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SHORT NOTE

OCCURRENCE OF *Biomphalaria glabrata* AND *Physa acuta* (GASTROPODA: MOLLUSCA) SNAILS IN QUINTA DA BOA VISTA MUNICIPAL PARK, RIO DE JANEIRO, RJ, BRAZIL

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ABSTRACT

The human species has an emotional connection with nature called "biophilia" (or, love for life). This connection goes from generation to generation, being one of the reasons why green spaces are present in large cities. Quinta da Boa Vista is a municipal park in Rio de Janeiro located in the São Cristóvão neighborhood in the northern part of the city. Its main attraction is the artificial lake system, a leisure attraction for the local community. This article reports the presence of *Biomphalaria glabrata* and *Physa acuta* snails around the main lake in Quinta da Boa Vista Municipal Park in Rio de Janeiro, RJ. Seventy-one *B. glabrata* and twenty-seven *P. acuta* snails were collected. The snails were measured and examined for the presence of trematode larvae according to standardized procedures. The prevalence rate of *B. glabrata* was estimated at 15.5%. Rediae and cercariae resembling those of the species *Typhlocoelum cucumerinum* (Typhlocoelidae, Digenea) which parasitizes aquatic birds of the order Anseriformes were found. This study reinforces the importance of monitoring park lakes and ponds to better understand the risk of helminth transmission by molluscan vectors.

KEY WORDS: Snails; Schistosomiasis; Digenea; Quinta da Boa Vista; Rio de Janeiro.

The human species has an emotional connection with nature. This link, called "biophilia" (or, love for life) by Wilson (1984), suggests that the tendency to seek connections with other forms of life is innate. Biophilia is present in all generations and has justified the opening and maintenance of public leisure areas in major cities of the world.

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Public parks are abundant in Rio de Janeiro. They are ideal spaces for leisure and physical activities, increasing quality of life. In addition, they bring the population closer to nature within the urban environment (Szeremeta & Zannin, 2008).

One of the most frequented parks in Rio de Janeiro is in the São Cristóvão neighborhood in the northern region of the city. Known as Quinta da Boa Vista Municipal Park, it is one of the largest urban parks in Rio de Janeiro, comprising an area of 155,000 m^2 (Rio de Janeiro Cityhall, 2013). The park is beautifully landscaped, with thriving vegetation in the predominantly tropical, hot and humid climate. It receives a large influx of visitors, especially on weekends. The fauna and flora located in the park are diverse thanks to its proximity to the city's zoo.

One of the main attractions of Quinta da Boa Vista Municipal Park is its artificial water system. This system, designed by French landscape architect Auguste François Marie Glaziou, consists of a series of artificial interconnected lakes, featuring water activities, such as paddling kayaks and pedalboats (Rio de Janeiro Cityhall, 2013). For this reason, frequent monitoring and analysis of the water is important to prevent parasitic diseases and infections.

The present work reports the presence of molluscan vectors of schistosomiasis in the main lake of the Quinta da Boa Vista Municipal Park. All snails were examined for the presence of digenetic trematodes according to standardized experimental procedures. The aim, thus, was to evaluate the risks of helminth infection to the local population.

The procedures adopted for the collection, transport, maintenance and identification of infected snails follow those described in Thiengo (1995). The malacological collections were carried out around the main lake of Quinta da Boa Vista Municipal Park located in the northern part of Rio de Janeiro city. The collection site bordered the lake (22°54'21.0"S / 43°13'21.9"W).

The snails were captured by the active search sampling technique with the aid of a capturing sieve and stainless steel tweezers. As these snails were found on aquatic vegetation and on the substrate, the capturing sieve was used to remove submerged plants and underwater sediment and bring the material to the surface, carefully washing the substrate away. Snails were then sampled with the tweezers. This procedure was repeated 10 times in different parts of the breeding site for up to 60 minutes.

Water from the breeding site was also collected to minimize the effects of experimental acclimatization. The snails were placed in plastic tubes (Falcon model 50 mL, Cralplast, SP) filled with water from the site and then transported to the laboratory.

