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# Zoomorphology

## New data on ultrastructure of proboscis in females of *Bonellia viridis* (Annelida: Bonellinae) --Manuscript Draft--

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**1 New data on ultrastructure of proboscis in females of *Bonellia viridis* (Annelida:**  
**2 *Bonellinae*)**

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## Abstract

Although *Bonellia viridis* is the best studied echiurid species, many aspects of its anatomy and ultrastructure are still unclear. In this study, detailed description of proboscis epithelium of females of *B. viridis* is done and illustrated by accurate schemes. Accordingly to histological specificities, four zones of proboscis stem can be distinguished: dorsal, ventral, and two lateral. These zones differ from each other in ciliation and abundance of bonellin-producing and gland cells. Each zone supplies certain functions: protective for dorsal, transport of food particles for ventral, and agglutination of food particles for lateral. Ciliated cells have been described in epithelium of the dorsal side of the proboscis stem for the first time. These cells, probably, supply slow ciliary movements of proboscis. For the first time, the organization of epithelial cells is described and the ability of epithelial cells to consume nutrients is detected. The absence of continuous basal lamina under the ventral epithelium is ascertained. This unusual state may be explained by activity of bonellin-producing cells, which constantly penetrate between epithelial cells. In bonellin-producing cells, voluminous perinuclear space and contribution of rough endoplasmic reticulum into formation of bonellin-containing granules are detected for the first time. Innervation of epithelial and gland cells occurs via nerve tracts originating from proboscis nerve cords. In general, echiurid proboscis epithelium exhibits many unusual features, which are unknown for any other food collecting apparatuses of annelids. The specificity of organization of proboscis correlates with unique evolutionary transformation, which echiurids underwent from initially segmented ancestor to recent non-segmented state.

**Key words:** echiurids, histology, transmission electron microscopy, epithelium, gland cells, functional morphology

## 1. Introduction

Accordingly to recent echiurid phylogeny (Goto et al. 2020), bonnelids form separate clade of Bonellinae, which also includes unusual ikedids. Bonellinae is the sister group to all rest echiurids, which form clade Thalassematinae and include two tribes Thalassematini and Echiurini (Goto et al. 2020). Echiurids are marine benthic invertebrates, which body is subdivided into trunk and proboscis (Pilger 1993; Maiorova and Adrianov 2020). The proboscis is usually extended on the surface of substratum and is used to collect and sort of food particles. Certain mechanisms of sorting of food particles are studied very poor (Jaccarini and Schembri 1977a). Moreover, for many echiurids, even position of the proboscis on the substratum is still unclear. For example, some specific structures were discovered in *Protobonellia zenkevitchi* and allowed to suggest the certain position of proboscis on the substratum (Temereva et al. 2017).

Organization of the proboscis differs in different species. Among echiurids, species from genus *Urechis* have the shortest proboscis: these echiurids produce the mucous net, which is used for capture of food particles (Fisher and MacGinitie 1928). Most of echiurids have long language-like proboscis. Females of *Bonellia viridis* have long proboscis, which terminated by two lobes (Jaccarini and Schembri 1977a). The proboscis of *B. viridis* is studied in details: there are data on location of proboscis on substratum (Jaccarini and Schembri 1977b), movements of proboscis (Jaccarini and Schembri 1977b), the work of cilia of terminal lobes and middle parts of proboscis (Jaccarini and Schembri 1979), organization of muscles of proboscis (Bosch 1981), the ultrastructure of integument (Bosch and Michel 1979), and blood vessels extended in the proboscis (Amor 1973; Bosch 1984). One of specific features of bonnelids is the presence of bonellin-producing cells, which is located in the connective tissue of body wall and proboscis and are able to penetrate into the integument (Bosch 1979; Bosch and Michel 1979). Bonellin is the pigment, which occurs in many echiuran species, but is well known in *B. viridis* because makes its body green. This unique pigment this unique pigment has been shown to have various

toxic and irreversible effects and therefore supplies protective function (Agius and Jaccarini 1981).

