальный срез апикального органа с секреторными паллиальными клетками на периферии (обратите внимание на секретируемый материал, формирующий тонкую кутикулярную ‘пленку’); D — сагиттальный срез через апикальный орган и супрапаллиальный эпителий под ним (граница показана пунктирной линией; обратите внимание на многочисленные впячивания мембран между соседними клетками и многочисленные цистерны ЭПР); E — косой фронтальный срез раковины и уплощенных основных паллиальных клеток на уровне желудочного отдела кишки (плоскость среза 1 как указано на A); F — косой фронтальный срез кубических основных паллиальных клеток выше глоточного отдела кишки (плоскость среза 1 как указано на A; обратите внимание на ‘верхнюю’ клетку в фазе митотического деления, на сложные контакты между двумя соседними клетками (отмечены пунктирной линией) и на толстую базальную пластинку (наконечники стрелок) между основным паллиальным эпителием и внутренней массой мышц и других мезенхимных клеток); G — косой фронтальный срез тендоцитов в месте прикрепления аддуктора (плоскость среза 2, как показано на A); H — сагиттальный срез выше грушевидного органа, показывающий две ’подушковидные‘ клетки и деформированную основную клетку паллиального эпителия (отмечена синим цветом), зажатую между ними; I — косой фронтальный срез тендоцитов на уровне ресничных гребней (плоскость среза 2, как показано на A); J — крупный план тендоцита, контактирующего с раковиной, показывающий полудесмосомы, обеспечивающие прикрепление к створке раковины, тонофиламенты и утолщенную базальную пластинку; K — поперечный срез на уровне нижних ‘подушковидных’ клеток, эпителиально-мышечных клеток и бицилиарных супракорональных клеток. Звездочки обозначают пространство между раковиной и паллиальным эпителием; белые наконечники указывают на базальную пластинку; черные стрелки указывают на цитоплазматические отростки основных паллиальных клеток.

Обозначения: a — передний конец; an — анальное отверстие; ao — апикальный орган; aor — ретракторы апикального органа; at — атриум; c — корона; ce — центриоль; cf — кутикулярная ‘плёнка’; cm — поперечно-полосатая эпителиально-мышечная клетка(и); cr — ресничные гребни; csc — ресничные секреторные клетки переднего и заднего участков эпителия между створками раковины; m — ротовое отверстие; me — мезенхимные клетки; mu — мышечные клетки; n — ядро; oa — орган адгезии (внутренний мешок); p — задний конец; pc — ‘подушковидная’ клетка(и); ph — глотка; po — грушевидный орган; ppc — основные паллиальные клетки; s — раковина; sc — супракорональная клетка(и); spc — супрапаллиальная секреторная клетка(и); st — желудок; t — пучок ресничек грушевидного органа; tc — тендоцит(ы); v — вакуоль.

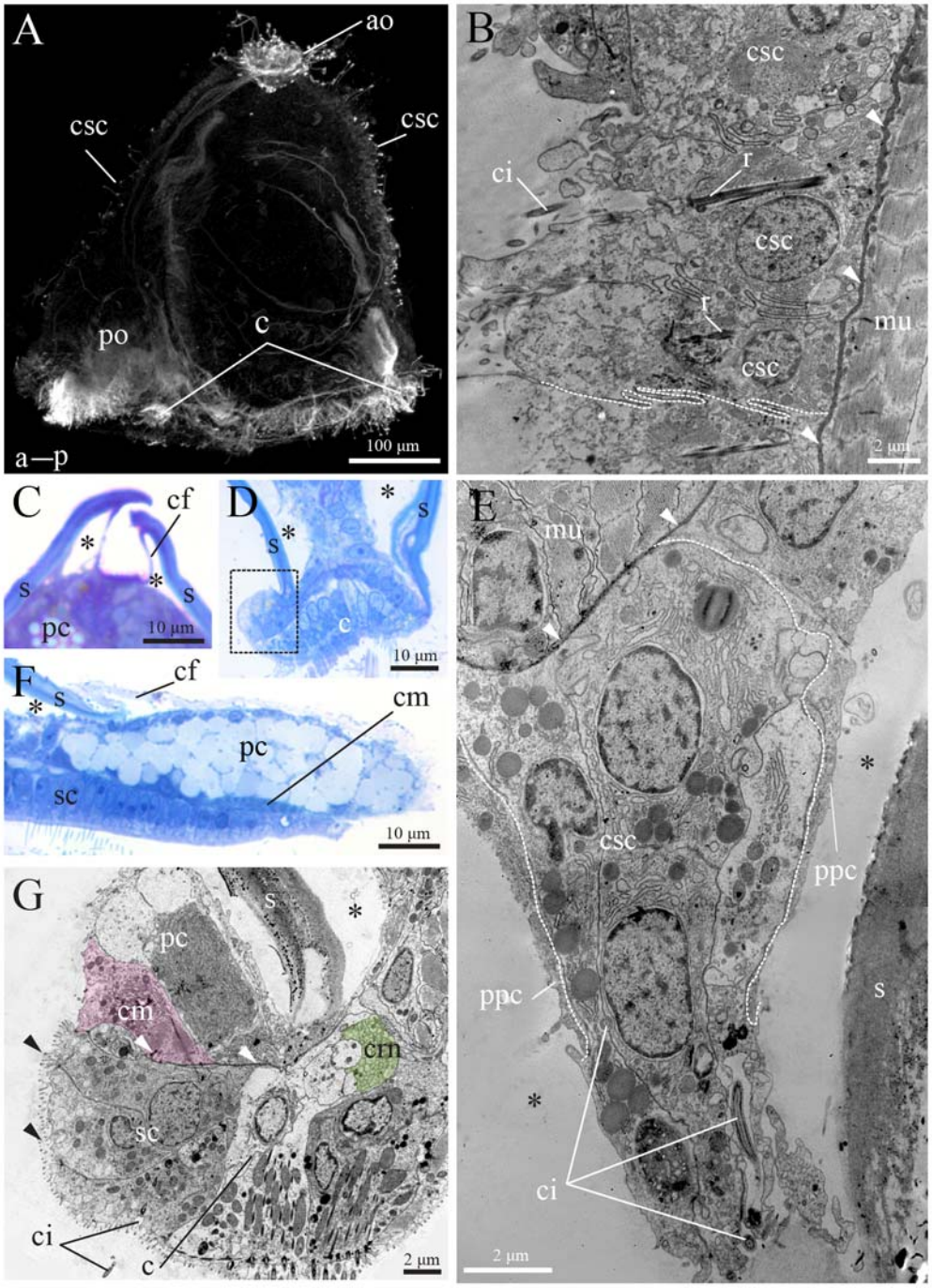

Fig. 2. Anatomy and ultrastructure of cyphonautes of *Electra pilosa* (Gymnolaemata: Cheilostomatida) (unless stated otherwise anterior end is oriented to *left*). A — CLSM, fluorescent labeling with antibodies against acetylated α-tubulin (confocal z-projection stack in middle part of larva); B, E, G — transmission electron microscopy; C–D, F — stained semithin sections, light microscopy. A — lateral view of cyphonautes; B — sagittal section through ciliated secretory cells forming anterior zone of larva between

B, E), (5) tendon cells at the sites of muscle attachment (Fig. 1G, I, J), (6) cross-striated epitheliomuscular cells (Figs 1K; 2F, G) and (7) biciliated supracoronal secretory cells above the corona (Fig. 2D, F, G). All pallial cells are positioned on a more or less thick basal lamina and, except for the supracoronal cells, lack a glycocalyx and microvilli (Figs 1; 2).

The ring zone around the apical organ base consists of up to four rows of trapezoidal (closer to the apical organ) and columnar suprapallial secretory cells with folded lateral walls (Figs 1C, D; 8A). Their electron-translucent cytoplasm contains a large central nucleus, numerous cisternae of rough endoplasmic reticulum (RER) and Golgi apparatus, as well as abundant vacuoles of various sizes; the latter release an amorphous substance around the periphery of the apical organ (visible as apocrine secretion on TEMs) (Fig. 1C).

Ciliated secretory cells form up to four rows along the anterior and posterior marginal areas between the valves (Figs 1B; 2A, B, E). They are columnar or cubic and equipped with one or two rigid cilia (each projecting from a deep pit) with at least one rootlet each, suggesting a sensory function (Fig. 2B, E). These cells are polarized with a basally located nucleus surrounded by numerous RER cisternae and, like suprapallial cells, contain prominent Golgi complexes and show an apocrine secretion. Laterally these cells are connected by interdigitations (Fig. 2B).

The pallial epithelium underlying the shell valves consists of two cell types. Principal pallial cells change their shape in the direction from the apical organ towards the corona along the larval surface (Fig. 8A) — they are cuboidal below the apical organ (Fig. 1F), becoming flatter and nearly squamous

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valves (note apocrine secretion and interdigitations between two neighbour cells (shown by dotted line)); C — oblique frontal section trough anterior part of the larva showing thin cuticular 'film' inbetween two valves; D — frontal section of posterior lower end of larva (rectangular area magnified in G); E — oblique frontal section of posterior zone of larva mostly showing biciliated secretory cells (section plane 1 as indicated in Fig. 1A; note cilia lying in deep pits; border between principal pallial cells is shown by dotted line); F — transversal section on the level of lower 'polster' cells, epitheliomuscular cells and biciliated supracoronal cells; G — frontal section of biciliated supracoronal secretory cells, some with cilium (corresponds to rectangular area indicated in D; cross-striated epitheliomuscular cell marked with rose color; coronal nerve ring at base of corona cells marked by green colour). Asterisks indicate cavity between shell and pallial epithelium; white arrowheads show basal lamina; black arrowheads indicate microvilli.

Abbreviations: a — anterior region; ao — apical organ; c — corona; cf — cuticular 'film'; ci — cilia; cm — cross-striated epitheliomuscular cell(s); crn — coronal ring nerve; csc — ciliated secretory cell(s) of anterior and posterior marginal areas between valves; mu — muscle cell(s); p — posterior region; pc — 'polster' cell(s); po — pyriform organ; ppc — principal pallial cell(s); r — ciliary rootlet(s); s — shell; sc — supracoronal secretory cell(s); spc — suprapallial secretory cell(s); v — vacuole.

Рис. 2. Анатомия и ультраструктура цифонаута *Electra pilosa* (Gymnolaemata: Cheilostomatida) (если не указано иное, передний конец ориентирован влево). A — CLSM, флуоресцентное мечение антителами против ацетилированного α-тубулина (z-стек проекций в средней части личинки); B, E, G — трансмиссионная электронная микроскопия; C–D, F — окрашенные полутонкие срезы, световая микроскопия. A — цифонаут (вид сбоку); B — сагиттальный срез через ресничные секреторные клетки, образующие передний участок эпителия между створками раковины (обратите внимание на апокриновую секрецию; сложные контакты между двумя соседними отмечены пунктирной линией); C — косой фронтальный срез передней части личинки с тонкой кутикулярной 'пленкой' между двумя створками; D — фронтальный срез заднего нижнего края личинки (черным пунктиром ограничена область, увеличенная на G); E — фронтальный срез задней зоны личинки, демонстрирующий ресничные секреторные клетки (плоскость среза 1 как показано на Рис. 1A; обратите внимание на реснички, лежащие в глубоких ямках; пунктирной линией показана граница между основными паллиальными клетками и ресничными секреторными клетками); F — поперечный срез на уровне 'подушковидных' клеток, эпителиально-мышечных клеток и бицилиарных супракорональных клеток; G — фронтальный срез супракорональных секреторных клеток (некоторые с ресничками) (участок среза соответствует прямоугольной области на D; поперечно-полосатая эпителиально-мускульная клетка отмечена розовым цветом; кольцевой нерв у основания клеток короны отмечен зеленым цветом). Звездочками обозначено пространство между раковиной и паллиальным эпителием; белые наконечники указывают на базальную пластинку; черные стрелки показывают на микроворсинки.

Обозначения: a — передний конец; ao — апикальный орган; c — корона; ci — реснички; cm — поперечнополосатая эпителиально-мышечная клетка(и); crn — кольцевой нерв короны; csc — ресничные секреторные клетки переднего и заднего участков эпителия между створками раковины; mu — мышечная клетка(и); p — задний конец; pc — 'подушковидная' клетка; po — грушевидный орган; ppc — основная паллиальная клетка(и); s — раковина; sc — супракорональные секреторные клетки; spc — супрапаллиальные секреторные клетки; v — вакуоль.

(Fig. 1E). Cuboidal cells have a central oval nucleus, numerous RER cisternae, some mitochondria scattered through the electron-lucent cytoplasm, and a few vacuoles in the apical part. These cells are interconnected by adherens junctions (zonula adherens) apically followed by (presumably) septate junctions, and via interdigitations (Fig. 1F). In some cases, principal pallial cells form a pseudo-stratified epithelium of overlapping cell parts occupying large areas (Fig. 8A). Principal pallial cells form thin cytoplasmic processes contacting the inner side of the shell (Fig. 1B).

The so-called pallial 'polster' cells are visible on both lateral sides of the mature cyphonautes, usually being basally inclined between the principal pallial cells, sometimes strongly deforming the latter and being covered by their processes (Figs 1H; 8A). Two lateral groups of large 'polster' cells flank the lower edge of the principal pallial epithelium on both sides, abutting the lower edges of each valve; these cells produce a thick layer of amorphous cuticular 'film' that continues to the valves (Figs 1K; 2F). 'Polster' cells are filled with tightly packed, electron-dense and translucent vacuoles of various sizes. The nucleus is situated basally, numerous RER cisterns are placed between vacuoles and in the basal part of the cell (Figs 1K; 2F, G).

Special tendon cells are grouped between the principal pallial cells directly above the point of convergence of two ciliary ridges, providing attachment of the adductor to the shell valves (Fig. 1F, I). Some tendon cells were also found along with muscle cells located beneath the ciliary ridges of the atrium (Fig. 1 I). They are flattened, sometimes forming deep folds and invaginations. Numerous tonofilament bundles cross the electron-translucent cytoplasm and insert into the thickened basal lamina via several hemidesmosomes (Fig. 1J). Tendon cells are attached to the shell by hemidesmosome-like structures (Fig. 1J).

