ISSN 1062-3590, Biology Bulletin, 2022, Vol. 49, No. 1, pp. 21–28. © Pleiades Publishing, Inc., 2022.
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ZOOLOGY

The First Data on Larvae of Trematodes from the Gastropod *Hydrobia acuta* in the Black Sea

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Received July 10, 2020; revised October 15, 2020; accepted October 15, 2020

Abstract—In this work, the first information on the species biodiversity of larvae of trematodes in the gastropod *Hydrobia acuta* is provided. As result of this research, the species diversity of trematodes of the most widespread species of mollusks *Hydrobia acuta* in bays of Sevastopol was studied. For the first time in the water area of Sevastopol, seven species of helminths from *Hydrobia* were discovered: five species of cercariae (*Timoniella imbutiformis*, *Cryptocotyle* sp., *Gynaecotyla adunca*, *Maritrema misenense*, and *Haplosplanchnus pachysoma*) and three species of metacercariae: (*Paratimonia* sp., *H. pachysoma*, and *Proctoeces maculatus*). *H. acuta* was first registered as the first intermediate host for *P. maculatus*.

Keywords: trematoda, larvae, mollusks, *Hydrobia acuta*, Black Sea
DOI: 10.1134/S1062359022020042

INTRODUCTION

The Black Sea mollusk *Hydrobia acuta* (Draparnaud, 1805) is the most abundant representative of malacofauna in freshened shallow waters. Despite its small size, this species of mollusk features prominently in the benthos, being an important food source for many species of fish in sea bays and coves.

In the Black Sea, in the waters of the Tuligul estuary, in the mollusks *H. ventrosa* (Montagu, 1803) were previously noted several species of cercariae of the family Microphallidae: *Cercaria xiphidocercaria* (II Sordi, 1959), *C. microphallidarum* (Reimer, 1963), and *C. caribbea* Cable, 1956 (Dolgikh, 1969), and off the coast of Novorossiysk Bay in the same species of gastropods, there were cercariae *S. kowalewski* (Dolgikh, 1966) and *C. xiphidocercaria* (Dolgikh, 1966).

There is no published information about *H. acuta* helminths in the Black Sea, beause no special helminthological studies of this type of mollusks have been conducted.

The purpose of this work was to show the species composition of the larval forms of helminths parasitizing the most abundant representative of the malacofauna *H. acuta* in the freshened water area (estuary of the Chernaya River) and shallow water (Kazach'ya Bay) of Sevastopol.

MATERIALS AND METHODS

The material for this work was our own collections of mollusks from the mouth of the Chernaya River (Sevastopol Bay) and Kazach'ya Bay in the period from November 2011 to November 2012. In total, 6790 specimens of the gastropod *H. acuta* were examined by the method of complete helminthological dissection. All tissues of mollusks were examined by the compressor method (Bykhovskaya-Pavlovskaya, 1969) under an MBS-10 binocular microscope with a magnification of ×98. Trematodes were stained with acetic carmine according to the standard method (Roskin and Levinson, 1957), and after dehydration with alcohols (70–100° C) and clarification in clove oil, the helminths were enclosed in Canadian balsam. Measurements of helminths are given in the following range: minimum–maximum, mean, and standard error. All drawings were made in a vector graphics editor in Inkscape 0.48.2.-1 (Scalable Vector Graphics, 2011). The following indicators of invasion were used: extensiveness of invasion (EI), intensity of invasion (II), and the abundance index (AI).

RESULTS

In 5053 specimens of *H. acuta,* seven species of trematode larvae were found in the surveyed water area of Sevastopol: cercariae *Maritrema misenense* (Palombi, 1940; Prévot, Bartoli and Deblock, 1976), *Gynaecotyla adunca* (Linton, 1905; Yamaguti, 1939), *Timoniella imbutiformis* (Molin, 1859; Brooks, 1980), *Haplosplanchnus pachysoma* (Eysenhardt, 1829; Looss, 1902), and *Cryptocotyle* sp. (Luhe, 1899), and the metacercariae *H. pachysoma*, *Paratimonia* sp. (Prevot and Bartoli, 1967), and *Proctoeces maculatus* (Looss, 1901; Odhner, 1911).