Most of ultrastructural data, which we know about echiurids are obtained from *B. viridis* and are extrapolated to other echiurids. At the same time, some of these ultrastructural data seem very unusual (i.e. syncytial nature of wall of the axial blood vessel (Bosch 1984)) and have to be reinvestigated.

Detailed data on organization of the proboscis may help to understand the echiurid biology and some aspects of their feeding behavior (Temereva et al. 2017; Kuznetsov et al. 2021).

The aim of this study is detailed description of ultrastructure of all organ systems of proboscis of *B. viridis* with special attention to new data concerning organization of integument and functional analysis of these data.

## **2. Materials and methods**

Three specimens of *Bonellia viridis* Rolando, 1822 were collected by SCUBA diving in the Frioul Archipelago on the coast of Provence, France, 43°16'38"N, 5°18'24"E. The morphology of all individuals was studied with Leica M165C stereomicroscope equipped with a Leica DFC420 digital camera (Leica Microsystems GmbH, Wetzlar, Germany). Proboscises were cut from the trunk and fixed in 2.5% glutaraldehyde in 0.05 M cacodylate buffer containing NaCl for 8 hours at +4°C. Materials were then washed in the cacodylate buffer (3 times for 4 hours) and were then post-fixed in 1% osmium tetroxide in the same buffer.

The fine morphology of proboscis was studied by scanning electron microscopy (SEM). Pieces of proboscis and trunk were dehydrated in increasing concentrations of ethanol and acetone; they were then critical point dried, mounted on stubs, and sputter coated with platinum-palladium.

Specimens were examined with JEOL JSM-6380LA scanning electron microscope (JEOL Ltd., Tokyo, Japan).

For semithin sectioning and transmission electron microscopy (TEM), specimens were dehydrated in increasing concentrations of ethanol and embedded in Embed-812 resin (Electron Microscopy Science, USA). Semi-thin and thin sections were prepared with a Leica UC7 ultramicrotome (Leica Microsystems GmbH, Wetzlar, Germany). Semithin sections were stained with methylene blue, observed with a Zeiss Axioplan2 microscope, and photographed with an AxioCam HRm camera (Carl Zeiss, Oberkochen, Germany). Ultrathin sections were stained with uranyl acetate (0.5%) and lead citrate (0.4%) and then examined with a JEOL JEM 100B electron microscope (JEOL Ltd., Tokyo, Japan).

### 3. Results

#### 3.1. Morphology and histology of proboscis

The body of females of *B. viridis* is subdivided into the trunk and proboscis (Fig. 1A). The proboscis is long projection of the dorsal side of the trunk, which is flattened in dorso-ventral direction and terminates with two large lobes (Fig. 1A, B). The proboscis length can exceed the length of the trunk in several times (Fig. 1A).

The margin of each lobe is swollen and is covered by numerous cilia (Fig. 1C, D). The margin is crossed by vertical grooves, which extend between dorsal and ventral sides of the lobe (Fig. 1C). The diameter of these grooves is about 20–30  $\mu\text{m}$ . The marginal edge of the lobe bears longitudinal wrinkle, which extends along the margin and divides it on two parts (ventral and dorsal). Accordingly to histological data, this wrinkle is formed by two small epithelial folds, which are associated with huge subepithelial aggregation of gland cells (Fig. 2A). The stem of proboscis is the longest part, which is able to twist and loop. The ventral side of proboscis stem bears shallow longitudinal grooves and is covered by cilia (Fig. 1E). At the proximal end, the

lateral edges of proboscis are usually directed ventrally and fuse together near the mouth, forming the short ventral collar (Fig. 1F). Near the mouth, proboscis is crossed by transverse grooves. In some specimens, the large bulb, which is probably form by the foregut, obstructs the mouth (Fig. 1F).

