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ORIGINAL PAPER

Microscopic anatomy of the axial complex and associated structures in the brittle star *Ophiura robusta* Ayres, 1854 (Echinodermata, Ophiuroidea)

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Abstract Several details of the anatomy of the axial complex in brittle stars remain unknown, and there are many mismatching descriptions of its structure in different sources. The comparison of the ophiuroid axial complex with other classes of echinoderms is important for understanding of the phylogenetic relations in Ophiuroidea. We describe the organization of the axial complex of *Ophiura robusta*, compare it with other Ophiuroidea and analyse how the specific structure of the brittle star axial complex could appear in evolution. The standard technique of dehydration of material in alcohols of increasing concentration was used, followed by embedding material in paraplast and dissection. In the main components, the axial complex of Ophiuroidea fits other Asterozoa. But there are some important differences. The stone canal connects with the ambulacral ring from the outside, not from the inside. The somatocoelomic perihaemal ring is closer to the mouth than the axocoelomic ring. The axial complex lies between the genital coelom and the digestive tract. The gastric haemal ring is located on the outer side of the axial complex. The "pericardial" part of the axial organ is shifted to the oral side, but all its anatomical connections are retained: with the genital haemal ring, with the haemocoel of the body wall, with the gastric haemal ring and the vessels of the axial part of the axial organ. All these features could be explained as a result of shifting of madreporic plate along interradius CD from the aboral side to the oral side.

Keywords Axial complex · Brittle stars · Ophiuroidea · Microscopical anatomy · Phylogeny

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Introduction

The morphology and anatomy of brittle stars (Echinodermata, Ophiuroidea) are considered to be well studied, although the majority of studies devoted to this group of echinoderms focus on their skeletal elements, which play an important role in ophiuroid taxonomy and facilitate understanding of the biomechanics of movement. Considerably less attention has been paid to the soft tissue anatomy of brittle stars, including the organization of the axial complex, a structure of importance in the identification of phylogenetic relationships between different higher echinoderm taxa (Byrne 1994; Stöhr 2012). The structure of the axial complex of echinoderms, the homology of its parts to organs of other Deuterostomia and the phylogenetic significance of these homologies have been actively discussed for more than a century (Bather 1900; Fedotov 1923, 1924, 1951, 1966; Cuénot 1948; Hyman 1955; Ivanova-Kazas 1978; Ivanov et al. 1985; Goldschmid 1996; Ruppert et al. 2004). The organization and development of the axial complex in Ophiuroidea have been described by several authors (Ludwig 1880; Cuénot 1888; Hamann 1889; MacBride 1892, 1907; Brooks and Grave 1899; Narasimhamurti 1933; Smith 1940; Olsen 1942). Nevertheless, several details of the anatomy of this organ remain unknown. For example, there are incoherent descriptions of its general structure and there is to date no complete

reconstruction of the axial complex for brittle stars. The role of the haemal system of ophiuroids and other echinoderms remains mysterious. It is involved in the storage and distribution of nutrients (Ferguson 1985; Byrne 1988). It is possible that haemal fluid could be manipulated to vary the viscosity of the echinoderm extracellular matrix (Byrne 1988, 1994).

The homology of the entire complex and its constituent parts within the Echinodermata or with other organs of Deuterostomia remains problematic (Hyman 1955; Ubaghs 1967; Ivanova-Kazas 1978; Goldschmid 1996; Ruppert et al. 2004; Ziegler et al. 2009). Generally the axial complex of echinoderms is believed to be homologues to heart–kidney complex of hemichordates. Various substructures forming the echinoderm axial complex are suggested to correspond with the constituents of hemichordate heart–kidney complex (Fedotov 1923; Balser 1990; Cameron 2000; Stach 2002; Ziegler et al. 2009).

Ophiuroids have undergone significant morphological and anatomical changes during the course of their evolution in comparison with other classes of echinoderms. Ophiuroids are believed to be closely related to the Asteroidea (Smith 1940; Ivanova-Kazas 1978; Goldschmid 1996; Ruppert et al. 2004) and to the Echinoidea (MacBride 1907; Narasimhamurti 1933). According to molecular analysis, support for the clade Asteroidea + Ophiuroidea is apparent, albeit not as strong as for other clades, such as Echinozoa (Echinoidea + Holothuroidea), Eleutherozoa, and Echinodermata (Janies 2001). However, in later works, was found support for a clade containing holothuroids and ophiuroids and was found the echinoids sister to that clade (Janies et al. 2011). In this context, a detailed study of the structure of the brittle star axial complex could be of particular interest. Therefore, we describe the organization of the axial complex of the brittle star *Ophiura robusta* Ayres, 1854, a species belonging to the Ophiuridae Müller and Troschel, 1840.

Materials and methods

Specimens of *O. robusta* with 2–4 cm in size (radius + interradius) were collected in July 2011 near the Pertsov White Sea Biological Station, which is located on the Kandalakshskii Bay (White Sea). Animals were collected at depths from 2 to 10 m from rocky substrates. For histological study, the animals were fixed in Bouin's fluid. To improve the penetration of the fixative, the arms were cut off near the base. Only the central disc with the bases of the arms was subjected to further processing. Until histological study, the material was preserved in 70 % ethanol. To prepare the material for histological processing, standard decalcification procedures were performed using EDTA solution (Trilon B, Helaton-3) (Valovaya and Kavtaradze 1993). The standard technique of dehydration of material in alcohols of increasing concentration was used, followed by embedding material in paraplast and cutting it into sections of 5 μm thickness. In total, five specimens were studied using light microscopy. Five series of sections of the disc in the vicinity of the axial region were made: two series in the sagittal plane and three series perpendicular to the oral-aboral axis. Photographs of histological sections were made using an Axioplan 2 photomicroscope ("Carl Zeiss Microscopy", Jena, Germany, 2003).

Results

The axial complex of *O. robusta* is located in the same interradius as in all other echinoderms. According to generally approved notation, this interradius is usually indicated as interradius CD (see for example, Ubaghs 1967; Goldschmid 1996). The pore of the madreporic plate is located on the oral side, and the entire axial complex is oriented towards the oral-aboral axis at an angle of about 45° (Fig. 1). The substructures of the axial complex can be classified as either axial (i.e. stone canal, "pericardial"[1] coelom, axial coelom, madreporic ampulla, pore canal, axial organ) or circular structures (i.e. water ring, genital ring, perihaemal ring coeloms and haemal vessels).

