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# Microscopic anatomy of the axial complex and associated structures in the sea cucumber *Chiridota laevis* Fabricius, 1780 (Echinodermata, Holothuroidea)

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**Abstract** The absence of axial and pericardial coeloms and the axial organ, presence of a mesocoelomic tentacular apparatus, correspondence of the larval sagittal plane with the madreporic plane of the adult, and some other peculiarities set holothurians apart from all other echinoderms. We studied the holothuroid Chiridota laevis with histological methods and constructed a 3D-model. The bilateral symmetry of the water-vascular system is clear in spite of a peculiar coelomic connection with one of the tentacles in radius D. The somatocoelomic and axocoelomic perihaemal coeloms are absent. There are perioral and watervascular circumoral coelomic rings. The hyponeural ring is absent. Each radial complex includes a water-vascular canal, a hyponeural canal, two nerve bands (ectoneural and hyponeural), and the non-coelomic epineural canal. The stone canal starts from the water ring on the aboral side in the CD interradius and ends with a madreporic ampulla, from which a few pores open into the perivisceral coelom, and one pore canal opens into the environment. This organization seems to be the intermediate state between most echinoderms, in which the madreporic pores open into the environment, and the typical organization of holothurians, in which the madreporic pores open into the perivisceral coelom. The histological structure of the oral haemal ring resembles the axial organ of other Eleutherozoa. The haemocoel lacune of the stone canal communicates with the haemal system of the gonad, the oral haemal ring, and

⊠ Olga V. Ezhova olga.ejova@gmail.com the gut haemocoel lacunae. The genital coelom is represented by narrow spaces surrounding the "genital rachis" of the gonad.

**Keywords** Axial complex · Water-vascular system · Nerves · *Chiridota laevis* · Sea cucumbers · Holothuroidea · Echinodermata · Morphology · Microscopic anatomy · Phylogeny

### Introduction

As is known, there are a few different opinions about the relationships of the modern classes of Eleutherozoa. There are two most popular hypotheses-"Cryptosyringida" and "Asterozoa-Echinozoa" (Janies et al. 2011) (Fig. 1). According to the first hypothesis, Asteroidea is a sister clade to the remaining three classes of Eleutherozoa-Ophiuroidea, Echinoidea, and Holothuroidea, which make up the Cryptosyringida group. These classes are characterized, in particular, by an ectoneural system, which is submerged into the body and is accompanied by an epineural canal. According to the second hypothesis, Eleutherozoa divides into two separate clades. The first one-Asterozoa, includes asteroids and ophiuroids, which have a "five-ray", stellate architecture of the body. The second clad-Echinozoa, includes echinoids and holothuroids, which are characterized by the lack of apparent rays and have more or less globose body. Further molecular studies mostly confirmed the "Asterozoa-Echinozoa" hypothesis (O'Hara et al. 2014; Telford et al. 2014; Reich et al. 2015). The researchers of echinoid and holothuroid morphology actually note a lot of common features in these echinoderm classes: a long convolute gut, general perivisceral coelom, and presence of the peripharyngeal and perianal coeloms, similarity in

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Fig. 1 "Cryptosyringida" and "Asterozoa–Echinozoa" hypotheses of the relationships of the recent classes of Eleutherozoa



the radial complex and structure of podia (Smith 1984). However, there are some considerable differences, exemplified by the ways of development of the epineural canal. In Echinoidea and Ophiuroidea, it appears to be a result of an enclosure of the epineural flaps (MacBride 1914), but in Holothuroidea, it forms otherwise, as a schizocoelic cavity within the connective tissue outside of the ectoneural nerves (Smiley 1986). Moreover, the holothuroid auricularia larva resembles the asteroid bipinnaria, and is very different from the echinoid and ophiuroid plutei (Ivanova-Kazas 1978; Smith 1997). The central structures of the echinoid axial complex (the haemal axial organ between the axial and pericardial coeloms) are arranged in the same way as in asteroids, whereas in holothuroids, these structures are absent.

