

## ***Centrocestus formosanus* (Trematoda: Heterophyidae) in *Melanoides tuberculata* (Gastropoda: Thiaridae) from Vila do Abraão, Ilha Grande, Rio de Janeiro, Brazil**

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### **Abstract**

Pleurolophocercous cercariae found in the invasive gastropod *Melanoides tuberculata* (Müller, 1774) collected in a stream of the Vila do Abraão, Ilha Grande, Angra dos Reis, Rio de Janeiro, Brazil were used for experimental infection that enabled the identification of the heterophyid trematode *Centrocestus formosanus* (Nishigori, 1924). The parasite has been found in the locality since 2007, after two years of the introduction of *M. tuberculata*. Recently, from a sample of 483 specimens collected in June 2013, 101 (21%) were found infected with parasite. The potential environmental impacts caused by the parasite occurrence could be underestimated in the country, and actions to monitor and control both the parasite and the mollusk are necessary.

**Keywords:** pleurolophocercous cercariae, invasion ecology, exotic freshwater snails.

## ***Centrocestus formosanus* (Trematoda: Heterophyidae) em *Melanoides tuberculata* (Gastropoda, Thiaridae) da Vila do Abraão, Ilha Grande, Rio de Janeiro, Brasil**

### **Resumo**

Cercárias do tipo pleurolophocerca encontradas no gastrópode invasor *Melanoides tuberculata* (Müller, 1774) coletados em um riacho da Vila do Abraão em Ilha Grande, Angra dos Reis, Rio de Janeiro, Brasil foram utilizadas em estudos de infecção experimental que possibilitaram a identificação do trematódeo heterofídeo *Centrocestus formosanus* (Nishigori, 1924). O parasito vem sendo encontrado na localidade desde 2007, dois anos após a introdução do molusco. Recentemente, de uma amostra de 483 espécimes coletados em junho de 2013, 101 (21%) apresentavam-se infectados pelo parasito. Os potenciais impactos ambientais ocasionados pela ocorrência deste parasito podem estar sendo subestimados no país, sendo necessárias ações visando o monitoramento e controle tanto do parasito quanto do molusco.

**Palavras-chave:** pleurolophocercous cercariae, ecologia da invasão, caramujos dulceaquícolas exóticos.

### **1. Introduction**

*Melanoides tuberculata* (Müller, 1774) is a limnic invasive gastropod from East Africa and South Asia (Pilsbry and Bequaert, 1927), probably introduced to Brazil by aquarium trade (Vaz et al., 1986). To date, it has been reported from 19 states and from the Federal District (Fernandez et al., 2003; Rocha-Miranda and Martins-Silva, 2006; Santos et al., 2013; Thiengo et al.,

2007). The species is associated with ecological problems, such as the modification of the community structure caused by the high population densities that it may reach (Santos and Eskinazi-Sant'Anna, 2010; Santos et al., 2012). *Melanoides tuberculata* is also the intermediate host for several parasites of medical and veterinary significance (Pinto and Melo, 2011; Santos et al., 2012).

Despite the expansion of *M. tuberculata* in Brazil for some time, the infection records of this thiarid snail with larval trematodes are relatively recent. During the last decade, the trematode transmission by this species was recorded in some localities in Brazil. Pleurolophocercous cercariae were firstly recorded in *M. tuberculata* in the state of Rio de Janeiro (Boaventura et al., 2002; Bogéa et al., 2005; Thiengo et al., 2001) and later, similar larvae were found in Minas Gerais state, which were identified as the heterophyid trematode *Centrocestus formosanus* (Nishigori, 1924), for the first time in Brazil (Pinto and Melo, 2010). This species was also found parasitizing *M. tuberculata* in the Federal District (Paula-Andrade et al., 2012) and in the states of Rio Grande do Norte (Pinto and Melo, 2012c) and Rio de Janeiro (Santos et al., 2013).

Long-time malacological studies have been conducted in a human-impacted stream in Vila do Abraão, Ilha Grande, Angra dos Reis city, state of Rio de Janeiro, Brazil (23°08'31"S, 44°10'10"W) (Figure 1), since the first record of *M. tuberculata* in 2005 (Braga et al., 2014; Santos et al., 2007, 2012). A previous research on helminths associated to *M. tuberculata*, revealed that pleurolophocercous cercariae have been present in this population since 2007 (personal observations), although the taxonomic identification of the parasite was not performed so far.

