= GENERAL BIOLOGY ====

Discovery of Trunk Coelomoducts in Hemichordata

A. I. Lukinykh^{*a*, *}, O. V. Ezhova^{*a*, *b*}, S. V. Krylenko^{*a*}, S. V. Galkin^{*c*}, A. V. Gebruk^{*c*}, and Corresponding Member of the RAS V. V. Malakhov^{*a*, *b*}

Received April 17, 2018

Abstract—Histological examination of a specimen of a deep-sea enteropneusts that belongs to a yet undescribed species (Torquaratoridae gen. sp.) revealed numerous trunk coelomoducts. They open into the genital wing coelom as a typical funnels; short ducts communicate with environment through pores located on the outer side of the genital wings. Total number of coelomoducts in a specimen is estimated at several thousand. Trunk coelomoducts have not been found earlier in any member of the phylum. We believe that the release of the male gonad products occurs through coelomoducts of Torquaratoridae gen. sp.

DOI: 10.1134/S0012496618060042

Hemichordata are marine invertebrates with traits similar to those of echinoderms and chordate animals. The Hemichordata phylum includes two classes: the sedentary colonial pterobranchs (Pterobranchia) and solitary worm-like enteropneusts (Enteropneusta). Hemichordata coelom comprises three distinct parts: the anterior proboscis (protocoel), short collar (mesocoel) and the long trunk (metacoel). The proboscis region opens with the only left coelomoduct on the dorsal side of proboscis; the collar region opens through the paired coelomoducts into the first pair of gill slits [2-4, 7]. No coelomoducts have been found in the trunk coelom. The pores through which mature gonads communicate with peribranchial cavity of enteropneusts are assumed to correspond to modified coelomoducts, namely, gonoducts [7].

Most researches on the enteropneust microscopic anatomy have been conducted on the representatives of Harrimaniidae, Ptychoderidae, and Spengelidae families inhabiting shallow depths. Recently, a new enteropneust family Torquaratoridae (Holland, Clague, Gordon, Gebruk, Pawson, and Vecchione, 2005) has been described, the members of which live at depths of 350–4500 m [5]. Their structure is clearly different as compared to that of enteropneusts from shallow depths [5, 6, 8–10].

We studied the Torquaratoridae gen. sp. specimens found at a depth of 2289 m in the Bering Sea. During microscopic examination of their anatomical features for the purposes of further detailed description of a

^a Moscow State University, Moscow, Russia

^b Far East Federal University, Vladivostok, Russia

new species, we have found new trunk coelomoducts. Since it is a new structural feature not only for the enteropneusts class, but also for the whole Hemichordata phylum, we believe that their coelomoduct structure should be described in a special report.

The specimens studied originated from the Commander Hollow, Bering Sea, and were sampled during the cruise 75 of the R/V Akademik M. A. Lavrentiev in 2016 using a Comanche 18 remote operated underwater vehicle (ROV). After fixation in formalin, the material obtained was dehydrated in a standard alcohol series of increasing concentrations [1]. The fragments prepared for histological analysis were embedded in paraplast and, serial 7-µm sections were prepared using a Leica RM 2125 microtome (Leica Biosystems, Germany). Two specimens were selected to make the cross and sagittal serial sections of the proboscis, collar and the anterior brancho-genital trunk portion (Table 1). The sections stained with hematoxylin and eosin alcohol solution were examined using an Axioplan 2 photomicroscope (Carl Zeiss Microscopy, Germany).

The members of the Torquaratoridae family have genital wings, which are body folds that turn onto the dorsal side of an animal [5, 6, 8-10]. In the studied species of Torquaratoridae gen. sp., the genital wings are narrow folds that originate on the ventral side and

Table 1. Characteristics of the material studied

Specimen number	Direction of section	Material features
1	Cross section	The absence of gonads in the genital wings
2	Sagittal section	Gonads (ovaries) are present in the genital wings

^c Shirshov Institute of Oceanology, Russian Academy

of Sciences, Moscow, Russia

^{*} e-mail:lukinyh.anastasiya@yandex.ru



Fig. 1. The genital-wing structure in Torquaratoridae gen. sp. studied: (a–c) specimen no. 1; (d) specimen no. 2. (a) General view of a cross section in the branchio-genital region (the left body part). (b) The scheme of the general view shown in (a). (c) Coelo-moduct funnel. (d) The oocyte-containing ovaries within the genital wing coelom. vn, ventral nerve cord; cdf, coelomoduct funnels; gc, gut cavity; cdc, coelomoduct channel; mes, ventral mesenterium; ml, ventral muscle layer; ooc, oocytes; pbc, peribranchial cavity; tc, trunk coelom; ep, epidermis. Scale: (a, d), 200 µm; (c), 20 µm.

rise up to the dorsal side to cover the body flanks (Figs. 1a, 1b). The inner cavities of the left and right genital wings are continuations of the left and right trunk coeloms, respectively, which are separated by the dorsal and ventral mesenteries (Figs. 1a, 1b).

