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**Two New Metacercariae  
of Genus *Austrodiplostomum* (Trematoda: Diplostomidae)  
from *Oreochromis niloticus* (Cichlidae)  
and *Varicorhinus beso* (Cyprinidae)  
in Tana Lake, Ethiopia**

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*Metacercariae of two new trematode species of the genus *Austrodiplostomum* are described from fishes of Tana Lake in Ethiopia: *Austrodiplostomum* sp. 1 from cranial cavity of *Oreochromis niloticus* and *Austrodiplostomum* sp. 2 from vitreous humor of *Varicorhinus beso*. New species metacercariae differs from *A. mordax* metacercariae in the shape of body, the size of body and organs, the shape of anterior extremity, and site of infection. This is the first record of *Austrodiplostomum* metacercariae from fish in Africa.*

**Keywords:** *Austrodiplostomum, Trematoda, new species, metacercaria, fish, Africa.*

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# Две новых метацеркарии рода *Austrodiplostomum* (Trematoda: Diplostomidae) из *Oreochromis niloticus* (Cichlidae) и *Varicorhinus beso* (Cyprinidae) в оз. Тана, Эфиопия

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Из рыб оз. Тана в Эфиопии описаны метацеркарии двух новых видов trematod рода *Austrodiplostomum*: метацеркария *Austrodiplostomum* sp. 1 из черепной полости *Oreochromis niloticus* и *Austrodiplostomum* sp. 2 из стекловидного тела глаза *Varicorhinus beso*. Новые виды метацеркарий отличаются от метацеркарии *A. mordax* формой тела, размерами тела и органов, формой переднего конца тела и локализацией в хозяине. Это первая регистрация метацеркарий рода *Austrodiplostomum* у рыб Африки.

Ключевые слова: *Austrodiplostomum*, Trematoda, новый вид, метацеркария, рыбы, Африка.

## Introduction

Genus *Austrodiplostomum* Szidat et Nani 1951 belong to the subfamily Diplostominae Poirier, 1886 of the family Diplostomidae Poirier, 1886 (Niewiadomska, 2002). Before this study, the genus *Austrodiplostomum* was represented by three species: *A. compactum* Lutz, 1928, *A. mordax* Szidat et Nani, 1951 (Szidat & Nani, 1951), and *A. ostrowskiae* Dronen, 2009 (Dronen, 2009). All of them as adult are endohelminthic parasites of cormorants (Lutz, 1928; Szidat & Nani, 1951; Ostrowski de Núñez, 1970; Dubois & Macko, 1972; Nasir & Díaz, 1972; Dubois, 1977; Rietschel & Werding, 1978; Fedynich et al., 1997; Dronen, 2009) and freshwater fishes (Machado et al., 2005; Salgado-Maldonado, 2006; Novaes et al., 2006; Violante-González et al., 2009) in the western hemisphere. A three-host life cycle of *Austrodiplostomum* includes gastropods of the genus *Biomphalaria* Preston, 1910 as first intermediate host (Rosser et al., 2016). As

metacercariae the genus *Austrodiplostomum* was represented by *A. compactum* (Lutz, 1928) Dubois, 1970, *A. mordax* (Szidat et Nani, 1951) n. comb., and *A. ostrowskiae* Dronen, 2009. These metacercariae are parasites of different South American, Central American and North American freshwater fishes.

During recent investigations into the parasites of some fishes in Tana Lake (Ethiopia), specimens of two *Austrodiplostomum* species were found in the brain and vitreous humor of *Oreochromis niloticus* and *Varicorhinus beso*, respectively. A closer examination of this material, made it possible to describe a two new species as metacercariae. The results of this study are presented herein.

## Materials and methods

In September–November 2006–2008, samples of Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758) (82 specimens, mean length

$\pm$ SE:  $12.9 \pm 0.7$  mm, range 9–260 mm) and Khramulya *Varicorhinus beso* Rüppell, 1835 (25 specimens,  $15.1 \pm 0.9$  mm, range 70–215 mm) were taken from Bahar-Dar Gulf of Tana Lake, Ethiopia ( $11^{\circ}33'$  N,  $37^{\circ}22'$  E). Fish captures were undertaken using gill nets. The eyes and brain of all individuals were examined. Live worms were rinsed in saline, briefly examined prior to fixation, killed with hot water, and fixed in 70 % ethanol. A total 56 specimens of metacercariae from *O. niloticus* and 35 specimens from *V. beso* were collected. Whole-mounts were stained with alum carmine, cleared in dimethyl phthalate and mounted in Canada balsam. Measurements were taken from whole-mounts and are expressed in micrometers ( $\mu\text{m}$ ).