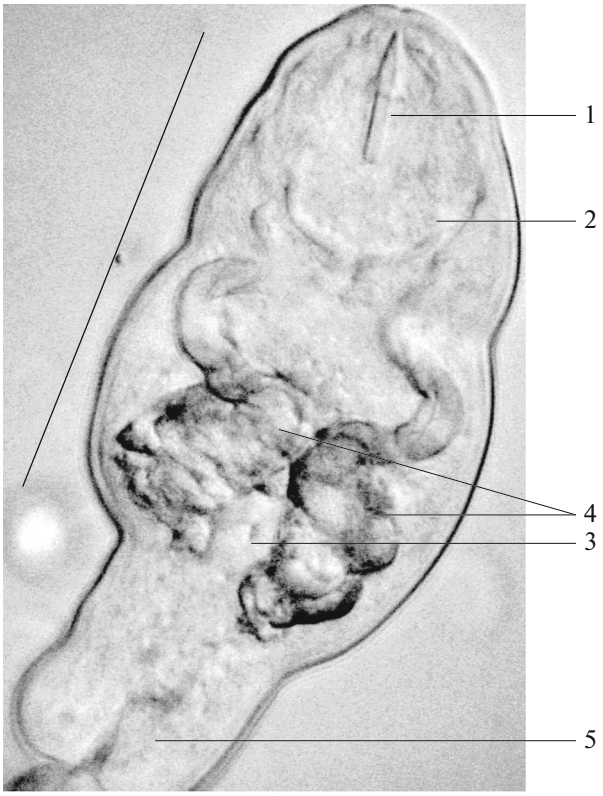

Fig. 1. Photomicrograph of cercaria *Maritrema misenense* in the water area of Kazach'ya Bay: 1, style; 2, oral sucker; 3, rudiments of the abdominal sucker; 4, glandular cells; 5, excretory bladder. Scale: 50 μm.

Below are the morphological descriptions of helminth larvae most often found in the *H. cuta* mollusks studied.

Family Microphallidae (Ward, 1901)

Maritrema misenense (Palombi, 1940; Prévot, Bartoli, and Deblock, 1976).

Localization: gonad; description: based on 20 specimens of cercariae.

Invasion indicators: EI, 1%; II 25 ± 5 specimens/individual, AI 0.01 ± 0.01.

Sporocysts *Maritrema misenense* are oval, contained 15–30 mature cercariae. The time of delivery has not been determined.

Small cercariae have an elongated body, flattened in the dorsoventral direction. The body length is 80 ± 3 μm, and the width is 51 ± 3 μm. The cuticle is covered with very small spines. Oral sucker, size 20 ± 2 × 18 ± 1, subterminal. The abdominal sucker (10 ± 1 × 9 ± 0.8 μm) is half the size of the oral one and is located in the middle of the body of the trematode. The suction cup is equipped with a 13 ± 1 μm cylindrical stylet. The conical part of the stylet is slightly shorter than its base (5 ± 0.3 μm), extending from the center of the oral sucker. The transverse slit in the suction cup indicates the location of the future mouth.

Four pairs of penetration glands open at the front of the body. The channels of the glands open in pairs in the area of the oral sucker. The glands of penetration, filled with granular inclusions, reach the level of the abdominal sucker. There are large unicellular glands. The boundaries between cells are poorly expressed. The ducts of the glands are represented by convoluted canals. The digestive system is not developed. The excretory bladder can be slit-like or bubble-like. The tail is of a simple structure, 60–105 μm long, tapering towards its end (Fig. 1).

The cercariae move with the body forward however, characteristically, they can easily discard the tail, crawling with the help of suckers.

Gynaecotyla adunca (Linton, 1905; Yamaguti, 1939).

Localization: gonad. Description based on ten samples of cercariae. Indicators of invasion by larvae: EI, 1%; II, 1–15 specimens/individual; AI, 0.007 ± 0.004.

Partenites are found in the gonads of gastropods. They are represented by several generations of sporocysts with a different composition of hemipopulations, depending on the season. No maternal sporocyst was found.

Cercariae are small. The body of the cercariae is oval. Their body length is 84 ± 3 μm, and width, 36 ± 2 μm. The oral sucker is 16 ± 1 × 15 ± 0.5 μm in size, located subterminally and armed with a large stylet (10 ± 0.5 μm) extending from the center of the oral sucker. The tip of the stylet may protrude slightly above the anterior edge of the cercariae body. Two pairs of stylet glands open in front of the body at the beginning of the oral sucker. The rudiments of the abdominal sucker are visible between the glands of penetration. The digestive system is not developed. The excretory bladder is Y-shaped. The excretory system of the trematode is well differentiated in living objects. The formula for the excretory system is 2 (2 + 2) = 8. The tail is 78 ± 3 μm long, tapering towards its end (Fig. 2).

Family Cryptogonimidae (Ward, 1917)

Timoniella imbutiformis (Molin, 1859; Brooks, 1980). Localization: liver. Description of two samples of cercariae. Indicators of invasion by larvae: EI, 2%; II, 1–1 specimen/individual; AI, 0.007 ± 0.004.