Furthermore, there are a few peculiarities, which noticeably distinguish Holothuroidea from all other Echinodermata. It is well known that holothuroids, in addition to the planctotrophic auricularia larva (which may be associated with the planctotrophic larvae of other Eleutherozoa), have another larval form—the doliolaria, which is very similar to the crinoid larva (MacBride 1914). It seems that there are two metamorphoses in the holothuroid life cycle: (1) transformation of the auricularia into the doliolaria; (2) transformation of the doliolaria into the pentactula. Holothuroidea not only have a water-vascular system, which is characteristic for all echinoderms, but also a tentacular apparatus, which is characteristic only for this class of Echinodermata. Both of these structures arise from the mesocoel. It has been shown that in Holothuroidea, the larval sagittal plane becomes the madreporic plane of the adult; thus, the bilateral symmetry of the adult holothurians directly originates from the larval bilateral symmetry (Smiley 1986; Malakhov and Cherkasova 1992). The absence of a connection of the stone canal with the environment and an internal madreporite are characteristic for most Holothuroidea; only in a few species, there is an additional pore canal opening into the exterior on the oral side of the body (Hyman 1955). All these peculiarities set holothurians apart from the Eleutherozoa and even from all echinoderms.

Earlier, we studied the morphology of the axial complex in Asteroidea (see Ezhova et al. 2013) and Ophiuroidea (see Ezhova et al. 2015), and concluded that, in spite of differences evident at first glance, the axial complexes of Asterozoa are arranged similarly. The differences in their arrangement are due to the evolution transformations caused by the ophiuroid life style (Ezhova et al. 2016a). If we consider the peculiar status of the holothurians among other echinoderms, the study of the holothuroid axial complex with modern methods

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Fig. 2 Axial complex and associated organs, and its location in the body of *Chiridota laevis*. **a** 3D-reconstruction of the coelomic structures of the axial complex. **b** Same with the haemal structures. **c** Diagram of a generalized section through the axial complex of *C. laevis* in the CD interradius (plane of the "gut mesentery"). *Arrow* shows the direction of oral-aboral axis. **d**, **e** Two frontal sections through the axial complex on the different levels. *amp* madreporic ampulla, *csr* 

calcareous ring, *ecn* ectoneural nerve ring, *ep* epidermic, *epr* epineural canal, *gm* "gut mesentery", *gn* gonad, *gnb* gonad haemal lacune, *gnd* gonoduct, *hmc* haemocoel, *hpc* hyponeural canal, *mp* madreporic plate (madreporite), *orb* oral haemal ring, *pc* pore canal, *pm* "perioral mesentery", *poc* perioral coelom, *pv* polian vesicle, *pvc* perivisceral coelom, *rwc* radial water-vascular canal, *sc* stone canal, *scb* haemal lacune of the stone canal, *tc* tentacle coelom, *wr* water-vascular ring

of reconstruction becomes relevant. As the object of our study we chose the White Sea holothuroid *Chiridota lae-vis* (O. Fabricius, 1780) from the order Apodida, which is considered by some authors to be the basal group of Holothuroidea (Smith 1997; Kerr and Kim 2001; Lacey et al. 2005; Janies et al. 2011).

## Materials and methods

Specimens of *C. laevis* 1.5–6 cm in size were collected in the summer of 2014 near the Pertsov White Sea Biological Station, which is located on the shore of Rugozerskaya Gulf of Kandalaksha Bay (White Sea). Animals were collected



with a trawl and by divers at depths from 4 to 40 m from sand and mud substrates. The animals were relaxed by a gradual addition of ethanol in a cold environment. After relaxation, the animals were fixed in 4% formaldehyde. Until histological study, the material was preserved in 70% ethanol. To prepare the material for histological studies, standard decalcification procedures were performed using nitric acid (Valovaya and Kavtaradze 1993). The standard technique of dehydration of material in alcohols of increasing concentration was used, followed by embedding the

