

The First Discovery of Trematodes (Digenea) in the Deep-Sea Acorn Worms Torquaratoridae (Hemichordata, Enteropneusta)

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Abstract—Trematodes found in the enteropneust hemichordates are described for the first time. Metacercariae have been found in the trunk coelom, in the collar coelom, in the proboscis coelom, and in the glomerulus of the deep-sea torquaratorid *Quatuoralisia malakhovi* Ezhova et Lukinykh, 2022. This is the first find of parasites in the glomerulus of acorn worms. The taxonomy of the found trematodes is discussed.

Keywords: Digenea, *Quatuoralisia malakhovi*, acorn worms, Torquaratoridae, Bering Sea, histology, morphology

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The new family of enteropneusts, Torquaratoridae, has been discovered only in 2005 [1]; it includes the species living at great depths, from 350 to 4000 m [1–3]. Deep-sea enteropneusts from the Torquaratoridae family differ significantly from shallow-water members of the class. Torquatorids usually lead an epibenthic way of life, slowly crawling along the bottom surface, they do not burrow. Their proboscis is short and does not contain strong muscles. On the contrary, the collar region, as a rule, grows laterally, forming pronounced lateral lips, which the animals use to collect sediments from the bottom surface [1–3].

The biology of enteropneust in general and, even more so, of deep-sea representatives of this class has been poorly studied. In particular, there are few data on the parasites and commensals of Enteropneusta. To date, infection of enteropneust by various endoparasites, both multicellular and unicellular, has been described only for representatives of the family Ptychoderidae. The lateral wings of *Ptychodera flava* Eschscholtz, 1825 are inhabited by comparatively large copepods [4]. Coccidia at different stages of the life cycle and other Apicomplexa have been found in the trunk coelom of the branchiogenital and hepatic regions [5, 6], as well as in the intestinal epithelium of *Glossobalanus minutus* (Kowalevsky, 1866) [7–9].

A case of commensalism or ectoparasitism (presumably leeches) on the lateral wings of enteropneust *Allaparus isidis* Priede, Osborn, Gebruk, Jones, Shale, Rogacheva, and Holland, 2012 from the Torquaratoridae family has been previously recorded [2]. For another torquaratorid, *Torquarator bullocki* Holland, Clague, Gordon, Gebruk, Pawson, and Vecchione, 2005, commensal Acoela have been found in the pharynx, and copepods from the order Harpacticoida, in the posthepatic intestine, which may also be parasites or commensals [1]. Until now, information about the habitation of such common parasites as trematodes in enteropneusts has been lacking.

Quatuoralisia malakhovi Ezhova et Lukinykh, 2022 was found in the northwestern part of the Pacific Ocean, in the Bering Sea, at depths of 1370–2470 m [3]. The specimens were collected on the slopes of the Volcanologists Massif in 2016 and 2018 during cruises of the RV *Akademik M.A. Lavrentiev* (Table 1), using the ROV Comanche 18. Trematode metacercariae were found in histological sections of *Q. malakhovi* specimens collected in 2018. The material for histological examination was fixed in 8% formalin buffered with sea water.

Washing off the fixative and dehydration of the material were carried out according to the standard method in alcohols of increasing concentration. The fragments prepared for histological examination were embedded in Paraplast blocks and dissected using a Leica RM 2125 microtome into a series of histological sections 10 µm thick. The sections were stained with Caracci's hematoxylin and eosin alcohol solution. The sections were photographed under a Mikmed-6 microscope (LOMO, St. Petersburg, Russia) by a

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Table 1. Sampling locations of examined specimens of *Quatuoralisia malakhovi*

Cruise no.	Station no.	Sampling date	Coordinates	Depth, m	Number of specimens studied
75	LV 75-17	June 27, 2016	55.4609 N 167.2688 E	2289	3
82	LV 82-9	June 18, 2018	55.3451–55.3466 N 167.2750–167.2752 E	1957–1933	4

Table 2. Location of the metacercariae in the body of *Quatuoralisia malakhovi*

<i>Q. malakhovi</i> specimen, collection no.	Location	Number of found metacercariae
2019-QM-04	Trunk coelom	1
2020-QM-05	Trunk coelom	2
	Proboscis coelom	7
	Glomerulus (heart–kidney)	11
2020-QM-06	Trunk coelom	1
	Collar coelom	5

mounted MC-12 digital camera. All histological studies were carried out in the Student Laboratory of Evolutionary Morphology of Animals (www.evolmorph.ru) of the Department of Invertebrate Zoology, Biological Faculty, Moscow State University. The collections of series of histological sections are stored there; collection numbers are given in Table 2.