The axial structures of the axial complex

At the centre of the axial complex is a tubular stone canal. Its walls are partly encrusted with calcium carbonate, and its lumen is lined with a columnar ciliated epithelium. The stone canal of *O. robusta* has a simple circular lumen without any additional structures. One end of the stone canal connects with the ambulacral ring, opening into it from the outside (Figs. 1, 2a, b). From the ambulacral ring, the stone canal continues at an angle of about 45° towards the oral-aboral axis into the direction of the oral surface. Near the oral side, it curves at an obtuse angle and connects with the madreporic ampulla (Figs. 1, 2c, d). The descending part of the stone canal is about twice as long as its horizontal part. The coelomic epithelium of the stone canal is formed by monociliated cells, which are elongated in the apical-basal direction. A basement membrane, easily visible in the histological sections, underlies the stone canal luminal epithelium. The coelothelium of the

[1] The term "pericardial" is not completely correct, because "pericardial" implies that it has been established that the axial organ is a heart, i.e., a circulatory pump, and this is not the case. Nevertheless, we use this term as it is traditional in literature and its replacing may produce many confusions. Therefore, we use this term in inverted commas.

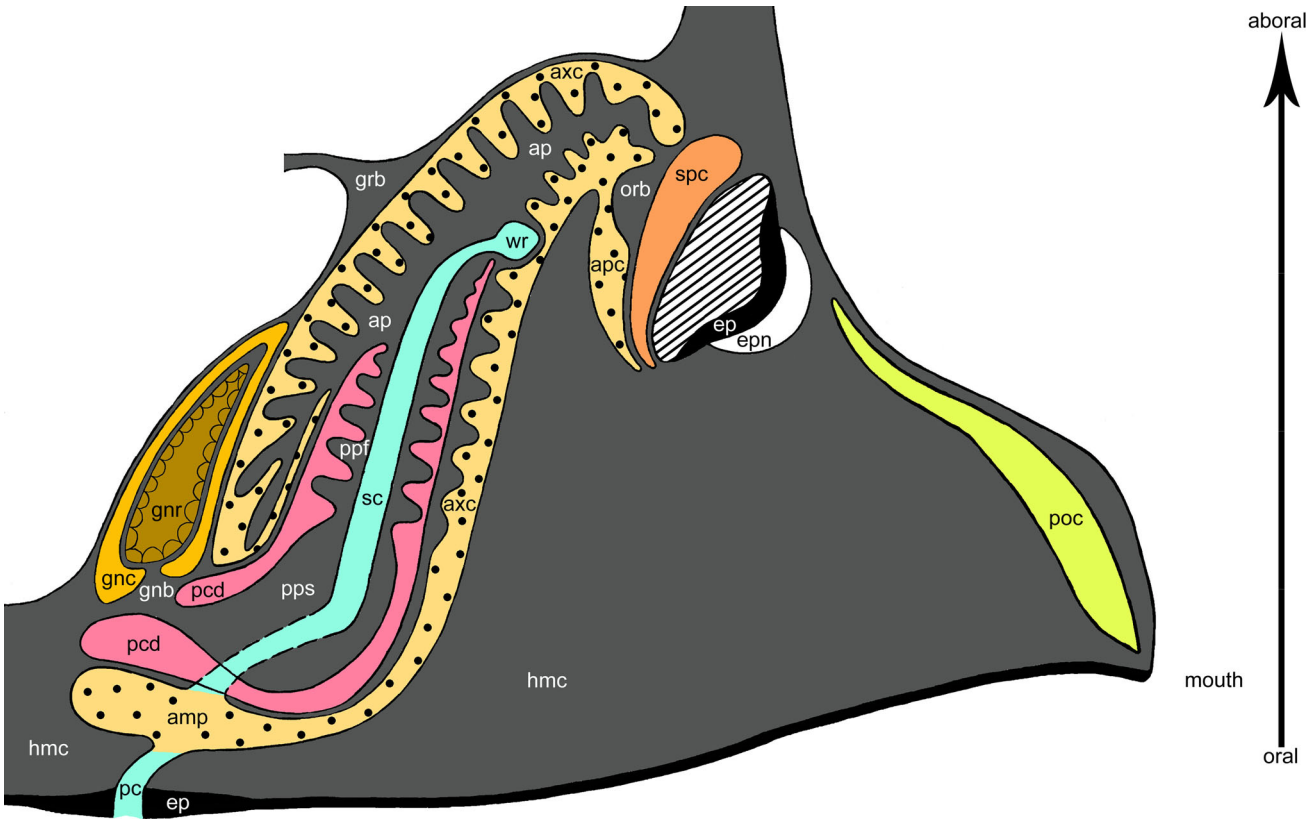
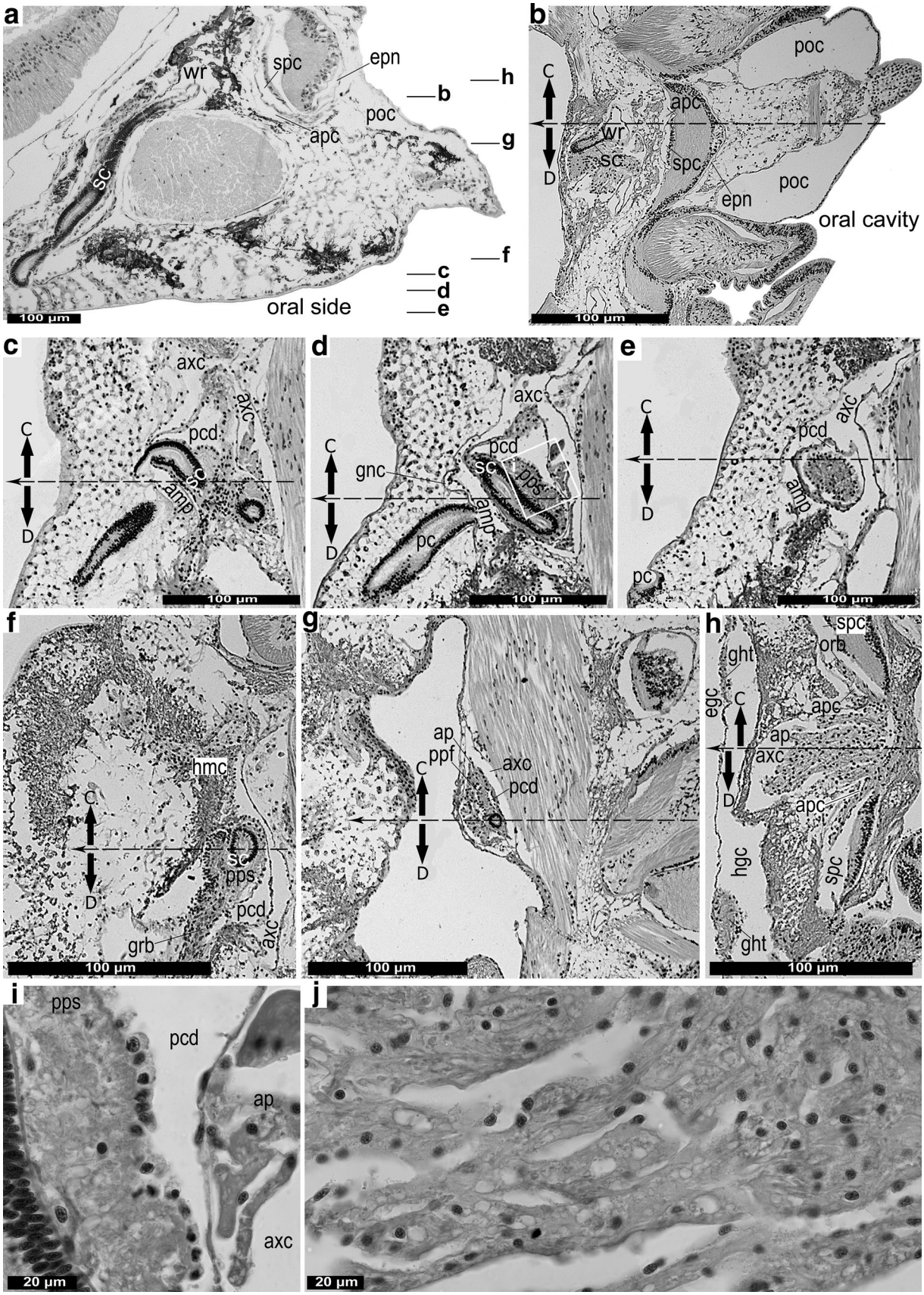


