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PARASITOLOGY OF HYDROBIONTS

Clinostomid Metacercariae (Clinostomidae Lühe, 1901) in Fishes of Lake Tana (Ethiopia)

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Abstract—A study of 2536 fish specimens of 22 species from Lake Tana in Ethiopia has revealed the presence of five species of clinostomid metacercariae represented by two genera: *Clinostomum complanatum* (from *Enteromius humilis*, *E. tanapelagius*, and *Garra tana*), *Euclinostomum* sp. and *Clinostomum brieni* (from *Clarias gariepinus*), and *C. phalacrocoracis* and *Euclinostomum heterostomum* (from *Oreochromis niloticus*). Morphological descriptions of the detected metacercariae, including the indication of their occurrence, mean invasion intensities, and tissue-use, are given.

Keywords: Trematoda, metacercariae, Clinostomidae, fish, Lake Tana, Ethiopia

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INTRODUCTION

As trematodes, representatives of the family Clinostomidae Lühe, 1901 have a typical heterogeneous life cycle, which involves gastropod mollusks as first intermediate hosts and fish (and amphibians in rare cases) as second intermediate hosts. Adult trematodes live in the oral cavity, pharynx, or esophagus of piscivorous birds, reptiles (less frequently), and mammals (sometimes), including humans. The family Clinostomidae is divided (Kanev et al., 2002) into four subfamilies of seven genera: *Clinostomoides* Dollfus, 1950; *Clinostomum* Leidy, 1856; and *Clinostomatopsis* Dollfus, 1932, *Euclinostomum* Travassos, 1928, *Ithyoclinostomum*, *Nephrocephalus* Odhner, 1902, and *Odhneriotrema* Travassos, 1928. The true number of species in the family is still unknown. Some species of the family are described according to their adult stage from birds; significantly more species are described according to their larvae from fish. The life cycles of trematodes, which make it possible to assign a larva and an adult stage to a certain species, are known for a limited number of species (Caffara et al., 2011; Dias et al., 2003; Dönges, 1974; Gustinelli et al., 2010; Jhansilakshmibai and Madhavi, 1997; Ukoli, 1966b).

The current taxonomic practice in parasitology involves the use of molecular markers in combination with a morphological study (Petkevičiūtė et al., 2018). The use of this approach can be particularly important during the study of the life cycle and identification of those clinostomid species which are described according to metacercariae. In our case, molecular data have confirmed the validity of seven species of the family: *Clinostomum complanatum* (Rudolphi, 1819), *C. marginatum* (Rudolphi, 1819), *C. tataxumui* (Sereno-Uribe, Pinacho-Pinacho, Garcia-Varela & Perez-Ponce de Leon, 2013), *C. cutaneum* Paperna, 1964, *C. phalacrocoracis* Dubois, 1930, *C. brieni* Dollfus, 1950 n. comb., and *Euclinostomum heterostomum* (Rudolphi, 1809); morphological differences between these species have also been revealed (Caffara et al., 2011, 2013, 2014, 2016, 2017; Dzikowski et al., 2004; Gustinelli et al., 2010; Sereno-Uribe et al., 2013). In Africa, the following clinostomid species have been recorded in birds and fish: *Clinostomum brieni* (Barson et al., 2008; Caffara et al., 2019; Fischthal and Thomas, 1970; Jansen van Rensburg et al., 2013; Manter and Pritchard, 1969; Prudhoe, 1957); *C. complanatum* (Abd-Al-Aal et al., 2008; Barson et al., 2008; Caffara et al., 2017; Echi et al., 2014; Khalil, 1969; Oliver et al., 2009); *C. tilapiae* Ukoli, 1966 (Britz et al., 2000; Caffara et al., 2017; Echi et al., 2012; Olurin and Somorin, 2006; Ukoli, 1966a); *C. cutaneum* (Akoll et al., 2012; Gustinelli et al., 2010); *C. phalacrocoracis* Dubois, 1930 (Caffara et al., 2014, 2017; Gustinelli et al., 2010; Kabunda and Sommerville, 1984; Taher, 2009); *Euclinostomum heterostomum* (Britz et al., 2000; Echi et al., 2012; Mashego and Saayman, 1989; Morenikeji and Adepeju, 2009; Oliver et al., 2009); and *Nephrocephalus bagriincapsulatus* (Wedl, 1861) (Scholz et al., 2018), as well as several doubtful species described according to metacercariae (Caffara et al., 2017; Scholz et al., 2018). The data on the trematodes of the family Clinostomidae in fish from Lake Tana (the largest lake in Ethiopia) are extremely limited.

The metacercariae of *Clinostomum* spp. and *Euclinostomum* sp. were found in fishes *Oreochromis niloticus* (L., 1758) and *Varincorhinus beso* (Rüppell, 1835) (Eshetu and Mulualem, 2003).

The purpose of this study was to morphologically describe the metacercariae of five clinostomid species that were found in fish from Lake Tana.

