



The intermediate hosts of *Wardium cirrosa* (Krabbe, 1869) Spassky, 1961 (Cestoda, Cyclophyllidea, Aploparaksidae) in Ukraine

O. Greben¹ · O. Kudlai² · V. V. Kornushin¹

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Abstract

The metacestodes of aploparaksid cestode *Wardium cirrosa* Krabbe, 1869 parasitic in gulls were found in polychaetes of the family Nereidae collected off the Black Sea coast, Ukraine. Two species of polychaetes, *Hediste diversicolor* (prevalence 5.3%; intensity 1–3 specimens) and *Neanthes succinea* (prevalence 9.9%; intensity 1–39 specimens), were infected with cysticercoids that were observed either individually or in accumulations. The preliminary identification of the material based on morphological characteristics was later confirmed by experimental infection of the definitive host, *Larus cachinnans* (Charadriiformes: Laridae) with metacestodes, and by the identity of the partial 28S rDNA sequences of cysticercoids and experimentally obtained adults. Although previous studies suggested freshwater leeches as the intermediate host for *W. cirrosa*, our study provides the evidence for marine polychaetes to serve as intermediate hosts. This study is the first to present the morphological characteristics of metacestodes of *W. cirrosa* in addition to molecular data for this species, as well as reporting the possibility of several cysticercoids developing from a single oncosphere. Morphology of the adult specimens obtained in the experiment was compared with adults of *W. cirrosa* previously collected from *L. cachinnans* in Ukraine. The results of our study suggest that further research focused on the elucidation of the life cycles of cestodes within the genus *Wardium* should consider marine invertebrates as potential intermediate hosts.

Keywords *Wardium cirrosa* · Cysticercoid · Life cycle · DNA · Polychaeta · Ukraine

Introduction

Wardium cirrosa (Krabbe, 1869) Spassky, 1961 is a widespread parasite of various gulls and terns in the Holarctic Region (Bondarenko and Kontrimavichus 2006). It was described by Krabbe (1869) as *Taenia cirrosa* from *Larus canus* Linnaeus, 1758 in Denmark. Deblock et al. (1960) published the first detailed description of this species from *Larus ridibundus* Linnaeus, 1766 and *L. canus* in France and assigned this species to the genus *Hymenolepis* Weinland, 1858. Spassky (1961) transferred *H. cirrosa* into

the genus *Wardium* Mayhew, 1925, which previously had been considered the junior synonym of the genus *Hymenolepis*. Bondarenko and Kontrimavichus (2006) in the revision of the family Aploparaksidae Mayhew, 1925 included the genus *Wardium* with *W. cirrosa* in this family.

The biology of *W. cirrosa* was reported by Pike (1968). The metacestodes found by Pike in freshwater leeches *Erpobdella octoculata* (Linnaeus, 1758) in England were identified as *Haploparaksis cirrosa* (= *W. cirrosa*) (Pike 1968). Illustrations and the description of the cysticercoids were provided. Specimens were identified based on the hook size. The presence of the definitive hosts, gulls, in the study localities served as further evidence. To the best of our knowledge, that is the only report regarding the life cycle of *W. cirrosa* to date. However, the species identification of members of the genus *Wardium* is considered possible only through examining the morphology of the strobila in adults (Bondarenko and Kontrimavichus 2006).

Wardium cirrosa was reported in countries neighboring Ukraine: Slovakia (Macko 1964), Poland (Bezubik 1956; Korpaczewska 1963; Pojmańska et al. 1984), Czechia

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✉ O. Greben
oksana1greben@gmail.com

¹ I. I. Schmalhausen Institute of Zoology, NAS of Ukraine, 15, Bogdan Khmelnytsky Str., Kyiv 01030, Ukraine

² Institute of Ecology, Nature Research Centre, Akademijos, 2, 08412 Vilnius, Lithuania

(Bušta et al. 1985; Ryšavý 1961), Kaliningrad and Murmansk Oblasts, Russia (Galkin 1986; Kuklina and Kuklin 2017), and Georgia (Petriashvili 1977). In Ukraine, *W. cirrosa* was found in *Larus cachinnans* (Pallas, 1811) from the Odessa, Mykolaiv, and Kherson Oblasts and in *L. ridibundus* and *Sterna hirundo* Linnaeus, 1758 collected from the Kherson Oblast (Bondarenko and Kontrimavichus 2006; Kornyushin and Greben 2013). However, *W. cirrosa* was absent in potential host specimens collected from Kyiv, Kaniv, and Kremenchuk Reservoirs of the Dnieper River (Smogorjevskaya 1976; Grushchinskaja 1978). The observance of this species of cestode parasitizing birds on the sea coast suggests that marine invertebrates rather than leeches may be the intermediate hosts of *W. cirrosa*.