We studied seven specimens of *Q. malakhovi* (Table 1). Trematodes were found in three specimens of *Q. malakhovi*. The maximum number of individual trematodes (metacercariae) found in one host specimen was 20. Single metacercariae were found in this specimen in the coelom of the branchiogenital region of the body and in the proboscis coelom; however, the largest number of parasites (11 spec.) was found in the glomerulus of *Q. malakhovi* (Fig. 1a, Table 2). When analyzing histological sections of other *Q. malakhovi* specimens, we have found metacercariae in the coelom of the branchiogenital region of the body (Fig. 1c) and in the collar coelom (Table 2).

The metacercariae are approximately 100–110 µm in length and about 90 µm in transverse diameter (Figs. 1a–1c). Each metacercaria carries two suckers, oral and ventral (Figs. 1a, 1c). The oral sucker leads into the pharynx, followed by a short esophagus (Fig. 1a). Behind the ventral sucker, there is a dense accumulation of dark cells, presumably a reproductive system primordium (Fig. 1a). Several pairs of unicellular glands are located in the anterior third of the metacercaria (Figs. 1a, 1b). Their ducts pass near the dorsal side of the body to its anterior end (Fig. 1b). In the posterior half of the body of the metacercaria there is a large light rounded cavity, which is the excretory bladder (Fig. 1a). The tegument of the metacercariae is thin (about 3 µm). Spines are visible in the tegument (Fig. 1d).

The metacercariae are surrounded by a thin-walled cyst (Fig. 1a), around which the host forms a capsule of scaly connective tissue cells (fibroblasts). In one case, the thickness of the capsule wall, which consisted of several layers of connective tissue cells, reached 100 µm (Fig. 1c).

It is known that adult trematodes usually parasitize vertebrate animals. In this case, the definitive host may be deep-sea fish. In fish living at depths of about 2000 m and deeper, representatives of only eight families of Digenea have been found by now [10, 11]: Derogenidae, Fellodistomidae, Gonocercidae, Hemiuridae, Lecithasteridae, Lepidapedidae, Opecoelidae, and Zoogonidae. Representatives of several other families (Bivesiculidae, Hirudinellidae, Ptychogonimidae, Sclerodistomidae, and Syncoeliidae) have been described from deep-sea fishes but without accurate bathymetric data. The Fellodistomidae, Gonocercidae, Hemiuridae, Lecithasteridae, Opecoelidae, Ptychogonimidae, Sclerodistomidae, and Syncoeliidae lack spines in the tegument [12, 13]. The only deep-sea representative of the Gorgoderidae (*Degeneria halosauri* (Bell, 1887) Campbell, 1977) has a Y-shaped excretory bladder [14], while in Bivesiculidae, it is V-shaped [12] rather than rounded, similar to that of the metacercariae we have found. In addition, the representatives of Bivesiculidae lack oral and ventral suckers. The excretory bladder in maritae of Lepidapedidae is tubular, but may be rounded in their cercariae and metacercariae [13, 15]. Zoogonidae also have a rounded excretory vesicle [14]. Representatives of both Lepidapedidae and Zoogonidae are characterized by thin, sharp spines in the tegument. Therefore, it is possible that the found metacercariae belong to the families Zoogonidae or Lepidapedidae, although a molecular study will help bring final answer to this issue.