MATERIALS AND METHODS

The material was collected in October–November 2006 and 2007 and in May and September–October 2008 in the southeastern part of Lake Tana (the Bahar Dar bay). A total of 2536 fish of different sizes (from adults to juveniles) belonging to 22 species were studied: *Oreochromis niloticus* L.; *Clarias gariepinus* (Burchell, 1822); *Enteromius tanapelagius* Graaf et al., 2000 ($n = 26$); *E. humilis* Boulenger, 1902; *E. pleurogramma* Boulenger, 1902 ($n = 5$); and *Garra tana* Stiassny, Getahun, 2007 ($n = 107$), as well as 15 large barbel species of the genus *Labeobarbus* ($n = 813$) and *Varicorhinus beso* (Rüppell, 1836) ($n = 36$). Fish were caught in the littoral zone of the lake at a depth of ≤5 m using nets and traps and kept alive until their examination at the laboratory. Wet preparations of gills, brain, eyes, kidneys, liver, intestine, spleen, fins, muscles, and gonads were studied for parasite infection under a light microscope. Prior to the analysis, the fish were examined externally.

Clinostomid metacercariae were extracted from cysts, transferred to a physiological solution, killed with hot water, and immediately fixed with 70% ethanol. They were then stained with alum carmine, dehydrated in alcohols, clarified in dimethyl phthalate, and enclosed in Canadian balsam. The worms were measured on total preparations and are given in millimeters.

RESULTS

Clinostomid metacercariae were found only in five of the 22 studied fish species from Lake Tana: *Enteromius humilis*, *E. tanapelagius*, *Garra dembecha*, *Oreochromis niloticus*, and *Clarias gariepinus*. The cyprinids of the genus *Labeobarbus* and African scraping feeder *Temulor Varicorhinus beso* were never recorded as clinostomid hosts within our research. In *Garra dembecha*, only one specimen, *Clinostomum complanatum*, was found over all study years.

Clinostomum complanatum (Rudolphi, 1819) metacercariae (Figs. 1a, 2a).

Hosts: *Enteromius humilis*, *E. tanapelagius*, and *Garra tana*.

Localization: abdominal cavity, under gill-cover skin.

Studied material: 45 specimens; voucher specimens (preparations nos. 6/442 (1–8) are stored in the Helminthological Collection, Institute for Biology of Inland Waters, Russian Academy of Sciences.

Description (12 specimens measured). Metacercariae covered with a soft, elastic, round cyst. Worms extracted from cyst highly mobile. Body tongue-shaped, 4.6–5.95 long (mean 5.53), 1.2–1.73 wide (1.5), and covered with very small spinules (absent at the anterior end). Oral sucker subterminal or terminal, 0.225–0.36 × 0.252–0.405 (0.306 × 0.334), surrounded by a wide peristome. Ventral sucker in the first third of body, 0.792–0.9 × 0.792–0.9 (0.846 × 0.853). Ratio of oral sucker length to ventral sucker length is 1 : 2.7–4.2 (1 : 3.6). Pharynx is 0.09–0.153 × 0.063–0.108 (0.113 × 0.081), no esophagus. Intestinal branches blind-ended at posterior end of body, not connected with excretory bladder. Excretory bladder horseshoe-shaped; excretory channels ascend from it and extend on sides of body to ventral sucker. Excretory pore subterminal and open to the ventral side (rarely to the terminal side). Genital primordia in the middle between ventral sucker and posterior end of body. Anterior testis is 0.216–0.54 × 0.153–0.54 (0.406 × 0.397), slightly shifted to the left of the midline; posterior testis is 0.252–0.396 × 0.315–0.612 (0.351 × 0.459), on the median line. Cirrus-sac is 0.18–0.315 × 0.135–0.396 (0.239 × 0.307), located on the right side between anterior testis and intestine, at the level of the middle of anterior testis. Genital pore to the right of the midline of body, at the right border of anterior testis. Ovary bean-shaped, less often having other forms, smooth-edged, 0.135–0.216 × 0.09–0.18 (0.183 × 0.153). Uterus around anterior testis on the left in the form of a narrow tubule, ascends forward without reaching ventral sucker and descends backward to genital pore. Metraterm muscular. Distance between ventral sucker and uterus is 0.216–0.657 (0.422).

Remarks. With respect to the description and size, this species of metacercariae from *Enteromius humilis* corresponds to *Clinostomum complanatum* larvae detected in different fish species. *C. complanatum* metacercariae have an extremely high specificity with respect to second intermediate hosts. Over 100 fish species of different orders were recorded as their hosts in different continents. In Africa, metacercariae of *C. complanatum* were found in 16 fish species. In *Enteromius humilis* from Lake Tana, metacercariae are always localized in the abdominal cavity, while its localization in different fish species from other regions include gills, fins, skin, muscles, oral cavity, eyes, gonads, gall bladder, and body cavity. Table 1 provides data on the infection of different-sized individuals of *Enteromius humilis* by *Clinostomum complanatum* metacercaria. Fish with a body length of 5–7 cm were most severely infected; the infection of the smallest and largest fish was extremely low. Metacercariae were rarely recorded in *Enteromius tanapelagius* (occurrence, 7.7%, abundance index, 0.12, invasion inten-

