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 трематод 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
±SE: 12.9±0.7 mm, range 9–260 mm) and Khramulya Varicorhinus beso Rüppell, 1835 (25 specimens, 15.1±0.9 mm, range 70–215 mm) were taken from Bahar-Dar Gulf of Tana Lake, Ethiopia (11°33′ N, 37°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 Oreochromis 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 (μm).

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°33′ N, 37°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 μm; B – 100 μm
**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

<table>
<thead>
<tr>
<th>Measurement</th>
<th><em>Austrodiplostomum</em> sp. 1 (present study) (n = 15)</th>
<th><em>Austrodiplostomum</em> sp. 2 (present study) (n = 15)</th>
<th><em>A. compactum</em> (Paes et al. 2010) (total variation for the hosts species)</th>
<th><em>A. mordax</em> (Szidat et Nani, 1951)</th>
<th><em>A. ostrowskiae</em> (García-Varela et al., 2015)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Min</td>
<td>Max</td>
<td>Mean</td>
<td>SD</td>
<td>Min</td>
</tr>
<tr>
<td>Body length</td>
<td>900</td>
<td>1260</td>
<td>1062</td>
<td>127.1</td>
<td>738</td>
</tr>
<tr>
<td>Body widtha</td>
<td>270</td>
<td>396</td>
<td>326.2</td>
<td>37.9</td>
<td>207</td>
</tr>
<tr>
<td>Anterior segment</td>
<td>792</td>
<td>1080</td>
<td>933.6</td>
<td>115.2</td>
<td>639</td>
</tr>
<tr>
<td>Posterior segment</td>
<td>78</td>
<td>180</td>
<td>137.6</td>
<td>29.3</td>
<td>78</td>
</tr>
<tr>
<td>Pseudosucker length</td>
<td>77</td>
<td>99</td>
<td>87.6</td>
<td>6.7</td>
<td>55</td>
</tr>
<tr>
<td>Pseudosucker width</td>
<td>37</td>
<td>55</td>
<td>47.7</td>
<td>6.4</td>
<td>33</td>
</tr>
<tr>
<td>Oral sucker length</td>
<td>44</td>
<td>60</td>
<td>52.3</td>
<td>5.3</td>
<td>44</td>
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<tr>
<td>Oral sucker width</td>
<td>54</td>
<td>72</td>
<td>59.3</td>
<td>4.8</td>
<td>44</td>
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<tr>
<td>Prepharynx length</td>
<td>Absent</td>
<td>Absent</td>
<td>–</td>
<td>–</td>
<td>2</td>
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<tr>
<td>Pharynx length</td>
<td>40</td>
<td>58</td>
<td>48.1</td>
<td>6.0</td>
<td>37</td>
</tr>
<tr>
<td>Pharynx width</td>
<td>30</td>
<td>40</td>
<td>34.2</td>
<td>2.5</td>
<td>24</td>
</tr>
<tr>
<td>Esophagus</td>
<td>42</td>
<td>77</td>
<td>58.9</td>
<td>10.7</td>
<td>44</td>
</tr>
<tr>
<td>Cecal bifurcation to anterior end</td>
<td>132</td>
<td>240</td>
<td>190.3</td>
<td>31.4</td>
<td>143</td>
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<tr>
<td>Brandes’ organ length</td>
<td>168</td>
<td>240</td>
<td>195.2</td>
<td>20.6</td>
<td>99</td>
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<tr>
<td>Brandes’ organ width</td>
<td>72</td>
<td>138</td>
<td>113.4</td>
<td>18.2</td>
<td>51</td>
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<td>Anterior testis length</td>
<td>31</td>
<td>42</td>
<td>36.7</td>
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<tr>
<td>Anterior testis width</td>
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<td>35.6</td>
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<tr>
<td>Posterior testis length</td>
<td>31</td>
<td>44</td>
<td>41.1</td>
<td>4.5</td>
<td>29</td>
</tr>
<tr>
<td>Posterior testis width</td>
<td>31</td>
<td>44</td>
<td>37.6</td>
<td>4.8</td>
<td>22</td>
</tr>
</tbody>
</table>

a Body width in the middle of body length for *Austrodiplostomum* sp. 1.
b Body width just anterior to Brandes’ organ for *Austrodiplostomum* sp. 2.
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. ostrowskia*. 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. ostrowskia*. 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 metacercaria 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. ostrowskia* 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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