Usually, after fixation, the lateral sides of proboscis bend ventrally (Fig. 2C). Accordingly to specificities of histology, four epithelial zones may be distinguished in the cross section of proboscis: ventral, dorsal, and two laterals. In additional, terminal lobes of proboscis also form specific zone. These zones differ from each other in thickness of epithelium, ciliation, and abundance of gland cells and bonellin-producing cells (Fig. 3A, B). Thus, ventral epithelium has 14–22  $\mu\text{m}$  in thickness, it is heavily ciliated, and contains many bonellin-producing cells and a few epithelial glands (Fig. 4A, B). The epithelium of dorsal side has 10–20  $\mu\text{m}$  in thickness, about lacks cilia, contains a few bonellin-secretory cells and many intraepithelial glands (Fig. 4C). The lateral zones of proboscis look similar to margin of the lateral lobes and are characterized by presence of numerous subepithelial glands (Fig. 4D). Lateral zones of proboscis stem bear ciliated grooves, which diameter is about 20  $\mu\text{m}$  (Fig. 2B).

The proboscis lacks spacious inner cavity and is filled with extracellular matrix and muscles (Fig. 2C, D). Muscles are mostly longitudinal and occupy the central and ventral parts of the proboscis, whereas the dorsal side is about lacks muscle cells (Fig. 2C). Fibers of extracellular matrix make a grid, which gather muscles in united construction (Fig. 2D). Rare dorso-ventral muscles are represented by separated muscle fibers. Transverse muscles are not observed on the dorsal side of proboscis, but form a prominent layer under the ventral epithelium (Fig. 2C). The proboscis contains the axial blood vessel extending in its center and two lateral blood vessels, which are incorporated into two lateral coelomic canals (Fig. 2C, D). Two lateral nerve cords extend along lateral sides of the proboscis above the coelomic canals (Fig. 2C, D). The nerve cord gives rise to the neurite bundles, which penetrate the connective tissue and extend to the subepithelial (Fig. 2A) and intraepithelial gland cells (Fig. 4A).



### 3.2. Ultrastructure

The epithelium consists of epithelial, intraepithelial and subepiermal gland cells of different types (Fig. 3C). All types of cells are organized similarly in all four zones of the proboscis.

**Epithelial cells** of ventral side bear numerous cilia, which form dense cover above the epithelium (Figs. 4B, 5A). Apical surface of epithelial cells bears branched microvilli and large apical protrusions, which are covered by flocculent material of cuticle (Fig. 5A, B). The kinetosome is represented by one centriole (basal body), which connects with two striated rootlets: transverse and longitudinal (Fig. 5C). Cells connect each other via adherence and septate junctions, which unite the apical parts of cells (Fig. 5C, D). The middle and basal parts of cells contact each other loosely; therefore voluminous intracellular spaces are visible (Fig. 5B, C). The cytoplasm of apical part of cells is electron dense and is armed by transverse bundles of actin filaments (Fig. 5B). The apical cytoplasm is filled with numerous vesicles of different diameter and multivesicular bodies (Fig. 5B, C). The middle and basal parts of cell contain large nucleus of irregular shape, several Golgi apparatuses, and phagosomes (Fig. 5A-C). Epithelium is not underlined by continuous basal lamina (Fig. 5A).

In epithelial cells of lateral sides of proboscis, because of presence of many cilia, the apical protrusions and microvilli are not such numerous as in the epithelium of ventral side (Fig. 6A). The epithelium of lateral sides is underlined by thin basal lamina. Epithelial cells of dorsal side are about lack cilia, nevertheless some ciliated cells can be detected (Fig. 6B). In cells, which lack cilia, the apical part is substantially protruded and forms wide lobes (Fig. 6C). Because of presence of apical protrusions, cell junctions are located in subapical zone. Apical membrane forms thick microvilli, which exposed into the layer of cuticle, and numerous thin canals, which penetrate the cytoplasm of apical protrusions. These canals terminate by pinocytotic vesicles, which fill the apical cytoplasm (Fig. 6C). In cells of dorsal epithelium, bundles of actin fibers extend are not only transverse, but are also longitudinal.

