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PARASITOLOGY

Description of *Echinobothrium typus* van Beneden, 1849 (Platyhelminthes: Diphyllidea) from *Raja clavata* Linnaeus, 1758 (Pisces: Rajidae) in the Black Sea

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Abstract—Mature specimens of the cestode *Echinobothrium typus* van Beneden, 1849 from the thornback ray *Raja clavata* Linnaeus, 1758 that inhabit the Black Sea are found and described in detail for the first time. New details of the scolex armature and reproductive system structures of *E. typus* are revealed. Infection indices of this cestode in the thornback rays are analyzed. It is shown that the occurrence of *E. typus* in the definitive host *Raja clavata* depends not only on the presence and the population density of the intermediate host, presumably, amphipods *Perioculodes longimanus* Bate et Westwood, 1868, but also on the age-related changes in the food spectrum of the thornback rays.

Keywords: cestodes, *Raja clavata*, *Echinobothrium typus*, intermediate host, occurrence, Black Sea
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INTRODUCTION

The genus *Echinobothrium* van Beneden, 1849 was established for the species *E. typus* van Beneden, 1849 from a thornback ray *Raja clavata* Linnaeus, that was caught at the Belgian coast of the North Sea [13]. Later, the author gave a brief diagnosis of the genus [14], but the description of *E. typus* remained incomplete, since there was no information on the number of testes and the sizes of the larger hooks and spikes. Pintner revised this genus in 1889 and slightly expanded the description of *E. typus* [30].

In the Black Sea, *E. typus* was first found by Borcea [15], but the author did not present its description. Later, Pogoreltseva [8] gave a brief description for immature specimens of the species. Further, a brief characteristic of the species and its schematic drawing from the study of T.P. Pogoreltseva were included in [5]. More information about the peculiarities of the morphology of *E. typus* was obtained by N.M. Biserova from the study on the ultrafine structure of the scolex and the strobila surface, as well as the detailed structure of the muscles of the proboscis apparatus [2].

Thus far, a complete description of *E. typus*, in accordance with modern requirements for the detailed description of cestodes of this taxon has not been made [22–24, 27, 31, 34]. An enhanced and detailed description of mature individuals of *E. typus* and discussion of infection of *R. clavata* with this cestode off the Crimea coast are presented in this paper.

MATERIALS AND METHODS

The material for this investigation of *Echinobothrium typus* was collected from 147 specimens of the thornback ray *Raja clavata*, which were caught during 2001–2012 in four localities of the water area near Sevastopol: at Kacha settlement, in the Karantinnaya and Kazach'ya bays, and at Cape Chersonesos. At the Kacha settlement, 14% of the thornback rays were infected with *E. typus* at the infection intensity of 1–11/8 ± 3 specimens per fish and the abundance index of 1.1 ± 0.7 spec. per fish; in Karantinnaya Bay, 19% of the fish were infected: 1–2119/246 ± 234 and 47 ± 45 spec. per fish, respectively; in Kazach'ya Bay it was 19%: 1–16/11 ± 5 and 2 ± 1 spec. per fish; and at Cape Chersonesos it was 29%: 1–48/13 ± 3 and 4 ± 1 spec. per fish. Cestodes were picked out using dental root needles of different thicknesses (nos. 1–3) and a woodcock pin feather ("painting feather"). Collected cestodes were kept in fresh water for 10–60 minutes for relaxation of the muscles and evagination of the cirrus before fixation in 70% ethanol and preparing of whole mount slides [3]. Helminths were stained with acetocarmine using the standard method [9], differentiated in "iron water" ($H_2O + Fe_2O_3$) and acid alcohol (70% ethanol with 3% HCl), dehydrated using an ethanol series (70–100%), cleared in clove oil, and mounted in Canada balsam. Measurements are given in micrometres (except where noted otherwise) as the range, mean and standard error, with the number of cestodes studied (n), and the number of measurements (n), if one specimen is a subject of more than