The body of the larva is elongated, flattened in the dorsoventral direction. The body length is 425 ± 25 μm, and the width, 176 ± 10 μm. Funnel-shaped oral sucker 132 ± 8 × 73 ± 3 μm in size, armed with 18 spines, twice as large as the abdominal one (73 ± 3 × 73 ± 3 μm). The prepharynx is relatively small, and the pharynx is small (30 ± 2 × 73 ± 3 μm). The short esophagus branches above the abdominal sucker. The intestinal branches reach the posterior end of the body.

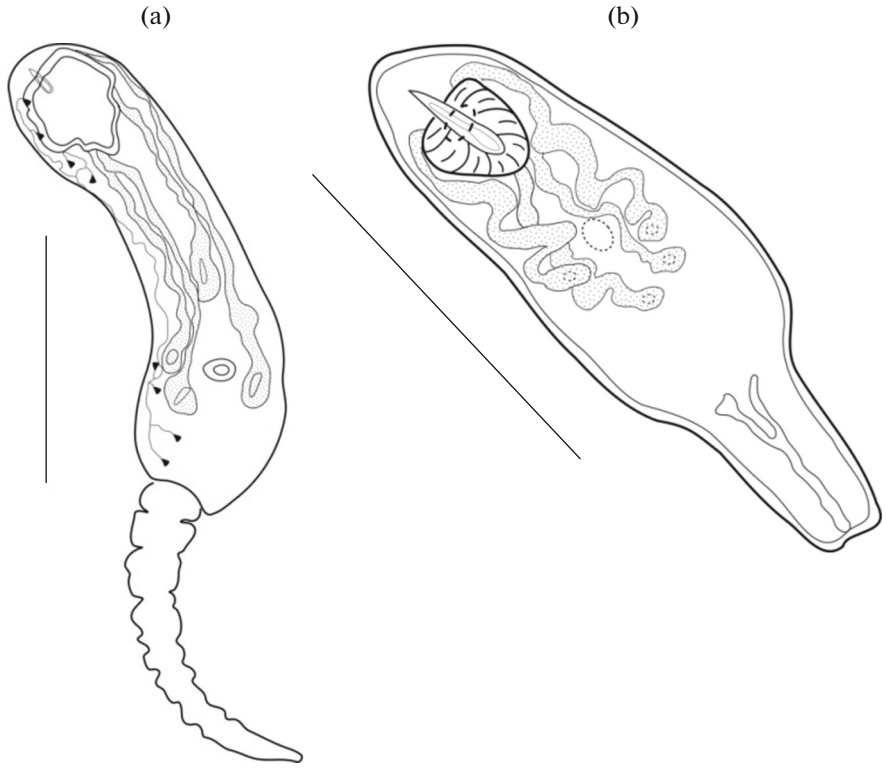

Fig. 2. Cercaria *Gynaecotyla adunca* (drawing from a live helminth) (a) flame cells of the excretory system; (b) the shape and position of the stylet. Scale: 50 μm.

At the level of the pharynx, there are two pigment eyes. The excretory system has the formula 2 [(2 + 2) + (2 + 2)] = 16. The excretory bladder is Y-shaped (Fig. 3).

Family Heterophyidae (Leiper, 1909)

***Cryptocotyle* sp.** (Lűhe, 1899). Localization: digestive gland.

Infestation rates: EI, 7%; EI, 25 ± 5 specimens/individual; AI, 0.08 ± 0.02. The description is based on 20 samples of cercariae from mollusks.

The body length of fixed cercariae is 143 ± 24 μm, and the width is 57 ± 10. The length of the oral sucker is 22 ± 5 μm, and the width is 23 ± 5. The pharynx is small, pronounced. At the level of the pharynx, there are two pigmented eyes. The esophagus and intestines are absent. The special structure of the system of glands of penetration is characteristic of cercariae of the genus *Cryptocotyle*. The glands of penetration (eight pairs) are located between the pharynx and the excretory bladder. The excretory bladder is cupped. The length of the tail of the cercariae is 200 ± 60 μm. It is 1.5 times the length of the body and has a swimming membrane on the sides (Fig. 4).

After leaving the host mollusk, the cercariae *Cryptocotyle* swim vigorously. The process of their movement is clearly divided into two alternating phases: active and passive. In the course of the first, the larva actively works with its tail, which ensures its forward