In specimen no. 1 (see Table 1), there were no gonads within the genital wing coelom cavity (Fig. 1a). Specimen no. 2 had ovaries containing oocytes (Fig. 1d). In specimen no. 1, numerous coelomoducts were along the entire outer side of each genital wing, which

a, 1b). uct consists of a funnel that opens into the coelom cavity, and a short duct communicating with the environment. The funnel edges are characteristically bent away (Fig. 1c). The funnel diameter measured from one bent edge to the other was approximately 100- $110 \ \mu m$, while the diameter of the funnel inlet was

opened into the coelom cavity from the one side and

into the environment from another side (Figs. 1a-1c).

In one histological section, six to eight coelomoducts

were observed on each side of the animal. Coelomod-

about 10 μ m. The coelomoduct length was 80–100 μ m. Both the funnel and the duct are lined with high epithelium (up to 20 μ m).

This coelomoduct structure is rather uncommon and has no analogues in Torquaratoridae described so far or in other members of Hemihordata phylum. Since 12–16 coelomoducts are seen on the cross section, their total amount is at least several thousands.

The genital wing coelom is a place where the enteropneust gonads develop. In specimen no. 2, the coelom contained numerous ovaries (Fig. 1d) which were adjacent to the genital wing wall facing the peribranchial cavity. The same is observed in other enteropneusts: gonadoducts are also opened through pores on the inner side of the genital wings [3-5, 11, 12]. The genital wing coelom of specimen no. 1, in which coelomoducts were found, contains no gonads, and the coelomoducts themselves are opened on the outer side of the genital wings. It may be that the genital products of specimen no. 1 have been already removed through the coelomoducts described. Probably, specimen no. 1 is a male, and specimen no. 2 is a female. Female gonoducts of the Torquaratoridae gen. sp. are opened into the peribranchial cavity, while the male gonoducts (= coelomoducts that are described) are opened on the outer side of the genital wings. Certainly, this assumption requires further studying.

ACKNOWLEDGMENTS

We are grateful to the National Scientific Center of Marine Biology, Far Eastern Branch of Russian Academy of Sciences, for organizing and conducting of the cruise 75 of the R/V *Akademik M.A. Lavrentiev*, as well as to the Federal Agency of Scientific Organizations of Russia for financial supporting of the expedition. Special thanks to the pilots and technicians who provided the works using a ROV Comanche 18.

Histological study was supported by the Russian Foundation for Basic Research (project no. 17-04-00482-a. The manuscript and illustration preparation was supported by the Russian Science Foundation, project no. 14-50-00034.

COMPLIANCE WITH ETHICAL STANDARDS

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

Statement on the welfare of animals. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

REFERENCES

- Valovaya, M.A. and Kavtaradze, D.N., *Mikrotekhnika*. *Pravila, priemy, iskusstvo, eksperiment* (Microtechnology: Rules, Techniques, Art, and Experiment), Moscow: Mos. Gos. Univ., 1993.
- 2. Benito, J. and Pardos, F., *Microsc. Anat. Invertebr.*, 1997, vol. 15, pp. 15–101.
- 3. Van der Horst, C.J., Nature, 1934, vol. 134, p. 852.
- 4. Van der Horst, C.J., in *Klassen und Ordnungen des Tierreichs*, Bronn, H.G., ed., Leipzig: Akademische, 1939, vol. 4, part 4, book 2, section 2.
- Holland, N.D., Clague, D.A., Gordon, D.P., Gebruk, A.V., Pawson, D.L., and Vecchione, M., *Nature*, 2005, vol. 434, pp. 374–376.
- Holland, N.D., Kuhnz, L.A., and Osborn, K.J., J. Morphol., 2012, vol. 273, pp. 661–671.
- 7. Hyman, L.H., *The Invertebrates: Smaller Coelomate Groups*, New York, 1959, vol. 5.
- Osborn, K.J., Kuhnz, L.A., Priede, I.G., Urata, M., Gebruk, A.V., and Holland, N.D., *Proc. R. Soc.*, 2012, vol. 279, pp. 1646–1654.
- Osborn, K.J., Gebruk, A.V., Rogacheva, A., and Holland, N.D., *Biol. Bull.*, 2013, vol. 225, pp. 113–123.
- Priede, I.G., Osborn, K.J., Gebruk, A.V., Jones, D., Shale, D., Rogacheva, A., and Holland, N.D., *Invertebr. Biol.*, 2012, vol. 131, pp. 244–257.
- Spengel, J.W., Fauna und Flora des Golfes von Neapel. Herausgegeben von der Zoologischen Station zu Neapel, 1893, vol. 18, pp. 1–757.
- 12. Willey, A., Zool. Res., 1899, part 3, pp. 223-334.

Translated by A. Nikolaeva