In the laboratory, the snails were kept in containers with approximately 1.5 L of water from the breeding site under natural cycles of light and temperature. They were fed dried lettuce *ad libitum* for up to 40 days. All snails were measured with the aid of a mechanical caliper (150 mm, Maclauren, SP).

The infected snails were identified and isolated in 10 mL glass beakers containing 5 mL of distilled water. The snails were exposed to artificial light at approximately 30ºC for 3 consecutive hours when the presence of cercariae was investigated in the water. Snails were also kept in the dark for approximately 12 hours. These screenings were repeated twice a week to induce cercarial emergence. The cercariae found were collected with the aid of Pasteur pipettes and processed according to standardized techniques (Bogéa & Caira, 2001).

The snails that remained negative for trematode larvae for up to 40 days after collection were dissected. The procedure consisted of two stages. First the shells were broken to remove the visceral mass which was then carefully sectioned utilizing needles and examined for larval trematodes under a stereoscopic microscope.

The prevalence of each molluscan sample was estimated after the conclusion of the dissections. This rate was defined as "the number of hosts infected with one or more individuals of a parasite species divided by the total number of hosts examined for that parasite species" (Bush et al., 1997).

Rediae and cercariae were studied *in vivo* (with and without vital Lugol´s iodine solution) under a light microscope. Approximately 10 larvae of each developmental stage were studied. All measurements are in micrometers and were performed with the aid of the Image J software (National Institutes of Health, version 1.52p).

Taxonomic identification of snails and trematodes found was conducted based on Combes et al. (1980), Schell (1985), Thatcher (1993), Barbosa (1995), Gibson et al. (2002), Jones et al. (2005), Paraense & Pointier (2003), Bray et al. (2008) and Pinto & Melo (2013a,b).

The malacological collection took place around the main lake of Quinta da Boa Vista Municipal Park in Rio de Janeiro, RJ. The mollusk breeding site consisted of a sunny shallow puddle filled with aquatic vegetation and grass.

Approximately 100 snails were sampled. They were identified as belonging to the species *Biomphalaria glabrata* (Say, 1818) (Planorbidae) and *Physa acuta* (Draparnaud, 1805) (Physidae). The presence of a renal crest and reddish hemolymph (Lima, 1995) confirmed the identification of *B. glabrata*. For the identification of *P. acuta*, recommendations contained in Thiengo et al. (2004) were followed. The species *P. acuta* and *Physa cubensis* Pfeiffer, 1839 were synonymized by Paraense & Pointier (2003) due to similar shell and general anatomy.

Seventy-one snails belonged to the species *B. glabrata* and 27 snails to the species *P. acuta*. Among *B. glabrata*, only 46 snails were measured due to the high mortality rate under laboratory conditions. Shell diameters ranged from 2 to 17 mm in *B. glabrata*. In *P. acuta*, shell lengths varied from 4 to 6 mm.

The screenings revealed the presence of larval stages of trematodes infecting *B. glabrata*. Eleven of the 71 snails collected were infected, resulting in a prevalence rate of 15.5%. Rediae and cercariae were found in different stages of development.

The morphology of the redia found in *B. glabrata* is described as follows: cylindrical body (0.03-0.44 mm length; 0.01-0.06 mm width) with the presence of an oral opening anterior to the muscular pharynx (Figure 1A). Intestine as elongated tube, with a dark brown pigmentation. Presence of at least one procrusculus in the lateroposterior region and one tail. With developing cercariae (Figure 1B).