This study was focused on marine invertebrates, specifically polychaetes collected from both Tendra Bay and Yahorlyk Bay (Black Sea, Kherson Oblast, Ukraine) with the specific goal of discovering the intermediate host of *W. cirrosa*. The results of this investigation along with the description of metacestodes of *W. cirrosa* are reported. An experimental infection of the definitive host using collected cysticercoids yielded gravid cestodes. The identities of the cestodes collected were confirmed using partial 28S nuclear ribosomal DNA sequencing.

Materials and methods

Material collection

A total of 286 specimens of the polychaetes of the family Nereidae were examined in June 2015 in the Hola Prystan Region of the Kherson Oblast (46° 39' N; 32°06' E), including 94 specimens of *Hediste diversicolor* (Müller, 1776) and 192 specimens of *Neanthes succinea* (Frey and Leuckart, 1847). The identification of the polychaetes was performed using the keys by Vinogradov and Losovskaya (1968) and Kiseleva (2004). Polychaetes were examined between two microscope slides under a dissecting microscope. Live cysticercoids were studied unstained in 0.9% NaCl under a microscope. Freshly collected cysticercoids were fixed in 70% ethanol. Several specimens were fixed in 96% ethanol for molecular analysis.

The adult cestodes obtained in the experiment were fixed in 70% ethanol for morphological analysis and in 96% ethanol for molecular analysis. The morphology of experimentally obtained specimens of *W. cirrosa* was compared with previously collected specimens initially found in *L. cachinnans* and residing in the Helminthological Collection of I. I. Schmalhausen Institute of Zoology, NAS of Ukraine, Kyiv (IZSHK). The specimens from the collection that were used for comparison during the current study are as follows: 2 specimens on 3 slides (No. 11) from the Mykolaiv Oblast (Ochakiv Region, 18.07.1992).

Morphological analysis

All adult cestode specimens were stained with either iron acetocarmine or Mayer's hematoxylin, dehydrated in an ascending alcohol series, cleared in clove oil and mounted in Canada balsam. Some fragments and scolices were mounted in Berlese's medium to facilitate the examination of the copulatory organs and hooks. The cysticercoids were stained with iron acetocarmine, dehydrated in an ascending alcohol series, cleared in clove oil, and mounted in either Canada balsam or Berlese's medium.

Morphology of adult cestodes and cysticercoids was examined under a Zeiss Axio Imager M1 and an AmScope T690B microscope, both equipped with digital cameras. The figures were made using drawing tube RA-6. All measurements are in micrometers unless otherwise stated and are given as a range followed by the mean and the number of measurements (*n*) in parentheses.

Experimental study

A specimen of *L. cachinnans* was experimentally infected with metacestodes whose identities were unknown in order to later compare morphological features between those observed at the metacestodes with those observed at the adult stage. The potential definitive host, a nestling of *L. cachinnans*, was collected from a bird colony at the Dnieper River near the town of Ukrainka (Kiev Oblast, Obukhiv Region) in June 2015. This bird colony was situated far from the sea coast (more than 400 km). Such a distance excluded the potential for contact between the nestlings and marine invertebrates. The nestling was inoculated with 68 cysticercoids in June 2015. During the experiment it was fed with boiled fish (sprat and notothenia) mixed with wheat porridge. The nestling was necropsied on the 13th day after inoculation. Gravid cestodes later identified as *W. cirrosa* were collected from the large intestine of the experimental host and fixed in 70% alcohol. Another nestling of *L. cachinnans* was collected from the same bird colony and was used as a control. Both the control and the experimental specimens of *L. cachinnans* were kept under the same conditions. Two adult *L. cachinnans* from the same colony were also necropsied additionally and appeared to be free of cestodes. Upon necropsy, the control nestling was also found to be free of cestodes.

Generation of molecular data

Following the protocol described by Georgieva et al. (2013), total genomic DNA was isolated from an alcohol-fixed cysticercoids and strobiles. The partial 28S nuclear ribosomal DNA was amplified using the forward primer LSU5 (5'-TAGGTGGACCCGCTGAAYTTAAGCA-3') (Littlewood

1994) and the reverse primer 1500R (5'-GCTA TCCTGAGGGAACTTCG-3') (Tkach et al. 2003) following the polymerase manufacturer's recommendations. The annealing temperature was 55 °C. Amplicons were purified using a QIAquick PCR purification KIT (Qiagen Ltd., UK) sequenced directly with ABI BigDye chemistry (ABI Perkin-Elmer, UK) alcohol-precipitated, and run on an ABI Prism 3130 × 1 automated sequencer. Sequencing primers included the amplification primers, LSU5 and 1500R, and three additional internal primers: 300F (5'-CAAGTACCGTGAGG GAAAGTTG-3'), ECD2 (5'-CTTGGTCCGTGTTT CAAGACGGG-3'), and 900F (5'-CCGTCTTGAAACAC GGACCAAG-3') (Tkach et al. 2003). Contiguous sequences were edited and assembled using Geneious ver. 11. (Biomatters, Auckland, New Zealand) and submitted to GenBank under the accession number MN401762 –MN401763.