**∢Fig. 3** Mesocoelomic (hydrocoelomic) derivatives in the axial complex of Chiridota laevis. Frontal sections. a Communication of the stone canal (sc) with the water-vascular ring (wr). The stone canal is surrounded by a haemocoel lacune (scb), which connects with the oral haemal ring (orb) and the haemal lacunae in the "gut mesentery" (gm). **b**, **h** Communication of the tentacle coelom (tc) with the watervascular ring (wr) by a tube outgrowth (to). csr Calcareous ring, ecn ectoneural nerve ring. c Madreporic plate (mp) with the ampulla (amp) communicating with the perivisceral coelom (pvc). The gonoduct (gnd) within the "gut mesentery" (gm) accompanies the stone canal, ampulla, and the pore canal (pc), which opens into the exterior (f). d 3D-reconstruction of the mesocoelomic derivatives. Capital letters indicate the radii. e Scheme of the water-vascular ring and its relations with the tube outgrowths to the tentacles and the radial water-vascular canals. g Communication of the polian vesicles (pv)with the water-vascular ring (wr). gn Gonad, orb oral haemal ring, poc perioral coelom, pvc perivisceral coelom

material in paraplast and cutting it into sections 5–9 µm thick. The sections were then stained with hematoxylin. In total, five specimens were studied using light microscopy. Five series of sections were made: four series of cross sections and one frontal section series. Photographs of the sections were made using an Axioplan 2 photomicroscope ("Carl Zeiss Microscopy", Jena, Germany, 2003) and a Leica DFC425 C photomicroscope ("Leica Microsystems", Heerbrugg, Switzerland, 2009). Using the photographs of the frontal series, a 3D model of the axial complex and associated structures was constructed using Adobe Photoshop and 3D-Doctor software.

All photographs in frontal plane and schemes are oriented mouth down for a clearer comparison with the other classes of Echinodermata.

#### Results

The axial complex of C. laevis (Fig. 2a-c) consists of the coelomic organs (water-vascular system, tentacular coeloms, hyponeural canals, perioral coelom, and gonad), and haemocoelic organs (haemal lacune of the stone canal, oral haemal ring, and haemal lacunae of gut and gonad). The structures associated with the axial complex are epineural canals, nerves, the calcareous ring, and the perivisceral coelom. Most of the organs of the axial complex are suspended on two mesenteries (Fig. 2c-e). The first is the "gut mesentery" (called the "dorsal mesentery" by Selenka 1867), which extends through almost the whole body in the perivisceral coelom in the interradius CD and supports the gut, stone canal, and gonad (Fig. 2d, e). The second mesentery is the "perioral mesentery", which is formed between the extensive perivisceral coelom on the aboral side and the perioral coelom on the oral side. It supports the coelomic water-vascular ring (Fig. 2d, e).

The central element of the axial complex of *C. lae-vis* is the water-vascular system. The water-vascular ring

surrounds the pharynx in its forepart and adjoins to the calcareous ring and perioral coelomic ring from the inner side (Figs. 2d, e, 3b, d). The water-vascular ring connects with polian vesicles (the studied specimens had 5). The polian vesicles branch from the water-vascular ring and extend towards the aboral side of the body (Fig. 3g). The thickness of the walls of the vesicles is 25  $\mu$ m in average.

There are 12 tentacles in C. laevis (according to the taxonomic descriptions, all chiridotids have 12 tentacles-Clark 1908). In the oral direction, the water-vascular ring gives start to ten outgrowths. One of them is the radial water-vascular canal to the radius A (on the opposite side to the stone canal in the interradius CD) (Fig. 3d, e). Others are the short tube outgrowths to the tentacular coeloms. Four of them dichotomically branch and also give rise to the radial water-vascular canals of the radii B, C, D, and E. On the right side of the animal, the order of these outgrowths is following (from bottom to top): (1) the single tube outgrowth to the tentacle, (2) the dichotomically branched outgrowth to the two tentacles and the radial canal of the radius B, (3) the single tube outgrowth to the tentacle, and (4) the dichotomically branched outgrowth to the two tentacles and the radial canal of the radius C. On the left side, the bilateral symmetry of the water-vacular outgrowths is broken only in radius D (Fig. 3e). We think that such disturbance of the left side may be formed as a result of the traumatic loss of one tentacle and subsequent regeneration.

Details of the connection of the tube outgrowths with the tentacular coelom are shown in Fig. 3h. The tentacular coelom in its basal part encloses the calcareous ring (Fig. 3b). The tube outgrowth extends along the calcareous ring on the inner side and fuses with the tentacular coelom. The coelomic cavities of the tentacles are lined with a coelothelium; under the coelothelium, there is a layer of the longitudinal musculature 20–80  $\mu$ m thick (Fig. 3b). The coelothelium of both the water-vascular ring and the tentacular coelomic cavity in the place of contact with the tube outgrowths consists of flat cells and is very thin—less than 10  $\mu$ m thick (Fig. 3h).