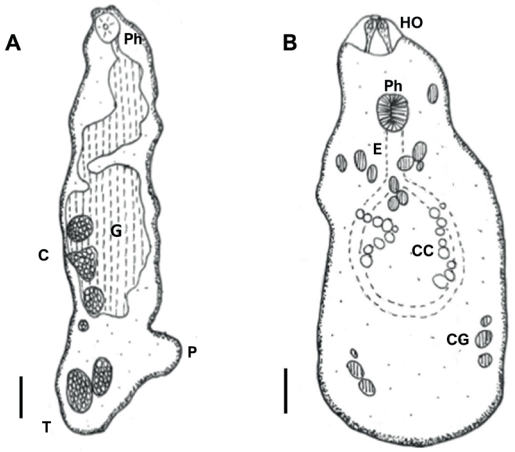

Figure 1. Redia (A) and cercaria (B) obtained in *Biomphalaria glabrata* snails collected in Quinta da Boa Vista Municipal Park, Rio de Janeiro, RJ. C= developing cercariae; CC= circular concretions; CG= cystogenous glands; E= esophagus; G= gut; HO= head organ; P= procrusculus; Ph= pharynx; T= tail. Scale bars= 0.9 mm (A) and 0.03 mm (B).

The morphology of the cercaria found in *B. glabrata* is described as follows: elongated oval body (0.25-0.28 mm length; 0.07-0.11 mm width), with tegument coated by minute spines. Presence of several glands, possibly cystogenous and penetration glands. Absence of stylet and ocelli. Prepharynx not observed. Presence of globular pharynx. Wide but short esophagus. Absence of ventral sucker. Intestinal ceca fused posteriorly. Presence of 11-12 circular concretions located in the main excretory tubules. Excretory vesicle and flame cells not observed. Absence of tail in the posteroventral part of the body (Figure 1B). Cercariae encysted promptly after removal from molluscan visceral mass.

Approximately 100 snails were collected around the pond in a public park in northern Rio de Janeiro. The importance of this finding is in the fact that the species found, *B. glabrata* and *P. acuta*, may harbor helminth parasites, including *Schistosoma mansoni*. Although *B. tenagophila* is the main species described in Rio de Janeiro (Lima, 1995), *B. glabrata* has been previously found in several sites in the city, including Oswaldo Cruz Foundation (FIOCRUZ) and Federal University of the State of Rio de Janeiro (UNIRIO) campi (Fernandez et al., 2001; Alexandre et al., 2017). Although there are no current risks of human infection with schistosomiasis as in other municipal parks (Massara et al., 2012), the present study reinforces the importance of monitoring park water bodies to better understand the risk of helminth transmission to local communities. In Quinta da Boa Vista Municipal Park, for instance, children are usually found swimming in the lake on warm days (Berthone, 2015).

The correlation between size and age is common among snails (Thiengo, 1995). In the present work, the class size frequency distributions of *B. glabrata* and *P. acuta* were unimodal, peaking at 4 mm in diameter and 5mm in length, respectively. Both samples are characterized as young (Paraense, 1987; Lima, 1995). This may explain the high mortality rate of *B. glabrata* under laboratory conditions as well as the absence of infected snails in *P. acuta*.

Among the 71 *B. glabrata* snails collected, 11 were infected with rediae and cercariae. The prevalence rate of *B. glabrata* snails was estimated at 15.5%. The possible taxonomic identification of the digenetic trematode found is discussed below.

The taxonomic identification of trematodes has focused on the morphology of adult and larval stages. Luhe (1901) suggested that the cercarial excretory system, particularly the distribution pattern of flame cells, would be useful for identifying cercariae at the family level. Later, Richard (1971) and Bayssade-Dufour (1979) demonstrated that the mapping of tegumentary sensory receptors is also of taxonomic value, especially when combined with the cercarial type (Schell, 1985), which is also specific at the family level (Bogéa et al., 2005).

The cercarial type of the trematode obtained in the present study is characterized by the absence of a posteroventral tail. This type is classified as "cercariaeum" (Pinto & Melo, 2013a), characteristic of digenetic trematodes whose means of transmission to the vertebrate host involve the ingestion of larval stages (Schell, 1985).

According to Pinto & Melo (2013b), the families Brachylaimidae, Eucotylidae, Leucochloriidae and Typhlocoelidae present "cercariaeum" infecting Brazilian snails. Of the families mentioned above, only the family Typhlocoelidae presents trematodes that develop in rediae (Gibson et al., 2002). Among typlocoelids, there are records of larval stages of the species *Thyphlocoelum cucumerinum* (Digenea) infecting Brazilian snails (Schafranski et al., 1975).