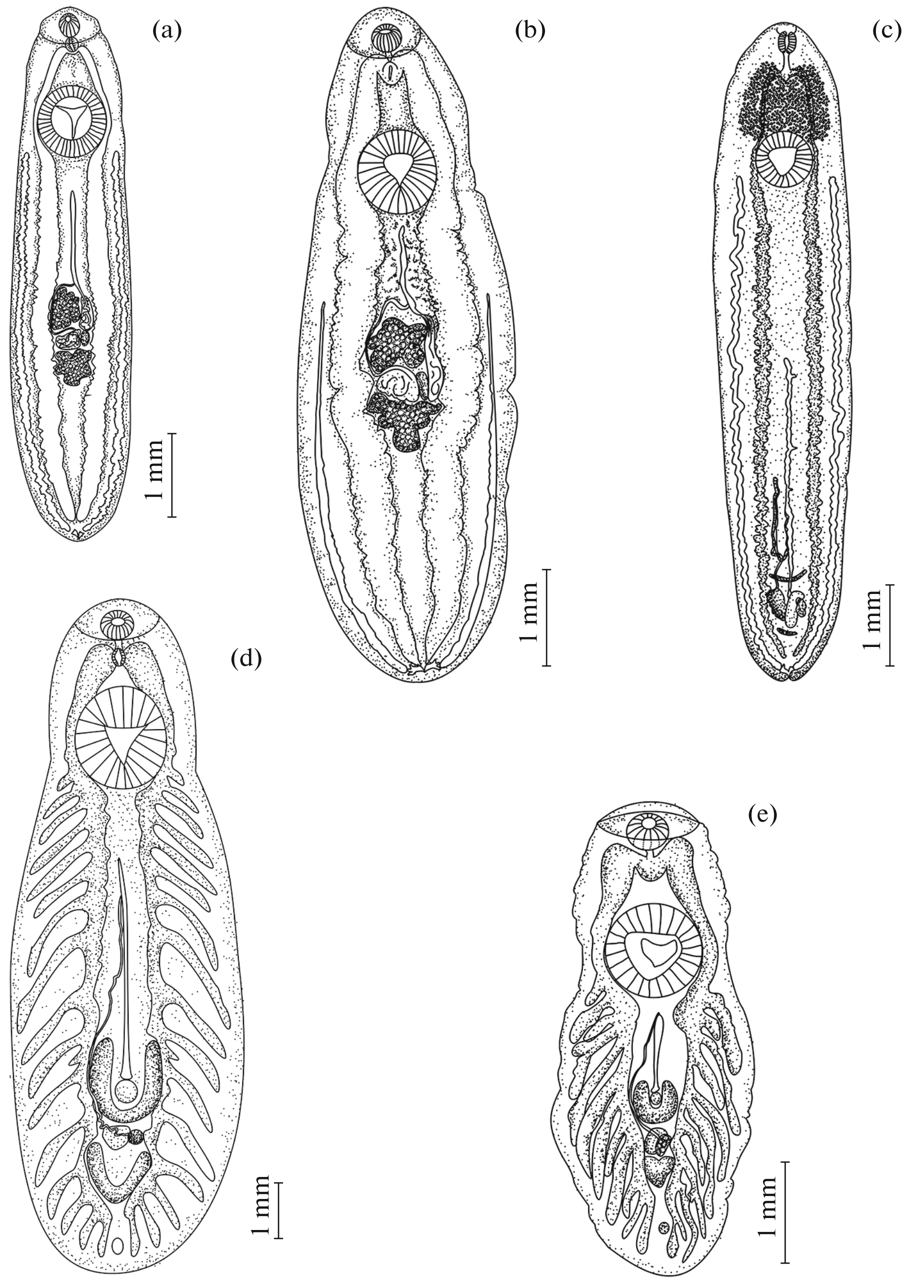

Fig. 1. Clinostomid metacercariae from fish of Lake Tana: (a) *Clinostomum complanatum*, (b) *C. phalacrocoracis*, (c) *C. brieni*, (d) *Euclinostomum* sp., and (e) *E. heterostomum*.

sity, 1–2); one larva of *Clinostomum complanatum* was found in *Garra tana*.

Clinostomum phalacrocoracis metacercariae (Figs. 1b, 2b).

Host: *Oreochromis niloticus*.

Localization: pericardial cavity and around it.

Studied material: 30 specimens; voucher specimens (preparations nos. 7/445 (1–6)) are stored in the Helminthological Collection, Institute for Biology of Inland Waters, Russian Academy of Sciences.

Description (ten specimens measured). The morphometric characteristics are given in Table 2.