The biciliated supracoronal secretory cells are columnar, with polarized cytoplasm (Figs 2F, G; 8A). Each cilium projects from the bottom of a small pit and have a ciliary rootlet (Fig. 2G). The cytoplasm in the apical third of these cells is filled with electron-translucent vacuoles of various sizes. A large, oval, sometimes lobed nucleus is situated in the central part of the cell, with mitochondria, RER, and vacuoles with electron-dense content clumped underneath. Unlike all other pallial cells, the apical membrane of the supracoronal cells forms many long, sometimes V-shaped (branching) microvilli, inserted into a layer of flocculent glycocalyx above them. When the larva is 'relaxed', the supracoronal cells protrude beyond the shell margin, and the cuticular 'film' extends above the microvillous layer. When the larva contracts, supracoronal cells (together with coronal cells) tuck under the 'polster' cells in such a way that their basal laminae come into contact.

The superficial ring of cross-striated epitheliomuscular cells is located directly between the lower group of 'polster' cells and the supracoronal biciliated cell ring (Figs 1K; 2G). Their cytoplasm is electron-lucent, with many small vesicles in the apical part, a large nucleus and a loose RER in the central part of the cell. The myofilaments are situated basally forming a series of sarcomeres which are visible in our transversal sections (Fig. 1K).

Flustrellidra hispida (Figs 3; 8B)

The pseudocyphonautes larva of *F. hispida* (d'Hondt, 1977a, c) is endotrophic, with a vestigial gut, and is presumably nourished by the maternal zooid during a 1–2 months long incubation in the modified tentacle sheath. After release, the larva lives up to three days in the water column before settlement (Prouho, 1892; our data; reviewed in Kvach *et al*., 2019).

The larva is bean-shaped, elongated along the anterior-posterior axis, and laterally flattened (Figs 3A; 8B) (average height 172 μm (N=4, range 157–186 μm), length 443 μm (N=8, range 343–692 μm), width 128 μm (N=4, range 115–122 μm)). Its hypospheral (oral) part is screwed inward, forming a small atrium under the episphere (aboral hemisphere). The apical organ is situated on the top, and the ciliary corona lies along the edge of the epispheral base. Laterally, the pseudocyphonautes is covered by two translucent non-mineralized shell valves (Fig. 3A, B). As in cyphonautes, the valves are interconnected by the thin cuticular 'film' along the anterior and posterior areas of the larval surface (Fig. 3B). This 'film' constitutes the outermost electron-denser layer of the shell valves and expands over the corona when the larva is 'contracted' (Fig. 3B, E).

The pallial epithelium comprises three cell types (Figs 3; 8B): (1) principal pallial cells constituting extensive fields underneath the shell valves and between them, along larval anterior and posterior areas (Fig. 3B–D), (2) tendon cells at the sites of muscle attachment (Fig. 3B, C, F, G), and (3) supracoronal cells forming several 'rows' above the corona (Fig. 3B, E). All pallial cells are interconnected by the zonula adherens in their apical parts and by numerous interdigitations (Fig. 3C, D).

The upper (except the apical organ) and lateral sides of the larva are covered by the columnar or trapezoidal principal pallial cells with long apical cytoplasmic processes expanding towards the shell (Figs 3B–D; 8B). These cells show noticeable polarity in organelle distribution, usually having a large, elongated, sometimes lobed nucleus that occupies the lower third of the cell. Most mitochondria are accumulated underneath the nucleus (Fig. 3D). The

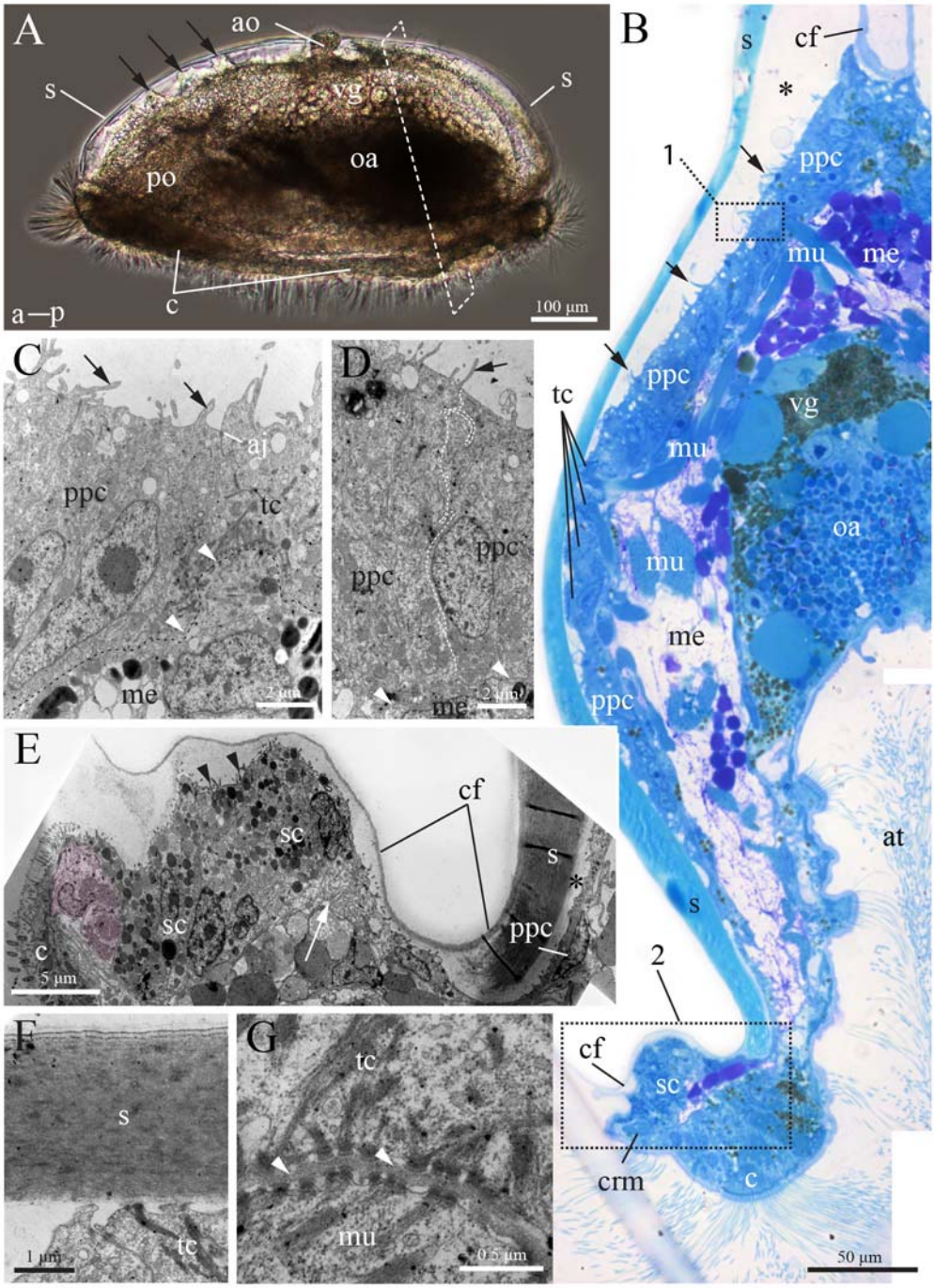

Fig. 3. Anatomy and ultrastructure of pseudocyphonautes of *Flustrellidra hispida* (Gymnolaemata: Ctenostomatida) (unless stated otherwise anterior end is oriented to *left*). A — light microscopy; B — stained semithin section, light microscopy; C–G — transmission electron microscopy. A — lateral view of living, newly released larva (plane shown by dotted-line corresponds to section plane in B); B — oblique frontal section through posterior region of larva showing principal pallial cells with long cytoplasmic processes,

cytoplasm in the upper part of the cell is filled with vacuoles, electron-lucent or with a flocculent content (Fig. 3B–D). Numerous cisterns of RER are visible throughout the cytoplasm. Principal pallial cells in the lower part of both lateral sides are flatter, sometimes squamous, non-polarized, and have shorter apical cytoplasmic processes, fewer vacuoles, and a centrally placed nucleus (Fig. 3E). Pallial epithelial cells situated along the anterior and posterior margins of the larva in between the shell valves contain a greater number of larger vacuoles than most of the principal pallial cells (Fig. 3B). Majority of the pallial cells are positioned on a very thin basal lamina; when the larva is contracted, their basal membrane can form folds that overlap each other.

Special tendon cells are situated between the principal pallial cells, attaching to the shell valves and also being the sites of muscle attachment (Figs 3B, C, F, G; 8B). They have an irregular shape, sometimes flattened, being sandwiched between the shell and the muscle cells whose tonofilaments are inserted to the thickened basal lamina below each tendon cell via several hemidesmosomes (Fig. 3G). On the opposite side of the basal lamina, thick bundles of tonofilaments of the tendon cell also attach to the hemidesmosomes (Fig. 3G). Some tonofilaments merge together, forming dense clusters in the cytoplasm. Some of them extend to the apical membrane and connect to hemidesmosome-like structures providing an attachment of the tendon

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tendon cells and supracoronal secretory cells (note cuticular ‘film’ connecting two valves; rectangular areas 1 and 2 magnified in C, D and E correspondingly); C, D — oblique frontal sections of principal pallial cells and tendon cell (correspond to rectangular area 1 in B; in C basal plate is underlined by black dotted line, in D interdigitations between two cells marked by white dotted line); E — supracoronal cells with cuticular ‘film’ above microvilli (note folded basal parts of cells (the area marked by white arrow) and squamous principal pallial cell just under shell valve; coronal ring muscle between coronal and supracoronal cells (also visible in B) marked by rose color); F — close-up of tendon cell contacting to shell showing hemidesmosomes providing attachment to shell valve; G — tendon cell contacting to muscle cell and showing tonofilaments, numerous hemidesmosomes and thickened basal lamina. Asterisk indicates cavity between shell and pallial epithelium; black arrows show cytoplasmic processes of principal pallial cells and tendon cells, black arrowheads — microvilli of supracoronal cells, white arrowheads — basal lamina.

Abbreviations: a — anterior region; aj — adherens junction (zonula adherens); ao — apical organ; at — atrium; c — corona; cf — cuticular ‘film’; crm — coronal ring muscle; me — mesenchymal cells; mu — muscle cells; oa — adhesive organ; p — posterior region; po — pyriform organ; ppc — principal pallial cell(s); s — shell; sc — supracoronal cell(s); sj — septate junction; tc — tendon cell(s); v — vacuole; vg — vestigial gut.

Рис. 3. Анатомия и ультраструктура псевдоцифонаута *Flustrellidra hispida* (Gymnolaemata: Ctenostomatida) (если не указано иное, передний конец ориентирован влево). A — световая микроскопия; B — окрашенный полутонкий срез, световая микроскопия; C–G — трансмиссионная электронная микроскопия. A — вид сбоку на живую, только что вышедшую из выводковой камеры личинку (пунктиром обозначена плоскость среза на B); B — косой фронтальный срез задней части личинки, показывающий основные паллиальные клетки с длинными цитоплазматическими отростками, тендоциты и супракорональные секреторные клетки (обратите внимание на кутикулярную ‘пленку’, соединяющую две створки раковины; прямоугольные области 1 и 2 увеличены на C, D и E, соответственно); C, D — косые фронтальные срезы основных паллиальных клеток и тендоцита (соответствуют прямоугольной области 1, отмеченной на B; на C базальная пластинка подчеркнута черной пунктирной линией; на D контакты между двумя клетками отмечены белой пунктирной линией); E — супракорональные клетки с кутикулярной ‘пленкой’ над микроворсинками (обратите внимание на складчатые базальные части клеток (участок отмечен белой стрелкой) и на уплощенную основную паллиальную клетку непосредственно под створкой раковины; кольцевая мышца короны между клетками короны и супракорональными клетками (также видна на B) отмечена розовым цветом); F — крупный план контакта тендоцита с раковиной, демонстрирующий полудесмосомы, обеспечивающие крепление тендоцита к створке раковины; G — крупный план контакта тендоцита и мышечной клетки, показывающий тонофиламенты, многочисленные полудесмосомы и утолщенную базальную пластинку. Звездочки указывают пространство между раковиной и паллиальным эпителием; черные стрелки указывают на цитоплазматические отростки основных паллиальных клеток и тендоцитов, черные наконечники — на микроворсинки супракорональных клеток, белые наконечники — на базальную пластинку.

Обозначения: a — передний конец; aj — адгезивный контакт (zonula adherens); ao — апикальный орган; at — атриум; c — корона; cf — кутикулярная ‘пленка’; crm — кольцевая мышца короны; me — мезенхимальные клетки; mu — мышечные клетки; oa — орган адгезии; p — задний отдел; po — грушевидный орган; ppc — основная паллиальная клетка(и); s — раковина; sc — супракорональная клетка(и); sj — септированный контакт; tc — тендоцит(ы); v — вакуоль; vg — рудиментарный кишечник.

cell to the shell (Fig. 3F). In most tendon cells such attachment zones are rather extensive (Fig. 3B). These cells have an electron-translucent cytoplasm with vacuoles of various sizes, numerous RER cisternae and mitochondria. Some tendon cells also form apical cytoplasmic processes.

In contrast to all other pallial cells, the supracoronal secretory cells have an electron-dense cytoplasm tightly packed with electron-dense and translucent vacuoles of various sizes in the apical part of the cell (Fig. 3E). These cells are columnar with a lobed nucleus situated basally in the cell. The apical membrane forms relatively rare filiform microvilli inserting into the flocculent glycocalyx above them. No signs of cilia were found. Similar to the cyphonautes, in the 'relaxed' larvae the supracoronal cells protrude beyond the shell, being covered by the cuticular 'film' extending above their microvilli (Fig. 3B, E).

Alcyonidium hirsutum (Figs 4; 8C)

The larva of *A. hirsutum* is lecithotrophic, with a vestigial gut, and is incubated in the modified tentacle sheath (Owrid, Ryland, 1991; Wood, Seed, 1992; our data). After release, the larva swims several hours in the water column before settlement.

The larva is hat-shaped (approximate height 195 μm, average length 283 (measured without cilia) / 340 (with cilia) μm (N=25, range 220–317 μm), width at the basal part 284/339 μm (N=25, range 213–310 μm) with the apical organ in the center of the convex aboral hemisphere surrounded by a narrow equatorial corona, and a slightly convex flattened oral hemisphere (Figs 4A, E; 8C). Larva has no shell valves.