The five long water-vascular canals with a diameter of about 10  $\mu$ m turn to the aboral direction and extend along the radii of the body (Fig. 4a, b). The radial water-vascular canals are adjacent to the hyponeural canals from the inner side (Fig. 4).

The wide perioral (peripharyngeal) coelom lies to the oral side from the water-vascular ring (Figs. 4, 5a). It is adjacent to the inner side of the calcareous ring (Figs. 2, 3a, b, g, 4a, d). From the oral side, the perioral coelomic ring is adjacent to the oral nerve ring (Figs. 2c, d, 3b, 4a, e), but does not form any radial outgrowths. The coelothelium of the perioral coelom consists of flat cells; the wall thickness is not more than 10 µm.



The perihaemal coelomic system (somatocoelomic and axocoelomic perihaemal coeloms) is absent. However, the hyponeural system lies in the radii, and consists of five radial canals without any circumoral ring (Fig. 4e–g). The diameter of these hyponeural canals is  $20–30 \mu m$ 

(Fig. 4a–c). On the histological sections, the hyponeural canals appear from the oral side of the perioral coelom (not communicating with it) and calcareous ring (Fig. 4a) and accompany the radial water-vascular canals (which lie to the inner side of the hyponeural canals), and radial nerve

**∢Fig. 4** Radial complex of *Chiridota laevis*. Frontal sections. **a** Communication of the radial water-vascular canal (rwc) with the watervascular ring (wr). The hyponeural canal (hpc) ends blindly near the perioral coelom (poc). The ectoneural ring and nerve (ecn) are accompanied with the epineural ring and radial canals (epn). csr Calcareous ring, pvc perivisceral coelom. b Longitudinal section of the radial complex: ecn ectoneural radial nerve, ep epidermis, epn epineural radial canal, hpc hyponeural canal, hpn hyponeural nerve, rwc radial water-vascular canal. c Transverse section of the radial complex; the abbreviations are the same as for **b. d-f** 3D-reconstruction of the circumoral and radial structures; the upper row shows the lateral view, the lower row shows the oral view. amp Madreporic ampulla, epn epineural ring and radial canals, pc pore canal, sc stone canal, tn tentacle nerves. g, h Scheme of the relations between the perioral coelom (poc), water-vascular ring, nerves, and hyponeural canals (*hpc*) around the pharynx (g) and in the radius (h); *hmc* haemocoel, other abbreviations are the same as for b

cords (which extend from the outer side of the hyponeural canals) (Fig. 4b, c, f–h). The inner side hyponeural epithelium is flat, 10  $\mu$ m thick, and consists of the flagellate cells, similar to the adjacent coelothelium of the water-vascular canals and perivisceral coelothelium.

It is known that the radial haemal vessel passes between the epithelia of the radial water-vascular canal and the hyponeural canal (Mashanov et al. 2009). In *C. laevis*, there is an expansion in the place of contact of these epithelia (Fig. 4c), although the lumen of the vessel is not distinguishable on the histological sections.

Each radial nerve consists of ectoneural and hyponeural bands, separated by a basal lamina (Fig. 4b, c, h). The ectoneural band is a thickening of the inner wall of the epineural canal (Fig. 4h). The hyponeural band is a thickening of the outer wall of the hyponeural canal (Fig. 4h). The neuropile adjoins to the basal lamina, which separates the ectoneural and hyponeural bands. The perikaryons of the ectoneural band are concentrated near the lumen of the epineural canal (Fig. 4a–c). The same is true for the hyponeural band—its perikaryons are concentrated near the lumen of the hyponeural canal (Fig. 4b–c).

The epineural radial canals fuse with the epineural ring canal which lies adjacent to the oral nerve ring (Fig. 4). Both radial and ring epineural canals are traditionally interpreted as part of the environment that sunk into the tissue (Goldschmid 1996). However, in Ophiuroidea and Echinoidea, the epineural radial canals and epineural circumoral ring are formed by the closing of the epineural flaps. However, in Holothuroidea, the epineural canal "...appears to be formed by cavitation of tissues superficial to the presumptive radial nerve" (Smiley 1986).