Metacercariae large, covered with a soft elastic yellowish–cream cyst; cyst rupture is accompanied by emission of grainy yellow substance into the water. Cyst oval and flattened. Living larvae with a whitish pink body and a bright orange-yellow intestine; they move actively, thereby stretching and contracting anterior part of body. Body without spinules, elongated-oval; anterior end of body narrower than posterior one, maximum width at the level of reproductive

Fig. 2. Clinostomid metacercariae from fish of Lake Tana: (a) *Clinostomum complanatum* from *Enteromius humilis*, (b) *C. phalacrocoracis* from *Oreochromis niloticus*, and (c) *C. brieni* from *Clarias gariepinus*.

system. Oral sucker subterminal, smaller than ventral one; oral frill weakly pronounced. Ventral sucker on the border of the first and second quarters of body length. Ratio of oral sucker length to ventral sucker length is 1 : 2.9–5.2 (1 : 4.1). Diameter of esophagus bulb is 0.126–0.288 × 0.135–0.252 (0.221 × 0.19). Intestinal trunks wide, with diverticula in central and posterior parts of body, blind-ended at posterior end of body, not connected with excretory bladder. Excretory bladder V-shaped, with two diverticula at each apex (Fig. 1b). Excretory canals ascend from bladder along sides of body and can be visible to the level of anterior testis. Excretory pore terminal. Reproductive system located in the middle third of body. Testes of most of the specimens delicate, in the form of slingshots with finger-shaped protrusions; slingshot handles directed forward and backward, respectively. The shape of anterior testis often irregular; testes of some individuals are massive, almost triangular, weakly and shallowly indented; posterior testis wider than anterior one. Both testes along the median line, one under the other; in rare cases, anterior testis slightly shifted to the left. Cirrus sac bean-shaped, located at posterior border of anterior testis between ovary and right intestinal branch. Genital pore to the right of the midline of body, always between the middle and posterior border of anterior testis. Ovary bean-shaped or oval, under cirrus sac. Uterus around anterior testis on the left, ascends forward not reaching ventral sucker and descends backward to genital pore. Metraterm present. Distance between ventral sucker and uterus is 0.135–0.495 (0.347).

Infection of tilapia by *C. phalacrocoracis* metacercariae increases with age; therefore, the largest fish are most significantly infected (Table 3). However, tilapia juveniles (1.0–2.9 cm) also have a high invasion intensity (up to ten cysts).

Remarks. The first detailed description of the morphology of *C. phalacrocoracis* metacercariae and their molecular analysis are given according to the material from cichlids in Lake Kinneret (Israel) (Caffara et al., 2014). These authors also report that the metacercaria of *C. phalacrocoracis* was previously described under

Table 1. Rate of infection of *Enteromius humilis* by *Clinostomum complanatum* metacercaria in Lake Tana with respect to the fish size

SL, cm	*N*	Parameter			
		Occurrence, %	*I*	Invasion intensity	*N*
2.0–2.9	104	1.0	0.010	1	1
3.0–3.9	91	1.1	0.011	1	1
4.0–4.9	130	1.5	0.020	1	2
5.0–5.9	206	8.3	0.800	1–61	154
6.0–6.9	186	9.1	0.440	1–17	81
7.0–7.9	127	4.7	0.130	1–6	16
8.0–8.9	43	7.0	0.580	2–20	25
9.0–10.0	5	0.0	0.000	0	0
Total, mean	892	5.3	0.310	1–61	280

Here and in Tables 3 and 5, *SL* is the standard fish length, *n* is the number of studied fish, *I* is the abundance index, and *N* is the number of collected parasites.

Table 2. Morphometric characteristics of *C. phalacrocoracis* metacercaria

Parameter	Lake Tana (according to authors' data)		Lake Kinneret, Israel (according to Caffara et al., 2014)	
	Min–max	*M*	Min–max	*M*
BL	8.840–11.400	10.100	9.500–15.200	12.06
BW	2.660–3.600	3.060	1.860–3.970	3.080
OSL	0.360–0.576	0.484	0.410–0.530	0.460
OSW	0.432–0.612	0.512	0.480–0.730	0.580
VSL	1.000–1.300	1.192	0.926–1.253	1.094
VSW	0.972–1.190	1.013	1.011–1.346	1.193
ATL	0.612–0.900	0.576	0.677–1.466	1.074
ATW	0.576–0.828	0.684	0.643–1.469	1.064
PTL	0.540–0.900	0.800	0.606–1.182	0.957
PTW	0.648–1.010	0.792	0.695–1.469	1.072
CSL	0.396–0.720	0.512	0.389–0.717	0.564
CSW	0.360–0.576	0.416	0.143–0.292	0.253
OL	0.180–0.360	0.292	0.119–0.378	0.280
OW	0.135–0.216	0.167	0.121–0.363	0.262

BL, body length; BW, body width; OSL, oral sucker length; OSW, oral sucker width; VSL, ventral sucker length; VSW, ventral sucker width; ATL, anterior testis length; ATW, anterior testis width; PTL, posterior testis length; PTW, posterior testis width; CSL, cirrus-sac length; CSW, cirrus-sac width; OL, ovary length; OW, ovary width; and *M*, mean value.

the name of *Clinostomum* sp. from *Oreochromis* sp. in Congo (Kabunda, Sommerville, 1984). *C. phalacrocoracis* larvae (without descriptions) were found in *O. niloticus* in Kenya (Gustinelli et al., 2010) and Egypt (Taher, 2009). According to the last research, cysts with a diameter of 2.7–5.2 have a thin transparent film with yellowish liquid inside; the metacercariae are yellowish orange and localized in the gill cavity and pharynx. *C. phalacrocoracis* larvae in fish from Lake Tana correspond to those in fish from Lake Kinneret in Israel with respect to their morphology and size (Table 2).