Fig. 1 Diagram of a generalized section through the axial complex of *Ophiura robusta*. Epidermis (*ep*) is *black*; haemocoel and its structures are *dark grey*; coelomic structures are *coloured*. The nerves are hatched. The axocoel derivatives are stippled. *Arrow* shows the direction of oral-aboral axis. *amp* madreporic ampulla, *ap* axial part of axial organ, *apc* axocoelomic perihaemal ring, *axc* axial coelom, *epn* epineural channel, *gnb* genital haemal ring, *gnc* genital coelom, *gnr* genital rachis, *grb* gastric haemal ring, *hmc* haemocoel of the body wall, *orb* oral haemal ring, *pc* pore canal, *pcd* "pericardial" coelom, *ppf* mesh (aboral) region of the "pericardial" part of axial organ, *pps* swollen (oral) region of the "pericardial" part of axial organ, *poc* perioral coelom, *sc* stone canal, *spc* somatocoelomic perihaemal ring, *wr* ambulacral ring (colour figure online)

"pericardial" coelom is also underlaid by own basal membrane. The thin layer of the extracellular matrix is visible between these two basal membranes. The madreporic ampulla is connected with the exterior through a single pore canal, which penetrates the madreporic plate (Fig. 2d, e). The pore canal connects with the madreporic ampulla in interradius CD, but the madreporic pore is shifted in the direction of radius D (Fig. 2e). The histological structure of the pore canal is similar to that of the stone canal. The walls of the ampulla are lined with a monociliated squamous epithelium (Fig. 2c–e).

Almost the entire stone canal is surrounded by the network of haemal vessels formed by the axial organ. The axial organ of brittle stars is here divided into two parts: the axial part and the "pericardial" part. The "pericardial" part of the axial organ surrounds almost the entire length of the stone canal and is enclosed in the cavity of the "pericardial" coelom (Fig. 1). The axial part of the axial organ is enclosed by the axial coelom and is located on the outside of the "pericardial" part and "pericardium", on the side of radius C.

The "pericardial" part of the axial organ is divided into a swollen region (oral) and a mesh region (aboral). The swollen region accompanies the horizontal part of the stone canal, while the mesh region accompanies the descending part (Fig. 1). Near the turn of the stone canal, the swollen "pericardial" region of the axial organ communicates with the genital haemal ring and haemocoel of the body wall (Figs. 2f, 3). Although the tissue of the axial organ surrounds the stone canal on all sides, it is thickened in radius C, because the aforementioned swelling (Fig. 2d) and mesh (Fig. 2g) are located on this side. The "pericardial" part of the axial organ is a network of haemocoelomic lacunae located within connective tissue that lie between the coelothelium lining the stone canal, and the coelothelium of the "pericardial" coelom located adjacent to the stone canal. The thickness of the "pericardial" coelothelium constituting the swollen region of the "pericardial" part of



◀**Fig. 2** Stone canal, pore canal and axial organ of *Ophiura robusta*. Sections in the plane of the madreporic plate (**a**), and in the plane perpendicular to oral-aboral axis (**b–j**). *Dotted arrow* indicates the direction of interradius CD, *bold arrows* show the direction to radii C and D. *Horizontal lines* on **a** labelled **b** to **h** indicate the planes of the sections shown in **b–h**. **a**, **b** Communication of the stone canal (*sc*) with the ambulacral ring (*wr*). *apc* Axocoelomic perihaemal ring, *epn* epineural ring channel, *poc* perioral coelom, *spc* somatocoelomic perihaemal ring. **c** Communication of the stone canal (*sc*) with the ampulla (*amp*). **d** Communication of the pore canal (*pc*) with the ampulla (*amp*). "Pericardial" coelom (*pcd*) and "pericardial" part of axial organ (*pps*) embrace the stone canal (*sc*). *gnc* Genital coelom. **e** Junction of the ampulla (*amp*) and the axial coelom (*axc*), and communication of pore canal (*pc*) with the exterior. **f** Communication of the "pericardial" part of axial organ (*pps*) with the genital haemal ring (*grb*) and the haemocoel of the body wall (*hmc*). **g** Central region of the axial organ. *ap* Axial part of axial organ, *axc* axial coelom, *pcd* "pericardial" coelom, *ppf* mesh region of "pericardial" part of axial organ. **h** Communication of the axial part of axial organ (*ap*) with the oral haemal ring (*orb*). *apc* Axocoelomic perihaemal ring, *egc* epigastric coelom, *ght* gastric haemal tufts, *hgc* hypogastric coelom, *spc* somatocoelomic perihaemal ring. **i** Histological structure of the "pericardial" coelom (*pcd*) and the "pericardial" part of axial organ (*pps*). *axc* Axial coelom. **j** Histological structure of the axial part of axial organ

the axial organ is about 30 μm (Fig. 2i). The coelothelium consists of monociliated cells, the nuclei of which are located apically. The remainder of the cell is formed by extensions of the coelothelial cells. Between these extensions lie haemocoel lacunae of different sizes and shapes. Directly adjacent to the stone canal coelothelium are large cells with voids. Perhaps, these cells are the sclerocytes, and the voids remained after the solution of the stereom during the preparation. The histological structure of the coelothelium of the "pericardial" coelom in the mesh area is similar.