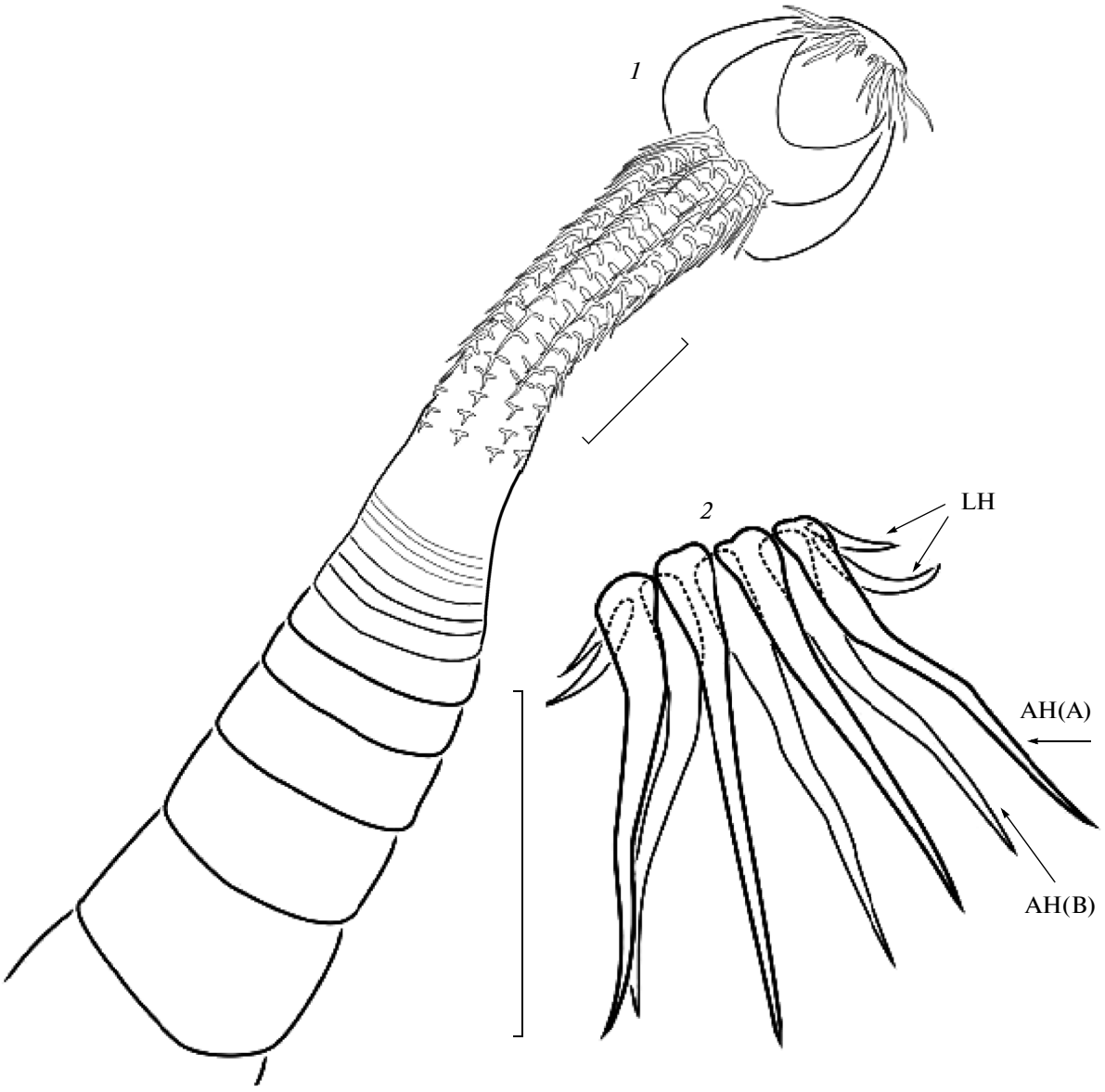

Fig. 1. *Echinobothrium typus* from *Raja clavata*. *1*, scolex and cephalic peduncle with spines, C.875.013.1; *2*, apical hooks and hooklets, C.877.013.4–6. Abbreviations: AH(A), dorsal and ventral hooks, type A symmetry; AH(B), dorsal and ventral hooks, type B symmetry; LH, lateral hooklets. Scale: *1*, 100, *2*, 50 μm.

one dimension. The drawings are based on photographs taken through a Carl Zeiss Axio Imager M1 microscope with an AxioCam HRc digital camera (Carl Zeiss). The descriptive statistics were calculated using the software package Statistica v. 6.0 for Windows. The material is deposited in the collection of the Department of Ecological Parasitology of the Kovalevsky Institute of Biology of the Southern Seas (IBSS, Sevastopol).

RESULTS

Echinobothrium typus van Beneden, 1849 (Figs. 1 and 2)

Type host: *Raja clavata* (family Rajidae).