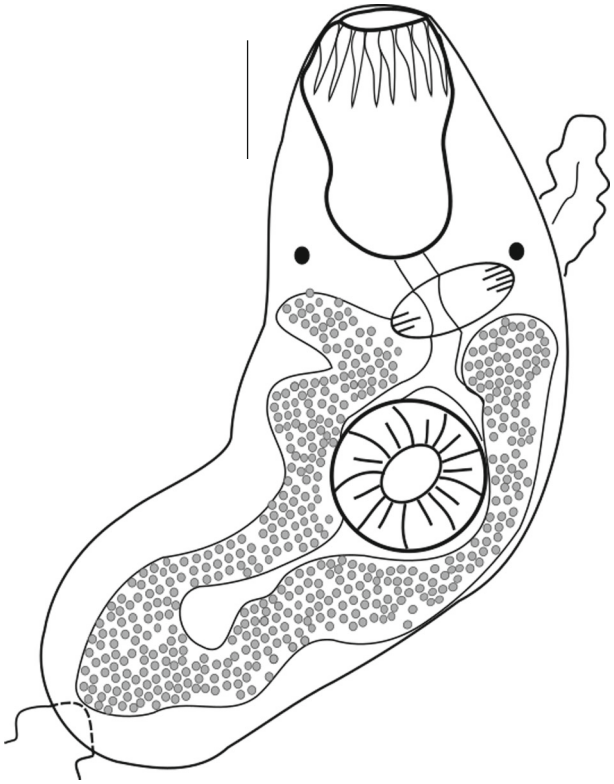

Fig. 3. Cercaria *Timoniella imbutiformis* (in the estuary of the Chernaya River (bay of Sevastopol). Scale: 50 μm.

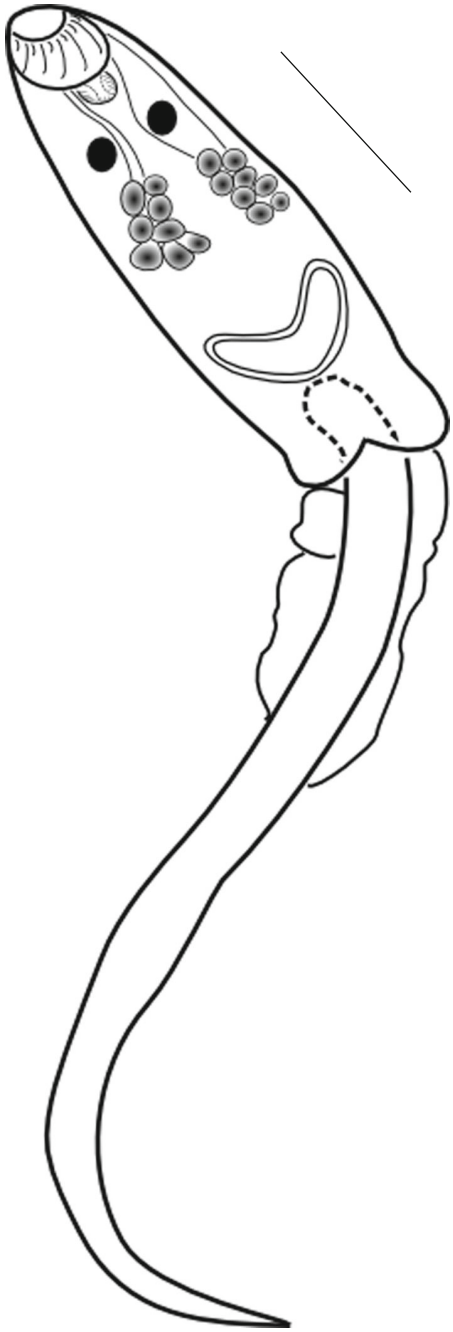

Fig. 4. Cercaria *Cryptocotyle* sp. in the estuary of the Chernaya River (bay of Sevastopol). Scale: 100 μm.

movement. During the second phase, it freezes and in this state floats in the water, slowly sinking down.

Family Haplosplanchnidae (Poche, 1926)

Haplosplanchnus pachysoma (Eysenhardt, 1829; Looss, 1902). Localization: body cavity. Description: three samples of adolescariae.

Large adolescariae, body length 1000 ± 40 μm and width 360 ± 40 μm. The body of the fluke is triangular. The suction cup (VS) is very powerful, measuring 294 × 168 μm. The oral sucker (OS) measures 120 ± 10 × 130 ± 10 μm. In living individuals it is located subterminally; in fixed individuals it is slightly displaced backward from the anterior end of the body. Ratio $2.5 \le VSL/OSL \le 3$. The prepharynx is short, 70 ± 10 μm long. The pharynx is oval, well developed, 70 ± 5 × 70 ± 8 μm. The intestine is unpaired, 215 ± 1 μm long, and extends to the posterior end of the abdominal sucker. A large number of relatively small glandular cells are located between the pharynx and the anterior edge of the abdominal sucker. It is possible that these are cystogenic glands. The rudiments of the sex glands are not marked (Fig. 5a). No cysts of metacercariae were found.

The cercarium *H. pachysoma* (one sample) was noted once in July 2019 (Fig. 5b).