## Results

Family DIPLOSTOMIDAE Poirier, 1886

Subfamily DIPLOSTOMINAE Poirier, 1886

Genus *AUSTRODIPLOSTOMUM* Szidat et Nani, 1951

*Austrodiplostomum* sp. 1. Fig. 1A, 2 (1–4)

*Host*: *Oreochromis niloticus* (Perciformes: Cichlidae).

*Site of infection*: cranial cavity.

*Material studied*: 56 specimens.

*Locality*: Tana Lake near Bahar-Dar, Ethiopia ( $11^{\circ}33'$  N,  $37^{\circ}22'$  E).

*Specimens deposited*: No. 5/407(1–4) (four slides), the Helminthological Collection of the Institute for Biology of Inland Waters RAS and No. 1113 (one slide), the Helminthological Museum of the Parasitological Center of the Institute of Problem of Ecology and Evolution, RAS.

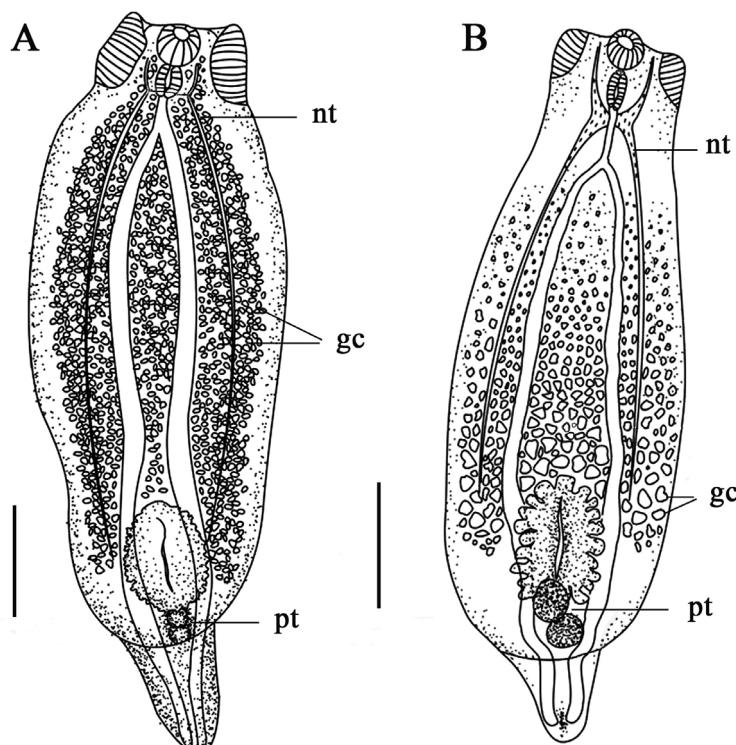


Fig. 1. *Austrodiplostomum* sp. 1 metacercaria (A), *Austrodiplostomum* sp. 2 metacercaria (B). Legend: pt – primordium of testes; nt – nerve trunk; gc – glandular cells. Scale bars: A – 200  $\mu\text{m}$ ; B – 100  $\mu\text{m}$

*Prevalence and intensity:* 21 of 82 (25.6 %), 1–17.

*Description* (based on 15 whole-mounted specimens): measurements of the entire series are given in Table. Metacercariae are large. Body elongate, bisegmented, with clear margin between segments, ventral cavity not well developed, maximum width of body at level of middle of anterior segment length. Anterior segment in form of elongated oval, posterior segment conical and elongated. Oral sucker scyphiform, terminal, width slightly greater than length. At the sides of oral sucker, there are well developed elongated pseudosuckers, situated always above level of oral sucker (Fig. 2). Pseudosuckers longer and narrower than oral sucker. Ventral sucker lacking. Prepharynx absent, pharynx oval, well developed, esophagus short. Intestinal bifurcation in anterior fourth of anterior segment. Intestinal caeca wide or narrow, reached to posterior extremity. Brandes' organ oval, with elongate, wide slit-like opening, lateral and posterior margins pinnately lobed. Primordium of two testes round or oval, one behind another or slightly diagonal, situated between Brandes' organ and posterior margin of anterior segment, in some specimens primordium of posterior testes lie in posterior segment of body. Anterior three fourth of body filled with numerous glandular cells, extending from cecal bifurcation to Brandes' organ. Laterally glandular cells subside to middle or anterior part of Brandes' organ. Nerve trunks and commissure are visible.