The stone canal starts from the aboral side of the watervascular ring in the CD interradius and is suspended from the "gut" and "perioral" mesenteries (Figs. 2d, e, 3a, d). The stone canal is a small wavy canal (Figs. 2e, 3d) with walls encrusted with carbonate. It ends with the madreporic ampulla, from which a few short processes arise (Fig. 3c). These processes open by pores into the cavity of the perivisceral coelom. One long process (the pore canal) extends from the madreporic ampulla and opens with a pore into the environment on the oral side of the body in CD interradius (Fig. 3d, f). The walls of both the stone canal and the pore canal are lined with columnar flagellate coelothelium  $25-60 \mu m$  thick (Fig. 3a, f).

The stone canal is surrounded by a haemocoel lacune on its whole extent (Figs. 2b, e, 3a, 5b). The lacune communicates with the haemal system of the gonad, the oral haemal ring, and the haemocoel lacunae of the gut in the "gut mesentery" (Figs. 2b, d, e, 3a, 5b, c).

The oral haemal ring surrounds the pharynx and lies in the haemocoel spaces between the perioral coelom, the "perioral mesentery", and the oral body wall (Figs. 2c–e, 3a, 5b, d). The coelothelium here forms multiple folds with the miniature haemal lacunae between them (Fig. 5f).

The only gonad is located in the CD interradius (Figs. 2c, 5a, 6a). It is suspended from the "gut mesentery" in the cavity of the perivisceral coelom (Fig. 2d, e). The gonad divides into two branches, extending into the rear part of the body (Fig. 6a). Each branch divides into numerous thin outgrowths. The gonad is enveloped by the perivisceral coelothelium 5-10 µm thick (Fig. 6d-f). It is known that in Echinodermata, the gonad is located in a special genital coelom. In the studied species, the genital coelom is represented by narrow, but clearly obvious, spaces surrounding the gonad (Fig. 6c-g). The coelothelium of the genital coelom is 3 µm thick and contains muscle cells, which are well observable on the cross section of the gonad (Fig. 6g). In addition, there is an evident coelomic cavity within the gonad (the cavity of the "genital rachis"). The wall of the gonad (i.e., the interval between the narrow genital coelom and relatively wide inner coelomic cavity of the gonad) is thick (20 µm in the thinnest areas) and forms numerous growths within the gonad cavity (Fig. 6c, d). The space in the gonad wall represents the haemocoel, which is crossed by the fibers of connective tissue (Fig. 6c, e, f). The growing oocytes are deeply sunk into the thickness of the haemocoel, though they never lose the connection with the coelothelium of the gonad's inner coelom (i.e., the coelothelium of the "genital rachis") (Fig. 6d-g). The gonad gives start to a single gonoduct. The gonoduct lies in the "gut mesentery" in close proximity to the stone canal and, extended, becomes adjacent to the pore canal (Figs. 3c, f, 6b). We did not find the gonoduct pore-possibly, it appears during the reproduction only.

#### Discussion

Before discussing the axial complex, which is the main objective of this study, let us note several features that we identified that were not previously known for *C. laevis*. Hyman (1955) says that "in the Apoda <...>, there are no radial [water] canals in the body wall" (p. 154). As can be seen in Figs. 3d, e, and 4, *C. laevis* has five radial watervascular canals that are arranged in the same way as in other holothurians. It is possible that earlier researchers of Apodida simply did not find the radial water-vascular canals. Another possibility is that the canals are present only in the genus Chiridota. We will be able to answer this question definitely only after the re-examination of other representatives of Apodida.

Most echinoderms have a genital coelom (derivative of the left somatocoel), enclosing the genital rachis with the inner coelomic cavity of the actual gonad (Fedotov 1951; Ivanova-Kazas 1978; Goldschmid 1996; Ezhova et al. 2013, 2014, 2015; Kalachev 2014). Holothurians are described to lack the genital rachis (Smiley and Cloney 1985) or genital coelom (Hyman 1955). Our study shows that the thickwalled genital rachis of *C. laevis* is enclosed in a very narrow, but well noticeable genital coelom (Fig. 6c–g). Hence, the reproductive system in sea cucumbers is the same as in other Eleutherozoa. Naturally, the final solution of this problem requires a study of the reproductive system of holothurians on an electron-microscopic level.