## 2. Material and Methods

In order to obtain experimentally the respective adult trematodes required for parasite identification, specimens of *M. tuberculata* were collected in Vila do Abraão (INEA

18/2007 and IBAMA/Sisbio 10812-1 licenses), in June 2013. After artificial photostimulation, cercariae were detected in some specimens of *M. tuberculata*, of which five specimens were transported to the Laboratório de Taxonomia e Biologia de Invertebrados, Universidade Federal de Minas Gerais (LTBI, UFMG - Belo Horizonte, state of Minas Gerais), where other developmental stages of the parasite were obtained, according to Pinto and Melo (2010). Briefly, 20 specimens of laboratory-reared *Poecilia reticulata* Peters, 1859 were individually exposed to a solution containing undetermined number of pleurolophocercous cercariae emerged from naturally infected *M. tuberculata*. Metacercariae recovered from the gills of fish 30 days after infection were force-fed to mice (50 metacercariae/animal) and adult parasites were recovered from their intestines seven days later. The procedures for experimental infection and euthanasia of vertebrate hosts were performed in accordance to the ethical principles of the Comitê de Ética em Experimentação Animal (CETEA/UFMG - 199/2009).

Adult parasites and cercariae obtained were killed in hot water (70 °C), fixed in 10% formalin, stained with alum acetocarmine, dehydrated using an ascending ethanol gradient, cleared in benchwood and mounted in Canada balsam. The parasites were examined under the microscope and measures were taken with the aid of a micrometer eyepiece. The results obtained were compared to the data provided by other studies (Pinto and Melo, 2010; Scholz and Salgado-Maldonado, 2000), as well as to the samples from other localities in Brazil. Specimens of cercariae and adult parasite were deposited in the collection of



**Figure 1.** Localization of Vila do Abraão, Ilha Grande, Angra dos Reis, RJ, Brazil where specimens of *Melanoides tuberculata* were found infected with *Centrocestus formosanus*.

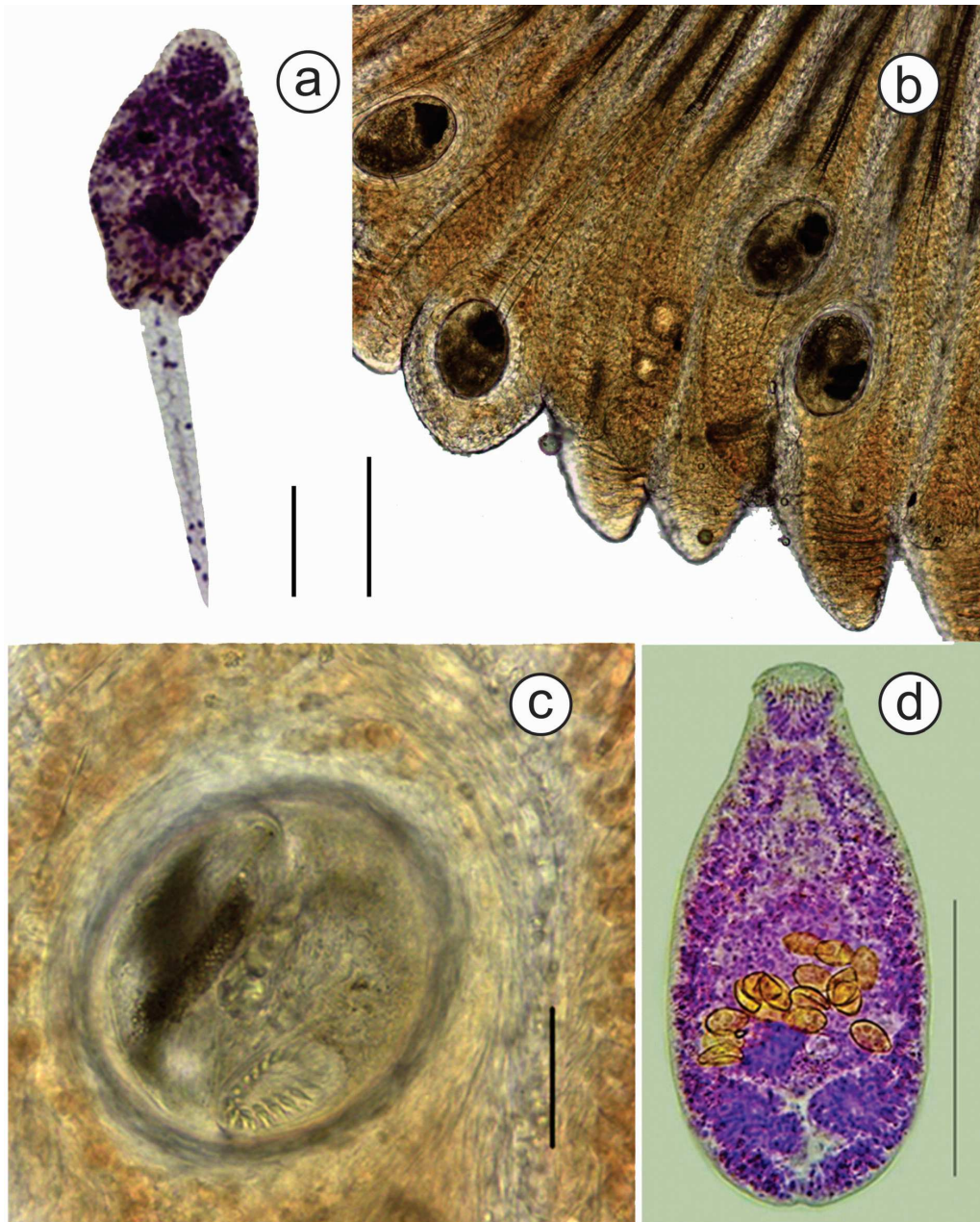
LTBI - UFMG, under the access number DPIC 8565-8566. The morphological analyzes performed made it possible to identify the parasite.