*Remarks.* The present form is identical in structure to *A. mordax*, *A. compactum*, and *A. ostrowskiae* metacercaria from different fishes in South America. The characters differentiating this species from their American congeners include the size of body and organs, absence of the prepharynx and shape of anterior extremity. The new species differs from *A. compactum* and

*A. ostrowskiae* by the smaller body, oral sucker, pseudosuckers, pharynx, esophagus and Brandes' organ, but longer posterior segment of body. The feature separating *Austrodiplostomum* sp. 1 and their American congeners is the different structure of anterior extremity. The pseudosuckers of *Austrodiplostomum* sp. 1 were situated above level of oral sucker, whereas those of American *Austrodiplostomum* were situated below level of oral sucker.

***Austrodiplostomum* sp. 2. Fig. 1B, 2 (5–8)**

*Host:* *Varicorhinus beso* (Cypriniformes: Cyprinidae).

*Site of infection:* vitreous humor.

*Material studied:* 35 specimens.

*Locality:* Tana Lake near Bahar-Dar, Ethiopia (11°33' N, 37°22' E).

*Specimens deposited:* No. 6/419 (1–5) (five slides), the Helminthological Collection of the Institute for Biology of Inland Waters RAS.

*Prevalence and intensity:* 2 of 17 (11.8 %), 12–18.

*Description* (based on 15 whole-mounted specimens): measurements of the entire series are given in Table. Body oblong, bipartite, expanded posterior to middle of anterior segment, maximum width of body near anterior margin of Brandes' organ. Ventral cavity not well developed. Anterior segment oval, posterior segment in form of elongate bulge. Oral sucker scyphoid, terminal, length slightly greater than width. Pseudosuckers oval, situated lateral and lower to oral sucker (Fig. 2). Pseudosuckers longer and wider than oral sucker. Ventral sucker lacking. Prepharynx absent. Pharynx oval, muscular, esophagus short. Intestinal bifurcation at approximately border of first and second fourth of anterior segment. Intestinal caeca wide with patent lumen, extends almost to posterior end. Brandes' organ oval, with median longitudinal slit, its margins deeply lobed. Testicular primordial round or oval, slightly

Table Metric data (μm) for African (*Austrodiplostomum* sp. 1 and *Austrodiplostomum* sp. 2) and American (*A. compactum* Lutz, 1928, *A. mordax* Szidat et Nani, 1951, and *A. ostrowskiae* Dronen, 2009) species of *Austrodiplostomum* metacercariae

Measurement	<i>Austrodiplostomum</i> sp. 1 (present study) (n = 15)				<i>Austrodiplostomum</i> sp. 2 (present study) (n = 15)				<i>A. compactum</i> (Paes et al. 2010) (total variation for the hosts species)				<i>A. mordax</i> (Szidat et Nani, 1951)				<i>A. ostrowskiae</i> (García-Varela et al., 2015)			
	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD				
Body length	900	1260	1062	127.1	738	864	802.2	34.2	880	2740	680	880	1300	1500	1400					
Body width <sup>a,b</sup>	270	396	326.2	37.9	207	288	254.8	19.7	400	1180	—	330	480	610	540					
Anterior segment	792	1080	933.6	115.2	639	756	709.2	35.7	—	—	—	—	—	—	—	—				
Posterior segment	78	180	137.6	29.3	78	121	106.1	13.8	41	97	—	—	—	—	—	—				
Pseudosucker length	77	99	87.6	6.7	55	88	63.6	8.3	93	148	—	—	—	—	—	—				
Pseudosucker width	37	55	47.7	6.4	33	51	40.1	5.4	65	146	—	—	—	—	—	—				
Oral sucker length	44	60	52.3	5.3	44	55	50.1	4.5	40	102	—	—	62	82	72					
Oral sucker width	54	72	59.3	4.8	44	51	47.4	2.3	30	116	50	80	50	75	66					
Pharynx length	40	58	48.1	6.0	37	55	42.8	4.5	44	98	—	50	52	75	64					
Pharynx width	30	40	34.2	2.5	24	37	27.8	3.5	38	80	—	30	42	60	53					
Esophagus	42	77	58.9	10.7	44	77	56.2	11.0	54	139	—	—	—	—	—					
Cecal bifurcation to anterior end	132	240	190.3	31.4	143	198	168.7	19.2	—	—	—	—	—	—	—					
Brandes' organ length	168	240	195.2	20.6	99	147	120.5	12.9	200	650	—	—	200	300	270					
Brandes' organ width	72	138	113.4	18.2	51	114	85.4	17.1	120	660	—	—	100	130	15					
Anterior testis length	31	42	36.7	3.9	31	44	35.7	4.0	—	—	—	—	—	—	—					
Anterior testis width	29	48	35.6	7.2	22	44	33.0	7.9	—	—	—	—	—	—	—					
Posterior testis length	31	44	41.1	4.5	29	44	35.3	4.9	—	—	—	—	—	—	—					
Posterior testis width	31	44	37.6	4.8	22	51	35.9	8.7	—	—	—	—	—	—	—					