The pallial epithelium comprises four cell types (Figs 4; 8C): (1) principal pallial cells (Fig. 4D–G), (2) vacuolated (presumably secretory) cells (Fig. 4B, E, F), (3) tendon cells associated with the muscle cells (Fig. 4B, E, G), and (4) ciliated supracoronal cells forming three to four 'rows' above the corona (Fig. 4E, H). Principal, tendon, and supracoronal cells are positioned on a rather thick basal lamina, whereas vacuolated cells are usually situated above it, intraepithelially (Fig. 4B, E). Except for the apical organ and supracoronal cells, the aboral hemisphere is covered by a rigid cuticle that has a filamentous structure with an electron-denser outer layer on TEM (Fig. 4B–E, G).

The principal pallial cells constituting most of the epithelium of the aboral surface are typically columnar, with apical cytoplasmic processes (some reaching the external cuticle) (Figs 4B, D, F, G; 8C). They have an electron-dense cytoplasm and show polarity, having basally situated mitochondria and nucleus, whereas the apical third is filled with numerous electron-dense 'granules' of the various sizes and shapes, with filamentous contents. Several RER cisterns are usually visible in the cell periphery. In addition, a few yolk droplets were recorded throughout the cytoplasm. In some of principal pallial cells, the cell basal membrane forms deep invaginations. The principal pallial cells on the border with the apical organ are squamous and show no polarity. No cuticular 'film' expands above these cells (Fig. 4D).

Round or oval 'intrapallial' vacuolated cells generally have a small, sometimes strongly deformed nucleus, almost totally filled with large vacuoles with light content which is clearly released outside the cell (Figs 4B, E, F; 8C).

As in *F. hispida* pseudocyphonautes, the tendon cells of *A. hirsutum* larvae are spread in between the principal pallial cells (Figs 4B, E, G; 8C). Their shape is irregular, sometimes warped by underlying muscle cells. The apical membrane forms microvilli that are supported by tonofilaments and connected with the external cuticle by very thin fibrils. Basally these cells anchor via hemidesmosomes on the basal lamina. The electron-translucent cytoplasm contains the tonofilament bundles connected with either apical or basal hemidesmosomes, sometimes with both. Electron-dense 'bodies' visible in the cytoplasm are presumed hemidesmosomes during their assembly and disassembly (Fig. 4G). Some tendon cells show large vacuoles with electron-translucent content.

Principal pallial cells are connected with each other and with tendon cells by adherens junctions (zonula adherens) apically, then by several interdigitations.

Supracoronal cells are typically columnar or bottle-shaped with one or two presumably rigid cilia and electron-dense cytoplasm (Figs 4E, H; 8C). The apical membrane forms many long, branching, clavate microvilli penetrating a filamentous glycocalyx above them. Each cell shows distinct apico-basal polarity, with a large nucleus at the cell base and several spindle-shaped and oval granules with heterogeneous content in the apical part of the cell, seemingly releasing their content that is visible in some TEMs. Some RER and mitochondria are scattered through the cytoplasm. Supracoronal cells are connected to neighboring cells by adherens junctions (zonula adherens) in the apical part and by interdigitations.

Tendra zostericola (Figs 5; 9A)

The larva of *T. zostericola* is lecithotrophic, with a vestigial gut, and is incubated in the acanthostegal brood chamber from less than 10 hours to two days. After release, the larva swims from 1–2 hours to two days in the water column before settlement (Paltschikowa-Ostroumowa, 1926; Braiko, 1967; our data).

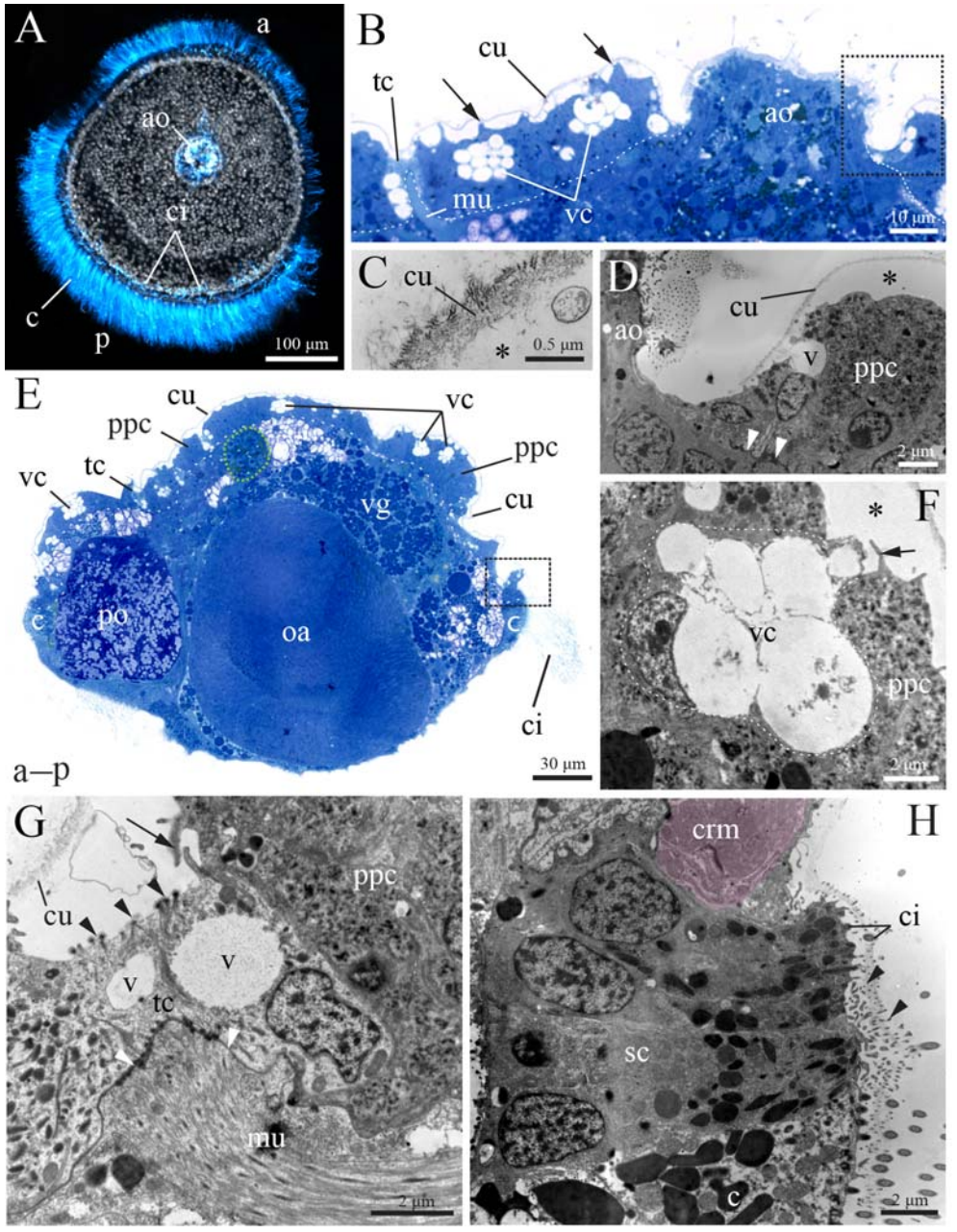

Fig. 4. Larval anatomy and ultrastructure of *Alcyonidium hirsutum* (Gymnolaemata: Ctenostomatida). A — CLSM, fluorescent labeling with antibodies against acetylated α-tubulin (blue) and with DAPI (white) (confocal z-projection stack); B, E — stained semithin sections, light microcsopy; C, D, F–H — transmission electron microscopy. A — general view of larva from above showing corona and apical organ (note darker arch-like area of adhesive organ in the posterior part of larvae and narrow ring of cilia of supracoronal cells above corona); B — sagittal section of the upper part of larva with apical organ (anterior end is oriented to *left*; white dotted-line indicates the border between pallial epithelium and underlying mesenchymal cells: rectangular area magnified in D); C — close-up of the cuticle (or cuticular 'film') showing two layers — filamentous and amorphous, and the vacuole underneath with filamentous content; D — sagittal section of

The larva is hat-shaped (approximate height 71µm, average length 104 (measured without cilia) /118 (with cilia) µm (N=11/5, range 62/105–127/128 µm), width 76/100 µm (N=11/8, range 47/73–95/119 µm)) with the convex aboral hemisphere bearing the apical organ, which is slightly displaced anteriorly, and a narrow equatorial corona (Figs 5B, C; 9A). The oral hemisphere is convex, with a massive adhesive organ (Figs 5C; 9A).

The pallial epithelium consists of (1) principal pallial cells, and (2) ciliated supracoronal cells forming at least two rows above the corona (Figs 5C–G; 9A). All cells are connected by a zonula adherens and septate junctions in the apical part and via interdigitations and are underlain by very thin basal lamina (Fig. 5D–G).

The principal epithelial cells form a relatively narrow 'ring' around the apical organ (Fig. 5C). They are typically columnar or somewhat bottle-shaped with electron-dense cytoplasm and polarized distribution of organelles (Fig. 5C, D). Their apical membrane is covered by a fuzzy, homogeneous, electron-translucent extracellular matrix (glycocalyx), pierced by numerous thin cytoplasmic processes of various sizes and shapes (Fig. 5D, F, G). Externally this fuzzy coat is covered by hemispher-

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upper part of larva showing principal pallial cells and their cuticular cover (correspond to rectangular area shown in B; note that cuticular 'film' above squamous pallial cell on the border with apical organ is absent); E — semithin sagittal section of the larva (anterior end is oriented to *left*; the green dotted line outlines cluster of presumptive blastemal cells; rectangular area magnified in H); F — close-up of vacuolated cell (outlined by dotted line); G — close-up of tendon cell contacting shell and muscle cell (tendon cell detached from cuticular layer during fixation; note tonofilaments, numerous hemidesmosomes and thickened basal lamina); H — sagittal section of supracoronal cells corresponding to rectangular area shown in E (note coronal ring muscle (marked by red colour) situated above supracoronal cells). Asterisk shows cavity between cuticular 'film' and pallial epithelium; black arrows indicate cytoplasmic processes of pallial cells, black arrowheads — hemidesmosomes of tendon cell in G and microvilli of supracoronal cells in H, white arrowheads indicate basal lamina.

Abbreviations: a — anterior region; ao — apical organ; c — corona; ci — cilia; crm — coronal ring muscle; cu — cuticular 'film'; mu — muscle cells; oa — adhesive organ; p — posterior region; po — pyriform organ; ppc — principal pallial cell(s); sc — supracoronal cell(s); tc — tendon cell(s); v — vacuole; vc — vacuolated cell; vg — vestigial gut.

Рис. 4. Анатомия и ультраструктура личинок *Alcyonidium hirsutum* (Gymnolaemata: Ctenostomatida). A — CLSM, флуоресцентное мечение антителами против ацетилированного α-тубулина (показано голубым) и окраска DAPI (показано белым) (z-стек проекций); B,E — окрашенные полутонкие срезы, световая микрокопия; C, D, F–H — трансмиссионная электронная микроскопия. A — общий вид личинки сверху, демонстрирующий корону и апикальный орган (обратите внимание на более темную область секреторных клеток адгезивного органа в виде арки в задней части личинки и на узкое кольцо ресничек супракорональных клеток над короной); B — сагиттальный срез верхней части личинки в области апикального органа (передний конец ориентирован влево; белая пунктирная линия показывает границу между паллиальным эпителием и лежащими ниже мезенхимными клетками; прямоугольная область увеличена на D); C — крупный план кутикулы или кутикулярной 'пленки', демонстрирующий два слоя — филаментозный и аморфный, и вакуоль снизу с филаментозным содержимым; D — сагиттальный срез верхней части личинки, демонстрирующий основные паллиальные клетки и их кутикулярный покров (соответствует прямоугольной области на B; обратите внимание, что кутикулярная 'пленка' отсутствует над плоской паллиальной клеткой на границе с апикальным органом); E — полутонкий сагиттальный срез личинки (передний конец ориентирован влево; зеленая пунктирная линия очерчивает скопление презумптивных бластемных клеток; черный прямоугольная область, увеличенная на H); F — крупный план вакуолизированной клетки (обведена пунктирной линией); G — крупный план тендоцита, контактирующего с раковиной и мышечной клеткой (тендоцит утратил контакт с кутикулой при фиксации; обратите внимание на тонофиламенты, многочисленные гемидесмосомы и утолщенную базальную пластинку); H — сагиттальный срез супракорональных клеток (соответствует прямоугольной области на E; обратите внимание на кольцевую мышцу короны (отмечена красным цветом), расположенную над супракорональными клетками). Звездочки указывают на пространство между кутикулой и паллиальным эпителием; черные стрелки указывают на цитоплазматические отростки основных паллиальных клеток, черные наконечники — на полудесмосомы тендоцитов на G и на микроворсинки супракорональных клеток на H, белые наконечники указывают на базальную пластинку.

Обозначения: a — передний конец; ao — апикальный орган; c — корона; ci — реснички; crm — кольцевая мышца короны; cu — кутикулярная 'пленка'; mu — мышечные клетки; oa — орган адгезии; p — задний конец; po — грушевидный орган; ppc — основная паллиальная клетка(и); sc — супракорональная клетка(и); tc — тендоцит; v — вакуоль; vc — вакуолизированная клетка; vg — рудиментарный кишечник.