**Intraepithelial gland cells** are incorporated into the epithelia of all four zones and do not form protrusions into extracellular matrix (Figs. 3, 7A, B). There are two types of intraepithelial gland cells: with electron-lucent vacuoles and with electron-dense flocculent granules. Cells of first type (with electron-lucent vacuoles) are the most abundant especially in dorsal epithelium (Figs. 4C, 7A). These cells are filled with vacuoles of different diameter, which contain electron-lucent material and many vesicles of smaller diameter. Apical surface of cells bears thin short microvilli with electron-dense cytoplasm. Usually, apical surface of gland cells is covered by apical projections of epithelial cells (Fig. 7A, B). Gland cells of first type connect with the epithelial cells via adherence and septate junctions (Fig. 7A). Secretory zone of cell is located in middle part of cell and is represented by Golgi apparatus, which is squeezed between vacuoles (Fig. 7A). Epithelial gland cells of second type contain granules of electron-dense flocculent material, which diameter is about 0.6–0.8  $\mu\text{m}$  (Fig. 7B). Cells of this type are rare and are usually occurred in dorsal epithelium.

**Subepithelial gland cells** are numerous in terminal lobes and in lateral zones of the proboscis stem. There are two types of subepithelial gland cells (Figs. 3, 8A, B). Cells of first type contain large roundish electron-dense granules, which diameter can reach up 1.5  $\mu\text{m}$  (Fig. 8A). The nucleus is relatively large, is irregular-shaped, and contains nucleolus (Fig. 8A). The secretory zone contains numerous Golgi apparatuses, which canals are filled with content of middle electron density (Fig. 8B). Vacuoles, which are produced by Golgi apparatuses, fuse together forming granules of middle electron density (Fig. 8A); then the density of granules becomes higher. Subepithelial gland cells of second type are filled with huge vacuole, which contains electron-lucent material and flocculent fibers (Fig. 8B). The nucleus is located in the central part of cell: it is relatively small in diameter and contains much heterochromatin and nucleolus. The secretory zone is also located in the central part of cell and is represented by numerous Golgi apparatuses, which canals are filled with electron-lucent content (Fig. 8B).

**Bonellin-producing cells** are specific type of subepithelial gland cells. These cells contain electron-dense granules of specific ovoid shape and maximal size 2x1  $\mu\text{m}$  (Fig. 9A). Bonellin-producing cells can also contain granules of irregular shape and middle electron density. These cells are mostly located in the connective tissues of the proboscis, but can be also incorporated into the epithelium (Figs. 3, 4, 9A). These cells have lengthy apical and swollen basal parts. Apical membrane forms thick short microvilli. Apical part of bonellin-producing cells does not form typical cells junctions with adjacent cells (Fig. 9A). Apical cytoplasm is filled with electron-dense granules. The secretory zone is located in the basal part of cell and contains two main elements: Golgi apparatuses and rough endoplasmic reticulum (RER) (Fig. 9C). Canals of Golgi apparatuses are filled with electron dense content (Fig. 9C). The RER connects the nuclear membrane: the perinuclear space is very wide and sophisticatedly lopped, thus, canals of RER penetrate the perinuclear space (Fig. 9C). The RER produce flocculent electron-dense material, which is incorporated in the secretory granules. Moreover, secretory granules are located into space, which is surrounded by RER canals (Fig. 9B). Some areas of cell membrane are very thick and electron dense (Fig. 9C). Cells are closely contacted the nerve tracts and some certain neurites (Fig. 9C).

Intraepithelial and subepithelial gland cells and bonellin-producing cells are well innervated by **nerve tracts**, which originate from the lateral nerve cords (Figs. 4A, 5A, 9B). Bundles of neurites also penetrate between epithelial cells (Fig. 6A). Usually, nerve tracts consist of neurites of two types. Neurites of first type have large diameter, electron-lucent cytoplasm, and contain dense-core synaptic vesicles (Figs. 3C, 5A). Neurites of second type are small in diameter, have electron-dense cytoplasm, and contain synaptic vesicles with electron-lucent content. Neurites connect each other via synapses (Fig. 9B).

