Research Note

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Metacercariae of *Centrocestus formosanus* (Trematoda: Heterophyidae) in *Australoheros facetus* (Pisces: Cichlidae) in Brazil

Metacercárias de *Centrocestus formosanus* (Trematoda: Heterophyidae) em *Australoheros facetus* (Pisces: Cichlidae) no Brasil

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Abstract

Heterophyid metacercariae were found in the gills of *Australoheros facetus* (Jenyns, 1842) collected from the Pampulha reservoir, Belo Horizonte, Minas Gerais, Brazil, between February and April 2010. The cysts were counted and used to perform experimental studies (artificial excystment and infection of mice). Fifty specimens of *A. facetus* were analyzed and it was found that the prevalence of infection was 100% and mean infection intensity was 134 metacercariae/fish (range: 4-2,510). Significant positive correlations were seen between total fish length and intensity of infection; between fish weight and intensity of infection, and between parasite density and fish length. Morphological analyses on metacercariae and adult parasites obtained from experimentally infected mice made it possible to identify *Centrocestus formosanus* (Nishigori, 1924). This is the first report of *C. formosanus* in *A. facetus* in Brazil.

Keywords: Heterophyidae, fish parasites, metacercariae, trematodes, Minas Gerais, Brazil.

Resumo

Metacercárias de Heterophyidae foram encontradas nas brânquias de *Australoheros facetus* (Jenyns, 1842) coletados na represa da Pampulha, Belo Horizonte, Minas Gerais, Brasil, entre fevereiro e abril de 2010. Os cistos obtidos foram contados e utilizados em estudos experimentais (desencistamento artificial e infecção de camundongos). Cinquenta exemplares de *A. facetus* foram analisados, sendo verificada prevalência de infecção de 100% e intensidade média de infecção de 134 metacercárias/peixe (variando entre 4 e 2510). Verificou-se correlação positiva entre a intensidade de infecção e comprimento total dos peixes; entre a intensidade de infecção e o peso dos peixes e entre a densidade parasitária e o comprimento total. A análise morfológica das metacercárias e dos parasitos adultos obtidos em camundongos experimentalmente infectados permitiu a identificação de *Centrocestus formosanus* (Nishigori, 1924). Este é o primeiro relato de *C. formosanus* em *A. facetus* no Brasil.

Palavras-chave: Heterophyidae, parasitos de peixes, metacercárias, trematódeos, Minas Gerais, Brasil.

Centrocestus formosanus (Nishigori, 1924) is a small intestinal trematode parasite of fish-eating birds and mammals, which has already been reported to infect human beings in Asia. Since it was first described in Taiwan, involvement of several species of fish as second intermediate hosts has been reported in different countries (CHEN, 1942; SCHOLZ; SALGADO-MALDONADO, 2000; MITCHELL et al., 2005). In these hosts, metacercariae are formed on the gill arches and filaments, causing pathological alterations such as cartilage proliferation and inflammatory reaction around the cyst (MITCHELL et al., 2000). Depending on factors such as worm burden, infection by *C. formosanus* can lead to reduced respiratory capacity and even fish mortality, thus resulting in potential damage to fish farming (MITCHELL et al., 2005; ARGUEDAS-CORTÉS et al., 2010).

The life cycle of *C. formosanus* was firstly elucidated in Asia (CHEN, 1942), and since then, experimental infection studies involving this parasite have been conducted in at least 12 countries (PINTO; MELO, 2011). In the late 1950s, *C. formosanus* was introduced to the Americas (MARTIN, 1958), and it has now been reported in more than 50 species of fish in Mexico (SCHOLZ; SALGADO-MALDONADO, 2000; ORTEGA et al., 2009;

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AGUILAR-AGUILAR et al., 2009), Colombia (VELÁSQUEZ et al., 2006), Venezuela (HERNÁNDEZ et al., 2003) and Costa Rica (ARGUEDAS-CORTÉS et al., 2010). Recently, Pinto and Melo (2010) observed that the invasive snail *Melanoides tuberculata* (Müller, 1774) was involved in the transmission of *C. formosanus* in Brazil. Nevertheless, the possible species of fish involved in the life cycle of this parasite are not well known in this country.

In the present study, occurrence of natural infection of *Australoheros facetus* (Jenyns, 1842) (= *Cichlasoma facetum*) by *C. formosanus* in Brazil is reported.