The "pericardial" part of the axial organ (along with the stone canal) is surrounded by the "pericardial" coelom, which in turn is surrounded by the axial coelom (Figs. 2g, 3). The "pericardial" coelom is a closed coelomic cavity with an elongated shape, expanding on its oral end (Figs. 1, 3). The "pericardial" coelom surrounds the stone canal and the axial organ from the side of radius C. In sections that pass perpendicular to the oral-aboral axis, the "pericardial" coelom has a horseshoe-shaped form, the ends of which are facing radius D (Fig. 2c–e). The lining of the "pericardial" coelom is non-homogeneous: part of the lining that surrounds the stone canal is thickened, and the part adjacent to the wall of the axial coelom is formed by squamous cells (Fig. 2d–i).

The axial coelom of *O. robusta* surrounds the stone canal and the "pericardial" coelom also from the side of radius C. In sections perpendicular to the oral-aboral axis, it also has a horseshoe-shaped form, the ends of which are facing radius D (Fig. 2g). The oral part of the axial coelom forms the madreporic ampulla, which, as mentioned above, connects with the oral end of the stone canal (Figs. 1, 2c, d, 3). The aboral part of the axial coelom communicates with the axocoelomic perihaemal ring (Figs. 1, 2h, 3).

The axial part of the axial organ is a network of haemocoelomic lacunae that are located within connective tissue between the coelothelium lining the "pericardial" coelom and the coelothelium of the axial coelom (Figs. 1, 2g). In sections of the axial part of the axial organ, numerous coelomic "tubules" can be seen. These "tubules" are the mutual interdigitations of the axial and "pericardial" coeloms (Fig. 2j). In the tissue septa between the coelomic canals lie numerous haemocoelomic lacunae. The coelothelium of the axial coelom (including the madreporic ampulla) is lined with a monociliated squamous epithelium (Fig. 2c–h).

The axial part of the axial organ extends towards the aboral direction from the horizontal part of the stone canal and reaches a maximum thickness in the region located above the confluence of the stone canal with the ambulacral ring (Fig. 2h). The vascular network of the axial organ takes up almost the entire volume of the axial coelom and on its oral side communicates with the oral haemal ring (Fig. 3).

Circular structures of the axial complex

The perihaemal system of *O. robusta* includes two ring coeloms, the axocoelomic and somatocoelomic rings, which are separated by a mesentery (Figs. 1, 2a, b, h, 3). In the mesentery between the axocoelomic and somatocoelomic rings of the perihaemal system lies the oral haemal ring, from which a single radial haemal vessel continues into each arm (Figs. 3, 4a, b). From the somatocoelomic perihaemal ring, two radial somatocoelomic processes continue into each arm. The radial haemal vessel lies in the mesentery separating the processes (Fig. 4b). The ambulacral ring lies outside of the coelom rings of the perihaemal system (Figs. 1, 3, 4a) and is shifted to the oral side of the animal (Fig. 2a). From the ambulacral ring, five radial coelomic processes continue into the arms. In sections across the arm, the ambulacral radial coelomic process is located on the aboral side of the somatocoelomic processes (Fig. 4b).

In total, there are four coelomic rings surrounded the mouth: the ambulacral ring (into which the stone canal opens), the axocoelomic perihaemal ring (into which the axial coelom opens), the somatocoelomic perihaemal ring (Fig. 3) and the perioral coelomic ring. The latter is often found adjacent to the mouth and forms five interradial tooth sacs (Fig. 2a, b).

In addition to these four rings, there is a canal surrounding the mouth in *O. robusta* called the epineural ring channel (Figs. 2a, b, 4a). This structure is not of coelomic nature and is essentially an external groove that has sunk into the body and enclosed part of the external