Results

A total of 125 metacestodes with rostellar hooks similar in size, number, and shape to the hooks of *W. cirrosa* were found in the examined polychaetes of the family Nereidae: *H. diversicolor* (prevalence 5.3%; intensity 1–3 specimens, mean intensity 1.6) and *N. succinea* (prevalence 9.9%; intensity 1–39 specimens, mean intensity 6.0). Gravid cysticercooids were localized in different parts of the polychaetes' coelom appearing most frequently in the posterior segments. In one *N. succinea*, one cysticercooid was localized at the anterior end of the body, and the second one in the middle part of the body. Due to the relatively long cercomer possessed by *W. cirrosa*, the cysticercooids were often localized in more than one segment of the host polychaete's coelom. Cysticercooids were observed both individually and in accumulations. These accumulations of cysticercooids formed "larvophorae" ranging in size from 2 to 39 individual specimens. Single cysticercooids were observed in roughly half of the infected polychaetes. All cysticercooids in a particular grouping of larvophorae were observed to be in the same stage of development as their cohorts.

The specimen of *L. cachinnans* that was experimentally infected with 68 cysticercooids collected from nereids was necropsied 13 days post inoculation. A total of 66 adult cestodes each possessing a pregravid uterus were collected from the posterior part of the digestive tract of the specimen of *L. cachinnans*. A comparison of the morphology between the metacestodes and the adult cestodes showed that both were *W. cirrosa*.

The description of cysticercooids and adult cestodes is provided below.

Family Aploparakidae Mayhew, 1925

Genus *Wardium* Mayhew, 1925

Wardium cirrosa (Krabbe, 1869) Spassky, 1961

Metacestodes (Fig. 1)

Morphological descriptions are based on 15 specimens collected in the Hola Prystan Region of the Kherson Oblast: Four specimens were stained with iron acetocarmine and 11 specimens mounted in Berlese's medium.

The total length measurements of the cysticercooids are 1.62–2.94 mm (2.26 mm, $n = 7$). Length and width measurements of the cysts are 260–480 × 170–380 (337 × 228, $n = 15$). No exocysts were observed. The thickness of the three-layered cyst wall measures 13–23 (17, $n = 13$). Calcareous corpuscles are numerous and concentrated in the anterior and posterior regions of the cyst cavity. The length and width of the scolex measures 100–160 × 120–150 (128 × 134, $n = 11$). The oval muscular suckers are 55–95 × 40–80 (69 × 53, $n = 32$). The rostellum measures 55–90 (70, $n = 12$), is armed with ten aploparakoid hooks, and possesses a maximum diameter where the hooks are located. The maximum diameter of the rostellum measures 55–90 (65, $n = 12$).

The anterior surface of rostellum usually is not invaginated. The hook blades on the rostellum are oriented anteriorly. The rostellar sheath measured 80–100 × 65–90 (92 × 75, $n = 9$). Measurements pertaining to the hooks are as follows: total length 23–26 (25, $n = 36$), handle 4–6 (5, $n = 36$), blade 14–16 (14, $n = 36$), guard 9–11 (10, $n = 36$), and base with guard 16–20 (19, $n = 36$).

The invagination pore is located anteriorly, appears as a narrow slit, and measures 55–110 (84, $n = 13$) in depth. The excretory canal is located posteriorly, is short in diameter, and measures 40–110 (59, $n = 12$). The cercomer is elongate 1.36–2.46 mm (1.86 mm, $n = 7$). It originates at the funnel-like mouth at posterior end of the cyst. The cercomer widens towards the posterior end of the cyst and measures 190–310 × 180–320 (258 × 272, $n = 14$). Along the length of the cyst, the cercomer forms globular protrusions. Single cysticercooids were observed to be connected with one another by way of a common cellular mass formed by cercomers. The resulting structure is termed "larvophorae." The cellular mass has the form of an irregular plate. Embryonic hooks are 10–18 long.