#### **4. Discussion**

Most echiurans are deposit-feeders, and the proboscis is used to collect food particles from the substrate (e.g., Ikeda 1904; Baltzer 1931; Hughes and Crisp 1976; Biseswar 1991; Hughes et al. 1993; Jumars 2015; Kharlamenko et al. 2018). Accordingly to available data, mucus ciliary mechanism works for capture of food particles: they adhere to the mucus, which is produced by gland cells, and then transport to the mouth due to activity of cilia (Biseswar 1991). Accordingly to many observations, the dorsal side of the proboscis usually faces the substratum and ventral side usually faces the water; the distal edge is submerged into the substratum and collects substrate particles (Biseswar 1991; Hughes et al. 1993; Goto et al. 2016). The specific position of the proboscis has been suggested for *Protobonellia zenkevitchi* due to specificities of morphology and histology of different zones of proboscis (Temereva et al. 2017). *B. viridis* uses the terminal lobes but not the lateral sides of the proboscis to collect food particles (Jaccarini and Schembri 1977a). Accordingly to our new data, the margin of terminal lobes of proboscis bears the huge aggregation of gland cells. The same cells are located along the lateral sides of the proboscis stem. The presence of similar gland cells in both terminal lobes and lateral sides of the proboscis stem may evidence the similar function of these zones, i.e. production of mucus for adherence of food particles. It probably means that the lateral edges of proboscis stem are involved into collection of food particles.

The size of food particles, which echiurids are able to capture, can vary greatly: 2-7  $\mu\text{m}$  in *Lissomiema mellita* (Kuznetsov et al. 2021), 7-45  $\mu\text{m}$  in *P. zenkevitchi* (Temereva et al. 2017), 90-300 in *B. viridis* (Jaccarini and Schembri 1977a). Usually, the size of food particles is determined by diameter of ciliary grooves, which extend on certain zones of proboscis (Temereva et al. 2017; Kuznetsov et al. 2021). In *B. viridis*, ciliary grooves are detected on the margin of the terminal lobes and on the lateral sides of the proboscis stem. In both parts of the proboscis, the diameter of ciliated grooves is similar and is about 20  $\mu\text{m}$ . The presence of ciliated grooves may evidence the sorting of food particles in *B. viridis*. The rejection of particles occurs on the ventral side of the proboscis and near the mouth (Jaccarini and Schembri 1977b; Pilger

1993). In studied specimens of *B. viridis*, the detected protrusion of the foregut may work as an obstacle for large particles.

Distribution of muscles, cells, and fibers in extracellular matrix of proboscis has functional meaning. Thus, in *P. zenkevitchi*, the terminal part of the proboscis is reinforced by large vacuolated cells (Temereva et al. 2017). In *L. mellita*, proboscis is very muscular and its ventral side is reinforced by thick layer of collagenous fibers (Kuznetsov et al. 2021). In *B. viridis*, muscles are mostly extended in central and ventral parts; moreover, the ventral epithelium is underlined by layer of transverse muscles. These muscles allow the proboscis to curve on the ventral side and form a kind of large groove (Fig. 2C).