The body of the larva is elongated. The oral sucker is small, slightly offset from the anterior end of the body. There are two pigmented eyes at the level of the oral sucker. The abdominal sucker is more powerful than the oral one. The prepharynx is very short, becoming a well-defined oval pharynx. The intestine is unpaired, extending to the posterior end of the abdominal sucker. Many relatively small glandular cells are located between the pharynx and the abdominal sucker. The excretory thin-walled bladder has a volumetric V-shape. Cercariae swim with the body forward with a simple tail.

Family Fellodistomidae (Nicoll, 1909)

Proctoeces maculatus (Looss, 1901; Odhner, 1911). Localization: body cavity. Invasion rates: EI, 1%; II, 2 ± 1 specimen/individual; AO, 0.002 ± 0.001. Description based on five samples of progenetic metacercariae.

The body of the fluke is elongated, narrowed towards the anterior and posterior ends. The body length is 1631 ± 75 μm, and the width, 550 ± 68 μm. The cuticle is smooth. The oral sucker, 141 ± 6 μm long, 163 ± 7 μm wide, is located subterminally. The muscular abdominal sucker, 243 ± 6 μm long, 256 ± 6 μm wide, is located in the anterior third of the body. The pharynx is well developed. The pharynx length is 113 ± 7 μm, and the width, 113 ± 7 μm. The esophagus is short. The intestinal branches almost reach the posterior end of the body. When fixing the trematode, it bent a little, so the genitals shifted slightly. Oval whole-edged testes, 146 ± 11 μm long, 95 ± 5 μm wide, are located one above the other. The rounded ovary is located in front of the testes. The ovary length is 119 ± 24 μm, and the width, 101 ± 23 μm. The uterus is strongly developed and fills the entire part of the body behind the abdominal sucker. Eggs are oblong–oval, yellowish in color. The length of the eggs is 48 ± 2 μm, and the width is 24 ± 1 μm (Fig. 6).

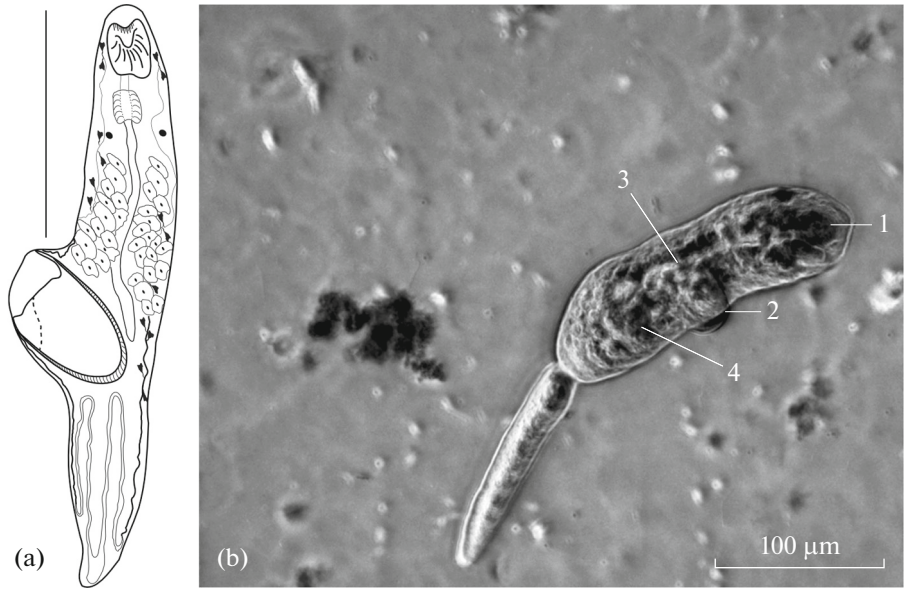

Fig. 5. *Haplosplanchnus pachysoma* in the estuary of the Chernaya River. (a) Larva (drawing of a living worm), scale 500 μm; (b) micrograph of cercariae (live helminth): 1, oral sucker; 2, abdominal sucker; 3, intestine; 4, glandular cells. Scale: 100 μm.

Family Monorchiidae (Odhner, 1911)

***Paratimonia* sp.** (Prevot and Bartoli, 1967). Localization: body cavity.

Invasion rates: EI, 1%; II, 1.33 ± 0.33 specimens/individual; AO, 0.014 ± 0.009. The description is based on two metacercariae.