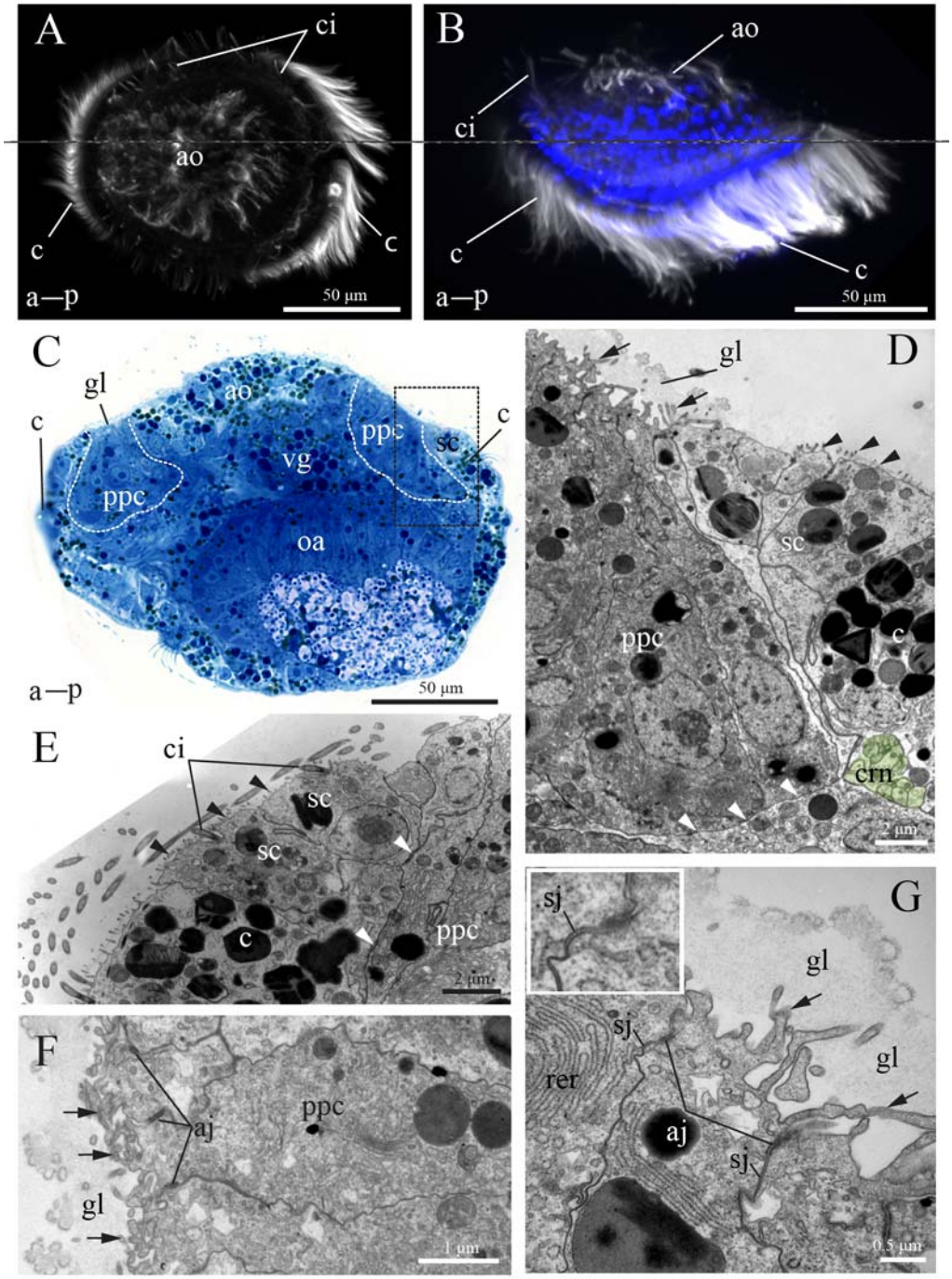

Fig. 5. Larval anatomy and ultrastructure of *Tendra zostericola* (Gymnolaemata: Cheilostomatida). A, B — CLSM, fluorescent labeling with antibodies against acetylated α-tubulin (A, B, white) and with DAPI (B, blue) (confocal z-projection stack); C — stained semithin section, light microscopy; D–G — transmission electron microscopy. A — general view of larva from above showing cilia of apical organ (in the center), rigid cilia of supracoronal cells and corona; B — lateral view of larva showing its hat-like shape (note cilia

ical ‘caps’ with peripheral ‘striation’; the cytoplasmic processes do not reach them (Fig. 5D, F, G). Many small vacuoles with electron-translucent content are present below the apical membrane (Fig. 5D, F, G). The elaborated RER forms plentiful strands and whorls in the basalmost as well apical parts of the principal pallial cells around the dense lipid droplets and elsewhere (Fig. 5D–F). The nucleus is situated basally in the cell. The apical part of these cells also contains prominent Golgi complexes and some mitochondria (Fig. 5G). Electron-dense and electron-translucent granules of various sizes and shapes, some with filamentous content, are scattered throughout the cytoplasm (Fig. 5D).

Supracoronal cells are typically cuboidal and presumably monociliated (Figs 5D, E; 9A). Rigid cilia project from the bottom of small ‘pits’ and have no ciliary rootlet. The apical membrane forms several short clavate microvilli embedded in a thin filamentous glycocalyx (Fig. 5E). The cytoplasm is electron-translucent with some RER, electron-light vacuoles, large granules with heterogeneous content, and scattered mitochondria. The oval nucleus occupies the basal part of the cell (Fig. 5D, E).

Juxtacribrilina annulata (Figs 6; 9B)

The larva of *J. annulata* is lecithotrophic, incubated in the ovicell. After release, it swims 1–2 hours before settlement (Ostrovsky, 1998a; Nekliudova *et al*., 2019b; our data).

The living larva is barrel-shaped, elongated along the oral-aboral axis (average height 182 μm (N=14, range149–220 μm), length 215 (measured without cilia) /264 (with cilia) μm (N=12/7, range 187/210–233/288 μm), width 191/238 μm (N=18/9, range 158/215–212/271 μm)), with densely ciliated wide

of apical organ and rigid cilia of supracoronal cells facing upwards relative to corona); C — sagittal section of larva (anterior end is oriented to *left*; white dotted line frames ‘ring’ of principal pallial cells between apical organ and supracoronal cells; note thick layer of extracellular matrix above principal pallial cells; rectangular area magnified in D); D — sagittal section of principal pallial cells and supracoronal cells (image corresponds to rectangular area in C; note cross-sectioned coronal nerve at base of corona cells marked by green colour); E — sagittal section of two supracoronal cells; F, G — sagittal section of principal pallial cells (note external extracellular matrix, consisting of glycocalyx and hemispherical “cups” (presumably, remnants of fertilization envelope)); G, inset — close-up of cell contacts between pallial cells. Black arrows show cytoplasmic processes of principal pallial cells, black arrowheads — microvilli of supracoronal cells, white arrowheads — basal lamina.

Abbreviations: a — anterior region; aj — adherens junction (zonula adherens); ao — apical organ; c — corona; ci — rigid cilia of supracoronal cells; crn — coronal ring nerve; gl — glycocalyx; oa — adhesive organ; p — posterior region; ppc — principal pallial cell(s); rer — rough endoplasmic reticulum; sc — supracoronal cell(s); sj — septate junction; vg — vestigial gut.

Рис. 5. Анатомия и ультраструктура личинок *Tendra zostericola* (Gymnolaemata: Cheilostomatida). A, B — CLSM, флуоресцентное мечение антителами против ацетилированного α-тубулина (A, B, показано белым) и DAPI (B, показано голубым) (z-стек проекций); B — окрашенный полутонкий срез, световая микроскопия; D–G — трансмиссионная электронная микроскопия. A — общий вид личинки сверху: реснички апикального органа (в центре), ригидные реснички супракорональных клеток и корона (на периферии); B — вид личинки сбоку, демонстрирующий ее шляповидную форму (обратите внимание на реснички апикального органа (вверху) и ригидные реснички супракорональных клеток, обращенные кверху относительно ресничек короны); C — сагиттальный срез личинки (передний конец ориентирован влево; белая пунктирная линия обрамляет “кольцо” из основных паллиальных клеток между апикальным органом и супракорональными клетками; обратите внимание на толстый слой экстрацеллюлярного матрикса (гликокаликса) над основными паллиальными клетками; прямоугольная область показывает участок, увеличенный на D); D — сагиттальный срез основных паллиальных клеток и супракорональных клеток (изображение соответствует прямоугольной области на C; обратите внимание на нерв короны, перерезанный поперёк (отмечен зеленым цветом)); E — сагиттальный срез двух супракорональных клеток; F, G — сагиттальный срез основных паллиальных клеток (обратите внимание на наружный экстрацеллюлярный матрикс, состоящий из гликокаликса и полусферических “чашечек” (предположительно, остатков оболочки оплодотворения)): G, вставка —. крупный план межклеточных контактов между паллиальными клетками. Черные стрелки указывают на цитоплазматические отростки основных паллиальных клеток, черные наконечники — на микроворсинки супракорональных клеток, белые наконечники — на базальную пластинку.

Обозначения: a — передний конец; aj — адгезивный контакт (zonula adherens); ao — апикальный орган; c — корона; ci — ригидные реснички супракорональных клеток; crn — кольцевой нерв короны; gl — гликокаликс; oa — орган адгезии; p — задний конец; ppc — основная паллиальная клетка(и); rer — шероховатая ЭПС; sc — супракорональная клетка(и); sj — септированный контакт; vg — рудиментарный кишечник.

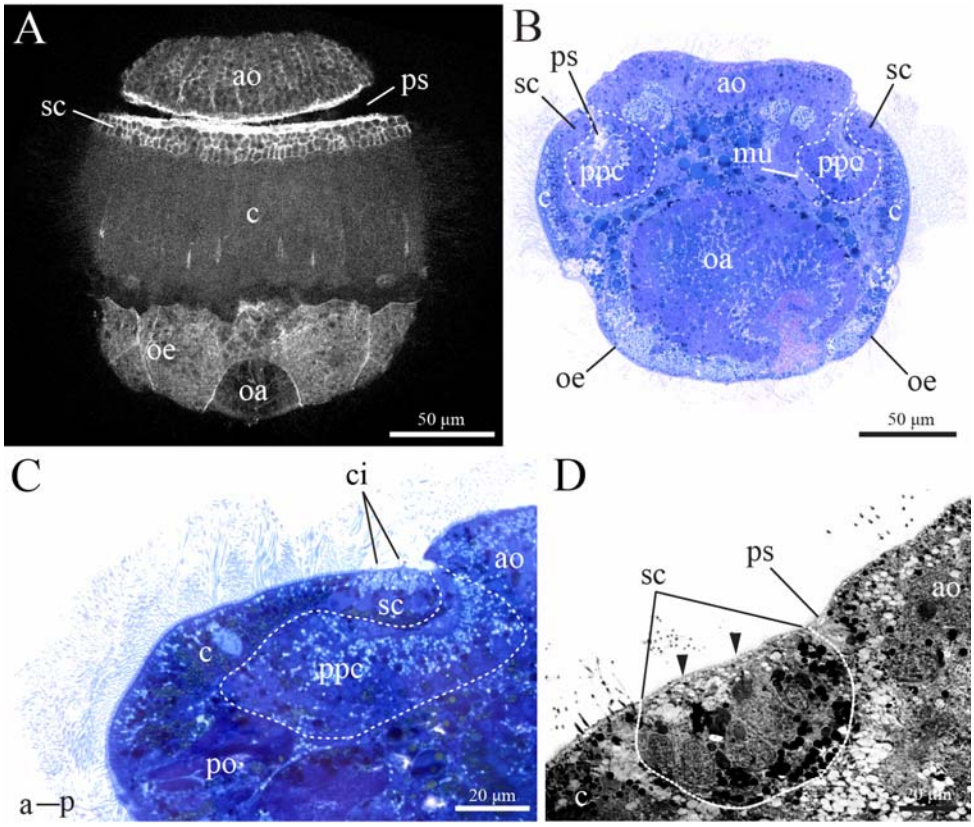

Fig. 6. Larval anatomy and ultrastructure of *Juxtacribrilina annulata* (Gymnolaemata: Cheilostomatida). A — CLSM, actin filaments staining with fluorescent phalloidin (confocal z-projection stack); B, C — stained semithin sections, light microscopy; D — transmission electron microscopy. A — general view of larval posterior side; B — frontal section of larva as in A (note part of muscle band attached to underside of pallial epithelium; white dotted line frames deep depression of principal pallial cells with slit-like pallial sinus (visible to left); C — sagittal section of larva (anterior end is oriented to *left*; white dotted line frames deep depression of principal pallial cells with slit-like pallial sinus; note short rigid cilia of supracoronal cells facing upwards); D — supracoronal cells (shown by white dotted line in same orientation as in C). Black arrowheads indicate microvilli of supracoronal cells.

Abbreviations: a — anterior region; ao — apical organ; c — corona; ci — short rigid cilia of supracoronal cells; mu — muscle cell(s); oa — adhesive organ; oe — oral epithelium; p — posterior region; po — pyriform organ; ppc — principal pallial cell(s); ps — pallial sinus; sc — supracoronal cell(s).

Рис. 6. Анатомия и ультраструктура личинок *Juxtacribrilina annulata* (Gymnolaemata: Cheilostomatida). A — CLSM, окраска актиновых филаментов флуоресцентным фаллоидином (z-стек проекций); B, C — окрашенные полутонкие срезы, световая микроскопия; D — трансмиссионная электронная микроскопия. A — общий вид личинки сзади; B — фронтальный срез личинки, как на A (обратите внимание на крепление мышечного пучка к нижней части паллиального эпителия; белая пунктирная линия показывает глубокое впячивание основных клеток паллиального эпителия, щелевидный синус виден слева); C — сагиттальный срез личинки (передний конец ориентирован влево; белая пунктирная линия показывает глубокое впячивание основных паллиальных клеток со щелевидным синусом; обратите внимание на короткие жесткие реснички супракорональных клеток, обращенные вверх); D — супракорональные клетки (показаны белой пунктирной линией, ориентация соответствует C). Черными наконечниками показаны микроворсинки супракорональных клеток.

Обозначения: a — передний конец; ao — апикальный орган; c — корона; ci — короткие ригидные реснички супракорональных клеток; mu — мышечная клетка(и); oa — орган адгезии; oe — оральный эпителий; p — задний конец; po — грушевидный орган; ppc — основная паллиальная клетка(и); ps — паллиальный синус; sc — супракорональная клетка(и).

corona occupying most of the larval surface except the large apical organ and oral hemisphere (Figs 6A, B; 9B). The apical organ and corona limit the pallial epithelium to a narrow ring zone composed of supracoronal cells, whereas the principal pallial cells are almost totally submerged forming a deep pallial sinus occupying the large part of the aboral hemisphere interior (Figs 6A–C; 9B).