Type locality: Belgian coast of the Northern Sea.

Additional localities: Atlantic, Mediterranean and Black seas (off Sevastopol: Kacha settlement (44°46′31″ N, 33°32′28″ E), Karantinnaya Bay (44°36′41″ N, 33°29′54″ E) and Kazachaya Bay (44°34′21″ N, 33°24′40″ E), Cape Chersonesos (44°34′59″ N, 33°22′49″ E).

Location: Spiral intestine.

Material: C.875.013.1; C.876.013.2; C.877.013.4–6; C.878.013.7; C.879.013.8–9; C.881.013.12; C.883.013.17; C. 887.013.21; C.888.013.22, C.890.013.24 from *R. clavata* from the Black Sea. The

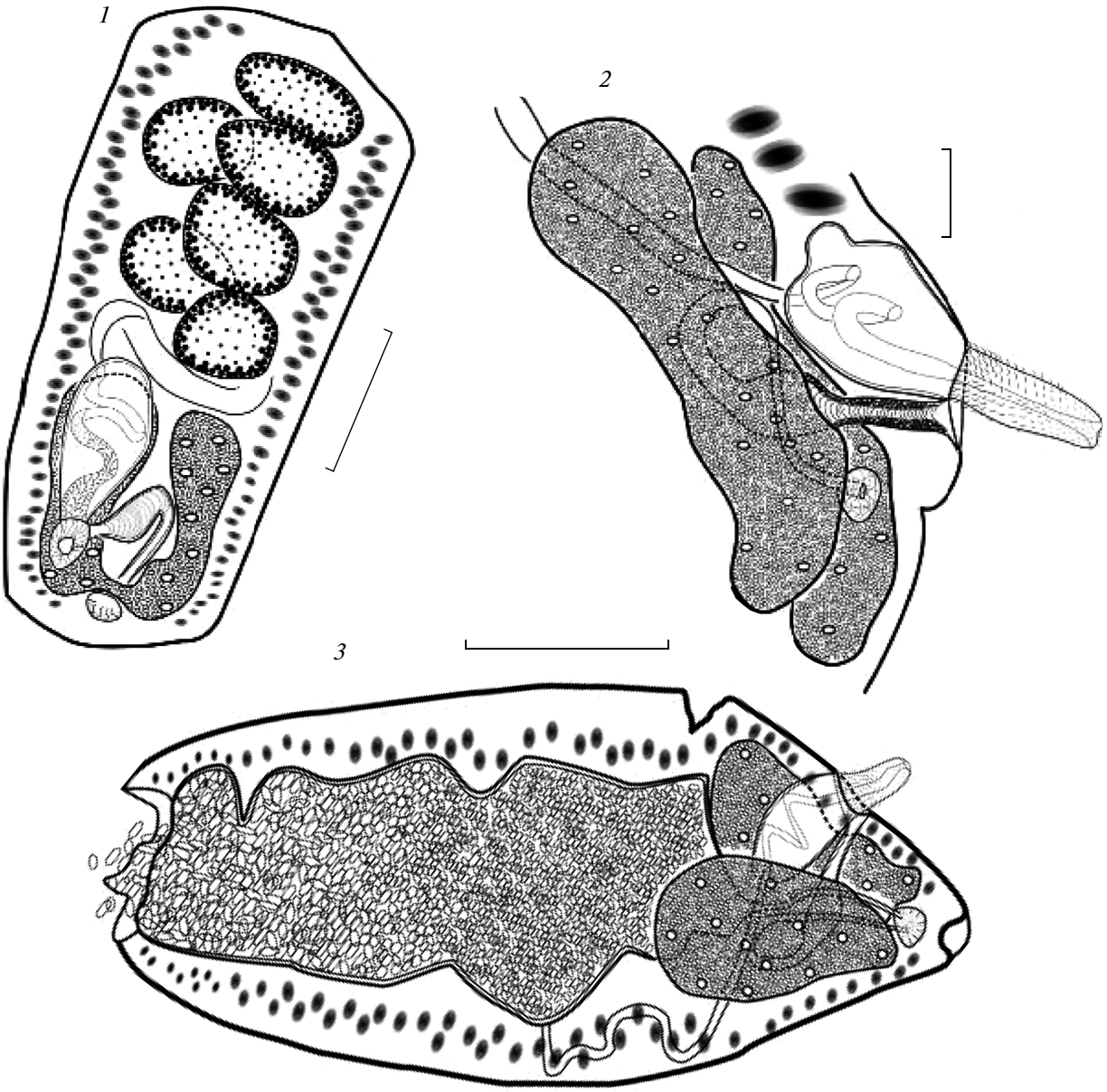

Fig. 2. *Echinobothrium typus* from *Raja clavata*. *1*, Frontal view of mature proglottids, C.876.013.2; *2*, Details of terminal genitals, lateral view, C.876.013.2; *3*, mature proglottid, C.878.013.7. Scale: *1*, 100, *2*, 50, *3*, 200 μm.

studied specimens: four immature, six sexually mature, and three adult individuals.