Clinostomum brieni **Dollfus**, 1950 n. comb. (Syn. *Clinostomoides brieni* Dollfus, 1950) (Figs. 1c, 2c).

Host: *Clarias gariepinus.*

Localization: gills.

Studied material: 22 specimens; voucher specimens (preparations no. 6/443 (1–5)) are stored in the Helminthological Collection, Institute for Biology of Inland Waters, Russian Academy of Sciences.

Description (17 specimens measured). The morphometric characteristics are given in Table 4.

Cysts white, with a soft thin film. Metacercariae extracted from cysts very mobile, crawling like

Table 3. Rate of infection of *Oreochromis niloticus* by *Clinostomum phalacrocoracis* metacercaria with respect to the fish size in Lake Tana

SL, cm	*n*	Parameter			
		occurrence, %	*I*	invasion intensity	*N*
1.0–2.9	74	13.3	0.40	1–10	30
3.0–4.9	19	10.5	0.11	1	2
5.0–11.9	15	13.3	0.67	3–7	10
12.0–15.9	27	48.1	1.00	1–8	27
16.0–20.9	27	55.6	3.30	1–25	90
21.0–24.9	29	96.6	4.60	1–19	134
25.0–30.0	13	92.3	12.20	1–89	158
Total, mean	484	46.3	2.90	1–89	451

Table 4. Morphometric characteristics of *Clinostomum brieni* metacercariae

Parameter	Lake Tana, Ethiopia (according to data of the authors)		South Africa, Kongo (according to Caffara et al., 2019)	
	Min–max	$M \pm SD$	Min–max	$M \pm SD$
BL	5.240–10.960	7.058 ± 1.333	6.760–10. 602	8.683 ± 1.425
BW	1.010–1.656	1.343 ± 0.208	1.250–1.530	1.392 ± 1.183
OSL	0.207–0.315	0.265 ± 0.029	0.172–0.260	0.218 ± 0.038
OSW	0.144–0.270	0.206 ± 0.036	0.172–0.280	0.233 ± 0.036
VSL	0.540–0.621	0.597 ± 0.046	0.622–0.750	0.676 ± 0.054
VSW	0.522–0.720	0.592 ± 0.058	0.678–0.794	0.733 ± 0.043
Distance between suckers	0.630–0.990	0.784 ± 0.126	0.531–1.709	1.277 ± 0.398
PL	0.078–0.216	0.137 ± 0.040	–	–
PW	0.081–0.180	0.124 ± 0.037	–	–
ATL	0.063–0.315	0.121 ± 0.073	0.104–0.128	0.115 ± 0.010
ATW	0.108–0.522	0.347 ± 0.108	0.317–0.526	0.426 ± 0.077
PTL	0.045–0.144	0.163 ± 0.230	0.114–0.190	0.145 ± 0.031
PTW	0.225–0.414	0.332 ± 0.058	0.254–0.444	0.326 ± 0.075
Distance between testes	0.369–0.720	0.563 ± 0.103	0.426–0.650	0.494 ± 0.083
CSL	0.324–0.540	0.440 ± 0.058	0.437–0.652	0.577 ± 0.077
CSW	0.990–0.153	0.121 ± 0.019	0.112–0.223	0.168 ± 0.039
OL	0.207–0.495	0.406 ± 0.071	0.165–0.20	0.178 ± 0.013
OW	0.162–0.243	0.210 ± 0.023	0.035–0.108	0.075 ± 0.025

PL, pharynx length and PW, pharynx width; see other notations in Table 2.

leeches; being placed into a Petri dish with water, they easily crawl out of it and can crawl along the substrate without water. Body tongue-shaped, covered with very small spinules; maximum width behind ventral sucker. Glandular cells in anterior part of body occupy the space between suckers. Oral sucker terminal. Ventral sucker in the middle of the first third of body, 2 times larger than oral one. Ratio of oral sucker length to ventral sucker length is 1 : 3.5–5.0 (1 : 4.3). Prepharynx present (0.045–0.099 (0.069)); pharynx poorly visible due to the enlarged esophagus overlying it. Intestine separated into two sections at the level of esophagus; intestinal branches with small diverticula throughout their length (diverticula are especially numerous behind ventral sucker), rarely with even borders. Excretory bladder Y-shaped. Reproductive system in the last third of body. Testes in the form of two narrow transverse, slightly curved strips. Ovary oval, on the right in the middle between testes, at the level of cirrus-sac. Uterus in the form of narrow tubes forms two

Table 5. Rate of infection of *Clarias gariepinus* by clinostomid metacercariae in Lake Tana with respect to the fish size