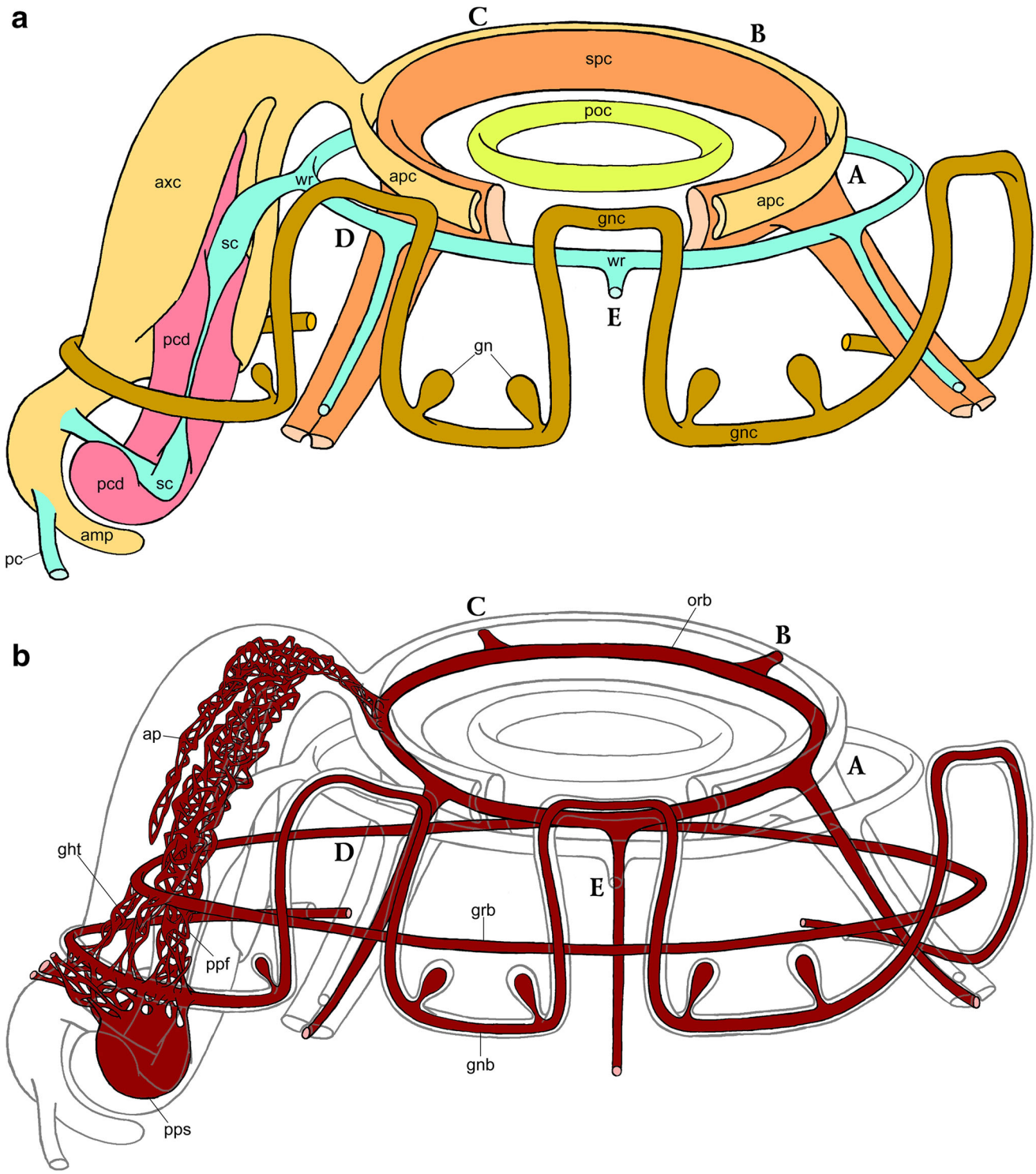


Fig. 3 Interrelations between different coeloms (**a**) and haemocoel structures (**b**) in the axial complex of *Ophiura robusta*. Capital letters indicate the radii. *amp* Madreporic ampulla, *ap* axial part of axial organ, *apc* axocoelomic perihaemal ring, *axc* axial coelom, *ght* gastric haemal tufts, *gn* gonads, *gnb* genital haemal ring, *gnc* genital coelom, *grb* gastric haemal ring, *orb* oral haemal ring, *pc* pore canal, *pcd* "pericardial" coelom, *poc* perioral coelom, *ppf* mesh (aboral) region of "pericardial" part of axial organ, *pps* swollen (oral) region of "pericardial" part of axial organ, *sc* stone canal, *spc* somatocoelomic perihaemal ring, *wr* ambulacral ring (colour figure online)

environment. The epineural ring channel accompanies the epineural ring of the nervous system and sends horizontal radial processes into each arm, which accompany each epineural radial nerve (Fig. 4b). In addition, the epineural ring channel sends out vertical interradial appendages that accompany nerves that innervate the teeth (Fig. 4a).

The genital coelom is in close contact with the axial complex (Figs. 1, 3). It also has the shape of a ring, and in *O. robusta* it is located in the peripheral part of the central disc and is not connected to any other coelomic cavity. In the radii, the genital coelomic ring forms loops, which rise to the aboral side. In the interradii, sections of the genital coelom are located oral to all other coelomic rings (Fig. 2d). The loops of the genital coelom continue into the bases of the arms and bend around the processes of the ambulacral and perihaemal coeloms on the aboral side (Fig. 4b). Inside the genital ring lies the ring genital rachis, the inner lining of which consists of the germinative epithelium. The cavity of the genital rachis is also a coelom. Between the basement membranes of the germinative epithelium of the genital rachis and the inner layer of the genital coelothelium lies the genital haemal ring composed of haemal lacunae (Fig. 4c). The gastric haemal ring lies in the horizontal mesentery dividing the body cavity into epigastric and hypogastric coeloms. In *O. robusta*, the "pericardial" part of the axial organ communicates with the gastric haemal ring through two tufts of haemal strands, but only in the area where it connects with the genital haemal ring (Figs. 1, 2h, and 3).

Discussion

Discussion of the structure of the axial complex is complicated by the fact that different authors have used different terms for the same components, as indicated in Table 1.

Early works on the structure and development of brittle stars contain a number of errors in the description of the axial complex. In some publications, only one perihaemal ring bordering directly with the peripharyngeal nerve ring is mentioned (Ludwig 1880; Hamann 1889). However, brittle stars have two perihaemal rings: an axocoelomic ring and a somatocoelomic ring. The descriptions of the cited authors probably refer only to the somatocoelomic perihaemal ring, while the axocoelomic perihaemal ring went unnoticed.

Bury (1889) believed that the stone canal opens into the ambulacral ring in interradius AB, but the stone canal opens into this ring in interradius CD. This author also interpreted the axial coelom as a derivative of the left posterior larval coelom (i.e. the left somatocoel), whereas it is in fact a derivative of the left anterior larval coelom (i.e. the axocoel). MacBride (1892, 1907) called the madreporic vesicle (i.e. the "pericardium") the "right hydrocoel", although it is a derivative of the right axocoel.

This author also tried to avoid the confusion in the interpretation of coeloms of the axial complex by using the terms "sinus *a*" (for the genital coelom), "sinus *b*" (for the axial coelom) and "sinus *c*" (for the ampulla). He also argued that in brittle stars "sinus *b*" (i.e. the axial coelom) is absent. Furthermore, in the works of Bury (1889) and MacBride (1892, 1907), the axial organ of brittle stars was called the "ovoid gland" and was considered to be the anlage of the genital rachis. However, the axial organ and genital rachis of brittle stars are separate structures, though closely located to each other.

Later, Fedotov (1924) described the brittle star axial organ and was the first to point out its division into a "right" and "left" part, enclosed into the cavity of the right and left axial sinus. He emphasized that the ampulla of the stone canal communicates with the "left axial sinus" (i.e. the axial coelom). The description of the axial complex of brittle stars made by Smith (1940) is similar to that given by Fedotov (1924), although with a slightly different terminology (Table 1). Smith (1940) noted the vascular nature of the axial organ and its close relationship with the "pericardium", the contractions of which contribute to fluid circulation in the vessels of the axial body (Smith 1940, p. 269).

In a paper about the development of "*Ophiocoma*" (now *Ophiocomina*) *nigra* Abildgaard, 1789, Narasimhamurti (1933) described the presence of a "heart" that contracted every 12 s. It "is formed by the invagination of the ventral wall of the pericardial vesicle" (i.e. the right axocoel) and corresponds to the swollen region of the "pericardial" part of the axial organ. Narasimhamurti (1933, p. 84) also noted that "all perihaemal spaces arise as… evaginations from the left posterior coelom", i.e. the left somatocoel. However, only the somatocoelomic perihaemal ring is formed by the left somatocoel, whereas the axocoelomic perihaemal ring originates from the left axocoel.