The pallial epithelium consists of (1) principal pallial cells (Figs 6B, C; 9B) and (2) supracoronal cells (Figs 6A–D; 9B). The apical organ is surrounded by a conspicuous, deep depression of the pallial epithelium (pallial sinus) with a slit-like lumen (Figs 6B–D; 9B). A collarette of approximately 2–4 rows of superficial supracoronal cells is situated between the principal pallial cells and coronal cells (Fig. 6A–D). Both pallial and supracoronal cells are underlain by the thin basal lamina.

Principal pallial cells are mostly columnar or bottle-shaped with distinct polarity (Fig. 6B, C). The basal nucleus opposes the apical part of the cell with well-developed RER and numerous electron-lucent vacuoles. These cells secrete a fuzzy extracellular organic matrix filling the sinus (Fig. 6B). The apical membranes of the pallial cells at the sinus opening form short, interfolded cytoplasmic processes. The peripheral pallial cells that adjoin the supracoronal cells are flat and lack polarity. Supracoronal cells are 'superimposed' on these flattened cells (Fig. 6C, D).

Supracoronal cells are columnar, also exhibiting apico-basal polarity with lipid granules at the cell base, a central elongated nucleus, and electron-dense yolk platelets above it; some small electron-dense and lucid vesicles are located in the apical part of the cell (Fig. 6B–D). Their apical membrane forms numerous branched clavate microvilli embedded in the glycocalyx (Fig. 6D). At least some supracoronal cells bear cilia (Fig. 6C). Peripheral supracoronal cells adjoin the coronal ciliated cells by zonula adherens in the apical part. Confocal images show distinct actin filaments beneath the plasmalemma in these cells (Fig. 6A).

Patinella verrucaria (Figs 7; 9C)

The endotrophic larva of *P. verrucaria* is incubated intracoelomically in the colonial incubation chamber, and is nourished via the placenta (Borg, 1926; Nekliudova *et al*., 2021). After release, it lives about 1 hour before settlement (our data).

The larva is round (the bilateral symmetry is obscure) (average height 105 μm (N=13, range 76–117 μm), width or diameter 99 (measured without cilia)/138 (with cilia) μm (N=16/12, range 82/107–112/154 μm)) with two invaginations on the opposite poles — the apical one and the adhesive organ (Figs 7A, B; 9C). The coronal ciliary epithelium occupies almost the entire larval surface except for the upper central part of the aboral hemisphere (Figs 7A, C; 9C). A deep apical invagination of the pallial epithelium is situated in the center of the upper hemisphere. The walls of this cavity are covered by a thick folded cuticle, leaving only a few slit-like spaces (Fig. 7C, F). Pallial cells are loosely packed; the basal plate is absent (Fig. 7D–F).

In the pallial epithelium at least four cell types can be recognized: (1) principal pallial cells (Figs 7D–F; 9C), (2) intrapallial cells with lysosomes (Figs 7C, D; 9C), and poorly differentiated (3) infrapallial cells (Figs 7C, D; 9C) and (4) subpallial cells (Fig. 7C, E).

The principal pallial epithelium lines a cupped invagination with inwardly curved edges and a convex bottom (Fig. 7C). The overall cell shape is prismatic, cuboidal, or columnar, whereby the apical membrane facing the cuticle forms numerous long, wide and narrow cytoplasmic 'processes' corresponding to the folds of the cuticle (Fig. 7F). Most of these cells exhibit an electron-dense cytoplasm and large nucleus, elaborated RER, free ribosomes, mitochondria, and numerous vacuoles of various sizes, especially in their apical part adjacent to the cuticle. The cuticle average width is 11 μm. It is stratified with an electron-denser outer layer (Fig. 7F).

Intraepithelial cells with characteristic oval and round lysosome-like and endosome-like vacuoles of various sizes, with dark and light-gray content (metachromatically stained with Richardson technique indicating the acidic content) and uneven boundaries, were recorded near the curved edges of the apical invagination between the principal pallial cells (Fig. 7C, D). These cells varied in shape and size, with their basal part containing the nucleus and most of the lysosomes, being embedded between other pallial cells, while the apical part reached the cuticle.

The basal parts of some principal pallial cells and intrapallial cells with lysosomes along the edges of the invagination were surrounded by numerous cubic infrapallial cells forming from two to several layers (Fig. 7C, D). They surround the invagination of the aboral hemisphere including the invagination 'neck' and obturating the opening of the apical invagination. No principal pallial cells are present around the 'neck'. The outermost infrapallial cells constitute the apical larval surface, also forming wide, flattened processes covered by the glycocalyx, which is penetrated by long thin sparse microvilli (not shown in Figure 7). Some of these cells are connected to the coronal cells. Some infrapallial cells from the deeper layers form cytoplasmic processes wedging between the neighboring cells including the coronal ones, and sometimes extending inside the cuticle (Fig. 7C). Except for the superficial infrapallial cells, most of these cells are apparently

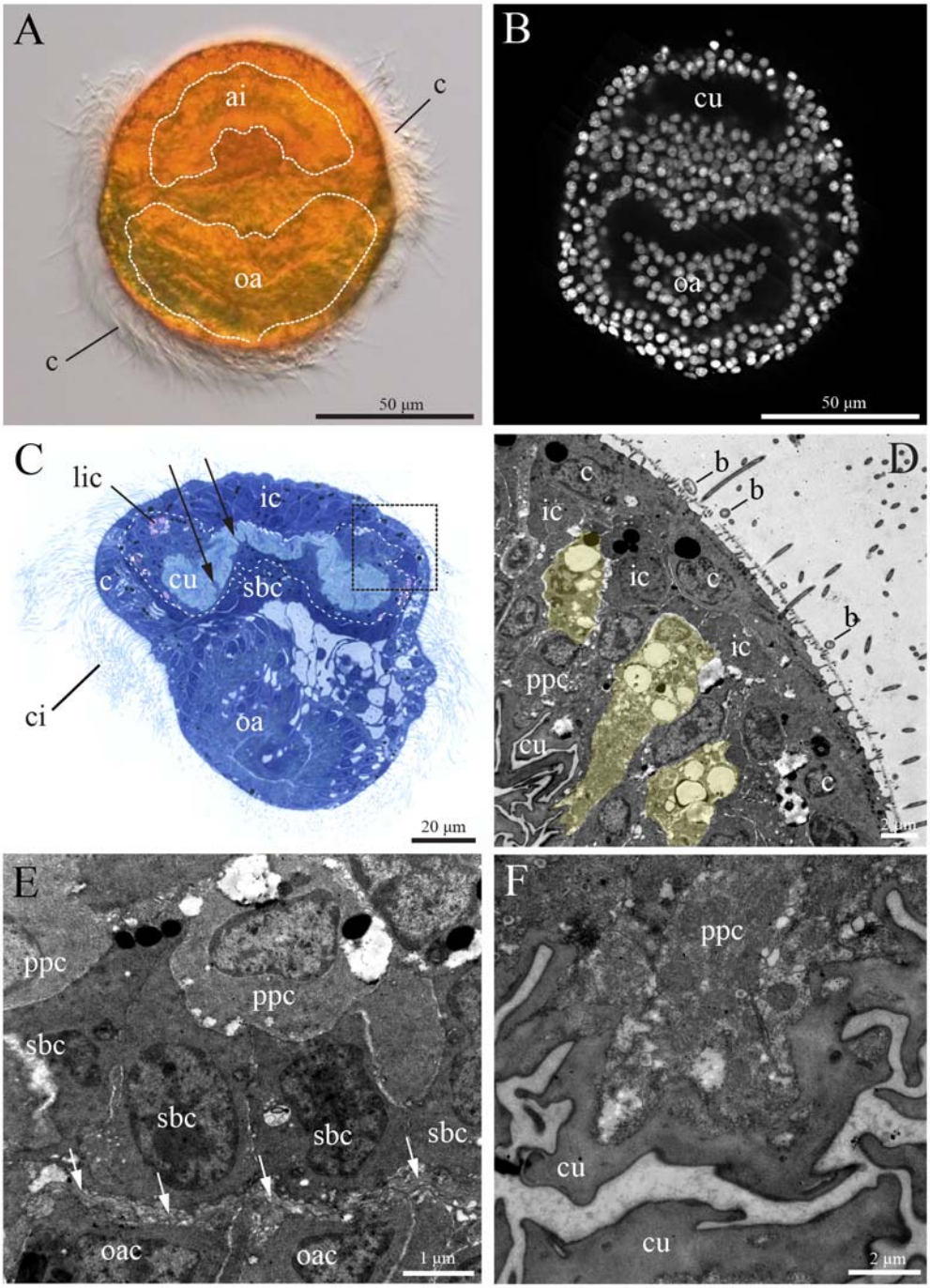

Fig. 7. Larval anatomy and ultrastructure of *Patinella verrucaria* (Stenolaemata: Cyclostomatida). A — light microscopy; B — CLSM, fluorescent labeling with DAPI (confocal z-projection stack); C — stained semithin section, light microscopy; D–F — transmission electron microscopy. A — general side view of larva showing apical invagination (note non-ciliated upper central part of aboral hemisphere), adhesive organ (both outlined by dotted lines) and corona; B — side view of the larva showing positions of apical

non-differentiated, having a high nuclear-cytoplasmic ratio, an electron-dense cytoplasm with numerous free ribosomes, and a large nucleus.

Similar non-differentiated cells are visible between the principal pallial cells and the adhesive organ (Fig. 7E). We were unable to establish their connection to infrapallial cells, so we called them subpallial, based on their position. Basally subpallial cells are separated from cells of the adhesive organ by a narrow zone filled with numerous cytoplasmic 'processes' of currently uncertain nature.

Numerous bacteria were detected between the cilia of the corona on our TEM images (Fig. 7D).

Discussion

Position of the pallial epithelium

In accord with the published data, our study showed that larvae from different clades markedly vary in size and shape as well as in shape and relative size of the aboral (epispheral) part of the outer epithelium, corona, and oral (hypospheral) part of the outer epithelium. In some gymnolaemates, larval episphere is convex, with the pallial epithelium sometimes secreting an organic (e.g., in *Flustrellidra. hispida*) or presumably slightly mineralized (e.g., *Electra pilosa*) protective shell (d'Hondt, 1977a, 2012; Zimmer, Woollacott, 1977a; Reed, 1991). In many myolaemate larvae, it is entirely or partly invaginated, forming a sinus between the apical organ and the corona or, in cyclostome larvae, a large invagination of the entire aboral epithelium (Nielsen, 1970; d'Hondt, 1977a, 1997; Zimmer, Woollacott, 1977a; Reed, 1991). Invaginated pallial epithelium clearly shows a derived state.

We found that in *Alcyonidium hirsutum*, similarly to *E.pilosa* and *F. hispida,* the pallial epithelium forms almost the entire larval episphere and never folds inwards/submerses.

In *Tendra zostericola*, the pallial epithelium is partially 'immersed' consisting of elongated, bottle-shaped cells with narrow apical parts facing the external environment and wider basal parts situated considerably deeper than the surrounding epithelial cells. Similar immersion is met in the larvae of some species from the genus *Alcyonidium* (see textfig. 3 in Seeliger, 1906; fig. 33 in Prouho, 1892; d'Hondt, 1973).

invagination and adhesive organ; C — longitudinal section of larva in position similar to A (white dotted line shows outer border of apical invagination; black arrows indicate cytoplasmic processes of principal pallial cells; black square shows area magnified in D); D — part of larva (corresponding to square area in C) showing various cell types (intrapallial cells with presumed lysosomes marked by yellow color; note numerous bacteria on larval surface); E — area beneath apical invagination and just above adhesive organ, showing principal pallial cells and subpallial undifferentiated cells (white arrows show numerous cytoplasmic "processes" in area between subpallial epithelium and adhesive organ); F — close-up of processes of principal pallial cell ajoined cuticle (note slit of apical invagination).

Abbreviations: ai — apical invagination; b — bacteria; c — corona; ci — cilia; cu — cuticle; ic — infrapallial cells; lic — intrapallial cell(s) with lysosomes; oa — adhesive organ; oac — cells of the adhesive organ; ppc — principal pallial cell(s); sbc — subpallial cell(s).

Рис. 7. Анатомия и ультраструктура личинок *Patinella verrucaria* (Stenolaemata: Cyclostomatida). A — световая микроскопия; B — CLSM, флуоресцентное мечение с использованием DAPI (z-стек проекции); C — окрашенный полутонкий срез, световая микроскопия; D–F — трансмиссионная электронная микроскопия. A — общий вид личинки, показывающий апикальное впячивание, орган адгезии (оба обведены пунктирными линиями) и корону (обратите внимание на верхнюю центральную часть аборального полушария, лишенную ресничек); B — общий вид личинки (ядра клеток помечены DAPI), показывающий положение апикальной инвагинации и адгезивного органа; C — продольный срез личинки в положении, аналогичном A (белая пунктирная линия ограничивает апикальное впячивание; черные стрелки указывают на цитоплазматические отростки основных паллиальных клеток; черным квадратом отмечен участок, увеличенный на D); D — часть личинки, соответствующая квадратной области на C; видны различные типы клеток (внутрипаллиальные клетки с предполагаемыми лизосомами, отмечены желтым цветом); обратите внимание на многочисленные бактерии на поверхности личинки; E — область под апикальным впячиванием чуть выше адгезивного органа; видны основные паллиальные клетки и субпалиальные недифференцированные клетки (белые стрелки указывают на многочисленные цитоплазматические "отростки" между субпаллиальным эпителием и адгезивным органом); F — крупный план отростков основных паллиальных клеток, прилегающих к кутикуле (обратите внимание на щель апикального впячивания).

Обозначения: ai — апикальное впячивание; b — бактерии; c — корона; cu — кутикула; ic — инфрапаллиальные клетки; lic — внутрипаллиальная клетка(и) с лизосомами; oa — орган адгезии; oac — клетки адгезивного органа; ppc — основная паллиальная клетка(и); sbc — субпаллиальная клетка(и).