The fish were collected between February and April 2010 from the Pampulha reservoir (19° 51' 771" S and 43° 58' 542" W), a eutrophic artificial urban lake located in the northern of Belo Horizonte, Minas Gerais, Brazil. The specimens were caught using a nylon net and were transported alive to the laboratory, where they were weighed on an analytical balance, measured with the aid of a pachymeter, killed by decapitation and then examined for parasites under a stereomicroscope. The gills were removed, transferred to glass slides containing saline solution (NaCl 0.85%) and analyzed by means of optical microscopy to determine whether metacercariae were present and to count them. Then, a sample of metacercariae was subjected to in vitro excystment by means of artificial digestion using 1% pepsin solution in 0.85% NaCl and 1% HCl (pH 2) for 1 hour at 37° C, followed by 0.5% trypsin solution in 0.85% NaCl and 1.5% $NaHCO_3$ (pH 9) for 15 minutes at the same temperature. Additionally, 50 encysted metacercariae were administered orally to five mice of the AKR/J strain. Ten days after infection, the mice were killed by means of cervical dislocation (in accordance with the procedures approved by the local ethics committee on animal experimentation - CETEA, UFMG), and the small intestines were removed, open longitudinally in Petri dishes containing saline, and examined under a stereomicroscope for the presence of adult parasites.

The developmental stages obtained (excysted metacercariae and adult parasites) were killed in water at 70° C, fixed in 10% formalin, stained with alum acetocarmine, dehydrated in ascending series of ethanol, clarified in beechwood creosote and mounted on permanent slides in Canada balsam. The morphological study was performed under an optical microscope, and at least 30 specimens of each developmental stage were measured with the aid of an ocular micrometer. The parasites were identified with the aid of taxonomic keys and descriptions in studies by different authors (CHEN, 1942; BRAY et al., 2008; HAN et al., 2008; PINTO; MELO, 2010).

Statistical analysis was performed using the BioEstat® software, version 5.0. Data normality was evaluated using the Shapiro-Wilk test. Possible correlations between the body measurements of the fish (total length, weight and parasite density) and the intensity of infection were determined using the Spearman's rank correlation test. The ecological terms were used in accordance with Bush et al. (1997). Specimens studied were deposited in the collection of the Invertebrate Taxonomy and Biology Laboratory, Department of Parasitology, (DPIC), Belo Horizonte, Minas Gerais, Brazil (accession number 5925a-g).

The fish sample analyzed was composed of 50 unsexed specimens of *A. facetus* (Figure 1a). Their mean total length was 26 mm (range: 14-80), and their mean weight was 0.9 g (range: 0.3-8.5). In all, 6,721 metacercariae were recovered from the gills of the fish necropsied. The observed prevalence of infection was 100%, the mean intensity of infection was 134 metacercariae/fish (range: 4-2,510) and the average density was 77 metacercariae/g body weight (range: 11-357). The metacercariae (Figure 1b-c) were found encysted in the gills of the fish, and were oval-shaped; the larvae presented an X-shaped excretory vesicle with dark granules inside, and also a crown of 32 circumoral spines in the anterior region of the body. The metacercariae were easily excysted with the treatments performed (Figure 1d); they were very active and their morphology was similar to that of adults, except for the absence of eggs. Significant positive correlations were observed between total fish length and intensity of infection ($r_s = 0.815$, $p < 0.0001$), between total fish weight and intensity of infection ($r_s = 0.724$, $p < 0.0001$) and between parasite density (metacercariae/g) and fish length ($r_s = 0.724$, $p < 0.0001$). Adult parasites were recovered from the proximal part of the small intestine of all the experimentally infected mice (Figure 1e), and the main differential characteristics were a small body with a double crown of 32 circumoral spines, entire ovary, two opposite testes, follicular vitellaria extending laterally along the body, fewer eggs in the uterus measuring on average 35 µm long × 18 µm wide, and an X-shaped excretory vesicle. Morphological analysis on the metacercariae (encysted and excysted) and adult parasites obtained experimentally made it possible to identify *C. formosanus*, an exotic trematode that is reported here for the first time infecting *A. facetus* in Brazil.

Centrocestus formosanus presents low specificity for the second intermediate host. However, the intensity of parasite infection reported in different fish species is variable, with reports in the literature ranging from 1 to 5,935 metacercariae (CHEN, 1942; SCHOLZ; SALGADO-MALDONADO, 2000). This wide range of intensity of infection is probably due to interspecies differences between fish species (e.g. structural features of gills), or even the transmission dynamics peculiar to the localities studied (e.g. temperature, number and intensity of infection of definitive and intermediate hosts).

The correlation between fish size and the intensity of infection found in this study is in agreement with studies on metacercariae of other species of trematodes (POULIN, 2000) and even on *Centrocestus* spp. (MADHAVI; RUKMINI, 1991; MITCHELL et al., 2000, KIMURA; UGA, 2005). This association probably results from the accumulation of repeated exposure, since previous studies have indicated that a single exposure of fish to a high number of cercariae of the parasite can cause the death of the host (MARTIN, 1958).