<sup>a</sup>Body width in the middle of body length for *Austrodiplostomum* sp. 1.

<sup>b</sup>Body width just anterior to Brandes' organ for *Austrodiplostomum* sp. 2.

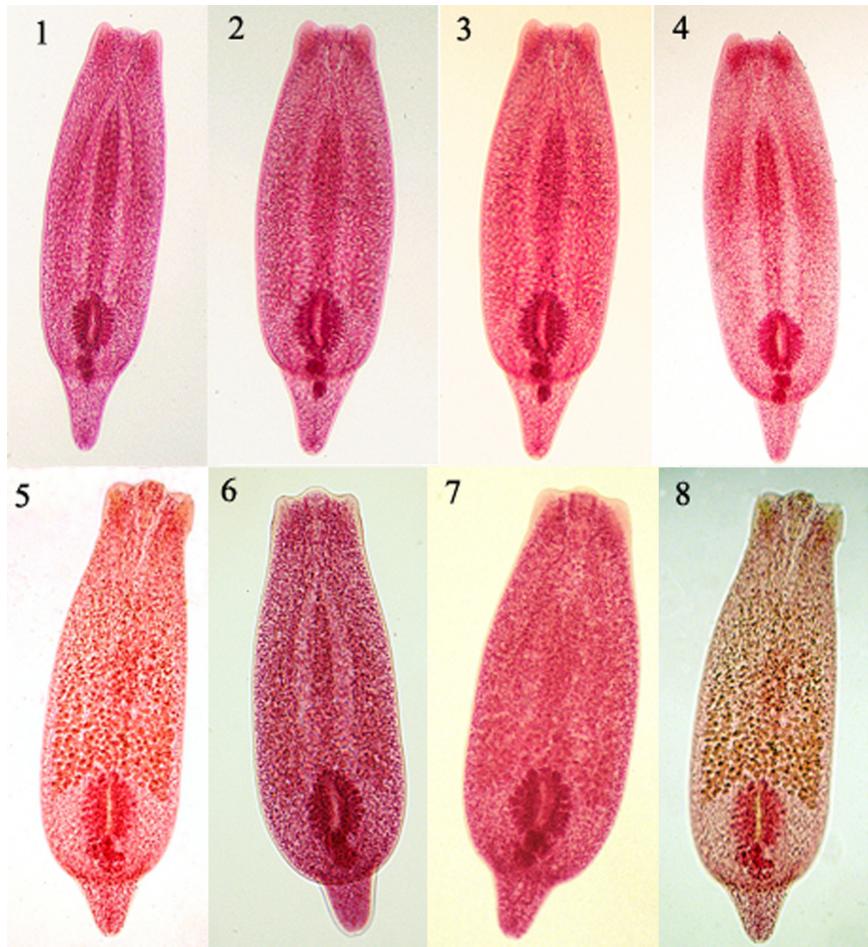


Fig. 2. *Austrodiplostomum* sp. 1 from cranial cavity of *Oreochromis niloticus*, 1–4, view of entire metacercaria; *Austrodiplostomum* sp. 2 from vitreous humor of *Varicorhinus beso*, 5–8, view of entire metacercaria

diagonal, situated between Brandes' organ and posterior margin of anterior segment. Numerous glandular cells fill most part of body anterior to Brandes' organ. Laterally glandular cells subside to level of the beginning of Brandes' organ. Size of glandular cells noticeably increases from cecal bifurcation to Brandes' organ. Nerve trunks and commissure are visible.