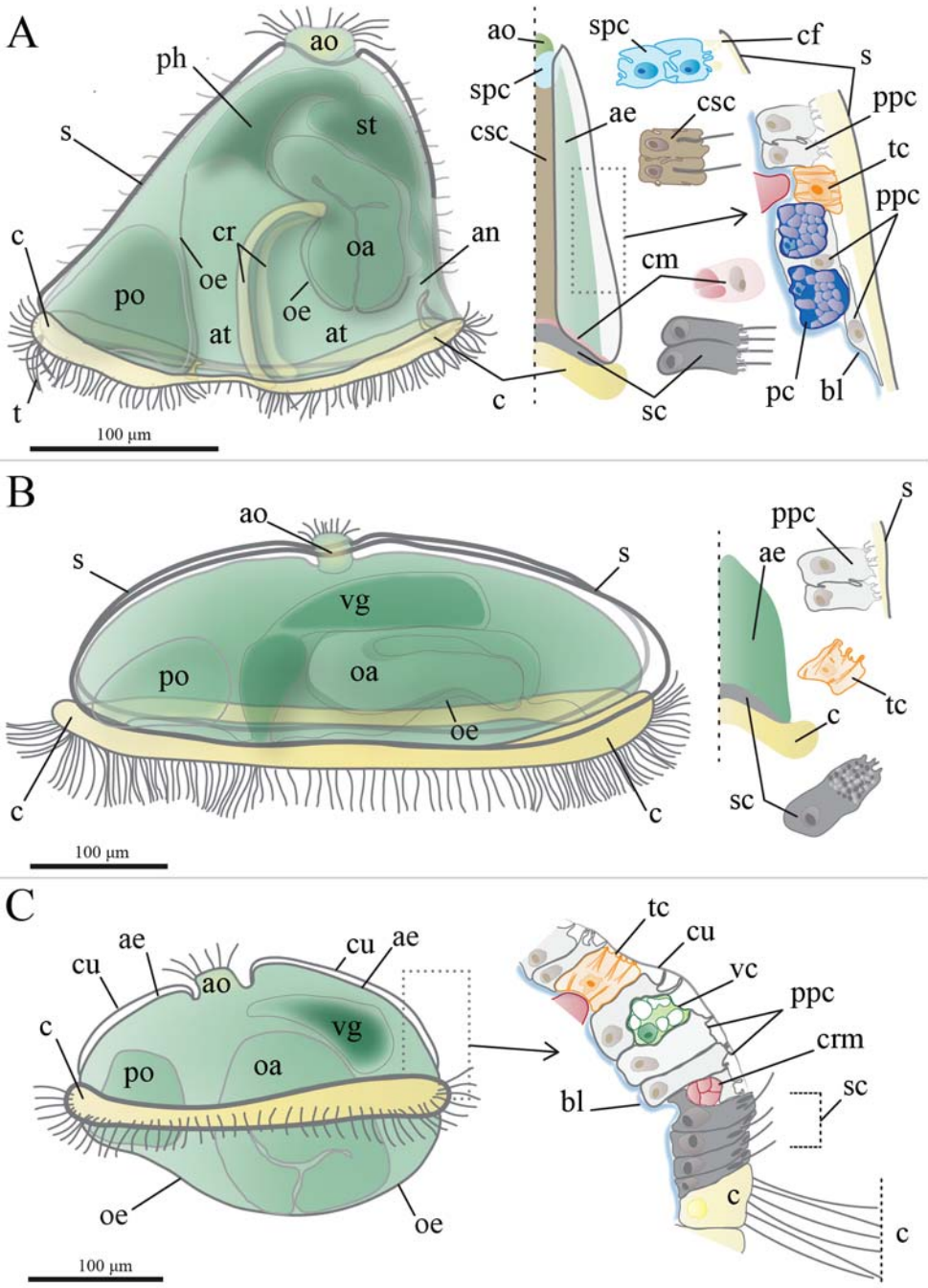

Fig. 8. Schematic representations of cyphonautes of *Electra pilosa* (A), pseudocyphonautes of *Flusrellidra hispida* (B) and hat-shaped larva of *Alcyonidium hirsutum* (C); anterior ends are oriented to *left*; internal cilia omitted; different colours show supposed homology of parts of pallial epithelium of larvae in different species, as well as supposed homology of different cell types. A — *left* — lateral view (left shell valve omitted), *right* — frontal view from posterior end (cilia omitted) showing various cell-types of pallial epithelium;

In contrast, in *Juxtacribrilina annulata* (as in closely related species *J. corbicula* (O'Donoghue et O'Donoghue, 1923) and many other coronate larvae, see d'Hondt, 1977a; Zimmer, Woollacott, 1977a; Reed, 1991), the pallial epithelium is almost totally invaginated, forming a more or less deep ring-shaped groove (pallial sinus) with a slit-like cavity that communicates with the external environment. The bottle-shaped principal pallial cells line this sinus and their apically narrowed parts face the slit.

In *Patinella verrucaria* and other studied cyclostome larvae, whose aboral hemisphere bears a deep invagination (d'Hondt, 1977b; Zimmer, Woolacott, 1977a; Nielsen, 1970; Nielsen *et al*., 2019), only a small hole remains on the top; the apical organ is missing. Similar to coronate larvae, the invagination is lined with the principal pallial cells.

Altogether, the immersion of the pallial epithelium in the myolaemate larvae can be considered as an evolutionary trend with cyclostome larvae showing its ultimate stage. It should be mentioned that at the initial stages of development the pallial epithelium is not invaginated in the cyclostome larva and a cuticular cap can be seen at its top (Nielsen, 1970).

The submersion of the pallial epithelium is accompanied by an enlargement of the corona that often spreads over almost entire larval surface. A noticeable increase in the area of locomotory corona relative to the rest of the larval outer epithelium is unlikely connected with a distant larval dispersal involving long-term swimming, since both coronate and cyclostome larvae spend in the water column from several minutes to a few hours before settlement. We hypothesize that these transformations of the larval outer epithelium presumably correlate with the multiple independed emergence of embryonic incubation in Bryozoa (for reviews see Ostrovsky, 1998a, b, 2013a; Ostrovsky, Taylor, 2004, 2005; Schwaha *et al*., 2018, 2019, 2020; Nekliudova *et al*., 2021). It was assumed that the acquisition of incubated lecithotrophic larvae, based on the shift from small oligolecithal to large macrolecithal oocytes could cause problems when large eggs had to be delivered to the brooding site via tiny coelomopore, at least in some species (Silén, 1945); Ström, 1977; Ostrovsky, Porter, 2011; Ostrovsky, 2013a). Note, however, large oocytes quickly pass through the coelomopore without squeezing in *Fenestrulina malusii* (Audouin, 1826) (Nielsen, 1981). One more problem is how the mature larva can release out of the incubation chamber with relatively small opening (Nielsen, 1981; Reed, 1991). It is also important to note that in

B — *left* — lateral view, *right* — frontal view from posterior end showing various cell-types of pallial epithelium; C — *left* — lateral view, dotted line marks part of pallial epithelium enlarged in *right*, *right* — part of the pallial epithelium showing various cell types; coronal cilia cut off.

Abbreviations: ae — aboral (pallial) epithelium; an — anus; ao — apical organ; at — atrium; bl — basal lamina; c — corona; cf — cuticular 'film'; cm — cross-striated epitheliomuscular cell(s); cr — ciliary ridge(s); crm — coronal ring muscle; csc — ciliary secretory cell(s) of anterior and posterior marginal areas between valves; cu — cuticle; oa — adhesive organ; oe — oral epithelium; pc — 'polster' cell; ph — pharynx; po — pyriform organ; ppc — principal pallial cell(s); s — shell; sc — supracoronal cell(s); spc — suprapallial secretory cell(s); st — stomach; t — ciliary tuft of pyriform organ; tc — tendon cell; vc — vacuolated cell; vg — vestigial gut.

Рис. 8. Схематические изображения цифонаута *Electra pilosa* (A), псевдоцифонаута *Flusrellidra hispida* (B) и шляповидной личинки *Alcyonidium hirsutum* (C); передние концы ориентированы влево; внутренние реснички не показаны; различными цветами показаны предполагаемая гомология частей паллиального эпителия личинок разных видов, а также предполагаемая гомология различных типов клеток. A — *слева* — вид сбоку, *справа* — вид с заднего конца (показаны различные типы клеток паллиального эпителия); B — *слева* — вид сбоку, *справа* — вид с заднего конца (показаны различные типы клеток паллиального эпителия); C — *слева* — вид сбоку, пунктирной линией отмечен участок паллиального эпителия, показанный справа, *справа* — участок паллиального эпителия (показаны различные типы клеток, реснички короны обрезаны).

Обозначения: ae — аборальный (паллиальный) эпителий; an — анальное отверстие; ao — апикальный орган; at — атриум; bl — базальная пластинка; c — корона; cf — кутикулярная 'пленка'; cm — поперечнополосатая эпителиально-мышечная клетка(и); cr — ресничные гребни; crm — кольцевая мышца короны; csc — ресничные секреторные клетки переднего и заднего участков эпителия между створками раковины; cu — кутикула; oa — орган адгезии; oe — оральный эпителий; pc — "подушковидная" клетка; ph — глотка; po — грушевидный орган; ppc — основная паллиальная клетка(и); s — раковина; sc — супракорональная клетка(и); spc — супрапаллиальные секреторные клетки; st — желудок; t — пучок ресничек грушевидного органа; tc — тендоцит; vc — вакуолизированная клетка; vg — рудиментарный кишечник.

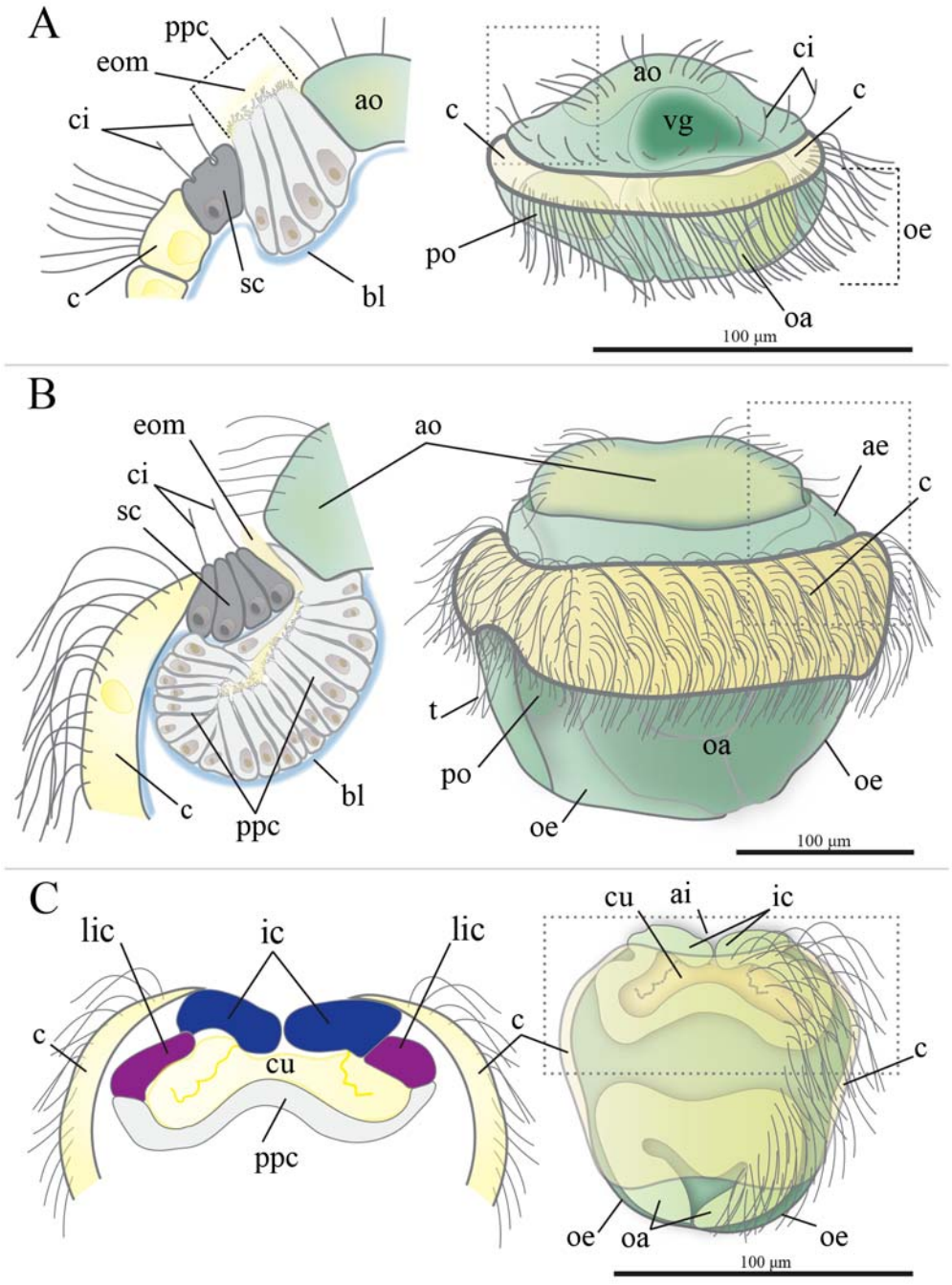

Fig. 9. Schematic representations of hat-shaped larva of *Tendra zostericola* (A), coronate larva of *Juxtacribrilina annulata* (B) and larva of cyclostome *Patinella verrucaria* (C); in A, B anterior ends are oriented to *left*, in C aboral pole is above (only coronal cilia on the right part of body are shown); different colors show the supposed homology of parts of pallial epithelium in larvae of different species, as well as supposed homology of different cell types. A, B — *right* — lateral view, rectangular area marks part of pallial

many myolaemate lineages, the larval development was supplemented by extraembryonic nutrition, resulting in a considerable increase in larval size (e.g., Borg, 1926; Woollacott, Zimmer, 1975; Dyrynda, King, 1982; Hughes, 1987; Moosbrugger *et al*., 2012; Ostrovsky, 2013b; Schwaha *et al*., 2019; Nekliudova *et al*., 2021; Bibermair *et al*., 2021). Thus, the oocyte-larval large size could conflict with the size of the much smaller opening of incubation chamber.

We suggest that the progressive immersion of the pallial epithelium accompanied by the development of a large and powerful locomotory corona, sometimes with the acquisition of well-developed body-wall musculature, provides (1) retaining the stable/smaller larval size corresponding to the size of the egg and the brooding space throughout postembryonic development, (2) a successful release of large non-feeding larvae through a much smaller opening of the incubation chamber and (3) active larval swimming and crawling (both rather short, however) in search of the suitable spot on the substrate for settlement. Similarly, some small-sized demersal larvae of epibiotic invertebrates that swim along the substrate in water layer of higher viscosity (Chia *et al*., 1984), are usually completely covered by cilia (e.g., crawling demersal planulae of some cnidarians, Piraino *et al*., 2011).