Ultrastructure of epithelium of proboscis is described for *B. viridis* in several papers (Pilger, 1977; Jaccarini and Schembri 1977a; Bosch and Michel 1979; Bosch 1979). These studies are mostly devoted to the description of gland cells, whereas epithelial cells have never been described in details. It is known that cells of ventral epithelium bear cilia, whereas cells of dorsal epithelium lack cilia (the exception is the dorsal side of terminal lobes of *B. viridis*) (Jaccarini and Schembri 1977a). Accordingly to our data, some cells of dorsal epithelium have cilia, that allow it to move slowly due to ciliary beating. In general, epithelial cells of all zones of proboscis are organized in similar way. It is very interesting that all epithelial cells are able to consume nutrients: the Figure 7B allows to realize the formation of pinocytotic vesicles, their aggregation into multivesicular bodies, and further transformation into the phagosomes. Because of pinocytotic activity epithelial cells contain large and well developed Golgi apparatuses, which produce lysosomes. The consumption activity of epithelial cells is consistent with suggestion that some intraepithelial glands produce proteins, which may be used for digestion of food prior to entering the mouth (Bosch and Michel 1979).

The subapical location of cells junction in dorsal epithelium allows it to stretch when the proboscis skirts on the ventral side. Another one interesting and unusual thing is the absence of

continuous basal lamina under the ventral epithelium. Strictly speaking, epithelium, which is not underlined by basal lamina, cannot be regarded as true epithelium (Vracko 1974). The absence of basal lamina in ventral epithelium may be caused by activity of bonellin-producing cells, which constantly penetrate between epithelial cells and change the structure of epithelium (see below).

Accordingly to literature, five types of gland cells can be distinguished in proboscis of females of *B. viridis*: two intraepithelial and three subepithelial (Bosch and Michel 1979). Intraepithelial gland cells had been detected in ventral epithelium only (Bosch and Michel 1979). As it was shown in our study, two types of intraepiermal gland cells are scattered in ventral and dorsal epithelia; moreover, dorsal epithelium contains many mucus-producing cells. The secretion of these cells is apparently used for protection of dorsal epithelium, which contacts the substratum.

Three types of subepithelial gland cells are associated with ventral (one type) and dorsal (two types) epithelia (Bosch and Michel 1979). Ventral mucus gland cells contain hypertrophied Golgi apparatus and produce large vacuoles, which then fuse together, forming the united mass with fibrillary network. The secretion of these cells contains sulfated mucus and is used for agglutination of food particles (Bosch and Michel 1979). Dorsal mucus cells contain ovoid granules, which consist of sulfated mucopolysaccharides and are used for protection of dorsal epithelium. The third type of subepithelial cells is represented by dorsal twisted mucus gland cells, which contain well developed RER, two Golgi apparatuses, and numerous metachromatic granules. The secretion of these glands may help to provide traction for the animal as it crawls over the surface (Bosch and Michel 1979). Accordingly to our data, there are two types of subepithelial gland cells and both types are associated with the terminal lobes and with the lateral sides of the proboscis stem. The secretion of these gland cells may be used for agglutination of food particles.

Ultrastructure of bonellin-producing cells has never been described in details (Pilger 1993). It is known that Golgi apparatus produces the material of bonellin-containing granules and then the granules are modified in cytoplasm (Kawaguti 1968). Our new data show that the bonellin-containing granules are produced by two ways (by Golgi apparatuses and by RER) and probably consist of at least two components. Unusual thing of bonellin-producing cells is the presence of wide perinuclear space, which is penetrated by canal of RER and contains the bonellin granules. The dense parts of cell membrane may play role for movements of bonellin-producing cells, which are able to move in connective tissue and form projections penetrating between epithelial cells (Bosch 1979). The abundance of bonellin-producing cells in the ventral epithelium is consistent with light sensitivity of bonellin: it releases due to mechanical or light stimuli. Because ventral epithelium is faced to the light, it contains much more bonellin-producing cells in comparison with dorsal epithelium, which is faced to the substratum.

For the first time, the innervation of epithelium, gland cells, and bonellin-producing cells has been shown in our study. In previous researches, the innervation of gland cells and bonellin-producing cells has not been detected (Pilger 1993).