The metacercariae *Paratimonia* sp. discovered by us have an oval body shape. The body length is 868–882 μm, and the width, 364–560 μm. In living individuals, small spines are visible on the integument. The powerful abdominal sucker, 252–280 μm long and 182–266 μm wide, is twice as large as the oral one (length 126–140 μm, width 154–182). The prepharynx is short, and the pharynx is large, round or oval in shape. The short esophagus forks above the abdominal sucker. The intestinal branches reach the lower border of the upper testis. At the level of the pharynx and the oral sucker, there are two large pigmented eyes, which are a systematic feature of the genus. The excretory vesicle is elongated, in the form of an ampoule. The testes lie diagonally one above the other, between the branches of the intestines. The ovary lies over the testes and is shifted to the right. The formed genital bursa is clearly visible in the back of the body. The genital pore is visible (Fig. 7).

Our studies of this larva have shown that, in all likelihood, an acceleration of the life cycle is possible for this species of trematodes, in which there is a premature development of all organ systems, especially the reproductive system, which characterizes the adult trematode. The gastropods *H. acuta*, acting in this case as a paratenic host, apparently could have become infected as a result of contact with cercariae that left the first intermediate host (bivalve mollusks

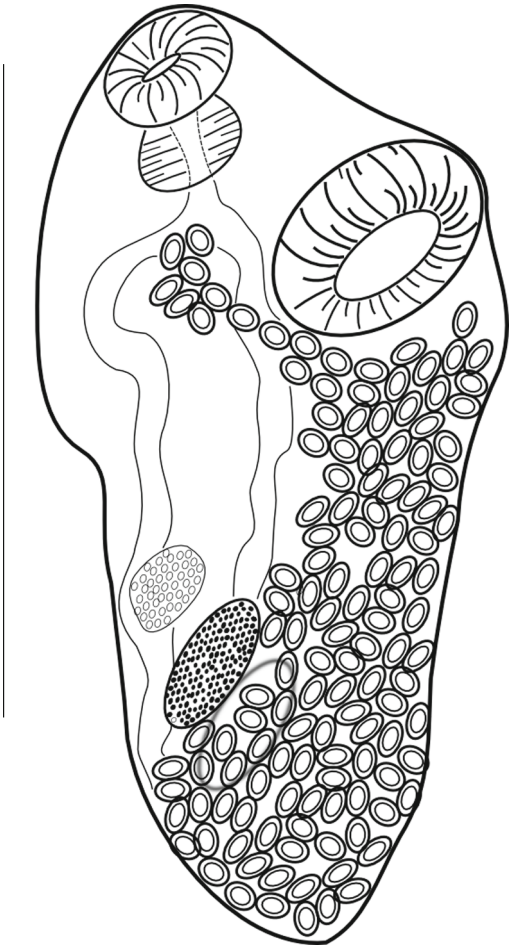

Fig. 6. Progenetic metacercaria *Proctoeces maculatus* in the estuary of the Chernaya River. Scale: 1500 μm.

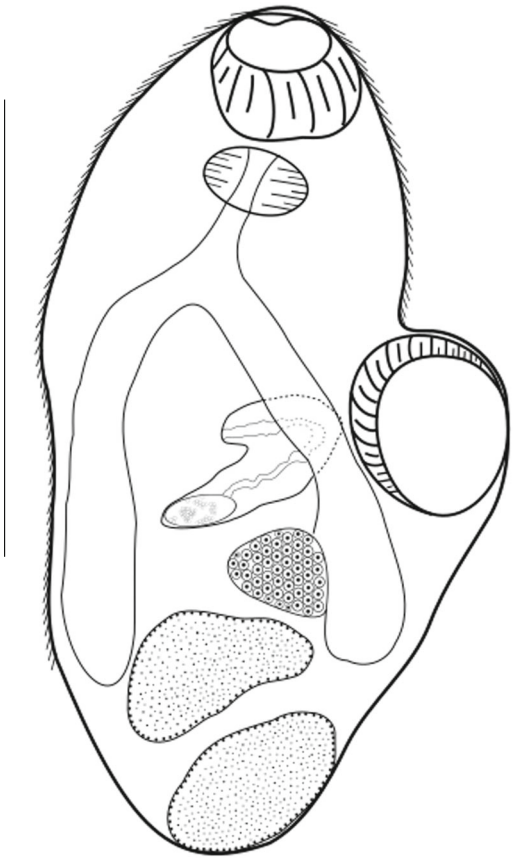

Fig. 7. Progenetic metacercaria *Paratimonia sp.* in the estuary of the Chernaya River. Scale: 500 μm.

Abra segmentum, sharing a common biotope with hydrobia).