In the larvae of some brooding ctenostomes that incubate several embryos on the surface of the zooid when lophophore is expanded and inside vestibulum when polypide is retracted (e.g. in *Bulbella abscondita* Braem, 1951), or in the voluminous space of the modified tentacular sheath (e.g., *F. hispida*, *A. hirsutum*, *Pherusella minima* Decker *et al*., 2021*,* and some others (Ström, 1977)), the pallial epithelium is expanded, and the corona remains narrow relative to the episphere. Also in ctenostomes the larval release from the modified tentacular sheath has not been studied in detail, but in *P. minima* (as *Pherusella* sp.) having pseudocyphonautes similar to *F. hispida*, it can take several days and requires contractions of the vestibular wall of the maternal zooid to release larvae through the peristome (Decker *et al*., 2020). In both *F. hispida* and *P. minima*, embryos grow during development (suggesting matrotrophy), which might further complicate the release of large larvae.

In contrast, the vast majority of incubating myolaemates possess larvae with a relatively large, well-developed corona and a deeply invaginated pallial epithelium, which we consider to be an advanced state (d'Hondt, 1977a; Zimmer, Woollacott, 1977a). This includes some ctenostomes, e.g., *Victorella muelleri* (Kraepelin, 1887) (as *Tanganella* in Zimmer, Reed, 1994) and *Amathia* (as *Bowerbankia* in Reed, 1978; Reed, Cloney, 1982a; reviewed in d'Hondt, 1997) that brood their embryos in the vestibulum or in the tentacle sheath, similar to *F. hispida* and *A. hirsutum*. During development, the embryo of matrotrophic *Amathia* greatly increases occupying most of the brooding zooid (see, e.g., Schwaha *et al*., 2019); thus, the expanded locomotory corona could be a major 'tool' for larval release (Reed, 1988).

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epithelium enlarged in *left*; C — *right* — side view, rectangular area marks part of pallial epithelium enlarged in *left; left* — only outer borders of epithelium are shown.

Abbreviations: ae — aboral (pallial) epithelium; ai — apical invagination; ao — apical organ; bl — basal lamina; c — corona; ci — cilia of supracoronal cells; cu — cuticle; eom — extracellular organic matrix; ic — infrapallial cells; lic — intrapallial cell(s) with lysosomes; oa — adhesive organ; oe — oral epithelium; po — pyriform organ; ppc — principal pallial cell; sc — supracoronal cell(s); t — ciliary tuft of pyriform organ; vg — vestigial gut.

Рис. 9. Схематические изображения шляповидной личинки *Tendra zostericola* (A), коронатной личинки *Juxtacribrilina annulata* (B) и личинки циклостомат *Patinella verrucaria* (C); на A, B передние концы ориентированы влево, на C аборальный полюс ориентирован вверх; разные цвета показывают предполагаемую гомологию частей паллиального эпителия личинок разных видов, а также предполагаемую гомологию разных типов клеток. A, B — *справа* — вид сбоку, прямоугольная область отмечает увеличенную часть паллиального эпителия *слева*; C — *справа* — общий вид, прямоугольной областью отмечена увеличенная часть паллиального эпителия *слева*.

Обозначения: ae — аборальный (паллиальный эпителий); ai — апикальное впячивание; ao — апикальный орган; bl — базальная пластинка; c — корона; ci — реснички супракорональных клеток; cu — кутикула; eom — внеклеточный органический матрикс; ic — инфрапаллиальные клетки; lic — интрапаллиальные клетки с лизосомами; oa — орган адгезии; oe — оральный эпителий; po — грушевидный орган; ppc — основная паллиальная клетка; sc — супракорональная клетка(и); t — пучок ресничек грушевидного органа; vg — рудиментарный кишечник.

Most incubating cheilostomes also have typical coronate larvae with a deep pallial sinus and a large corona covering the whole body except apical organ and small part of the oral hemisphere (d'Hondt, 1977a; Zimmer, Woollacott, 1977a). The few known exceptions fall in two groups. The first group comprises clades that acquired embryonic incubation and non-feeding larvae independently from the remaining so-called 'neocheilostomes' (Ostrovsky, 2013a). Among them the larvae of only three genera — *Tendra*, *Scruparia*, and *Thalamoporella*, were described showing such plesiomorphic traits as convex episphere and relatively narrow corona (Repiachoff, 1875; Barrois, 1877; Marcus, 1939; d'Hondt, 1997, 2016, 2018). We found, that the pallial epithelium of *T. zostericola* larvae does not form a sinus, although it shows signs of 'cell immersion'. In this species, several larvae develop in the voluminous space (epistege) between the frontal spines and the frontal zooidal membrane of the brooding zooid (the so-called acanthostegal brood chamber). The larval release takes 10–20 minutes, whereby the larva flattens and slowly squeezes through the brood chamber opening (Braiko, 1967). Noteworthy, despite its rather narrow corona, *T. zostericola*, unlike other studied larvae, has the longest cilia of the corona cells in relation to the body size. The second group of exceptions comprises some 'neocheilostomes' with the relatively narrow corona (e.g., *Watersipora*). Although their larvae retain the deep pallial sinus, the epithelium of the oral hemisphere is (secondarily?) expanded (Zimmer, Woollacott, 1989; Reed, 1991). The larvae of *J. annulata* we described, have similarly 'narrow' corona and large oral ciliated hemisphere. The same was mentioned by Reed in 1991 for the larvae of *J. corbicula* (as *Cribrilina*). Activity of cilia of the oral hemisphere potentially can compensate the narrow corona

Finally, we suggest that phylactolaemates (Reed, 1991; Gruhl, 2021) and cyclostomes followed the same evolutionary vectors, enlarging locomotory ciliary cover and immersion of the pallial epithelium.

The development of an extensive corona did not prevent the nutrient absorption and endocytosis in phylactolaemate, cyclostome, and some coronate larvae from the fluid of the incubation cavity during development. Although endocytosis occurs from all sides of the developing embryo via its superficial cells (Moosbrugger *et al*., 2012; Ostrovsky, 2013b; Schwaha *et al*., 2019; Nekliudova *et al*., 2019a, 2021; Bibermair *et al*., 2021), the pallial epithelium probably is the least suited for this function, as it is either covered by a protective cuticle or shell, or immersed.

Fine structure of the pallial epithelium

Several cell types were described in the pallial epithelium of the myolaemate larvae (Stricker *et al*., 1988; d'Hondt, 1975, 2012), and d'Hondt (1977a) considered their position to be one of the key features of bryozoan larval types. In the larvae we studied the pallial epithelium is composed of 2–7 cell types. The principal pallial cells, tendon and supracoronal sensory cells are the most common. Other types are confined to certain species and/or larval types. All the aboral epithelial cells are connected by a typical set of contacts characteristic for other invertebrates (Tyler, 2003), with the apicalmost zonula adherens. In addition, in *E.pilosa, F. hispida, A. hirsutum*, and other bryozoan larvae with a convex episphere, the pallial cells often form interdigitations that provide a large contact area, and, presumably, allow the epithelium to stretch and shrink during larval contractions.

I. Principal pallial cells

The most common type is principal pallial cells, usually forming a typical palisade epithelium with apico-basal polarity, and underlain (except *P. verrucaria*) by a variously developed basal lamina. This epithelium was recorded in all studied larvae and considered as homologous across Myolaemata (Mukai *et al*., 1997; see also Supplementary Files 1, 2 and references therein). The absence of the basal lamina in *Patinella* (and probably other cyclostomates) is possibly associated with the unique features of their early development (i.e., irregular cleavage, fission of embryos).

In the larvae of *E. pilosa* and *F. hispida*, the principal pallial cells have different shapes, being columnar, cuboidal (in the upper part of the larva), or flattened, sometimes nearly squamous (Kupelwieser, 1905; d'Hondt, 1977c; our study). In the latter case, these cells usually lose their polarity, which is most likely accompanied by a change in function, for example, the loss of

secretory ability. In both cyphonautes and pseudocyphonautes, cell deformation can be explained by larval growth, which involves a significant increase in the size of the larvae compared to the initial embryo (*E. pilosa* — four times, *F. hispida* — three times). The cell deformation was also observed to be a result of the 'pressure' of the neighboring 'polster' (*E. pilosa*), vacuolated (*A. hirsutum*), or infrapallial cells (*P. verrucaria*). This also happens along the margins of the pallial epithelium zone as a result of its immersion (in *T. zostericola*) or invagination (in *J. annulata* and *P. verrucaria*).

When the principal pallial cells are interspaced with the aforementioned specialized cells that have an intraepithelial position and neither contact the basal lamina nor reach the larval surface, the epithelium becomes pseudo-stratified. In *E. pilosa*, the principal pallial cells are partially overlapped in some areas, giving the impression of a bi- or even three-layered epithelium. This might provide higher mechanical stability, especially in the areas where cells are flattened.

Our data confirmed the statement of d'Hondt (1979) that principal pallial cells are devoid of cilia. Noteworthy, Zimmer & Woollacott (1977a) and Gruhl (2021) depicted cilia on the surface of the episphere of the hypothetical gymnolaemate larva, presumably suggesting this as a plesiomorphic state. Indeed, some bryozoan larvae have ciliated cells in the epispheral part of the body, for example, cells between valves (in cyphonautes) and supracoronal cells. Since they never form the entire episphere, we suggested they evolved secondarily as sensory cells.

The ultrastructure of principal pallial cells (primarily the presence of RER and various vacuoles) indicates high synthetic activity. In all larvae we studied, these cells (except flattened) produce the variously developed external extracellular matrix; usually, their apical membrane forms irregular cytoplasmic processes that contact, but did not penetrate it. The extracellular matrix can resemble a fuzzy coat (*T. zostericola, J. annulata,* and most coronate larvae studied) or can form a thick, folded (but flexible?) cuticular cover as in *P. verrucaria* and other cyclostome larvae (d'Hondt, 1977b; Nekliudova *et al*., 2021). It can also form a more or less rigid cuticle (*A. hirsutum*) or even a shell (*E. pilosa*, *F. hispida*, *P. minima*, *B. abscondita* and other cheilostome and ctenostome shelled cyphonautes and pseudocyphonautes (Braem, 1951; Stricker *et al*., 1988; d'Hondt, 2012)), mineralized in some cheilostome larvae. We assume that the suprapallial cells in cyphonautes of *E. pilosa*, as well as secretory cells of the anterior and posterior regions between shell valves in this species and in pseudocyphonautes of *F. hispida* (see below), can be involved in the shell formation. In the cyphonautes of *E. pilosa*, most of the cells of the pallial epithelium on the lateral sides are flattened and lost their synthetic activity.

The genus *Alcyonidium* represents the most striking example of the diversity of the extracellular matrix. The cyphonautes-like larva of the zygote-spawning *A. albidum* has a rigid cuticle or a shell (Prouho, 1892), the apical hemisphere of the brooded larvae of *A. hirsutum* is covered by a cuticular 'film' (this study), whereas the brooded larvae of *A. polyoum* (Hassall, 1841) (with slightly immersed pallial epithelium) have no recognizable cuticle (d'Hondt, 1972, 1973), which could be a result of a secondary loss.

We found that in all larvae except *J. annulata* the ultrastructure of the uppermost layer of the cuticle or shell differs from the rest in being more electron-dense (although not always 'solid'). In *E. pilosa*, *A. hirsutum*, and *T. zostericola* this layer resembles remnants of the fertilization envelope, which corresponds to the observations by Mawatari & Mawatari (1974) and by Hageman (cited in Reed, 1991) on the cyphonautes of *Membranipora villosa* Hincks, 1880 (as *M. serrilamella)*. These authors suggested that the shell valves in the cyphonautes are formed from the fertilization envelope broken up by apical and coronal cilia. Possibly later it is used as a base for a developing shell, although in much larger adult cyphonautes it cannot cover the entire valve. Whether the fertilization envelope is resistant in this and other larvae (except cyclostomes, whose larvae develop from the secondary embryos that lack such an envelope) requires further study.

In the absence of paleontological data, we can only speculate whether the larvae of the ancestral bryozoans had a shell. Since the two-valved shell in the larvae of some cheilostome and ctenostome bryozoans is fundamentally the same (except its slight mineralization in cheilostomes), possibly it has evolved just once in the originally non-shelled (although, covered by

cuticle) larva of the ancestral form. Potentially the cuticle could be secreted due to the heterochronous activity of the larval pallial cells although originally should appear much later in development, in the ancestrular stage (see p. 133 in Zimmer, Woollacott, 1977b). In some myolaemates the larval cuticle is later used for the protection of forming ancestrula. We further suggest that the protective mineralized shell of the cyphonautes has evolved because of its extended free-swimming period. In most species that have acquired an embryonic incubation, the cuticle is usually markedly reduced due to the submersion of the pallial epithelium. The exceptions are cyclostomes and some ctenostomes with prolonged brooding — *Flustrellidra*, *Pherusella* and a few others (e.g., *A. hirsutum*) with non-invaginated pallial epithelium.

II. Additional pallial cell types

Additional specialized cell types were recognized in the larvae of most of the studied species. These include intrapallial cells (tendon cells, 'polster' cells, vacuolated cells, and cells with lysosomes), and supra-, infra- and subpallial cells.

Tendon cells

In bryozoan larvae, tendon cells originally were described by d'Hondt (1979) as muscular insertion cells in *F. hispida* and *Alcyonidium polyoum*. They are very similar to the tendon cells found in the integument of other invertebrates (e.g., Lai-Fook, 1967; Bairati, Gioria, 2008; Ponder *et al*., 2020). We redescribed these cells in the pseudocyphonautes of *F. hispida* and recorded them in *E. pilosa* and *A. hirsutum*. In the larvae of these three species, tonofilaments and hemidesmosomes are very prominent, providing both stable anchor points for muscle attachment to the tendon cells and a strong connection of the pallial epithelium to the shell/cuticle. According to Tyler (2003), invertebrate hemiadherens junction may be misinterpreted as hemidesmosomes, but this requires further study.