## **5. Conclusion**

For the first time for echiurans, detailed description of epithelium of proboscis is done and visualized as accurate schemes. Four functional zones are distinguished in proboscis stem: dorsal, ventral, and two lateral. These zones differ from each other in ciliation and abundance of bonellin-producing and gland cells. Accordingly to specificities of histology, each zone provides certain function. Thus, dorsal side works for protection of proboscis, ventral – for transport of food particles, and lateral for agglutination of food particles. Many unusual and previously undescribed features are discovered: the capability of epithelial cells to consume nutrients via pinocytosis, the absence of continuous basal lamina under the ventral epithelium, the presence of ciliary cells in dorsal epithelium of the proboscis stem, the innervation of epithelial cells and

gland cells by nerve tracts, and the specificities of bonellin-producing cells. In sum, epithelium of proboscis exhibits many unusual features, which are unknown for any food collecting apparatuses of annelids. The specificity of organization of proboscis correlates with unique evolutionary transformation, which echiurids underwent from initially segmented ancestor to recent non-segmented state.

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## **Conflict of Interest Statement**

The authors declare that they do not have any conflict of interest. All authors have read the text and approved the final version of the manuscript.

## **Author contributions**



PK performed the histology, prepared the serial sections, made some schemes, and photographed the sections. AE collected and fixed the animals, helped interpret the results, and helped write the manuscript. ET prepared all figures, wrote the manuscript, and interpreted the results.

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## Figure legends

Fig. 1. Morphology of females of *Bonellia viridis*. **a-b** Photographs of fixed animals; **c-f** SEM. **a** Whole animal viewed from the left. **b** Terminal lobes of proboscis. **c** A part of one terminal lobe; longitudinal wrinkle of margin is pointed by arrowheads. **d** A portion of margin of terminal lobe, which is heavily ciliated. **e** A portion of ventral side of the proboscis stem. **f** Anterior part of the body viewed from the top; large bulb of foregut obstructs the mouth. *ci* cilia, *cg* ciliated groove, *ch* chaetae, *ds* dorsal side of terminal lobe, *fb* foregut bulb, *lg* longitudinal groove, *m* mouth, *me* marginal edge of terminal lobes, *ps* proboscis stem, *tg* transverse groove, *tl* terminal lobe, *tr* trunk, *vc* ventral collar.

Fig. 2. Anatomy proboscis of female of *Bonellia viridis*. **a-c** Photographs of transverse semithin sections; **d** SEM. **a** A portion of margin of terminal lobe: huge aggregation of subepidermal glands is visible. **b** Ciliated groove of lateral side of the proboscis stem. **c** Whole proboscis stem. **d** A portion of proboscis stem: grid, which is formed collagenous fibers, is evident. *abv* axial blood vessel, *agc* aggregation of gland cells, *bpc* bonellin-producing cells, *cf* collagenous fibers, *cg* ciliated groove, *dep* dorsal epithelium, *dvm* dorso-ventral muscles, *lbc* lateral blood vessel, *lc* lateral coelomic canal, *lm* longitudinal muscles, *lnc* lateral nerve cord, *ls* lateral side, *nb* neurite bundle, *tm* transverse muscles, *vep* ventral epithelium.

Fig. 3. Schemes of histological and ultrastructural organization of proboscis of female of *Bonellia viridis*. **a** Portion of terminal lobe. **b** Portion of the proboscis stem. **c** Ultrastructure of ventral epithelium of proboscis stem. *bpc* bonellin-producing cells, *ci* cilia, *cu* cuticle, *igc-I* intraepidermal gland cell type I, *igc-II* intraepidermal gland cell type II, *mc* muscle cells, *mi* microvilli, *n* nucleus, *nb* neurite bundle, *pha* phagosome, *pns* perinuclear space, *sgc-I* subepidermal gland cell type I, *sgc-II* subepidermal gland cell type II.

Fig. 4. Histological organization of proboscis of female of *Bonellia viridis*; photographs of transverse semithin sections. **a** Ventral epithelium of terminal lobe. **b** Ventral epithelium of

proboscis stem. **c** Dorsal epithelium of proboscis stem. **d** Lateral epithelium of proboscis stem.  
*bpc* bonellin-producing cells, *ci* cilia, *ct* connective tissue, *igc-I* intraepidermal gland cell type I,  
*igc-II* intraepidermal gland cell type II, *mc* muscle cells, *nb* neurite bundle, *sgc-I* subepidermal  
gland cell type I, *sgc-II* subepidermal gland cell type II.