The cysticercooids connect with one another by way of their cercomers during the early stages of larval development. It was not possible to isolate individual cercomers in the cellular mass. Embryonic hooks are more often localized within the mass of cercomers compared with individual cercomers. In order to determine whether the cysticercooids developed from one cestode egg or through budding the number of the embryonic hooks present within, several "larvophorae" were counted and compared with the number of cysticercooids observed within the relevant number of "larvophorae." Cysticercooids and embryonic hooks were observed in the following numbers: 4 cysticercooids—6 embryonic hooks; 4 cysticercooids—18 embryonic hooks; 9 cysticercooids—36 embryonic hooks. The oncosphere of a cestode contains six hooks. The number

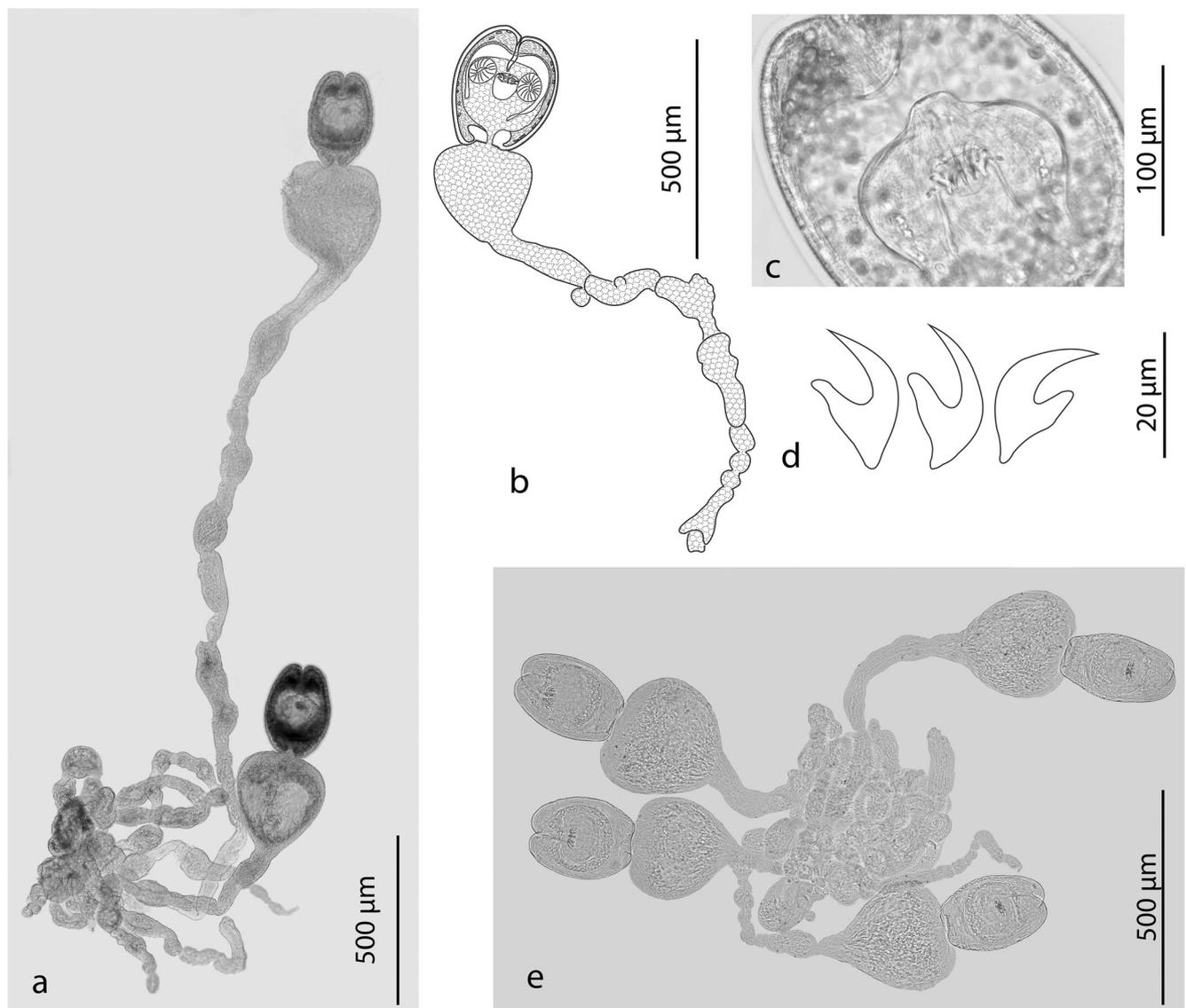


Fig. 1 Metacystodes of *Wardium cirrosa* from *Neanthes succinea* and *Hediste diversicolor*. **a** Alive cysticercoids in “larvophorae.” **b** Fixed

and stained cysticercoid. **c** Hooks of alive cysticercoid, in profile. **d** Rostellar hooks in fixed cysticercoid in profile. **e** Fixed “larvophorae”