We did not find tendon cells in larvae of other studied species. However, our semi-thin sections show that, at least in the larvae of *J. annulata*, muscle bundles are attached directly to the pallial epithelium. Since the larva of *J. annulata* has no cuticle or shell, the pallial epithelium is probably unable to resist muscle contractions. Most likely, it is these muscles that pull it inwards, so that it eventually completely involutes by the time the larva leaves the brood chamber. The same is typical for *P. verrucaria* and other cyclostome larvae, in which the pallial epithelium is not invaginated at the initial stages of development (Nielsen, 1970). It invaginates later (Nielsen, 1970, Nielsen *et al*., 2019), probably after the formation of longitudinal muscle cells and prior to the hardening of the cuticle.

'Polster' cells

These cells were first described in the cyphonautes of *E. pilosa* by Kupelweiser (1905), who thought that they protect the apical organ and corona (from 'polster' (German) — pad). Later, Stricker and co-authors (1988) found them in the cyphonautes of related *Membranipora membranacea* (Linnaeus, 1767), suggesting that their main function is to resist muscle contraction during metamorphosis, along with tendon cells. However, we believe that the 'polster' cells can also function as storage cells for metamorphosis, since *Electra* and other free-spawning species have oligolecithal eggs and must accumulate reserves for successful development. This is confirmed by the fact that the number and volume of 'polster' cells increase during the feeding period of cyphonautes in the plankton. It is also possible that these cells may be involved in the secretion of the larval shell or ancestrular pellicula.

Vacuolated cells

Pallial cells with characteristic clumped vacuoles originally were described by d'Hondt (1973) in the larva of *A. polyoum*. In our turn, we found similar cells in the larva of *A. hirsutum*. Considering position and presence of vacuoles, these cells are similar to the 'polster' cells of cyphonautes. We think that these vacuoles can participate in the formation of a dense ancestrular pellicula after the larva has settled. Interesingly, similar-looking cells are present in the preoral lobe of actinotroch larvae of some phoronids (Temereva, Tsitrin, 2014).

Cells with lysosomes

Some intrapallial cells seemingly containing lysosomes were found in the larvae of *P. verrucaria*. We suggest that these cells could

perform a cell 'digestion' since the larvae of this species grow being supported by the placental nutritive tissue, acquiring nutrition via endocytosis (see Nekliudova *et al*., 2021).

Supra-, infra- and subpallial cells

In the cyphonautes of *E. pilosa* the principal pallial cells are flanked from above by the suprapallial secretory cells that presumably participate in the formation of the shell cuticle (see above).

Remarkably, we found that in *E. pilosa* cyphonautes, the aboral pallial epithelium includes cross-striated epitheliomuscular cells that form an infrapallial ring just above the supracoronal sensory cells. These cells appear to correspond to the coronal ring muscle, described by previous authors as the muscle running underneath the coronal cells (Gruhl, 2008).

In the larvae of *P. verrucaria* non-differentiated infrapallial cells and the additional layer of subpallial undifferentiated cells were found. The latter may represent the mesoderm of incipient polypide rudiment (see Nielsen, 1970 and Nielsen *et al*., 2019 for another cyclostome *Crisia eburnea* (Linnaeus, 1758)).

Sensory cells

One of the most striking features of the aboral epithelium of gymnolaemate larvae is the presence of mono- or biciliated supracoronal cells, usually forming one to several rows in episphere just above the corona (Stricker *et al*., 1988; Zimmer, Woollacott, 1989; Reed, 1991; Zimmer, Reed, 1994; Santagata, Zimmer, 2000). Using TEM, we redescribed them in *E. pilosa* cyphonautes, confirmed their presence in *J. annulata* coronate larva (see Kupelwieser, 1905 and Nikulina, 1998 for original description), and found them in the larvae of *A. hirsutum* and *T. zostericola.* Interestingly, in contrast to *J. annulata*, in a closely related species *J. corbicula*, supracoronal cells were described as showing signs of non-differentiated cells with no cilia (see Addendum in Reed, 1991).

Although ultrastructural characteristics of supracoronal cells differ slightly between the larvae of the studied species, they are presumably primary sensory cells possessing mechanoreceptors. These cells usually have the non-motile cilia projecting from the deep pit and facing upwards relative to the cilia of the corona. In *T. zostericola,* supracoronal cell cilia seem to lack a ciliary root as was described in the uniciliate sensory cells of some oligochaetes (Welsch *et al*., 1984); therefore, their sensory function is obscure. We should stress that in some cheilostome coronate larvae (e.g., in *Celleporaria brunnea* and *Schizoporella errata*), complex sensory organs have evolved from the supracoronal cells some of which produce a shielding pigment for the primary eyespots (Santagata, Zimmer, 2000; Reed, 1991).

Ciliated secretory cells are also known to develop between shell valves in the cheilostome cyphonautes (Stricker *et al*., 1988 and our data). Presumably, they are multifunctional; in addition to the rigid cilia, they are characterized by many secretory granules with flocculent content occupying the upper half of the cell and well-developed RER and Golgi apparatus. These cells are presumed to be neuro-secretory or glandular, possibly providing the growth of mineralized shell along anterior (predominantly) and posterior valve margins. Interestingly, in some invertebrates (Gnathostomulida) mucous cells are presumably formed from monociliated cells since they are found to possess degenerating cilia, ciliary pits, and rootlets (Storch, 1984). We should point out, that in pseudocyphonautes of *F. hispida* pallial epithelial cells along the anterior and posterior margins in between the shell valves contain a greater number of secretory vacuoles than most of the principal pallial cells, suggesting their participation in the shell formation. Unlike in cyphonautes of *E. pilosa*, these cells have no cilia, thus their homology to ciliated secretory cells forming anterior and posterior marginal zones between the valve edges in cyphonautes is not clear.

In contrast to the principal pallial cells, the supracoronal and ciliated secretory cells generally form typical microvilli. They are normally embedded in the glycocalyx layer and have inflated tips, that extend beyond the cuticle, like in sensory cells in many other invertebrates (e.g., kamptozoans (Storch, 1984)).

The fate of pallial epithelial cells during metamorphosis

Bryozoans have a catastrophic metamorphosis with the reduction of most larval organs and tissues (reviewed in Reed, 1991; Zimmer,

Woollacott, 1977b; Mukai *et al*., 1997). Nevertheless, some of the ectodermal derivatives including aboral epithelium are definitely involved in the formation of the body wall of ancestrula.

The post-metamorphic fate of ectodermal derivatives has been studied for at least seven myolaemate genera (including *Electra*, *Flustrellidra*, and *Alcyonidium*) (reviewed in Zimmer, Woollacott, 1977b; d'Hondt, 1979; Reed, 1991; Mukai *et al*., 1997). In most cases, the principal pallial cells of the aboral outer epithelium constitute the prospective upper wall of the preancestrular cystid, primarily the frontal wall. This was recorded in *E. pilosa*, *F. hispida*, and several other gymnolaemate species with convex larval episphere (d'Hondt, 1974, 1977c, 1997; Zimmer, Woollacott, 1977b). In some larvae with convex episphere, the aboral outer epithelium forms the whole ancestrular cystid (e.g., *B. abscondita* (Braem, 1951)). It is also involved in the formation of the tentacular sheath, and/or vestibulum in some species (d'Hondt, 1974b, 1977a, c).

As to the coronate larvae with a pallial sinus, its cells can form the entire ancestrular wall in some ctenostomes (e.g., in *Amathia* (as *Bowerbankia* in d'Hondt, 1977d; Reed, Cloney, 1982b; Lyke *et al*., 1983) and *Victorella muelleri* (as *Tanganella* in Zimmer, Reed, 1994)). In cyclostomes (Nielsen, 1970; d'Hondt, 1977b), and in most cheilostomes, the larval pallial epithelium (principal pallial cells) usually gives rise to the frontal wall of the ancestrular cystid, and to a thin squamous epithelium of tentacular sheath and/or vestibulum (e.g., in *Bugula*, Woollacott, Zimmer, 1971, 1978; Reed, Woollacott, 1982; *Watersipora* sp. and *Juxtacribrilina* (as *Cribrilina*) *corbicula* (Lyke *et al*., 1983; Reed, 1991, Addendum).

Thus, in cyclostome and gymnolaemate larvae with a deep pallial sinus, principal pallial cells seem to play no role during the larval stage, developing within the epispheral part of the larval body as pre-patterned adult tissues. In contrast, in the larvae of other lophotrochozoan groups, the adult rudiments/blastemas more often develop within the hyposphere. The examples are postlarval segments of various polychaete endolarvae, the shell gland of gastropod and bivalve veligers. Yet, the epithelium of the episphere in lophotrochozoan larvae usually does not form submersed/invaginated rudiments (for example, see Ivanova-Kazas, 1977). In this respect, the pilidium larvae of nemerteans and the larvae of some bryozoans are exceptions.

Considering that in the larvae of *A. hirsutum* and *T. zostericola* the principal pallial cells form a convex episphere as in cyphonautes and pseudocyphonautes, we can hypothesize that they give rise to the frontal ancestrular wall (with the cuticular covering) too. We do not know the fate of the principal pallial cells of other studied species yet. Also, little is known about the destiny of other cell types constituting the epispheral region in the bryozoan larvae. Addressing these issues helps us to clarify tissue homology between various larval types (d'Hondt, 1975).

Conclusions

In bryozoan larvae, the pallial epithelium is among the most variable structures in terms of position to the other tissues, number of cell types, and functions. We suggest that this variation could be related to the acquisition of incubation and a concomitant change in larval biology in various bryozoan lineages. Indeed, exo- and endotrophic larvae develop in the totally different environment: either in plankton or inside the incubation chambers of different morphotypes (in some cases acquiring nutrition from maternal zooid).

In all myolaemate larvae studied to date, principal pallial cells are covered by an external extracellular matrix. It can be more or less rigid and, together with the pallial epithelium itself, can act as a muscle antagonist. Following Zimmer & Woollacott (1977b) we consider the presence of a cuticle above the aboral larval epithelium as a plesiomorphic trait since it is found in larvae of different clades among Myolaemata. The formation of the unique two-valved shell presumably occurred before the separation of cheilostomes and ctenostomes, but still, the presence of the shell in the ancestral form cannot be stated with certainty. We propose a new schematic representation of hypothetical 'ancestral' larva of Myolaemata with unciliated principal pallial epithelium covered with a cuticle (Fig. 10).

In some incubated myolaemate larvae, the submersed pallial epithelium lost the protective function. Nevertheless, we would like to point

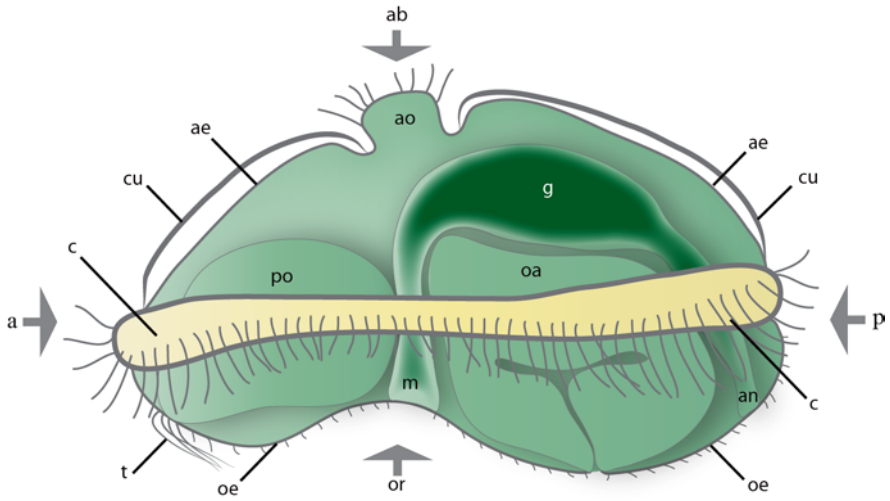

Fig. 10. Schematic representation of hypothetical 'ancestral' larva of Myolaemata. Aboral part of larva corresponds to episphere, oral part to hyposphere of typical trochophore. Internal cilia omitted.
Abbreviations: a — anterior; ab — aboral pole; ae — aboral pallial epithelium; an — anus; ao — apical organ; c — corona; cu — cuticle; g — gut; m — mouth opening; oa — adhesive organ (internal sac); oe — oral epithelium; or — oral pole; p — posterior; po — pyriform organ; t — ciliary tuft of the pyriform organ.
Рис. 10. Схематическое изображение гипотетической 'предковой' личинки миолемат. Верхняя (аборальная) часть личинки соответствует эписфере, нижняя (оральная) часть — гипосфере типичных трохофорных личинок. Реснички внутри личинки не показаны.
Обозначения: a — передний конец; ab — аборальный полюс; ae — аборальный паллиальный эпителий; an — анус; ao — апикальный орган; c — корона; cu — кутикула; g — кишка; m — ротовое отверстие; oa — адгезивный орган (внутренний мешок); oe — оральный эпителий; or — оральный полюс; p — задний конец; po — грушевидный орган; t — пучок ресничек грушевидного органа.

out that in mature larvae it is always well-developed (even in a submersed state) further participating in the development of the outer epithelium of the ancestrula. We hypothesize that invagination of the pallial epithelium, which is typical for most myolaemates with incubation, is achieved independently in evolution of different lineages since among both cteno- and cheilostomes we found brooded larvae with exposed and submersed pallial epithelium. This is applicable to viviparous cyclostomes too if we believe that they originated from ctenostomes.

We should stress that larval epithelia in bryozoans are surprisingly complex, and perform several functions. The number of the cell types of the pallial epithelium varies in larvae from different phylogenetic lineages (having from two to seven cell types). In total, we recorded 11 various cell types by TEM, and functional interpretation has been proposed for some of them. Except for principal pallial cells, tendon cells, and probably ciliated supracoronal cells, true homology could not be stated for most cell types at the moment.

Compliance with ethical standards

CONFLICTS OF INTEREST: The authors declare that they have no conflicts of interest.

Supplementary data. The following materials are available online.

Supplementary File 1. List of references on published data on the myolaemate larval pallial epithelium.

Supplementary File 2. List of examined bryozoan species with data on their larval structure and reproductive biology.

Supplementary File 3. List of cell types and ultrastructural details of the larval pallial epithelium of examined bryozoan species.

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