The axial complex of holothurians is significantly simplified due to the reduction of its main components: the axial and pericardial coeloms with the haemal axial organ between their coelothelia. The left axocoel of Echinoidea gives rise to the axial coelom and the madreporic ampulla and in sea stars and brittle stars—also the axocoel perihaemal ring (Brooks and Grave 1899; MacBride 1903; Ubisch 1913; Gemmill 1914, 1915; Ivanova-Kazas 1978). Holothurians generally do not have an axial coelom, but a small remainder of the left axocoel apparently remains in adults. The studied *C. laevis* has a pronounced madreporic ampulla, which derives from the left axocoel. However, in the holothurian larvae, the left axocoel develops as a small vesicle connected with the hydropore (Balser et al. 1993).

The madreporite ampulla in most Echinodermata is connected with the exterior (Fig. 7a). In the majority of Holothuroidea (order Dendrochirotida, Aspidochirotida, Molpadida, and the Synaptidae family from the order Apodida), the pores of the madreporic plate open not into the environment, but into the perivisceral coelom (Fig. 7c). Moreover, ontogenetically, the stone canal opens into the exterior via a hydropore (McEuen and Chia 1991; Malakhov and Cherkasova 1992; Dolmatov and Yushin 1993; Mashanov and Dolmatov 2000), but in adults, this connection in most cases is lost. In Elasipodida, madreporic plate is connected with the environment by several pores (Théel 1882; Hyman 1955). In the studied *C. laevis* (Chiridotidae, Apodida), the madreporic ampulla is connected with the perivisceral coelom by several short canals and with the environment by a longer pore canal. This organization seems to be the middle state between most echinoderms (in which all madreporic canals open into the environment) and the typical organization of holothurians from orders Aspidochirotida, Dendrochirotida, and Molpadida (in which all madreporic canals open into the perivisceral coelom) (Fig. 7b). According to modern views, Apodida occupies the basal position in the phylogenetic tree of the class Holothuroidea (Kerr and Kim 2001).

How can we explain the shift of the madreporite inside the body? The madreporic opening connects the watervascular system and the axial coelom with the environment. At the same time, the wall of the axial coelom is an ultrafiltration site, through which the liquid is filtered from the haemal axial organ into the axial coelom (Welsch and Rehkämper 1987; Ziegler et al. 2009; Ezhova et al. 2016b). Thus, the pores of the madreporite are the excretory openings in echinoderms. Probably, the body of the holoturoid ancestor was almost entirely submerged in the soil except for the tentacles. In this case, the madreporite cannot provide the excretion. Some Apodida have a madreporite, which is partially submerged into the body, but maintains a canal and pore, which opens into the environment on the oral side under the tentacles. In this case, the pore can perform the excretory function. In the further evolution of holothurians, the madreporite loses touch with the environment completely. These forms have no excretory orifice. We can assume that in this case, the diffusion of byproducts from the perivisceral coelom occurs through the extensively branched surface of the respiratory trees, and from the water-vascular system through large branched tentacles.

The loss of the excretory functions by the axial coelom apparently led to the reduction of this coelom in all holothurians. Together with the axial coelom, the axial organ is also reduced. However, holothurians have a haemal lacune around the stone canal, which is connected with the gonad haemal system, gut haemal vessels in the "gut mesentery", and the oral haemal ring. This situation is similar to that observed in the other classes of Eleutherozoa: they have an axial organ which is always linked to three structures: (1) the genital haemal lacune and genital haemal ring, i.e., gonad haemal system, (2) the gastric haemal ring, i.e., gut haemal system, and (3) the oral haemal ring (Ezhova et al. 2013, 2014, 2015). Thus, the topographic interconnections of the axial complex haemal system are preserved in holothurians. Histologically, the structure of the haemal lacune of the stone canal of holothurians is more simple than the axial organ of other Eleutherozoa. In sea stars, brittle stars, and sea urchins, the lacunae of the axial organ lie in numerous interdigitations of the coelothelium of the axial and pericardial coeloms (Ezhova et al. 2013, 2014, 2015). In holothurians, these coeloms have disappeared, but a structure similar Author's personal copy