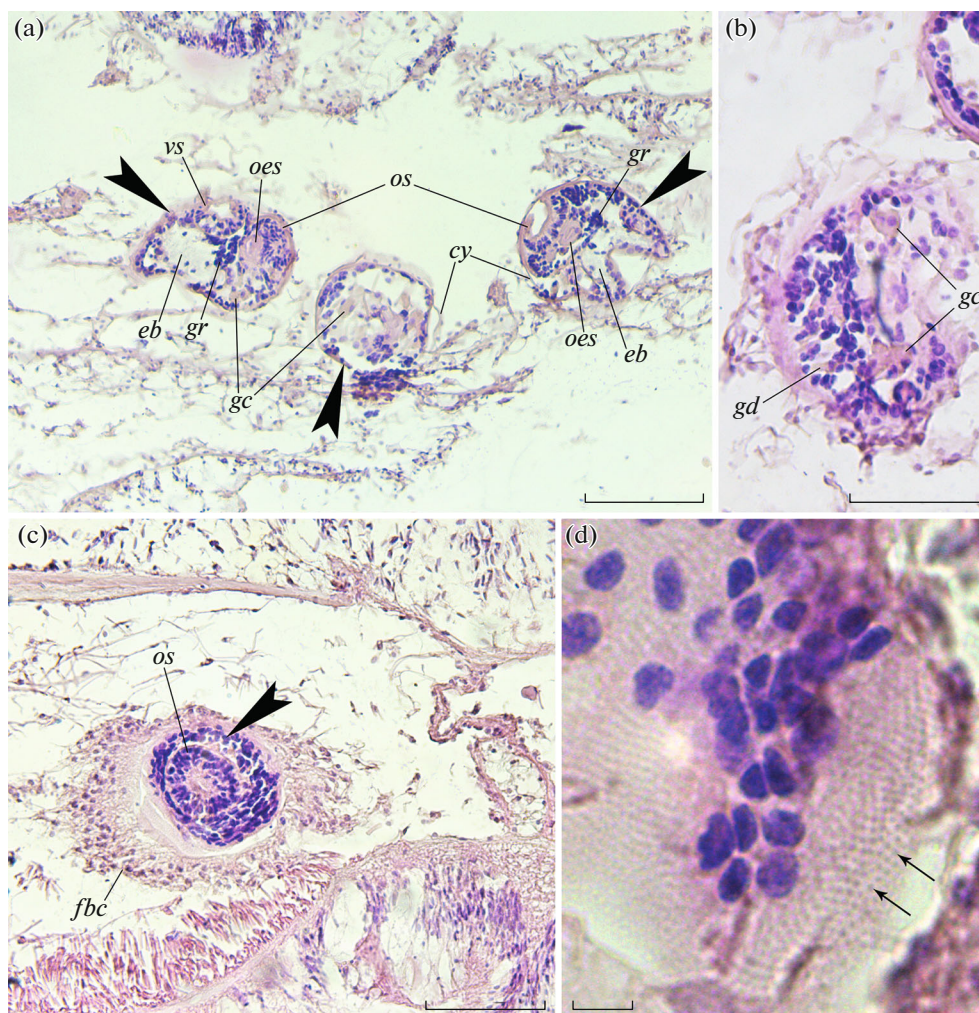


Fig. 1. Location of metacercariae (arrowheads) in different part of the body of *Quatuoralisia malakhovi*: (a, b) in the glomerulus and (c) in the trunk coelom. (d) The structure of the spinous tegument of the metacercariae studied in the tangential section. The tegumental spines are marked by arrows; *cy*, cyst; *eb*, excretory bladder; *fbc*, fibrous capsule; *gc*, gland cells; *gd*, ducts of the gland cells; *gr*, reproductive system primordium (genital rudiment); *oes*, oesophagus; *os*, oral sucker; *vs*, ventral sucker. Scale: (a, c) 100, (b) 40, and (d) 5 μ m.

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COMPLIANCE WITH ETHICAL STANDARDS

All authors read the final version of the manuscript. All applicable international, national and institutional guidelines for the use of animals have been followed.

CONFLICT OF INTEREST

The authors have no financial or any other conflicts of interest.

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