DISCUSSION

Purposeful faunistic study of trematodes of Black Sea mollusks in natural settlements in the water area of Sevastopol was first begun in 1909–1911 by D.O. Sinitsyn (Sinitsyn, 1911). He published the first report "Parthenogenetic Generation of Trematodes and its Offspring in Black Sea Mollusks." In this work, parasitization of 25 species of cercariae and metacercariae in 11 species of Black Sea mollusks is noted and morphological descriptions of the found larvae of trematodes are given. Only half a century later were these studies resumed by A.V. Dolgikh (Dolgikh, 1965). She examined 40 species of mollusks from 20 families (17% of the Black Sea mollusk fauna), and 32 species of trematode larvae were recorded in 21 species (two species from the family Bucephalidae, one species from the family Diplostomatidae, four species from the family Opecoelidae, one species from the family Acanthocolpidae, one species from the family Zoogonidae, four species from the family Fellodistomatidae, two species from the family Gymnophallidae, one species from the family Plagiorchioidea, three species from the family Microphallidae, two species from the family Opisthorchioidae, one species from the family Monorchidae, two species from the family Lepocreadiidae, one species from the family Haploporidae, and seven species from the family Hemiuroidae), including cercariae of 27 species of trematodes and metacercariae of five species of trematodes (of which two are progenetic). Of these, 12 species of trematode larvae were described as new to science; nine species were recorded by her for the first time in mollusks of the Black Sea (Dolgikh, 1965). From an oral communication by A.V. Gaevskaya (Dolgikh), it is known that in the 1960s work began on the study of trematodes of *H. acuta,* but the results of her research were not published. Thus, this work is a continuation of helminthological monitoring on the one hand and the first work on the study of trematode fauna of *H. acuta* for this area.

From the family Heterophyidae, we marked the cercariae of the genus *Cryptocotyle,* first described by F. Creplin (1825). However, the greatest contribution to the study of the morphology and life cycle of this trematode was made by G. Stunkard (1930). In his now classic work, Stancard examined the history of the genus and its life cycle and described a number of stages of the parasite, parasite–host relationships, and the morphological features of cercariae and metacercariae. He set up a number of experiments to describe the early stages of the development of the parasite. The cercariae found by us are the closest *Cryptocotyle* according to the main morphological characteristics (body shape, tail structure, structure of the system of penetration glands) and measurements.

From the family Microphallidae, we found two types of cercariae—*Maritrema misenense* and *Gynaecotyla adunca*. The latter were first described by Palombi (1940) from the mollusks *Cerithium vulgatum* in the Mediterranean Sea. F. Ankel (1962) notes these cercariae as *Cercaria misenensis* in two types of aquatic organisms—*Hydrobia ulvae* (Pennant, 1777) and *H. ventrosa* (Montagu, 1803) recovered off the coast of Denmark. Trematodes similar in morphological features were discovered by M. Sordi (1959) from *Paludestrina salinasi* Aradas Calcara, 1842 from the vicinity of Livorno (Italy). Prior to us A.V. Dolgikh (1965) found the microphallidae cercariae *G. adunca* in the Black Sea, which was called *C. misenensis*. The cercariae *G. adunca* (syn. *Cercaria sevillana*) from Portugal from the mollusk *Nassarius reticulatus* (Linnaeus, 1758) (Russel-Pinto and Bartoli, 2002) are identical to the cercariae found by Dolgikh (1965) in the same mollusk species in the Black Sea; however, they are erroneously called *Cercaria misenensis,* which are parasites of the mollusks *Cerithium rupeste*, Risso, 1826. *Cercaria sevillana* differs from *Cercaria misenensis* in the shape and size of the excretory bladder, the length and shape of the tail, and the behavioral responses. These morphological differences were proven in the

work of Portuguese authors (Russel-Pinto and Bartoli, 2002). Our find of cercariae in all respects coincides with the latest morphological description (Russel-Pinto and Bartoli, 2002): the Y-shaped bladder of the detected cercaria *G. adunca* vs. the pear-shaped *C. misenensis* (Dolgikh, 1965), the ribbed tail structure vs. the smooth structure of *C. misenensis* (Dolgikh, 1965), and the position and shape of the stylet.

The cercariae of the second type of microphallidae *Maritrema misenense* discovered by us is similar in size and basic morphological features, including the shape of the stylet, the configuration of which is the most important diagnostic feature of cercariae microphallidae, to the shape of the stylet of *M. misenense* individuals previously described in the literature (Dolgikh, 1965): position and form of the stylet, pear-shaped body, smooth structure vs. the ribbed structure of the tail in other representatives of the family Microphallidae (Dolgikh, 1965). These morphological characteristics allow us to classify the discovered microphallidae cercariae as *Maritrema misenense.*

The larval stage of *H. pachysoma* was first recorded by us in the water area of Sevastopol (Sevastopol Bay, Chernaya River). Larva at the cercaria stage and life cycle of *H. pachysoma* were first studied in detail by Cable (Cable, 1954). Later Feres and Maillard (1975) described the life cycle of *H. pachysoma.* The authors note that cercariae develop in the gastropod mollusk *H. ventrosa*, and, after a while, the cercariae leave their first intermediate host and are encysted. For the fluke *H. pachysoma,* mullet are the final host and fish infection occurs through adolescaria (Fares and Mailard, 1975). In the examined mollusks of *H. acuta,* in early spring and autumn, large nonencysted larvae of trematodes were found, and in July 2019, a larva was recorded at the cercaria stage in the same species of mollusks. The morphology of both types of larvae corresponded to the diagnosis of the genus *Haplosplanchnus*: elongated-oval oral sucker, large abdominal sucker of a peculiar structure (it is deeply immersed in the body, when viewed from the side it has a saccular or cylindrical shape, and it is equipped with a muscular sphincter), unpaired intestine.