Fig. 5 Interrelations between different coeloms (a) and haemocoel structures (b) in the axial complex of *Chiridota laevis*. *Capital letters* indicate the radii. *amp* Madreporic ampulla, *gb* beginning of the haemal lacune in the "gut mesentery", *gn* gonad, *gnb* gonad haemal lacune, *gnd* gonoduct, *hpc* hyponeural canal, *orb* oral haemal ring, *pc* pore canal, *poc* perioral coelom, *rbv* radial haemal vessels, *rwc* radial water-vascular canals, *sc* stone canal, *scb* haemal lacune of the stone canal, *tc* tentacle coelom, *to* tube outgrowths from the water-vascular

ring to the tentacle coelom, wr water-vascular ring. **c–e** 3D-reconstruction of the oral haemal ring (*orb*) and its relations with the gonad haemal lacune (*gnb*), haemal lacune of the stone canal (*scb*), the haemal lacune of the gut (*gb*), and some circumoral structures: calcic skeletal ring (*csr*), and perioral coelom (*poc*). *gn* Gonad, *gnd* gonoduct. The *upper row* shows the lateral view; the *lower row* shows the oral view. **f** Histological structure of the oral haemal ring



Fig. 6 Gonad of *Chiridota laevis*. **a**, **b** 3D-reconstruction of the gonad (gn) with the gonduct (gnd) in the CD interradius (**a**) passing along the stone canal (sc) (**b**). *Upper row* shows the oral view; the *lower row* shows the lateral view. *Capital letters* indicate the radii. *amp* Madreporic ampulla. **c** Section through the branches of the gonad (gn), lying in the perivisceral coelom (pvc). *gnc* Genital coelom, lined its own coelothelium, *gnr* coelomic cavity within the

gonad (the cavity of the "genital rachis"). **d**, **g** Transverse section through the branch of the gonad. *mf* Muscle fibers, *ooc* oocyte, *pvc* perivisceral coelothelium, other abbreviations are the same as for **c**. **e**, **f** Scheme of the gonad structure. *hmc* Haemocoelic wall of the gonad, forming the growths within the inner cavity of the gonad (*gnr*), other abbreviations are the same as for **d** and **g** 

to the axial organ of other Eleutherozoa is present. It is the oral haemal ring, which is a network of small haemal lacunae that lie in the numerous folds of the coelothelium of the perioral coelom (in the studied *C. laevis* and some other holoturians). In histological sections, the holothuroid oral haemal ring looks like the axial organ of other classes of Eleutherozoa (Ezhova et al. 2013, p. 649, Fig. 5a, c; 2015, p. 250, Fig. 2h–j). Interestingly, the axial organ of other Eleutherozoa is supported by the stone canal, while the oral haemal ring of holothurians is supported by different structure—the calcareous ring. Apparently, the oral haemal ring is not a homologue of the axial haemal organ, although it probably functions as axial organ which is lacking in holothurians, and that explains the histological similarities. Note that the oral haemal ring of sea cucumbers cannot be considered as



🗯 epidermis, 📁 axial coelom and madreporic ampulla, 📩 stone canal, 📁 somatocoel cavity, 📁 connective tissue (haemocoel)

Fig. 7 Scheme of the location of the madreporte in most echinoderms ( $\mathbf{a}$ ), in holothuroid Elasipodida and Chiridotidae (Apodida) ( $\mathbf{b}$ ), and in holothuroid Dendrochirotida, Aspidochirotida, Molpadida, and Synaptidae (Apodida) ( $\mathbf{c}$ )

a homologue of the oral haemal ring of sea stars and brittle stars. In these classes, the oral haemal ring lies between the ring coeloms of the perihaemal system (the axocoelomic and somatocoelomic perihaemal rings), and in holothurians, the perihaemal system is absent and the oral haemal ring lies between the perioral coelom and the water-vascular ring.