SL, cm	*n*	Parameter			
		occurrence, %	*I*	invasion intensity	*N*
5.0–20.9	15	6.70 / 6.67	0.270 / 0.070	4 / 1	4 / 1
21.0–30.9	69	4.30 / 8.70	0.070 / 0.087	1 – 3 / 1	5 / 6
31.0–40.9	33	6.10 / 6.10	0.610 / 0.061	1 – 19 / 1	20 / 2
41.0–50.9	15	0.00 / 6.67	0.000 / 0.070	0 / 1	0 / 1
51.0–60.9	15	13.30 / 0.00	0.270 / 0.000	1 – 3 / 0	4 / 0
61.0–80.9	18	5.60 / 0.00	0.060 / 0.000	1 / 0	1 / 0
81.0–120.0	8	0.00 / 0.00	0.00 / 0.00	0 / 0	0 / 0
Total, mean	173	5.40 / 6.78	0.220 / 0.058	1 – 19 / 1	34 / 10

Clinostomum brieni is above the line; *C.* sp. is below the line.

longitudinal, parallel blind loops in the interintestinal space. Right loop of uterus 2 times shorter than left loop on the median line along body. Seminal receptacle oval, located on the left in the space between cirrus sac and intestinal trunk, 0.108–0.297 × 0.017–0.225 (0.211 × 0.113). Cirrus sac in the form of a club on the median line along the longitudinal axis of body. Genital pore at anterior border of posterior testis, along the median line. Excretory bladder Y-shaped, excretory pore terminal.

On the whole, catfish in Lake Tana are slightly infected by *C. brieni* metacercariae; there is no clear dynamics of the infection of the fish with their growth (Table 5). Young fish (≤40-cm-long) are more severely infected than old ones, although the larvae are also found in large catfish.

Remarks. This species differs from other clinostomids by its large size and location of its reproductive system in the posterior part of the body. Therefore, it has been distinguished as a separate genus, *Clinostomoides*. However, molecular and phylogenetic analyzes have shown that *Clinostoides brieni* belongs to the genus *Clinostomum* (Caffara et al., 2019). *Clinostomum brieni* n. comb. metacercariae were found in sharptooth catfish (*Clarias gariepinus*, *C. lazera*, *C. senegalensis*, and *C.* sp.) in Africa (Barson et al., 2008; Fischthal and Thomas, 1970; Jansen van Rensburg et al., 2013; Manter and Pritchard, 1969; Prudhoe, 1957). *C. brieni* metacercariae used for molecular analysis (Caffara et al., 2019) were collected in a typical region. Morphological differences between these specimens from other areas in Africa are considered intraspecific variations (Caffara et al., 2019). Specimens from Lake Tana also slightly differ from the above-described specimens (Table 4): the distance between their suckers are lower and their ovaries are larger.

Genus *Euclinostomum* Travassos, 1928.

Euclinostomum heterostomum Rudolphi, 1809 (Figs. 1e, 3a).

Host: *Oreochromis niloticus*.

Localization: under serous membrane of kidney.

Studied material: three specimens; voucher specimens (preparations nos. 2/45 (1–7)) are stored in the Helminthological Collection, Institute for Biology of Inland Waters, Russian Academy of Sciences.

Description (three specimens measured). Metacercariae form white cysts with a soft thin film. Body of metacercaria cylindrical, thick, and fleshy; lateral borders folded, 4.59–6.29 × 1.69–3.24 (5.16 × 2.28). Oral sucker spherical, 0.279–0.297 × 0.261–0.315 (0.288 × 0.288), protruding above peristome, terminal. Ventral sucker round, 0.9–1.368 × 0.9–1.44 (1.09 × 1.116), larger than oral one. No pharynx; esophagus narrow and short, forming an expansion in region of intestinal bifurcation. Intestinal branches long, close to posterior end of body. Testes on the border of the second and last third of body. Anterior testis in the form of a horseshoe is 0.324–0.576 × 0.432–0.756 (0.414 × 0.54); posterior testis V-shaped or triangular, 0.252–0.468 × 0.27–0.54 (0.336 × 0.378). Cirrus sac round, 0.288–0.405 × 0.18–0.36 (0.33 × 0.255). Ovary oval, 0.162–0.243 × 0.099–0.225 (0.195 × 0.168). Uterus in the form of a narrow tube ascends

Fig. 3. Clinostomid metacercariae from fish of Lake Tana: (a) *Euclinostomum heterostomum* from *O. niloticus* and (b) *Euclinostomum* sp. from *Clarias gariepinus*.

forward along left edge of anterior testis and descends backwards, not reaching the posterior edge of ventral sucker, thereby forming a loop. Posterior part of uterus loop wider than anterior one. Genital pore median, located strictly against cirrus sac. Excretory pore at the level of ends of last blind intestinal processes. The number of intestinal diverticula is 8–13 (11).

Remarks. In Africa, metacercariae of *E. heterostomum* were found in 12 fish species in many countries (Britz et al., 2000; Caffara, Locke, Cristanini, et al., 2016; Echi, Eyo, Okafor, et al., 2012; Mashego and Saayman, 1989; Morenikeji and Adepeju, 2009; Oliver, Luus-Powell, and Saayman, 2009; Taher, 2009). In the Bahar Dar bay of Lake Tana, this larva occurs very rarely in tilapia. Only four metacercariae were found over three study years. In other areas of the lake, metacercariae of *Euclinostomum* sp. (probably *E. heterostomum* (Eshetu and Mulualem, 2003)) were found in tilapia. The fish infection was also low.