The cercariae *Timoniella imbutiformis,* the second intermediate host of which are the gobies *Neogobius melanostomum,* were registered in the water area of Sevastopol. The first description of cercariae of this kind was given by Rebecq in 1960 (Rebecq, 1960) from the Mediterranean Sea. In the work by Maillard (1973) it is mentioned that the first intermediate host for this species of trematodes are gastropods of the genus *Hydrobia*, and the second intermediate host for *T. imbutiformis* is four species of gobies (Kvach, 2004). The most recent revision of the genus *Timoniella* (Kvach., 2017) showed that, to identify the species *T. imbutiformis,* the number of spines on the oral sucker is indicated as the main diagnostic sign (17–20), along with the location of the intestinal branch. The cercariae of this species of trematodes found by us are the closest to the species *T. imbutiformis* in the number of spines on the oral sucker (18) and the location of the intestinal branch, which ends blindly and reaches the posterior end of the body.

A brief description of metacercariae and sexually mature individuals of *Paratimonia* in the Black Sea was first presented by N.N. Naydenova in 1970 (Naydenova, 1974). One species of this genus has been registered in the Black Sea—*Paratimonia gobii.* The most recent information on the morphology of the genus marite *Paratimonia* are given in Bray et al. (Bray et al., 2008). According to the literature (Naydenova, 1974; Bray et al., 2008), the absence of a prepharynx, the presence of a short esophagus and the intestinal branches reaching the lower border of the testis, and the structure of the reproductive system allow us to attribute the discovered metacercariae to the genus *Paratimonia*.

In the conditions of the Black Sea, the mussel *Mytillus galloprovincialis* was determined as the intermediate host for *Proctoeces maculatus* (Machkevsky, 1984). Benthic mollusks were identified as additional hosts, such as *Rissoa labiosa*, *R. splendida*, *Tricolia pulla*, and *M. galloprovincialis* (Dolgikh, 1965) and the polychaetes *Nereis diversicolor* and *N. succinea.* Fish of the families Labridae and Gobiidae are definitive hosts in the Black Sea. For the first time in 1959 (Stunkard and Uzmann, 1969) the progenetic stage was described for *Proctoeces maculatus* in the mussel *Mytillus edulis*. In the same mussel specimen, the authors noted sporocysts with cercariae, noncystic metacercariae, and marita species. Cases of finding progenetic metacercariae in mollusks have been reported in other works (Dolfus, 1964; Dolgikh, 1965, 1967). Off the Crimean coast, the progenetic metacercariae *Proctoeces maculatus* was first discovered by Dolgikh (1967) in the mollusks *Rissoa splendida. H. acuta* was registered for the first time as an intermediate host for *P. maculatus.*

Thus, as a result of the study of the mollusks *H. acuta*, new information was obtained on the composition of their trematode fauna in the Black Sea. It was found that, in the water area of Sevastopol, the trematode fauna of the studied gastropods is represented by seven species belonging to six families: Microphallidae, Monorchiidae, Cryptogonimidae, Fellodistomidae, Haplosplanchidae, and Heterophyidae.

A significant part of all mollusk trematodes are cercariae, for which fish are the final hosts. The larvae of trematodes of the families Heterophyidae and Microphallidae are most invasive, while larvae of *Paratimonia* sp. and *Haplosplanchnus pachysoma* are extremely rare.

ACKNOWLEDGMENTS

The author is grateful to M.V. Makarov (Kovalevskii Institute of Biology of the Southern Seas, Russian Academy

of Sciences) for help in collecting and determining the species composition of mollusks and Yu.M. Korniychuk for scientific advice when preparing the article for publication.

FUNDING

The work was carried out within the framework of a State Assignment of Kovalevskii Institute of Biology of the Southern Seas, Russian Academy of Sciences, topic no. 121030100028-0.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest. The author declares that he has no conflicts of interest.

Statement on the welfare of animals. This research was conducted in accordance with animal ethics and welfare. All studies were performed in accordance with the Law on Veterinary and Medical Activities and the National Law on the Protection of Animals.

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