In the illustrations of the axial complex of *Ophiura sarsii* Lütken, 1855, Ivanov et al. (1985) label a region of the "pericardial" coelom as "cavity of the axial sinus", but without specifying whether it is the right or left axocoel (i.e. the "pericardium" or the axial coelom). However, the "pericardial" coelom is derived from the right larval axocoel. In *O. sarsi*, the radial perihaemal coelomic outgrowths are described as unpaired structures (Ivanov et al. 1985, p. 563, Fig. 759), whereas the present data suggest that in *O. robusta* a pair of these outgrowths continues into each arm and that between the mesentery of the outgrowths lies the radial haemal vessel. Furthermore, Ivanov et al. (1985, p. 565) mention that the sexual stolon has a "direct connection with aboral department of the axial organ". We argue that there can be no "direct connection" between these two bodies, because the sexual stolon (i.e. the genital rachis) is a coelomic formation and the axial organ is part of the haemocoel.

Earlier, Ivanova-Kazas (1978) indicated that in different genera of brittle stars the location of the confluence of the

stone canal and the ambulacral ring may differ: in *Ophioderma* Müller and Troschel, 1840 it is found in the area of radius B, in *Ophiopholis* Müller and Troschel, 1842 in the interradius AB, and in *Ophiothrix* Nägeli, 1849 and *Ophiocoma* L. Agassiz, 1835 in interradius CD.

Cuénot (1948, p. 257, Fig. 290) stated that in brittle stars the madreporic ampulla opens with a pore canal into the nearest bursal slit. However, in *O. robusta*, the pore canal opens into the external environment independently of a bursal slit. In addition, only one perihaemal coelom is mentioned by Cuénot, although in the species studied here and in most of other studied brittle stars there are two perihaemal coelomic rings: the axocoelomic and somatocoelomic rings. On the figures provided by Cuénot (1948, p. 251, Fig. 284. p. 252, Fig. 285, p. 254, Fig. 288, p. 258, Fig. 293), the radial perihaemal coelomic process is shown unpaired, but *O. robusta* has a pair of coelomic processes in each arm, and in the mesentery between these processes lies the radial haemal vessel. Cuénot (1948, p. 254) stated that brittle stars may have one or two closed perioral cavities, but actually in all investigated brittle stars (including *O. robusta*) there is only one perioral coelom. Furthermore, Cuénot (1948) described a "wavy sinus", which accommodates a lacuna, "which leads to the genitals". Presumably, the "wavy sinus" of Cuénot is the wavy genital coelom (Table 1). With regard to the axial coelom he states that "closer to the end of the ventral side" it is divided into two cavities: the "proximal", which communicates with the stone canal, and the "distal", which does not connect with any other structure. These findings are consistent with our data, if we assume that the "proximal" cavity corresponds to the axial coelom and the "distal" to the "pericardium" (Table 1).

Hyman (1955) concluded that brittle stars have only one perihaemal coelomic ring. In an illustration of a section through the madreporic plate of *Ophiothrix* (Hyman 1955, Fig. 253B) only a single perihaemal coelomic ring is shown lies between the mouth and nerve cord, while the epineural canal is shown on the opposite side of the nerve cord. Our research, as well as data from other authors (Ivanova-Kazas 1978; Goldschmid 1996), shows that brittle stars have two perihaemal coelomic rings, with the epineural canal adjacent to the nerve cord on the side of the mouth and to the perihaemal coelomic ring on the opposite side (Fig. 1).

The structure and topography of individual structures of the axial complex are vary between different brittle star species. In the studied *O. robusta*, there is just a single pore in the madreporic plate, whereas in *Ophionereis annulata* Le Conte, 1851 there are eight pores and eight pore canals (Cuénot 1948), and in *Ophiopsila annulosa* M. Sars, 1859 from three to twelve pores, and a corresponding number of pore canals (Reichensperger 1908).

Fig. 4 Circular structures of the axial complex of *Ophiura robusta*. ▶ **a** Section through one of the radii in the plane perpendicular to oral-aboral axis. *apc* Axocoelomic perihaemal ring, *en* epineural appendages accompanying the teeth nerves, *epn* epineural ring channel, *spc* somatocoelomic perihaemal ring, *wr* ambulacral ring. **b** Section through the arm. *bv* Single radial haemal vessel, *egc* epigastric coelom, *epn* radial process of the epineural channel, *gnc* genital coelom, *hgc* hypogastric coelom, *spc* paired processes of the somatocoelomic perihaemal ring, *wr* radial coelomic process of the ambulacral ring. **c** Histological structure of the gonad. *gnb* gonad haemal lacuna, *gnc* genital coelomic ring, *gnr* genital rachis. **d** Location of the gonads (*gn*) in the body; section in the plane perpendicular to oral-aboral axis. *brs* Bursa

In *Gorgonocephalus* Leach, 1815 a true madreporic plate develops, containing up to 250 pores (Ludwig 1878). In addition, there can be more than one madreporic plate in ophiuroids. For example, *Ophiactis virens* M. Sars, 1857 has up to five madreporic plates due to its ability to reproduce asexually by fission (Hyman 1955). Some members of the genus *Astrophyton* Fleming, 1828 have madreporic plates in all interradii, and every madreporic plate is pierced by 15–20 pores. In all brittle stars with multiple madreporic plates, a separate stone canal connects each madreporic plate with the water ring. The number of axial organs is the same as the number of stone canals (Cuénot 1948, p. 259). Finally, in *Trichaster elegans* Ludwig, 1878 there is a single pore in each interradius, which is not associated with skeletal elements (Ludwig 1878).

The stone canal of *O. robusta* opens into the ambulacral ring from the outside (Fig. 2a, b). In *Ophiothrix fragilis* Abildgaard, in O.F. Müller, 1789 the stone canal makes a loop and communicates with the ambulacral ring on the inside (MacBride 1907, p. 579; Smith 1940, p. 272). In his description of *Ophiocomina nigra* Abildgaard, in O.F. Müller, 1789, Narasimhamurti (1933, p. 73) also describes the communication of the stone canal with the ambulacral ring on the inner side.

The organization of the axial complex in brittle stars suggests that during the evolution of the group this structure gradually moved along interradius CD and rotated by 135° as compared to its position in other Asterozoa (Fig. 5). As a consequence, the madreporic plate and the pore canal have shifted to the oral side of the animal. In sea stars and sea urchins, the stone canal runs from the aboral side to the ambulacral ring, which is located on the oral side. However, in brittle stars the stone canal runs from the madreporic ampulla, which is shifted to the oral side, to the ambulacral ring, which is located closer to the aboral side. In sea stars, the stone canal connects with the ambulacral ring from the inside. It is interesting to note that in some brittle stars the stone canal makes a loop, so that it connects with the ambulacral ring from the inside (MacBride 1907; Narasimhamurti 1933; Smith 1940).