Adult cestodes small, acraspedote, euapolytic, 0.3–4.95 mm (2.06 ± 0.5; n = 13) long by 0.11–0.425 mm (0.21 ± 0.01; n = 13; *n* = 49) wide. Proglottids: 7–17 (13 ± 1; n = 13) in strobila. Scolex consisting of scolex proper and cephalic peduncle (Fig. 1, *1*). Scolex proper 150–250 (198 ± 10; n = 13) long by 100–200 (157 ± 10; n = 13) wide, composed of armed apical rostellum and one dorsal and one ventral bothrium; bothria 120–200 (163 ± 5; n = 13; *n* = 22) long by 50–100 (74 ± 3.4; n = 13; *n* = 22) wide. Rostellum bearing one dorsal and one ventral group of seven solid apical hooks, arranged in two rows, flanked on each side by separate lateral groups of two small hooklets similar to scalpel blades, 15–50 (25.3 ± 1.2; n = 13; n = 44) long (Fig. 1, *2*). Hook formula {2 4/3 2}. Apical hooks gradually decreasing in length from center of the group to edges. The first hooks of type A symmetry 43–75 (64 ± 2.1; n = 13; *n* = 21) long, 39% longer than lateral hooklet; the second hooks 55–90 (73 ± 2.3; n = 13; *n* = 21) long; the third hooks 60–95 (75 ± 2.03; n = 13; *n* = 21) long, and the fourth ones 50–75 (63 ± 1.4; n = 13; *n* = 21) in length. First hooks of type B symmetry 50–63 (56 ± 1.2; n = 13; *n* = 21) long; second hooks 55–75 (64.3 ± 1.1; n = 13; *n* = 21) long,

and third hooks 50–65 (56 ± 1.1; n = 13; *n* = 21) in length. Cephalic peduncle 75–500 (340 ± 32.2; n = 13) long by 50–175 (92.3 ± 7; n = 13; *n* = 20) wide, armed with eight longitudinal columns of 14–19 (16 ± 0.5; n = 13) spines. Spines with broad Y-shaped bases, reducing in length to the posterior margin of peduncle; free prong of first three anterior spines 35–93 (60 ± 1.4; n = 13; *n* = 99) long, and free prong of last three posterior spines (21 ± 0.7; n = 13; *n* = 99) long (Fig. 1, *1*).

Immature proglottids 7–14 (11 ± 0.6; n = 13) in strobila, 30–320 (93 ± 10; n = 13; *n* = 32) long by 110–270 (169 ± 6.3; n = 13; *n* = 32) wide; mature proglottids 2–3 (2.7 ± 0.2; n = 6) in strobila, 200–700 (505 ± 40; n = 6; *n* = 14) long by 220–310 (263 ± 8; n = 6; *n* = 14) wide (Fig. 2, *1*); length : width ratio 0.77–*3*.1 (2 ± 0.2; n = 6; *n* = 14) : 1; adult proglottids 1–2 (n = 3), 620–900 long by 200–425 wide (Fig. 2, *3*), length : width ratio 2–4.3 : 1. Testes 6–7 (6.4 ± 0.1; n = 13; *n* = 17) in proglottid, 25–80 (59 ± 4; n = 13; *n* = 19) long by 50–125 (70 ± 6; n = 13; *n* = 19) wide, arranged in two columns from anterior margin of proglottid to anterior margin of ovary (Fig. 2, *1*). Cirrus-sac ovoid, tapering distally, 50–100 (81 ± 4.3; n = 11) long by 100–150 (119 ± 4.5; n = 11) wide, length : width ratio 0.6–0.9 (0.71 ± 0.03; n = 11) : 1; located between ovary wings, opening into common genital atrium anteriorly to vagina, containing robust cirrus armed with small thin spinitriches (Fig. 2, *2*). Internal and external seminal vesicles are absent. Vas deferens 20–30 (26 ± 2; n = 5) in diameter. Ovary near posterior margin of proglottid, H-shaped in front view, 100–320 (219 ± 11; n = 13; *n* = 26) long by 90–170 (140 ± 5.3; n = 13; *n* = 16) wide (Fig. 2, *2*). Mehlis' gland 35–50 (48 ± 3; n = 6) long by 50–75 (54 ± 4.2; n = 6) wide. Vagina short, muscular, from Mehlis' gland to anterior margin of proglottid, turning back at the level of anterior margin of cirrus-sac; and posteriorly to the latter opening into genital atrium. The genital pore midventral, 11–26% (19 ± 2.5; n = 6) from posterior margin of mature proglottid. Vitellarium follicular; located in two lateral fields extending throughout the length of proglottid, uninterrupted at the level of ovary and continued from one proglottid to another. Uterus saccate, beginning as a uterine duct in ootype region, extending anterodorsally to cirrus-sac and ventrally to testes (Fig. 2, *3*). Eggs oval, 13–15 (13 ± 0.2; n = 3; *n* = 18) long by 13–20 (15 ± 0.5; n = 3; *n* = 18) wide, without a terminal filament; unembryonated.