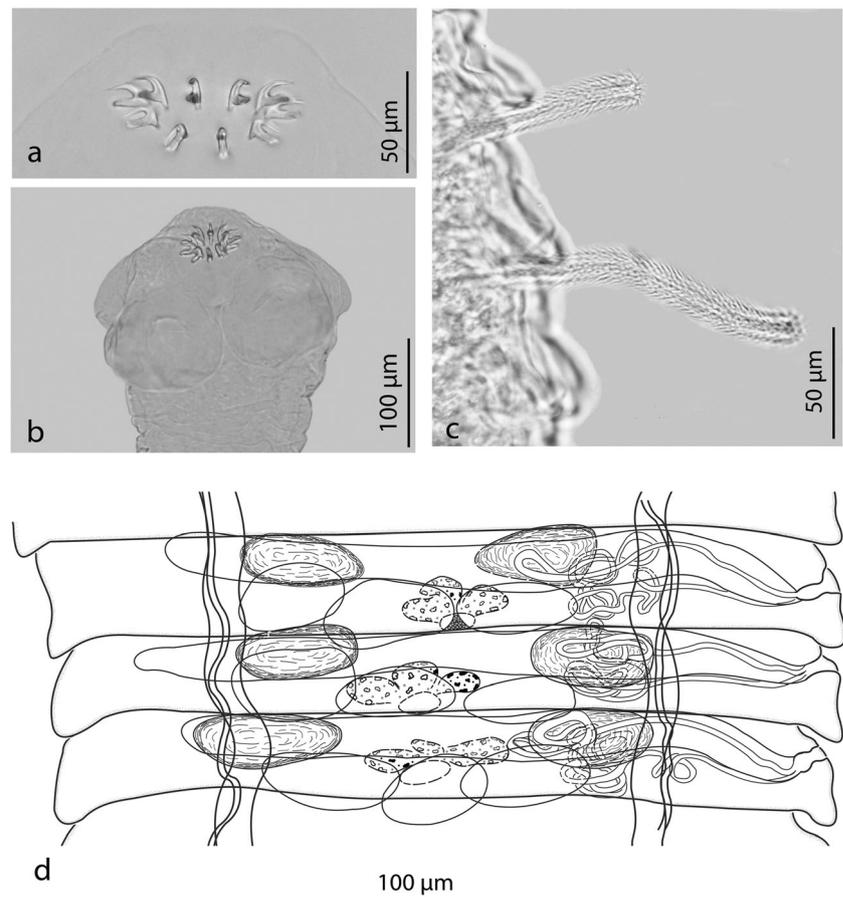
of cysts observed was larger than the presumed number of oncospheres infecting the intermediate hosts based on the number of embryonic hooks counted. This observation suggests that several cysticercoids may develop from one oncosphere.

Remarks The metacystodes of *W. cirrosa* differ from all known cysticercoids of cestodes of the family Aploparaksidae. Polymorphism is typical for the metacystodes of this family (Bondarenko and Kontrimavichus 1976). Bondarenko and Kontrimavichus (2006) described six modifications observed in the cysticercoids of the Aploparaksidae: autotomicercus, ramicercus, florificercus, tailed diplocyst, ovoid diplocyst, and typical diplocyst. Cysticercoids of *W. cirrosa* differ from

autotomicercus and ramicercus by the presence of oval massive (equal in volume to the cyst itself) widening of the proximal part of the cercomer, without any lateral processes. Cysts of cysticercoids of florificercus are submerged by a half in bed formed by a proliferated tail appendix. These traits are not present for cysts of cysticercoids of *W. cirrosa*. Cysticercoids of *W. cirrosa* lack an exocyst, setting them apart from tailed, ovoid, and typical diplocysts, all of which possess an exocyst. Additionally, it differs from the typical diplocyst by the presence of long tail.

Metacystodes of another cestode species *Ophryocotyle proteus* Friis, 1870 were found by Burt (1962) in polychaetes *H. diversicolor* in Scotland. The adults are parasites of charadriiform birds. Cysticercoid of *W. cirrosa* differs from

Fig. 2 Adult of *Wardium cirrosa* from *Larus cachinnans* (obtained in the experiment). **a** Rostellar hooks in profile. **b** Scolex, dorsal view. **c** Cirrus, partly evaginated. **d** Hermaphroditic proglottids, dorsal view



cysticercoid of *O. proteus* by the shape and size of the rostellar hooks, the presence of cercomer, and the absence of armed suckers.

Adults (Fig. 2)

Of the six adult specimens of *W. cirrosa* obtained during the study, four specimens possessing a scolex were stained with Mayer's hematoxylin and iron acetocarmine (one scolex and some fragments in Berlese's medium), and two specimens without scolices were stained with iron acetocarmine.