### 3. Results

In the present study, 483 specimens of *M. tuberculata* were collected. Pleurolophocercous cercariae were detected in 101 (21%) specimens of *M. tuberculata*. Thus, the parasite found in Ilha Grande was identified as *Centrocestus formosanus* (Figure 2).

### 4. Discussion

*Centrocestus formosanus* is a small intestinal parasite of birds and mammals which has also been recorded in humans from Asia (Chai et al., 2013). The species was introduced into American continent in the 1950s (Martin, 1958) and it may be found in the USA, Mexico, Colombia, Venezuela and Brazil, nowadays (Pinto and Melo, 2011). Its occurrence is very likely underestimated due the lack of studies. *Centrocestus formosanus* has a complex life cycle, involving a thiarid snail and fishes



**Figure 2.** *Centrocestus formosanus*. (a) Cercariae obtained from *Melanoides tuberculata* collected in Ilha Grande, Brazil. (b) Gills of *Poecilia reticulata* experimentally infected with metacercariae. (c) Detail of metacercariae obtained from the gills of *Poecilia reticulata* experimentally infected. (d) Adult parasite obtained from the intestine of laboratory infected mice. Scales: (a) 50µm; (b) 200 µm; (c) 50µm; (d) 200µm.

as intermediate hosts (Aguilar-Aguilar et al., 2009; Scholz and Salgado-Maldonado, 2000). The presence of metacercariae in gills can lead to the death of fishes, resulting in losses for fish-farming (Aguilar-Aguilar et al., 2009; Mitchell et al., 2005; Ortega et al., 2009; Scholz and Salgado-Maldonado, 2000).

In Brazil, *C. formosanus* was first identified in its mollusc intermediate host in 2010 (Pinto and Melo, 2010), although cercariae of the same species have been recognized since 2000's (Boaventura et al., 2002; Bógea et al., 2005; Thiengo et al., 2001). Recently, the infection of some fish species by *C. formosanus* was discovered in the state of Minas Gerais (Pinto and Melo, 2012a, b; Pinto et al., 2014). Moreover, there is only one conclusive record of a bird, *Butorides striata* (L.), harboring this heterophyid in Brazil, (Pinto et al., 2013). However, given the known non-specificity host for the parasite, it probably also occurs in other waterfowl species.

The first record for *M. tuberculata* in Ilha Grande dates to 2005 and its infection with pleurolophocercous cercariae, here identified as *C. formosanus*, was first detected in 2007, indicating a relative short time between the introduction of the intermediate host and its involvement in the transmission of parasites. Other factors concerning the introduction and dispersion of this parasite to previously unaffected areas are still unknown. However, it may be introduced by infected molluscs or fishes; it is more likely that the movement of birds between places with established populations of mollusc hosts is the main form of introduction. Due to the diversity of fishes and birds occurring on Ilha Grande (Alves and Vecchi, 2009; INEA, 2013; Rocha et al., 2009), it is likely that the life cycle of *C. formosanus* is established in Vila do Abraão stream.

Actions to prevent the dispersal of molluscs and parasites are important to control negative effects on the native fauna, as well as to limit the occurrence of future cases of centrocestiasis and other parasite diseases transmitted by *M. tuberculata*.

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