***Euclinostomum* sp.** (Figs. 1d, 3b).

Host. *Clarias gariepinus*.

Localization. Orbital cavity.

Studied material: nine specimens; voucher specimens (preparations nos. 7/444 (1–6)) are stored in the Helminthological Collection, Institute for Biology of Inland Waters, Russian Academy of Sciences.

Description (six specimens measured). Metacercariae without cysts. Worms very large, body insole-shaped, narrowing in the ventral sucker, without spinules; lateral borders of body even or slightly folded, both ends of body smoothly rounded. Maximum width at the level of uterus mid-length. Oral sucker terminal, surrounded by a wide oral fold; ventral sucker round, 4–5 times larger than oral sucker, located in the anterior third of body. Esophagus short, esophagus bulb in region of intestinal bifurcation, large. Intestinal trunks carry long processes on the outside; their number on both sides varies from 13 to 17. Testes in the last third of body. Anterior testis U-shaped, with a round cirrus sac along the median line between its branches. Posterior testis V-shaped, smaller than anterior one. Ovary oval, located to the right of the midline of body, contacting with right intestinal branch. Uterus in the form of a narrow channel ascends between anterior testis and left intestinal branch along left edge of anterior testis, not reaching posterior edge of ventral sucker, and descends backward, thereby forming a narrow loop. Genital pore strictly against cirrus sac, median. Excretory pore near posterior end of body between ends of intestine processes.

Remarks. A similar larva (one specimen) was found in *Clarias gariepinus* in South Africa (Prudhoe and Hussey, 1977). The size and description of this larva are similar to those of metacercariae that we detected (Table 6). The metacercaria of *Euclinostomum* sp. (Prudhoe and Hussey, 1977) was found in the body cavity of catfish. The authors do not report whether it was found in a cyst or not. Metacercariae that we detected in the catfish orbital cavities were always unencysted. This is the second largest metacercaria species after *Clinostomum cutaneum* (Gustinelli et al., 2010). Several other species are described in the genus *Euclinostomum*; however, all specific features of these species fall within the range of variability of *E. heterostomum* (Caffara et al., 2016). The species that we found differs from the metacercariae of *E. heterostomum* by the following features. The testes of *Euclinostomum* sp. are shifted to the posterior end of the body and located in the last third of the body; the testes of *E. heterostomum* are between the second third and last third of the body; *Euclinostomum* sp. has a large muscular expansion (bulb) of the esophagus, which is absent in *E. heterostomum*; the number of lateral branches of the intestine is larger in *Euclinostomum* sp. (13–17) than in *E. heterostomum* (8–13); however, the latter occasionally has more branches (9–16); the ratio of the ventral sucker width to the oral sucker width is higher in *Euclinostomum* sp. (mean 4.09) than in *E. heterostomum* (mean 1.66); the metacercariae of *Euclinostomum* sp. do not form cysts, while those of *E. heterostomum* form cysts with a few exceptions (Echi et al., 2012); *Euclinostomum* sp. is 3 times larger than *E. heterostomum*. All nine metacercariae of *Euclinostomum sp.* were extracted from catfish orbital cavities, while *E. heterostomum* was localized in other regions: body wall near kidneys, serous membrane of kidneys, kidneys, liver, muscles, body cavity, skin, and eyes. These morphological and size differences give reason to consider the metacercaria of *Euclinostomum* sp. a separate species; however, it is necessary to carry out a molecular analysis to confirm its validity. This species is rare in Lake Tana; only one specimen of this species was always found among small catfish.

DISCUSSION

A total of five species of clinostomid metacercariae (*Clinostomum complanatum*, *C. brieni* n. comb., *C. phalacrocoracis*, *Euclinostomum heterostomum*, and *Euclinostomum* sp.) were found in fish from Lake Tana. These species give a fairly complete picture of the diversity of this group of trematodes in the lake. The ichthyofauna of Lake Tana is not very diverse; only 24 species are recorded here (Vijverberg et al., 2009). We found different species of clinostomid metacercariae in *Oreochromis niloticus*, *Clarias gariepinus*, *Enteromius humilis*, *E. tanapelagius*, and *Garra tana*. Metacercariae of *Euclinostomum* sp. (Eshetu and Mulualem, 2003) were found in African scraping feeder *Varicorhinus beso* (Eshetu and Mulualem, 2003). All these fish species have a benthic lifestyle in the littoral and sublittoral zone of macrophyte thickets, where they come into contact with infected mollusks. The first intermediate hosts of clinostomids are gastropod mollusks of the families Limnaeidae and Planorbidae. In Africa, mollusks *Bulinus truncatus* (Audoin, 1827) and *B. globosus* Morelet, 1866 (Planorbidae) (Dönges, 1974; El-Naffar and Khalofa, 1981) were recorded as hosts for *E. heterostomum*. It is noteworthy that large barbels of the genus *Labeobarbus* (juvenile and adult fish) in the littoral part of the lake have no clinostomid metacercariae. Among the three species of small barbels of the genus *Enteromius* in Lake Tana, clinostomid metacercariae are common only in *Enteromius humilis*. The pelagic species *E. tanapelagius* lives in the open part of the lake and is biotopically unrelated to gastropods; therefore, it is very slightly infected by *C. complanatum*.