Fig. 5. Ultrastructure of ventral epithelium of proboscis stem in female of *Bonellia viridis*; TEM.  
**a** General view of epithelium and neurite bundles under the epithelium. **b** Apical parts of  
epithelial cells; space between to adjacent cells is marked by asterisks. **c** Organization of  
kinetosome and rootlets of cilia; wide spaces between cells are marked by asterisks. **d** Septate  
junction between adjacent cells. *af* actin filaments, *aj* adherence junction, *ap* apical projection,  
*bpc* bonellin-producing cells, *ce* centriole, *ci* cilia, *ct* connective tissue, *cu* cuticle, *igc-I*  
intraepidermal gland cell type I, *lr* lateral rootlet, *mi* microvilli, *mvb* multivesicular body, *n*  
nucleus, *nb* neurite bundle, *pha* phagosome, *pv* pinocytotic vesicle, *sj* septate junction, *tr*  
transverse rootlet.

Fig. 6. Ultrastructure of lateral and dorsal epithelia of proboscis stem in female of *Bonellia*  
*viridis*; TEM. **a** General view of epithelium of lateral side. **b** Apical part of ciliated cell of dorsal  
epithelium. **c** Apical and middle parts of epithelial cell of dorsal epithelium. *af* actin filaments, *aj*  
adherence junction, *ap* apical projection, *bl* basal lamina, *bpc* bonellin-producing cells, *ce*  
centriole, *ci* cilia, *cu* cuticle, *G* Golgi apparatus, *igc-II* intraepidermal gland cell type II, *lr* lateral  
rootlet, *mc* muscle cell, *mi* microvilli, *mvb* multivesicular body, *n* nucleus, *nb* neurite bundle, *pha*  
phagosome, *sj* septate junction, *tr* transverse rootlet.

Fig. 7. Organization of intraepidermal gland cells in dorsal epithelia of proboscis stem in female  
of *Bonellia viridis*; TEM. **a** Gland cell of first type contains large vesicles with electron-lucent  
content. **b** Gland cells of second type contains granules of electron dense flocculent material. *aj*  
adherence junction, *ap* apical projection, *cu* cuticle, *G* Golgi apparatus, *igc-I* intraepidermal

459 gland cell type I, *igc-II* intraepidermal gland cell type II, *mi* microvilli, *m vb* multivesicular body,  
460 *n* nucleus, *pha* phagosome, *v* vesicle.

461 Fig. 8. Organization of subepidermal gland cells in lateral zones of proboscis stem in female of  
462 *Bonellia viridis*; TEM. **a** Gland cell of first type contains large electron dense granules. **b** Gland  
463 cell of second type contains mucous material and many Golgi apparatuses. *ecm* extracellular  
464 matrix, *G* Golgi apparatus, *gr* granule of secretion, *ms* mucus secretion, *n* nucleus, *nu* nucleolus,  
465 *ngr* newly formed granule, *sgc-I* subepidermal gland cell type I, *sgc-II* subepidermal gland cell  
466 type II, *sve* secretory vesicles.

467 Fig. 9. Organization of bonellin-producing cells of proboscis stem in female of *Bonellia viridis*;  
468 TEM. Electron dense portions of cell membrane are pointed by arrowheads. **a** Cell incorporated  
469 into ventral epithelium. **b** Cell in connective tissue. *G* Golgi apparatus, *gr* granule of secretion,  
470 *mi* microvilli, *n* nucleus, *nb* neurite bundle, *ngr* newly formed granule, *pha* phagosome, *pns*  
471 perinuclear space, *RER* rough endoplasmic reticulum, *sy* synapse.

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