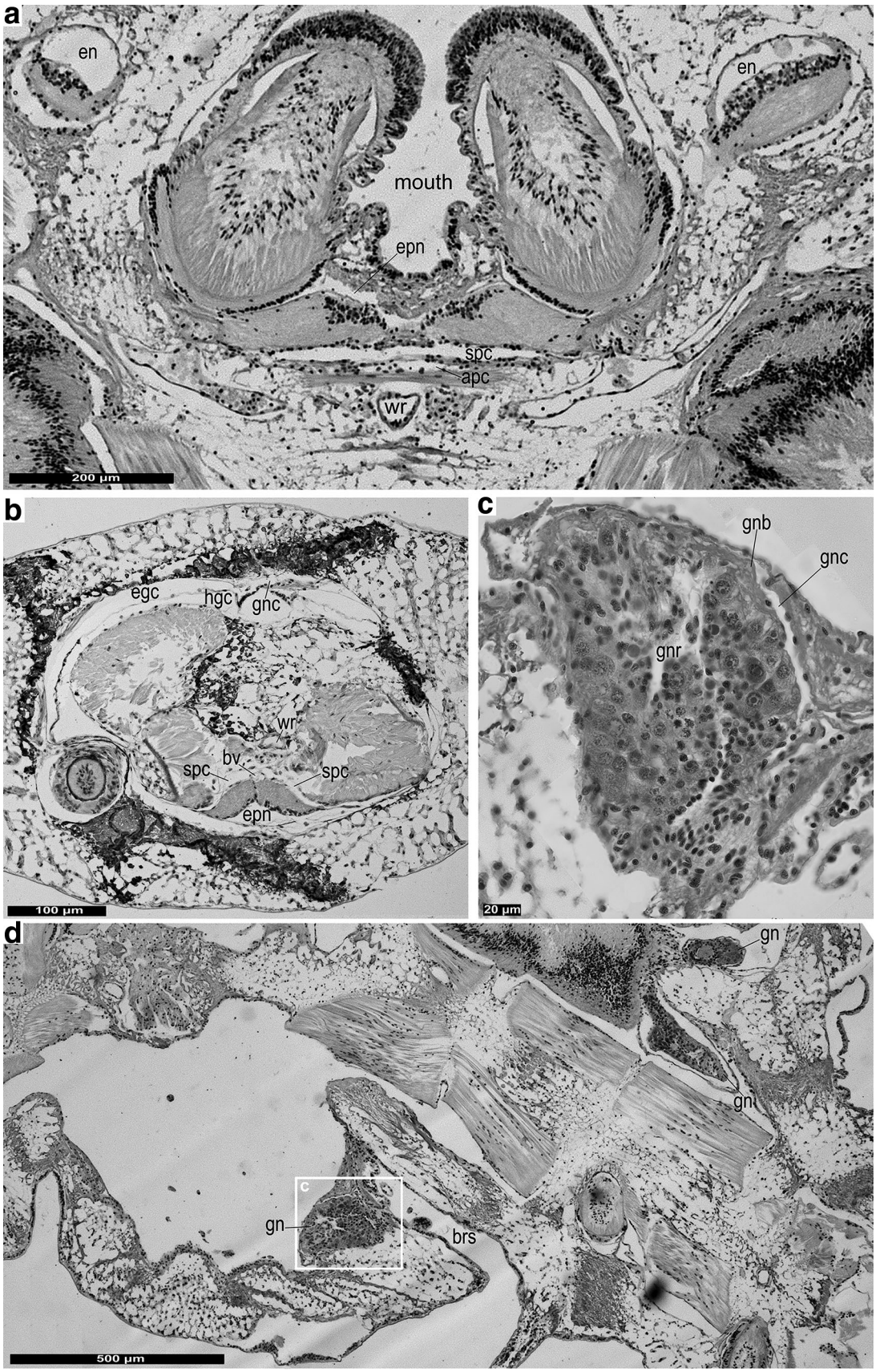


Table 1 Terms used for designation of structural elements of the ophiuroid axial complex

MacBride (1892, 1907)	Narasimhamurti (1933)	Smith (1940)	Cuénot (1948)	Hyman (1955)	Ivanova-Kazas (1978)	Ivanov et al. (1985)	Goldschmid (1996)	Ruppert et al. (2004)	Ziegler et al. (2009)[a]	Terms used in the present paper
Stone-canal	Stone canal	Stone canal	Tube aquifère	Stone canal	Stone canal	Stone canal	Steinkanal	Stone canal	Stone canal	Stone canal
Pore-canal	Pore canal	Pore canal	Pore aquifère	Pore canal	Pore canal	Pore canal	Hydroporuskanal	Pore canal	–	Pore canal
Ampulla = sinus *c*; axial sinus = sinus *b*	Ampulla of the stone canal	Left axial sinus	Sinus glandulaire; sinus entérocœlien	Axial sinus	Left axocoel; ampulla of stone canal	Left axial sinus	Axocoel	Axial canal (sinus)	Axial coelom	Axial coelom
Perihæmal system Perihæmal space = sinus *b* Perihæmal cavities	Perihaemal system of cavities	–	Sinus hyponeural – Sinus hyponeural	Oral perihaemal ring – Hyponeural ring canal (sinus)	Oral perihaemal system Inner perihaemal ring Outer perihaemal ring	Perihaemal ring (canal)	Hyponeuralkanal Ringkanal des Axocoels Hyponeuralkanal (Somatocoel) Ringkanal	Hyponeural canal (coelom)	–	Perihaemal coeloms - Axocoelomic perihaemal coelom - Somatocoelomic perihaemal coelom
Peri-oral cœlom	Peri-oral coelom	–	Espace périœsophagienne; cavité péristomienne	Peristomial ring sinus	Perioral coelom	–	–	–	–	Perioral coelom
Right hydrocœle; axial sinus = sinus *b*	Pericardial vesicle	Madreporic vesicle; right axial sinus	Sinus clos correspondant au sinus terminal des Astéries	Right part of the axial sinus	Right axocoel; pericardium; madreporic vesicle	Right axial sinus	Dorsalblase	Pericardial cavity; dorsal sac	Dorsal sac	Pericardial coelom
Ovoid gland	–	Axial organ Left axial organ Right axial organ	Glande brune Glande brune Extrémité ventrale de la gland brune	Axial gland Darker thicker aboral part Lighter slender oral part	–	Axial organ Oral part Aboral part	Axialorgan – Fortsatzsinus	Axial hemal vessel (gland)	Axial organ – Head process	Axial organ Axial part of the axial organ Pericardial part of the axial organ
Aboral sinus = sinus *a*	–	Genital sinus	Anneau périhémal aboral; sinus ondulé; sinus gonadique; sinus périgénital	Aboral (coelomic) sinus; genital sinus	–	Genital sinus	Aboraler Genitalkanal; Genitalcoelom; Aboraler Somatocoelring	Genital coelom	–	Genital coelom
Genital rachis	Genital rachis	Genital rachis	Cordon génital	Genital rachis	Genital cord	Genital stolon	Genitalrhachis; Genitalstrang	–	–	Genital rachis
Epineural canal	Epineural canal	–	Cavité épineurale; sinus épineural	Epineural (ring) sinus	Epineural canal	Epineural canal	Epineuralkanal; versenktes Ambulacralsystem	Epineural canal	–	Epineural channel

[a] Terms used in this paper relate to sea urchins (Echinoidea)

The "pericardial" part of the axial organ in sea stars and sea urchins is located on the aboral side (Fig. 5a), whereas in brittle stars it is shifted to the oral side (Fig. 5c). All the anatomical connections of the "pericardial" part of the axial organ are retained: its vessels communicate with the genital haemal ring, with the haemocoel of the body wall and the vessels of the axial part of the axial organ (Figs. 1, 3).