Mature strobila is delicate and measures 10.4 cm long with a maximum width of 720. Proglottids are numerous and are wider than long. Scolex measures 120–140 × 155–180 (128 × 165, $n = 3$) in stained specimens and 220 × 300 in the specimen mounted in Berlese's medium. The four weakly muscular rounded suckers measure 60–80 × 45–70 (66 × 54, $n = 8$). The sac-like rostellar sheath measures 85–125 × 65–70 (108 × 68, $n = 3$) and extends beyond the posterior borders of the suckers. The rostellum is armed with 10 apolaraksoid hooks. It measures 65–80 (73, $n = 3$) long and has a maximum diameter, where the hooks are located, of 50–65 (57, $n = 3$). Measurements pertaining to the hooks are as follows: total length 23–25 (24, $n = 13$), handle 4–6 (5, $n = 13$), blade 13–

16 (14, $n = 13$), guard 9–13 (10, $n = 13$), and base with guard 16–20 (18, $n = 13$).

The neck measures 80–110 (90, $n = 3$) in diameter. Genital pores are unilateral and are open at the middle of the lateral proglottis margin. The genital atrium is considered simple with numerous bundles of inner longitudinal muscle present. The osmoregulatory canals are in two pairs. No transverse anastomoses were observed. The diameter of the ventral osmoregulatory canals is 15–30. The diameter of the dorsal canals measures 5–9.

The genital primordia appears 15–20 mm from the scolex. Development of proglottids is protandrous. The testes are round, exist in groups of three, and measure 50–85 × 35–55 (63 × 39, $n = 28$). They are positioned in a transverse row. In male proglottids, the testes occupy a median field between the osmoregulatory canals. In hermaphroditic proglottids, the testes are oval and located in a posterior part of the median field. The cirrus sac is long and narrow—310–420 × 25–35 (375 × 28, $n = 20$)—and reaches the aporal osmoregulatory canals, occasionally crossing the aporal osmoregulatory canals and penetrating the lateral fields of the proglottids. The external seminal vesicle is oval 55–110 × 30–90 (87 × 59, $n = 40$) and overlaps the antiporal region of the cirrus sac. The evaginated cirrus is cylindrical and long with large spines present. The

length of the maximum evaginated cirrus is 360. The base of the cirrus is 10–13 (12, $n = 20$) in diameter and unarmed. The armed part of the cirrus is 8–10 (9, $n = 20$) in diameter. The invaginated cirrus forms some loops within the cirrus sac.

The female genitalia appear as oval primordia and develop later than the male genitalia (protandry). Testes are present in proglottids with well-developed female genitalia. The mature ovary is trilobed and measures 190–250 (213, $n = 20$) wide. It is localized in the median field. Oval vitellarium measures 50–80 × 30–45 (71 × 37, $n = 20$) and is located posteriorly to the ovary. The vagina is posterior to the cirrus sac. The copulatory part of the vagina measures 10–18 (15, $n = 12$) in diameter. The conductive part of the vagina is tubular, has thin walls, and measures 10–13 (12, $n = 10$) in diameter. The conductive part of the vagina forms numerous curves and turns at the poral osmoregulatory canals and poral testis and eventually leads to the seminal receptacle. The seminal receptacle measures 60–100 × 45–55 (80 × 49, $n = 20$) and is found in the median field near the poral osmoregulatory canals. The uterus first appears as a transverse tubule and is ventral to the testes. The gravid uterus is sacciform and occupies nearly the entire proglottis, including the lateral fields. Oval eggs measure 40–75 × 30–70 (52 × 44, $n = 20$) in stained specimens. The oncosphere measures 30–40 × 23–35 (35 × 29, $n = 20$). Median pairs of the embryonic hooks are 18–20 (19, $n = 10$) long; lateral pairs of the embryonic hooks are 15–16 (15, $n = 21$).

Remarks Cestodes studied were determined to be *W. cirrosa* based on the presence of 10 aploparaksoid hooks, 23–25 in length, a long, cylindrical, armed cirrus, and twisted vagina. The specimens obtained in the present study were compared with the specimens originating from naturally infected hosts from the Mykolaiv Oblast stored at IZSHK Helminthological collection. Descriptions of *W. cirrosa* by Deblock et al. (1960) and Macko (1964) were also utilized. The morphologies of the specimens obtained experimentally were very similar to those of the specimens collected from *L. cachinnans* in the Mykolaiv Oblast (Table 1). However, specimens obtained experimentally exhibited distinctly shorter cirri due to the different degrees of evagination, wider ovaries, large sizes of the vitellarium, and gravid eggs (see Table 1). It should be noted that the distal part of the cirrus of *W. cirrosa* from naturally infected *L. cachinnans* was a maximum 100 µm long, unarmed, and gradually converged from 8 to 2 µm in diameter (Fig. 3). The morphology and morphometry of the specimens obtained in the current study generally corresponded well to the morphology of the specimens reported by Deblock et al. (1960) and Macko (1964) (see Table 1 for details). Specimens from Ukraine exhibited substantially smaller length and width measurements of the strobila that may be explained by the presence of more gravid proglottids in specimens described by Deblock et al. (1960) and Macko (1964). The observation

