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Research note

Metacercariae of *Renifer heterocoelium* (Trematoda: Reniferidae) in tadpoles of *Rhinella schneideri* (Anura: Bufonidae) in Brazil

Metacercarias de *Renifer heterocoelium* (Trematoda: Reniferidae) en renacuajos de *Rhinella schneideri* (Anura: Bufonidae) en Brasil

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Abstract. *Renifer heterocoelium* is a parasite of the oral cavity of Neotropical snakes for which larval stages and the life cycle are still unknown. During studies of parasites of *Rhinella schneideri*, tadpoles collected in a lake from the municipality of Santa Luzia, Minas Gerais, Brazil, yielded specimens with metacercariae adhered to the external intestinal wall. After morphological characterization, the metacercariae were identified as *R. heterocoelium*. This is the first record and morphological characterization of metacercariae of *R. heterocoelium* for Brazil.

Key words: *Rhinella schneideri*, Amphibia, Bufonidae, *Renifer heterocoelium*, Digenea.

Resumen. *Renifer heterocoelium* es un parásito de la cavidad bucal de las serpientes neotropicales cuyas formas larvales y ciclo de vida son todavía desconocidos. En un estudio de los parásitos de renacuajos de *Rhinella schneideri* recogidos en un estanque ubicado en el municipio de Santa Luzia, Minas Gerais, Brasil, se encontraron ejemplares con metacercarias en la pared intestinal. Después de caracterizarlos morfológicamente, los quistes fueron identificados como *R. heterocoelium*. Este es el primer registro y caracterización morfológica de las metacercarias de esta especie en Brasil.

Palabras clave: *Rhinella schneideri*, Amphibia, Bufonidae, *Renifer heterocoelium*, Digenea.

Recently, studies on associations between larval digeneans and tadpoles have become more important, considering that, for example, *Ribeiroia* Travassos, 1939, may be involved in malformation and possibly mortality among some species of amphibians in some localities in the United States (Johnson et al., 1999, 2004; Skelly et al., 2007).

In South America, few studies have reported tadpoles of different species of anurans as second intermediate hosts of digenetic trematodes, among them *Clinostomum heluans* Braun, 1899, *Episthmium suspensum* (Braun, 1901), *Paryphostomum segregatum* Dietz, 1909 (Lutz, 1935; Lie and Basch, 1967; Ostrowski de Nuñez, 1974). Recently, in Argentina, tadpoles of different species of amphibians were found infected with metacercariae of several species of digeneans, and some of the parasites found (*Opisthogonimus* Lühe, 1900, *Styphlodora* Looss,

1899, *Travtrema* Pereira, 1929) have snakes as the definitive hosts (Kehr and Hamann, 2003; Hamann and González, 2009).

In the present study, metacercariae of *Renifer* Pratt, 1902 (= *Ochetosoma* Braun, 1901, *Pseudorenifer* Price, 1935, *Neorenifer* Byrd and Denton, 1938, *Neochetosoma* Caballero, 1949) are reported in tadpoles of *Rhinella schneideri* (Werner, 1894) from Brazil.

Renifer is a trematode parasite of the upper digestive tract of snakes from Neotropical and Nearctic regions. Nine species are currently considered valid, among more than 30 nominal species that have been described (Byrd and Denton, 1938; Leão, 1945; Kagan, 1947; Dubois and Mahon, 1959; Tkack, 2008).

Aspects of the life cycle of these parasites have been elucidated with regard to some species in the United States. The life cycle involves gastropod molluscs (*Physa* Draparnaud, 1801, and *Physella* Haldeman, 1842), in which larvae of the xiphidiocercariae group are formed. These emerge and encyst in a second intermediate host,

mainly amphibian tadpoles. Metacercarial excystment occurs after ingestion of the infected amphibians by snakes, and the adult parasites develop in the oral cavity and esophagus of these reptilian hosts (Byrd, 1935; Byrd and Scofield, 1952; Sogandares-Bernal and Grenier, 1971). Metacercariae of *Renifer* have been reported in different species of amphibians and fish in North America (Pulido-Flores, 1994; Sánchez-Nava et al., 2004; Muzzall, 2005; Aguilar-Aguilar and Salgado-Maldonado, 2006; Pérez-Ponce de León et al., 2007; McKenzie, 2007; Lira-Guerrero et al., 2008).

Tadpoles of *R. schneideri* at different stages of development, ranging from level 25 to level 30 according to Gosner (1960), were collected between 1993 and 1994 during malacological surveys conducted in bodies of water from the rural area of the municipality of Santa Luzia (19°46'11" S, 43°51'05" W), state of Minas Gerais, Brazil. The specimens were captured with the aid of a metal scoop, and were packed and transported to the laboratory, where they were identified. The tadpoles were then necropsied in Petri dishes containing physiological saline solution and examined under a stereomicroscope for the presence of parasites. The metacercariae were found in the body cavity of the larval amphibians and were fixed in 10% formalin at 70 °C, stained with acetic-alum carmine, dehydrated in increasing alcohol series, cleared in Beechwood creosote, and mounted in Canada balsam. The parasites were examined under a light microscope, and measurements (in μm) were made on 20 specimens using an eyepiece graduated in millimeters. Data are presented as means followed by standard deviation, and amplitude between parentheses. Drawings were made with the aid of a camera lucida attached to a microscope. Specific identification was performed by comparison with the morphology of adult parasites (Travassos, 1921; Dubois and Mahon, 1959; Travassos et al., 1969; Bray et al., 2008), and the association between the morphology of metacercariae and adult worms, in accordance with Byrd (1935). The slides studied were deposited in the collection of the Department of Parasitology, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brasil, under accession number 6152 a-v.