The rearrangement of the madreporic plate and organs related to it resulted in a change of the location of ring structures of the axial complex. Thus, in sea stars the axocoelomic perihaemal ring is located closer to the mouth than the somatocoelomic perihaemal ring (Fig. 5a). In brittle stars, a result of the shift is that the somatocoelomic perihaemal ring is located closer to the mouth than the axocoelomic ring (Fig. 5c). The mutual arrangement of radial coelomic outgrowths in the arms of brittle stars remains the same as in sea stars (Ezhova et al. 2013): single unpaired ambulacral outgrowth is located aborally to the paired perihaemal processes.

The genital coelom and genital haemal ring in sea stars are located on the aboral side (Fig. 5a). However, in brittle stars those parts of the genital coelom and the genital haemal ring that are located in the interradii descend to the oral side, while those parts that are located in the radii remain on the aboral side (Fig. 3). As a result, the genital coelom and the genital haemal ring form five aboral loops, which are located in the radii (Fig. 3). A section through the madreporic plate reveals that in sea stars the genital coelom is located between the digestive tract and the axial complex (Fig. 5a), while in brittle stars the axial complex lies between the genital coelom and the digestive tract (Fig. 5c). Furthermore, the gastric haemal ring of sea stars is located on the inner side of the axial complex (Fig. 5a). In contrast, in brittle stars the gastric haemal ring is located on the outer side of the axial complex (Figs. 2h, 3, 5c).

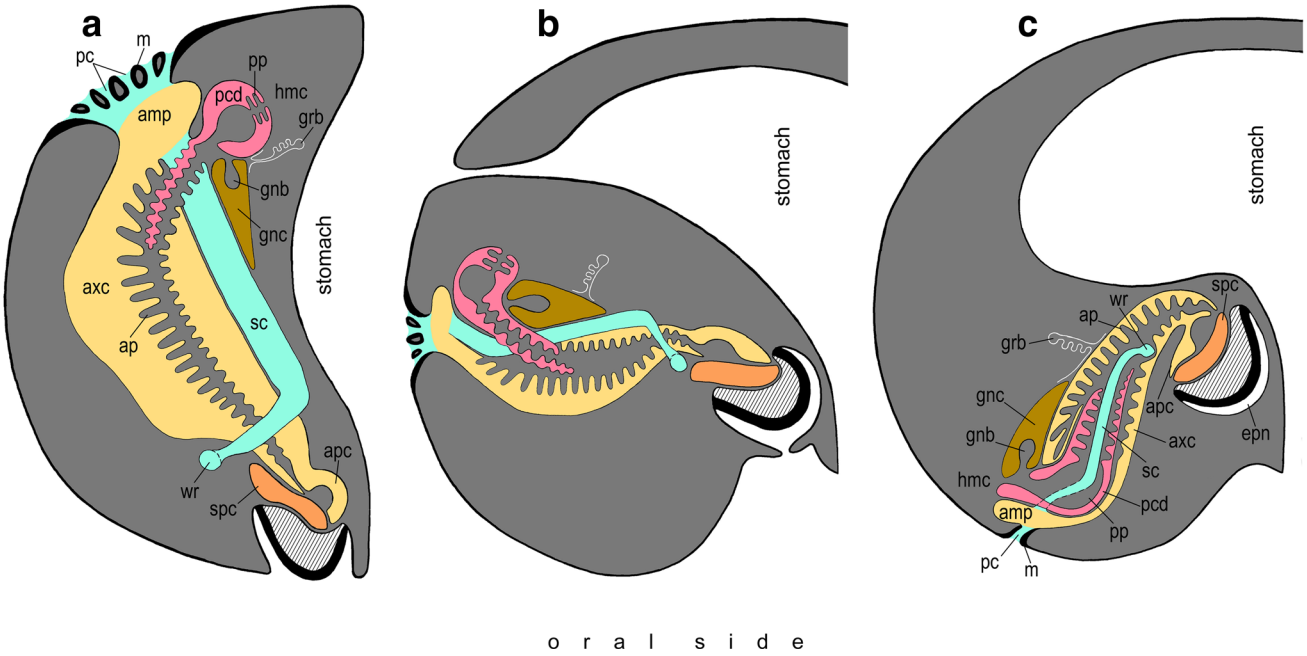


Fig. 5 Diagram of the shift and rotation of the axial structures of axial complex. Epidermis is *black*; haemocoel and its structures are *dark grey*; coelomic structures are *coloured*. The nerves are hatched. **a** Location of the axial complex in the sea star body (after Ezhova et al. 2013). **b** Hypothetic intermediate form. **c** Location of the axial complex in the brittle star body. Madreporic ampulla (*amp*), madreporic plate (*m*), pore canal (*pc*), "pericardial" coelom (*pcd*) and "pericardial" part of the axial organ (*pp*) are shifted from the aboral side to the oral side. The "pericardial" part of the axial organ (*pp*) communicates with genital haemal ring (*gnb*), with haemocoel of the body wall (*hmc*), with gastric haemal ring (*grb*), and with the axial part of the axial organ (*ap*). In sea stars the stone canal (*sc*) flows into the ambulacral ring (*wr*) from the side of the mouth, the axocoelomic perihaemal ring (*apc*) is closer to the mouth than the somatocoelomic perihaemal ring (*spc*), and in *O. robusta* vice versa. In sea stars the genital coelom (*gnc*) is located between the digestive tract and the axial complex, while in brittle stars the axial complex lies between the genital coelom (*gnc*) and the digestive tract. The gastric haemal ring (*grb*) of sea stars is located on the inner side of the axial complex, and in brittle stars, on the outer side of the axial complex. *axc* Axial coelom, *epn* epineural channel (colour figure online)

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