Clinostomid metacercariae in Lake Tana show a narrow specificity to hosts, which is possibly due to a poor fish species composition. Thus, *C. complanatum* was found in *Enteromius humilis*, *E. tanapelagius* (rarely), and *Garra tana* (casually); however, on the whole, this species is extremely highly specific to second intermediate hosts. *Clinostomum phalacrocoracis* was recorded as a parasite only for Nile tilapia, although it is cichlids for which this species is specific (Caffara et al., 2014; Finkelman, 1988; Gustinelli et al., 2010; Kabunda and Sommerville, 1984; Taher, 2009; Yekutiel, 1985). Being rare in the lake, *Euclinostomum heterostomum* was found only in tilapia. Two other species (*Clinostomum brieni* and *Euclinostomum* sp.) invade only sharptooth catfish in the lake; in other African regions, both species are recorded as parasites of catfishes of the genus *Clarias* (Barson et al., 2008; Fischthal and Thomas, 1970; Jansen van Rensburg et al., 2013; Prudhoe and Hussey, 1977).

Analysis of the localization of clinostomid metacercariae in the body of fish from Lake Tana revealed that none of the species used fish muscles as a parasitic

Table 6. Morphometric characteristics of metacercariae of *Euclinostomum* sp. from *Clarias gariepinus*

Parameter	Lake Tana, Ethiopia		South Africa (according to Prudhoe and Hussey, 1977)
	Min–max	*M*	*M*
BL	13.600–18.000	15.80	19.300
BW	4.000–6.000	4.930	5.370
OSL	0.360–0.468	0.396	0.400
OSW	0.432–0.612	0.510	0.400
VSL	1.800–2.250	2.030	1.750
VSW	1.800–2.250	2.030	1.750
VSW/OSW	2.940–5.000	4.090	4.400
Distance between suckers	0.900–1.620	1.162	–
Esophagus	0.180–0.360	0.252	0.270
VSL	0.360	0.360	0.275
VSW	0.216–0.284	0.237	0.155
ATL	1.650–2.250	1.936	2.200
ATW	1.250–1.750	1.600	0.300
PTL	1.000–1.350	1.207	1.060
PTW	1.100–1.700	1.342	0.200
CSL	0.288–0.360	0.317	0.430
CSW	0.252–0.432	0.317	0.430
OL	0.252–0.360	0.306	0.340
OW	0.216–0.288	0.252	0.160
UL	4.250–6.300	5.414	6.900
Distance between PT and posterior end of body	1.296–2.772	1.805	2.240
Number of:			
right diverticula	13–17		16
left diverticula	15–17		14

Note: UL, uterus length; PT, posterior testis. See other notations in Table 2.

site. This is an important fact, since trematode larva can enter the human body together with raw fish consumed and then develop into adult trematodes and parasitize the human body. In some areas of Ethiopia, it is common to eat raw fish. In addition to other organs, fish muscles are parasitized by the larvae of *C. complanatum* (in cyprinids and *Oreochromis niloticus*), *C. cutaneum* (in cichlids), *C. tilapiae* (in cichlids), and *E. heterostomum* (in *O. mossambicus* and *O. niloticus*) (Britz et al., 2000; Caffara et al., 2014; Kabunda and Sommerville, 1984; Oliver et al., 2009; Taher, 2009). However, this localization of metacercariae in fish is not observed everywhere. Clinostomid metacercariae are known as yellow grubs due to the color of the intestinal contents. In most cases, parasitization of these trematodes in humans resulted from consuming *Clinostomum complanatum* and was observed in Japan, Korea, Thailand, India, and Israel. In infected people, trematodes are localized deep in the pharyngeal mucosa and cause acute laryngopharyngitis with different symptoms: congestion and irritation of the mucous membrane of the larynx and pharynx, nausea, pain and difficulty in swallowing, coughing, blood loss, voice changes, short breath, and (in serious cases) death due to suffocation (Kamo et al., 1962). In Africa, there are still no reported cases of human disease caused by clinostomids. The significance of *C. cutaneum*, *C. tilapiae*, and *Euclinostomum heterostomum* as zoonotic species needs to be confirmed.

CONCLUSIONS

The study of 22 fish species from Lake Tana revealed that five of them contained five trematode metacercariae of the family Clinostomidae: *Clinostomum complanatum* (in *Enteromius humilis*, *E. tanapelagius*, and *Garra tana*), *Euclinostomum* sp. and *Clinostomum brieni* (in *Clarias gariepinus*), and *Clinostomum phalacrocoracis* and *Euclinostomum heterostomum* (in

Oreochromis niloticus); this characterizes the lake as a unique water body with the richest (according to the currently available data) species composition of trematode larvae of this family.

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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.

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