The metacercariae (Fig. 1) found adhered to the external intestinal wall are characterized below:

Encysted metacercariae ($n = 20$): oval, 551 ± 92 (375-751) long by 247 ± 23 (218-294) wide; cystic membrane transparent, 3 ± 1 (2-5) in thickness. Larvae with spinous tegument. Oral sucker subterminal, 125 ± 13 (106-158) by 124 ± 9 (103-137). Ventral sucker equatorial, 92 ± 17 (65-115) by 110 ± 14 (86-128). Suckers ratio 1:1.4 \pm 0.2 (1.0-1.8). Pre-pharynx short; pharynx oval, 42 ± 4 (34-

51) by 35 ± 4 (29-43); esophagus short, 43 ± 9 (26-56) in length, bifurcated in preacetabular region; intestinal caeca sacciform, reaching level of ventral sucker. In some specimens ($n = 8$) 2 groups of 9 cephalic glands arranged in fields lateral to the pharynx and esophagus are visible, with tiny ducts directed to the oral sucker. Genital primordium formed by 3 spherical masses located in post-acetabular region. Testes round, symmetrical, postovarian; right testis 17 ± 3 (12-26) by 24 ± 6 (15-34); left testis 18 ± 4 (12-26) by 20 ± 5 (12-26). Ovary rounded, submedian, located anterior to left testis, 18 ± 4 (12-26) by 22 ± 5 (17-34). Cirrus pouch primordium elongate, oblique, pre-acetabular, 97 ± 12 (77-120) by 23 ± 3 (17-26), in some specimens showing the posterior portion overlapping the anterior margin of the ventral sucker. Uterine primordium transverse to ventral sucker with the distal portion parallel to the cirrus pouch, opening into genital pore. Genital pore lateral, on the right side of the body at level of intestinal caeca. Excretory vesicle Y shaped.

The morphological features of the metacercariae found were compatible with digeneans of the genus *Renifer*; the extent of the intestinal caeca and the biometric relationship enabled the identification of our specimens as *Renifer heterocoelium* (Travassos, 1921) Tkach, 2008. The finding of adult parasites of this species in Wagler's snake, *Xenodon merremii* (Wagler, 1854), from the same region (Pinto et al., 2012), suggests that these adults are conspecific with the metacercariae found in the tadpoles. In fact, a correlation between the morphology of the metacercariae and adult parasites of *Renifer aniarium* (Leidy, 1890) from experimentally infected tadpoles and *Natrix* spp. was verified by Byrd (1935). Moreover, the general morphology of metacercariae reported in *R. schneideri* in the present study is similar to that of the young specimen of *R. heterocoelium* reported in snake by Lenis et al. (2009), although the latter is larger and has more developed vitellaria and uterus.

Renifer heterocoelium was described parasitizing Neuwied's lancehead, *Bothrops neuwiedii* (Wagler, 1824), from the state of São Paulo and was subsequently recorded in 21 other snake species from several Brazilian localities (Travassos, 1921; Travassos et al., 1969; Corrêa, 1980; Corrêa et al., 1990; Thatcher, 1993; Silva et al., 1999; Pinto et al., 2012). It has also been reported in Venezuela (Thatcher, 1993), and redescribed as *Ochetosoma heterocoelium* (Travassos, 1921) from adult specimens obtained from snakes in Colombia (Lenis et al., 2009).

New studies relating to the participation of amphibians in the life cycle of Neotropical trematodes are needed. This is the first report and morphometric characterization of metacercariae of *R. heterocoelium*, as well as the first report on the participation of amphibians in the biological cycle of this parasite in Brazil.

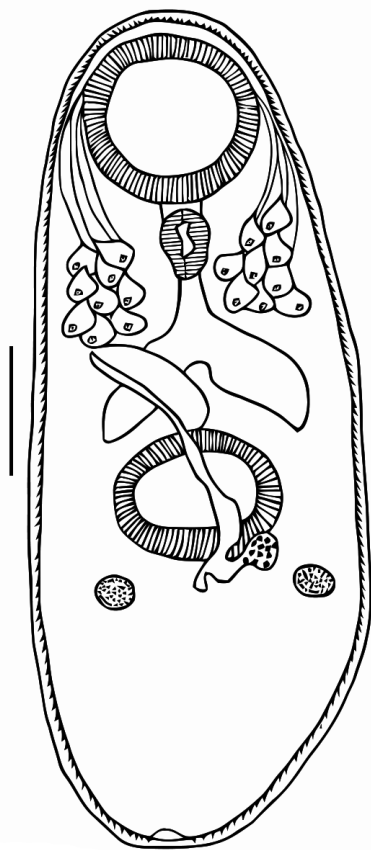


Figure 1. Metacercaria of *Renifer heterocoelium* (Travassos, 1921) from tadpole of *Rhinella schneideri* (Werner, 1894), dorsal view. Scale bar: 100 μ m.

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