DISCUSSION

Cestodes of the order Diphyllidea represent a small but unique group of parasites of the elasmobranch fish. The recent molecular studies of phylogenetic relationships of this group of tapeworms have revealed a close relationship of diphyllideans with the order Trypanorhyncha [16, 17, 28, 29]. The systematics of diphyllideans was significantly changed in recent years and five genera comprising fifty species were recognized as valid: *Echinobothrium* van Beneden, 1849 s. str.; *Ditrachybothridium* Rees, 1959; *Ahamulina* Marques, Jensen et Caira, 2012; *Halysioncum* Caira et al., 2013, and *Coronocestus* Caira et al., 2013 [17, 23, 24].

The modern diphyllidean taxonomy is based mainly on the morphology of the scolex armature. The latter is described with the use of a formula that represents the number and arrangement of hooks and hooklets on the rostelium proposed by Neifar et al. [27]. In addition, Jones and Beveridge [22] have suggested defining the anterior apical hooks in the dorsal and ventral groups as type A symmetry and the posterior apical hooks in both groups as type B symmetry. Thus, the formula of Neifar et al. is as follows: {LH AH(A)/AH(B) LH}, where AH(A) is the number of apical hooks (type A) on the bothrium surface; AN(B), the number of apical hooks (type B) on the bothrium surface; and LH is the number of lateral hooklets surrounding apical hooks on each side, since the apical hooks on the dorsal and the ventral surfaces of bothria are symmetrical. However, this formula is applicable only to those species that have apical hooks that are clearly distinguishable from lateral hooklets in their size and/or shape, and lateral hooklets are arranged on each side in separate dorsal and ventral groups.

A modified formula {(LH) AN (A)/AN (B)}, where the total number of lateral hooklets in a continuous group is indicated in parentheses as a single number (or its range) and followed by the number of apical hooks, was proposed for species in which the apical hooks and lateral hooklets cannot be clearly distinguished by any feature, particularly in the site of transition between the smaller apical hooks and lateral hooklets [24].

Only one species of this order, viz., *E. typus*, which is a representative of the genus *Echinobothrium* s. str., with lateral hooks arranged on each side in separate dorsal and ventral groups, occurs in *Raja clavata* of the Black Sea. For description of this species, we used the formula {LH AH(A)/AH(B) LH} [27]. Since descriptions of *E. typus* from other seas [13, 14, 30] are too brief (see table), we cannot conduct a complete comparison of our materials with the available descriptions. However, we note that *E. typus* from *R. clavata* inhabiting the Black Sea is distinguished by a shorter strobila, longer hooks and spines, a greater number of spines, and fewer testes (see table). In addition, only two small hooklets were found in the Black Sea *E. typus*, while the descriptions cited above [13, 14, 30] include two to four hooklets per specimen. In spite of the revealed differences, the Black Sea specimens belong to *E. typus* according to a majority of the significant characters.