The lack of the somatocoelomic perihaemal coelom is important for the analysis of the structure of the radial complex of organs in holothurians and Eleutherozoa, in general. In the Introduction, we noted that the similarities of the radial complex (the presence of epineural canals above ectoneural nerves in particular) have allowed to unite holothurians with sea urchins and brittle stars as the Cryptosyringida. However, the hyponeural and epineural canals of holothurians are formed differently than in other Eleutherozoa and have different organization. In Asteroidea and Ophiuroidea, a pair of somatocoelomic perihaemal outgrowths accompanies the radial hyponeural nerve (Ezhova et al. 2013, 2015). In Holothuroidea, the somatocoelomic perihaemal ring and its radial outgrowths do not develop at all (Ivanova-Kazas 1978; Malakhov and Cherkasova 1992), and the hyponeural nerve is accompanied with the hyponeural canal, which develops after the metamorphosis (Smiley 1986) in 1-month old juveniles and appears neither in anterior, nor in posterior, but in the central part of the body, first in the mid-ventral nerve cord (Dolmatov et al. 2016). According to some recent research, the hyponeural canal is not a coelom, but a cavity surrounded by glial cells (Mashanov et al. 2013). There is no hyponeural circumoral ring in holothurians and the radial hyponeural canals are adjacent to the perioral coelomic ring but do not connect with it. Epineural radial canals in holothurians are formed by a gap in the tissue along the ectoneural nerve but not by the closing of the epineural folds like in brittle stars and sea urchins (Smiley 1986). It is possible that the epineural canals of holothurians appeared independently from the epineural canals of brittle stars and sea urchins.

Holothurians are the only group of echinoderms, which have circumoral tentacles. The tentacular coeloms develop from the left mesocoel (hydrocoel) same as the radial water-vascular canals (Ivanova-Kazas 1978; Smiley 1986; Malakhov and Cherkasova 1992). Some authors have suggested that the canals of the five primary tentacles are homologous with the radial water-vascular canals of other echinoderms, and the holothuroid water-vascular canals are a new formation (Semon 1888; David and Mooi 1996, 1998). Dolmatov et al. (2016) consider that the tentacles of holothurians are homologous with the ambulacral podia. Smiley (1986) believed that the tentacle coeloms of holothurians are homologous with the axocoel of the other echinoderms, because their coelomic lining comes from the axohydrocoel in the embryogenesis. He also postulated the homology between brachiolar podia of asteroids and buccal tube-feet of holothurians (Smiley 1988). However, Smith (1997) opposed this, stressing that the brachiolar podia of asteroids originates entirely from axocoel and buccal tube-feet of holothurians originates exclusively from the hydrocoel.

Most likely, the tentacles of sea cucumbers are homologous to neither the ambulacral podia nor the brachiolar podia. Five tentacles are initially formed in the development of holothurians (Smiley 1986; Malakhov and Cherkasova 1992). The tentacles and their coeloms are formed before the radial water-vascular canals and ambulacral podia (tentacles of holothurians are often called "primary tentacles" or "primary buccal podia"—Hyman 1955; Smiley 1986). The tentacles are formed in the interradii (Smiley 1986; Malakhov and Cherkasova 1992; Dolmatov and Yushin 1993; Mashanov and Dolmatov 2000; Dolmatov et al. 2016). In *Leptosynapta*, the five primary tentacles retain their positions in the interradii (Runnström 1927). In *Cucumaria* and *Apostichopus*, the five primary tentacles shift: two move closer to the mid-ventral water-vascular canal, other two—to the left dorsal water-vascular canal, and the last—to the right dorsal water-vascular canal (Runnström 1927; Dolmatov et al. 2016). The studied *C. laevis* has 12 tentacles, the coeloms of which are connected by tube outgrowths not with the radial water-vascular canals, but with the water-vascular ring. The water-vascular canal in radius A is not associated with the tentacles (Fig. 3d, e).

We can assume that the tentacles of sea cucumbers are homologous with the mesocoel tentacles of the common deuterostome ancestor. Coeloms of the arms of Pterobranchia develop from the mesocoel (Schepotieff 1909; John 1932). The coelom of the circumoral tentacles of a lancelet also develops from the second pair of coeloms (MacBride 1898). Echinoderms have retained only the left mesocoel, and thus, tentacles of holothurians are homologous to only the left half of the tentacular apparatus of other Deuterostomia.

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#### Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** All applicable international, national, and institutional guidelines for the care and use of animals were followed. All authors read and approved the final manuscript.

**Informed consent** Informed consent was obtained from all individual participants included in the study.

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