Regarding the transmission dynamics of the parasite in the Pampulha reservoir, it is important to emphasize that it currently has a high population density of fish, due mainly to the high degree of eutrophication. With this factor, in association with the presence of populations of waterfowl species (potential definitive hosts) and *M. tuberculata* naturally infected by the parasite (PINTO; MELO, 2010), the Pampulha reservoir has becomes an environment suitable for maintenance of the life cycle of *C. formosanus*. This would partly explain the high prevalence and intensity of infection observed in *A. facetus* in the present study.

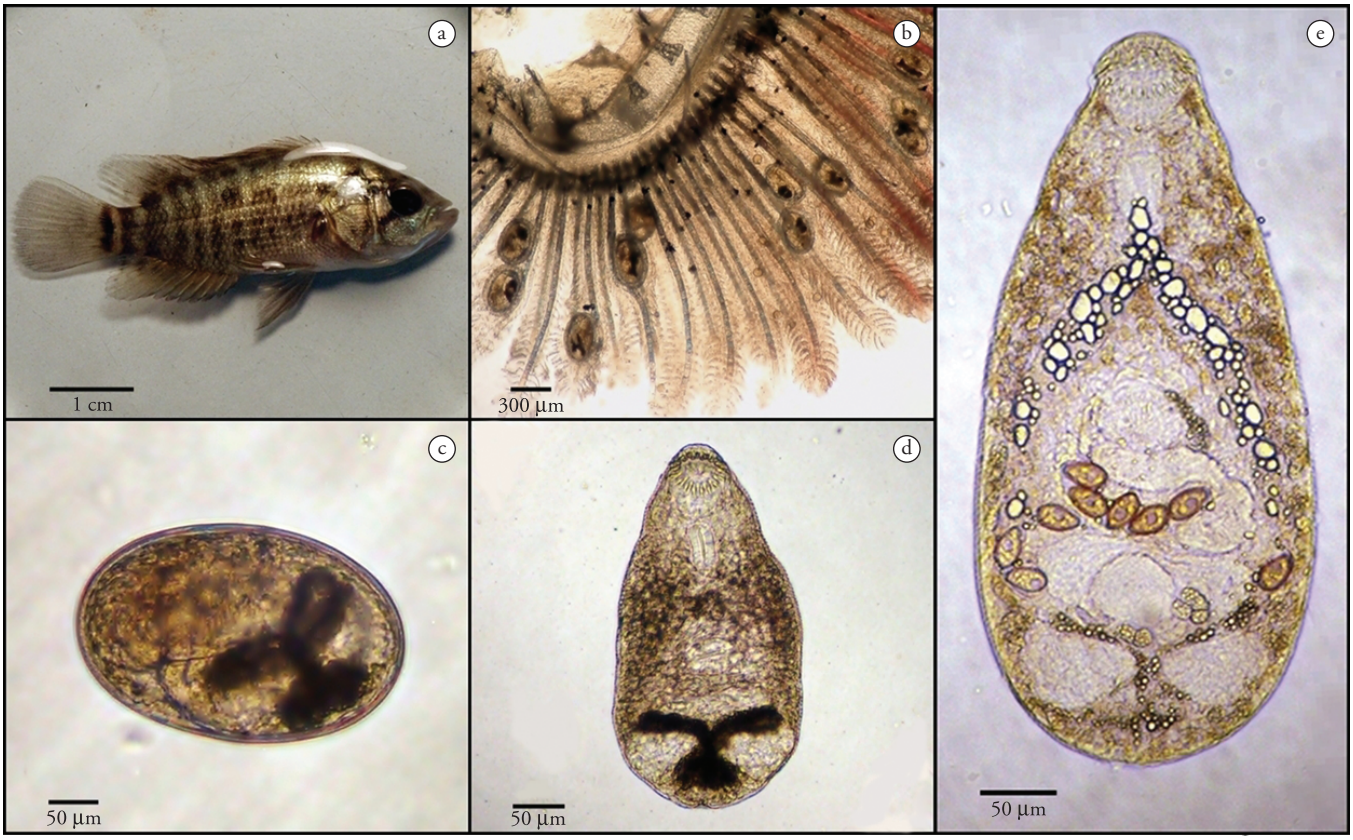

Figure 1. (a) *Australoheros facetus*, a natural host of *Centrocestus formosanus* in the Pampulha reservoir, Belo Horizonte, Minas Gerais, Brazil. (b) Metacercariae of *C. formosanus* adhering to gill filaments. (c) Encysted and (d) excysted metacercariae. (e) Adult parasite recovered from experimentally infected mice.

Knowledge about the distribution of *C. formosanus* in Brazil needs to be expanded in order to prevent the possible impacts resulting from the establishment of this invasive species. In the context of fish farming, the most effective control measure is to prevent the establishment of the transmitter snail, *M. tuberculata*.

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