of a larger scolex may be due to the use of Berlese's medium for clearing specimens as soft tissues swell in this medium. A larger rostellar sheath was observed, compared with that reported by Deblock et al. (1960), but was smaller than reported by Macko (1964). A larger rostellum was observed compared with that which was reported by Deblock et al. (1960). Larger suckers, a shorter seminal receptacle, and a greater upper limit in egg size were observed in the specimens collected during the present study compared with those reported by Deblock et al. (1960). A smaller ovary width was observed in specimens collected in the current study, compared with those reported by Deblock et al. (1960), but were larger than those reported by Macko (1964). The differences in size of the rostellar sheath, rostellum, and suckers between the specimens collected during the present study and those reported by Deblock et al. (1960) may be due to the method of fixation utilized. The difference of the ovary width may be due to the different degrees of maturity of the proglottids.

Molecular analysis

The partial 28S rDNA sequences (1490 nucleotides) for the isolate of metacestode collected from polychaetes as well as the isolate of adult cestode collected from *L. cachinnans* were identical. Both the metacestodes and adult cestodes represented *W. cirrosa*.

Discussion

Marine polychaetes were shown to be an intermediate host for *W. cirrosa*. The metacestodes that were reported by Pike (1968) in leeches *E. octoculata* in England in fact belong to a different species and are not *W. cirrosa*. Pike (1968) identified metacestodes by the presence of 24.4-long aploparaksoid hooks. However, the hooks of cysticercoids observed by Pike (1968) differed in shape from those of *W. cirrosa* (see Bondarenko and Kontrimavichus 2006). The latter authors considered Pike's identification to be erroneous. The size and shape of the rostellar hooks alone are not a reliable metric for the species identification of cestodes belonging to the family Aploparaksidae, as the number of species within this family possessing hooks of the same length as in *W. cirrosa* is quite large (Bondarenko and Kontrimavichus 2006). Since the life cycle of many of the Aploparaskidae remains unknown, species identification within this group should be based on the morphology of the strobila and molecular analyses. Metacestodes with 18–21-long aploparaksoid hooks were recorded in the same leech species, *E. octoculata* in the Kolyma River basin in Russia (Regel 2016). Regel (2016) supposed that those cysticercoids belonged to *Aploparaksis shigini* Bondarenko et Kontrimavichus, 2006, a parasite of gulls. The life cycle of *A. shigini* is unknown.

Table 1 Comparative metrical data for adults of *Wardium cirrosa*

Host	<i>Larus cachinnans</i> Pallas, 1811			<i>Larus cachinnans</i> Pallas, 1811			<i>Chroicocephalus</i> (= <i>Larus</i>) <i>ridibundus</i> (Linnaeus 1766), <i>Larus canus</i> Linnaeus, 1758	<i>Larus canus</i> Linnaeus, 1758
Locality	Experimentally obtained			Mykolaiv Oblast			France	Slovakia
Source	Present study			Present study			Deblock, Capron et Rose (1960)	Macko (1964)
	Range	Mean	<i>n</i>	Range	Mean	<i>n</i>	Range	Range
Total body length (mm)	104	–	–	117	–	–	110–140	134–158
Body width (mm)	0.720	–	–	0.700	–	–	1	0.927–0.973
Scolex length	120–220	151	4	200–220	210	2	90–100	–
Scolex width	155–300	199	4	160–240	200	2	115–150	178–212
Sucker length	60–80	66	8	60–75	67	6	50–60	75
Sucker width	45–70	54	8	55–70	60	6	–	75–80
Rostellar sheath length	85–125	108	3	100–135	118	2	70	178
Rostellar sheath width	65–70	68	3	70	70	2	45	109
Rostellum length	65–80	73	3	70–90	80	2	50	–
Rostellum width	50–65	57	3	50–95	73	2	25	82 (in diameter)
Total length of hooks	23–25	24	13	24–26	25	10	22–23	22–24
Blade length	13–16	14	13	14–18	15	10	–	–
Handle length	4–6	13	13	4–5	5	10	–	–
Guard length	9–13	13	13	9–12	10	10	–	–
Base with guard length	16–20	18	13	18–20	18	10	–	–
Testes length	50–85	63	28	50–90	70	20	90–100	36–42
Testes width	35–55	39	28	40–60	53	20	45–50	45–63
Cirrus sac length	310–420	375	20	320–400	352	20	380	–
Cirrus sac width	25–35	28	20	30–45	36	20	45	–
Cirrus total length	360 (evaginated)	–	–	540 (fully evaginated)	–	–	500–630	540
Cirrus width	8–10	9	20	8–10	8	14	8–10	7–10
External seminal vesicle length	55–110	87	40	70–90	79	10	80–90	55–110
External seminal vesicle width	30–90	59	40	45–80	58	10	80	30–90
Copulatory part of vagina (diameter)	10–15	12	12	15–18	16	10	–	–
Ovary width	190–250	213	20	160–200	176	15	480	112–150
Vitellarium length	50–80	71	20	40–60	48	15	60–80	39–51
Vitellarium width	30–45	37	20	25–35	28	15	30–45	–
Seminal receptacle length	60–100	80	20	60–105	83	12	120	–
Seminal receptacle width	45–55	49	20	40–55	48	12	40	–
Egg length	40–75	52	20	40–55	46	12	38–50	51–60
Egg width	30–70	44	20	35–50	42	12	38–50	51–60
Oncosphere length	30–40	35	20	25–35	32	16	–	–
Oncosphere width	23–35	29	20	20–34	26	16	–	–
Embryonal hook length	15–20	–	12	13–18	–	20	14–15	18