Thyphlocoelum cucumerinum has been reported in Rio de Janeiro State since the 19th century. The first record is by Magalhães (1888), followed by detailed descriptions as *Thyphlocoelum obovale* by Travassos (1921). Travassos also reported that Magalhães attributed to *T. cucumerinum*, formerly *Monostoma flavum*, a suffocating, sometimes fatal, parasitic infection in mallards found in Rio de Janeiro. Fernandes (1980) registered records of this trematode in the city of Niterói, RJ.

Schafranski et al. (1975) described larval forms of *T. cucumeriunum* in *B. glabrata* and *Biomphalaria tenagophila* infected experimentally with miracidia obtained from duck feces collected in the municipality of Jequitibá, Minas Gerais State. The redia described by Schafranski and colleagues were cylindrical, with an oral opening anterior to the long muscular pharynx. It had two pairs of procrusculi, with the anterior one less developed than the posterior one. Several embryonic cells were seen, and the posterior extremity ended in a wide rather than long tail. The rediae described in the present study are morphologically similar to that of *T. cucumerinum* except for the number of procusculi observed. The variation in length is mostly due to different developmental stages as observed by Schafranski and collaborators.

The cercaria of *T. cucumerinum* was also described by Schafranski and collaborators. It presented a dorsum-ventrally flattened ovoid body without a tail. This cercaria was characterized by the absence of oral and ventral suckers, a head organ and a tegument coated with minute spines, concentrated in the anterior end. A muscular globular pharynx was observed. A short esophagus bifurcated into two ceca that merge posteriorly. The excretory system included a globose excretory vesicle located in the posterior region of the cercarial body (Schafranski et al., 1975).

The cercaria described in the present work resembles that of *T. cucumerinum*. It also develops in rediae inside freshwater snails, presenting an ovoid body structure with tegumentary spines, absence of oral and ventral suckers and posteroventral tail. A structure, possibly glandular, was observed in the anterior part of the cercarial body. Its excretory system presents a small globular vesicle in the posterior part of the body. The presence of a muscular pharynx, a short esophagus and numerous glands dispersed throughout the body of the cercaria were also noted. The intestinal ceca, characteristically joined at the posterior end of the cercarial body, were also visible.

Schafranski and collaborators (1975) also described the biological cycle of *T. cucumerinum*. The egg containing the miracidium is eliminated in the duck and mallard feces. After 30-60 minutes in contact with water at 28°C, the miracidium hatches from the egg. When finding *B. glabrata* and *B. tenagophila*, it adheres to the molluscan foot, allowing the penetration of the redia ant its migration to the pericardial cavity. After a few days, the cercariae were observed at different stages of development within the rediae. From the thirteenth day of infection, the cercariae encysted into metacercariae, ready to

infect the definitive host, usually ducks, mallards and waterfowl that feed on snails. The parasite reaches its maturity in the airways of the vertebrate hosts after 70 to 75 days. Studies report adults of *T. cucumerinum* parasitizing nasal cavities, trachea, air sacs, ventricles and, more rarely, intestines of waterfowl *Cairina moschata domestica* and *Anas boschas domestica* (Travassos, 1921, Fernandes, 1980).

Several species of aquatic birds are present in the Quinta da Boa Vista Municipal Park. Besides the various representatives of the order Anseriformes, other species may also act as definitive hosts. The large white heron *Ardea alba* and the Moorish grace *Ardea cocoi*, for instance, are found in the park (Museu Nacional, 2020). It is worth noting that the species *A. cocoi* usually feeds on snails. Future studies are particularly encouraged to confirm the taxonomic classification of this trematode. Such confirmation can be obtained by experimental infection of birds with cercariae obtained from infected *B. glabrata* snails and/or *B. glabrata* snails with miracidia obtained from eggs collected in the feces of infected birds. Molecular biology methods, such as PCR-RFLP and nested PCR, have also been particularly useful in identifying cercariae shed by their molluscan hosts (Abath et al., 2006).

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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