The life cycles of diphyllidean cestodes are completed via invertebrates (molluscs, amphipods, copep-

Comparison of the morphological traits of the cestode *Echinobothrium typus* from *Raja clavata* (Black Sea) with the data of other authors

Traits	Our data	van Beneden (1849, 1858)	Pintner (1889)
Cestode length, mm	0.25–4.95	5–6	6
Scolex with cephalic peduncle, mm	0.225–0.75	–	0.65
Number of large hooks	7	7	7
Length of large hooks, mm	0.043–0.095	–	0.044–0.046
Number of spines	14–19	12–13	–
Spin length, mm	0.01–0.09	–	0.05
Number of proglottid	7–17	8–10	14
Number of testes	6–7	–	8–10
Egg length, mm	0.013–0.015	0.01	0.01

ods, and decapods) as the first intermediate hosts, through teleost fish as the second intermediate hosts, and cartilaginous fish as the definitive hosts [34]. Procercoides of the cestodes of the genus *Echinobothrium* have been found in amphipods and decapods, as well as bivalve and gastropod mollusks: *Perioculodes longimanus* Bate et Westwood, 1868; *Gammarus locusta* (L., 1758); *Penaeus longistylus* Kubo, 1943; *Hippolyte varians* Leach, 1814; *Leukoma laciniata* (Carperter, 1864); *Solen vagina* L., 1758; *Solenosteira cancellarius* (Conrad, 1846); *Nassarius vibex* (Say, 1822); *N. reticulate* (L., 1758)] in the Indian Ocean, in the Mediterranean and the North seas, in the southern Great Barrier Reef, and in the northern Gulf of Mexico [12, 14, 18, 22, 25, 26, 32, 33].

For the first time, the larval *E. typus* were found in the amphipod *P. longimanus* that were collected at the Italian coast [26]. In the Black Sea, this amphipod occurs down to 100-m depths (mostly at depths of 10–25 m) on sandy bottoms [7], which are the preferred habitats of the thornback ray *R. clavata*. The amphipod *P. longimanus* was recorded in the northern Black Sea from the Bolgarian and Crimean coasts [6] and to the Caucasus shores [1]. It is worth noting that the parasitic fauna of this amphipod in the Black Sea has not yet been studied, therefore we cannot unambiguously state that *P. longimanus* is the first intermediate host of *E. typus*. However, the wide occurrence of *P. longimanus* in the Black Sea habitats allows us to suggest that this amphipod is a candidate for the role of the first intermediate host.

Only scarce information is available on the nutrition of *R. clavata* in the Black Sea [4, 10]. The basis of the thornback ray nutrition is made of crabs of the genus *Liocarcinus* Stimpson, 1871, crayfish of the genus *Upogebia* Borradaile, 1903, decapods *Callianassa pontica* Czerniavsky, 1884 and *Pachygrapsus marmoratus* (Fabricius, 1793), and various fish species (pickerel, mackerel, mullets, herring, and anchovies), less frequently mollusks, worms, and sea anemones. For example, the intestinal contents of the thornback rays caught near Sevastopol and in Karkinitsky Bay, included predominantly large crabs of the genus *Liocarcinus* (42%), decapod crustaceans of the genus *Upogebia* (25%), and benthic fish (22%), as well as single mollusks [10]. No data on the age dynamics of the nutrition of the thornback rays of the Black Sea are available. In the Mediterranean Sea, young and immature individuals of *R. clavata* feed mostly on small crustaceans, viz., amphipods, shrimp, and small crabs, while adult rays prey on larger crustaceans and fish [20].

According to the data on the size–age structure of *R. clavata* populations in the Mediterranean, North, Irish, and Adriatic seas [20, 21], rays of both sexes reach their sexual maturity around 8.8 years on average, with a total body length of 77–80 cm. In the Black Sea, the rays mature at smaller sizes, with a total body length of 51–55 cm, corresponding to 9–11 years [11]. Thus, among the studied rays from the Black Sea with a size range from 33 to 70 cm, 90 specimens with sizes of 33–45 cm were immature (ages of about 6–8 years) and 57 specimens with sizes of 50–70 cm were mature (ages of about 9–14 years). The cestode *E. typus* was found in rays of the both age groups, but the greatest abundance of this parasite was recorded in immature fish (abundance index of 541 ± 4 spec./fish individual). These data suggest that the first intermediate host of *E. typus* in the Black Sea is some small crustacean that predominates in the feed of young rays.

Thus, the occurrence of *E. typus* in *R. clavata* of the Black Sea depends on the presence and the population density of the intermediate host, presumably the amphipod *P. longimanus*, and on the age-related changes in the food spectrum of the ray.

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