The life cycle of only one species of *Wardium*, parasitic in gulls, *Wardium fryei* Mayhew, 1925 was known prior to the present study (Bondarenko 1997). Based on a review of the Aploparaksidae by Bondarenko and Kontrimavichus (2006), the family includes three genera: *Aploparaksis* Clerc, 1903;

Monorcholepis Oschmarin, 1963; and *Wardium*. The intermediate hosts of these cestodes are annelids. Oligochaetes are intermediate hosts for cestodes belonging to the genera *Aploparaksis* and *Monorcholepis* as well as for the cestode *Wardium chaunense* Bondarenko et Kontrimavichus, 1997

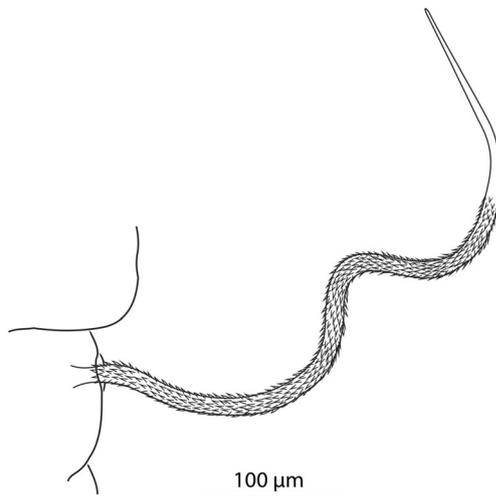


Fig. 3 Adult of *Wardium cirrosa* from *Larus cachinnans* from Mykolaiv Oblast (collection of I. I. Schmalhausen Institute of Zoology, NAS of Ukraine, Kyiv (IZSHK). Cirrus, fully evaginated

and *Wardium paraclavicirrus* Oschmarin, 1963. Species of the genus *Wardium*—*W. fryei* and *W. cirrosa*—are known to exploit marine polychaetes as the intermediate hosts. Similarly to *W. fryei*, *W. cirrosa* forms “larvophorae” where cysticercoids are connected with a common cellular mass. However, metacestodes of *W. cirrosa* differ from those of *W. fryei* in that the exocyst is absent.

Polychaetes are rarely recorded as intermediate hosts of cestodes. Peoples (2013) searched the Institute for Scientific Information Web of Science online database and found three cestode species utilizing polychaetes as hosts. Only one of them was identified to the species level. It was the metacestode of *O. proteus* from *H. diversicolor*. This metacestode was described by Burt (1962) from Scotland. Despite numerous registrations of *O. proteus* in definitive hosts in the Hola Prystan Region, Kherson Oblast (Leonov 1958; Korniyushin 1967; Spasskij and Korniyushin 1977), the metacestodes of this species expected to be found in nereids were not observed in either 2010 or 2015.

The study conducted by Peoples (2013) was based on two comprehensive reviews published by Margolis (1971, 1973). In these reviews, the data on helminths using polychaetes as intermediate hosts known at that time were summarized. Besides *Ophryocotyle proteus*, Margolis (1971, 1973) reported two unidentified metacestodes parasitizing polychaetes—an unidentified trypanorhynchid metacestode from *Polycirrus denticulatus* Saint-Joseph, 1894 and “*Taenia myzostoma*” from *Myzostomum graffi* Nansen, 1885 (see Margolis (1971) for more details).

According to Burt (1962), Margolis (1971), Bondarenko (1997), Peoples (2013), and the data currently presented, the metacestodes of five cestode species are known to use polychaetes as the intermediate hosts. *Ophryocotyle proteus*, *W. fryei*, and *W. cirrosa* are parasitic of polychaetes in the

family Nereidae. The remaining two metacestodes found in polychaetes of the families Terebellidae and Myzostomidae have only been identified to the family level. There have been no further updates regarding the larval stages of the aforementioned cestodes and their relationships with their polychaete hosts since they were first reported.

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Compliance with ethical standards All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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

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