**Remarks.** The metacercariae of *Austrodiplostomum* sp. 2 closely resemble those of *Austrodiplostomum* sp. 1 described above. *Austrodiplostomum* sp. 2 differs from *Austrodiplostomum* sp. 1 in its body shape,

relatively shorter posterior segment, smaller body size and organs size, larger glandular cells and the structure of anterior extremity. The pseudosuckers of *Austrodiplostomum* sp. 2 situate below level of oral sucker, whereas those of *Austrodiplostomum* sp. 1 situate above level of oral sucker. In addition, the pseudosuckers of *Austrodiplostomum* sp. 2 are relatively rounded and shorter than those of *Austrodiplostomum* sp. 1. The maximum of body width is positioned near anterior margin of Brandes' organ in *Austrodiplostomum* sp. 2 compared with at level of middle of anterior segment length in *Austrodiplostomum* sp. 1.

The larva of *Austrodiplostomum* sp. 2 morphologically corresponds to metacercaria of *Austrodiplostomum* from American fishes. Both of them have the relatively rounded pseudosuckers situated below level of oral sucker and relatively shorter posterior segment. This species differs from American congeners by having a smaller body size. The body size, pseudosuckers and pharynx size, oral sucker and Brandes' organ size in *Austrodiplostomum* sp. 2 are approximately two times smaller than those in *A. compactum* and *A. ostrowskiae*. Furthermore, in *A. mordax* and *A. compactum* the glandular cells just extend to apex of Brandes' organ, in *Austrodiplostomum* sp. 2 they extend markedly below of apex of Brandes' organ.

## Discussion

This is the first record of the genus *Austrodiplostomum* from Africa. The description of two new species increases the number of species in the genus to a total of five; the two previously described species being *A. mordax*, *A. compactum*, and *A. ostrowskiae*. The finding of new species of *Austrodiplostomum* in Africa means that the genus has now been found from both hemispheres of the world.

Metacercariae of *Austrodiplostomum* sp. 1 and *Austrodiplostomum* sp. 2 are clearly distinguishable. The characters differentiating these species one from another include the shape of body, the size of body and organs, the shape of anterior extremity, and these differences are fundamental. At the same time, the metacercariae of American species (*A. mordax* and *A. compactum*) have not clear morphological differences (Dubois, 1970; Ostrowski de Núñez, 1977).

American metacercariae of *Austrodiplostomum* have wide specificity and occur in a broad spectrum of fish hosts (65 fish species) (Kohn et al., 1995; Viozzi &

Flores, 2002; Machado et al., 2005; Salgado-Maldonado, 2006; Violante-González & Aguirre-Macedo, 2007; Violante-González et al., 2007; Paes et al., 2010; Zica et al., 2010; García-Varela et al., 2015). In contrast to American species *Austrodiplostomum* African species *Austrodiplostomum* exhibit narrow host specificity. Of the eight fish species (*Clarias gariepinus* Burchell, 1822, *Barbus tanapelagius* Graaf et al., 2000, *B. humilis* Boulenger, 1902, *B. pleurogramma* Boulenger, 1902, *Garra dembecha* Getahun et Stiassny, 2007, *Labeobarbus intermedius* Rüppell, 1835, *V. beso*, *O. niloticus*) studied in Tana Lake, only *V. beso* and *O. niloticus* harboured metacercariae *Austrodiplostomum* sp. 1 and *Austrodiplostomum* sp. 2, respectively. The absence of infection in other fish species may be related to specificity of these metacercariae.

Herewith site of infection with African species of *Austrodiplostomum* metacercariae differs from the site infection of American species. Species of *Austrodiplostomum* sp. 1 and *Austrodiplostomum* sp. 2 are confined to the vitreous humor and cranial cavity, respectively, whereas that of metacercariae of *A. mordax*, *A. compactum*, and *A. ostrowskiae* are the vitreous humor (mainly), brain, mesentery, swim bladder, fins, muscles (Salgado-Maldonado, 2006; García-Varela et al., 2015; Zica et al., 2010).

The potential definitive hosts of *Austrodiplostomum* sp. 1 and *Austrodiplostomum* sp. 2 may be cormorants *Phalacrocorax carbo* (Linnaeus, 1758) and *P. africanus* (Gmelin, 1789) and darter *Anhinga rufa* (Daudin, 1802) which are residents in Tana Lake (Nagelkerke, 1997).

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