



## RESEARCH ARTICLE

# Larval trematodes hosted by *Biomphalaria straminea* in the Brazilian semiarid region: implications for schistosomiasis control

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## ARTICLE HISTORY

Received: 29 May 2021  
Revised: 8 November 2021  
Accepted: 8 November 2021  
Published: 20 February 2022

## ABSTRACT

The present study aimed to identify larval trematodes shed by snails found in water bodies used by urban communities in a former schistosomiasis endemic area in the state of Piauí, in the Brazilian semiarid region. A malacological survey was performed followed by analysis of the cercariae shed by the snails after light exposure. *Biomphalaria straminea* specimens (n=1,224) were obtained from all seven collection sites. Cercariae shed by snails were i) single tailed, in which one type of cercariae was identified (*Echinostoma* cercariae), and ii) with bifurcated tail (brevifurcate apharyngeate distome, brevifurcate pharyngeate distome, and longifurcate pharyngeate distome [strigeocercaria]). Brevifurcate apharyngeate distome were further examined and the presence of spikes in swimming membranes enabled the identification of Spirorchidae cercariae in all individuals, demonstrating the absence of cercariae compatible with *Schistosoma mansoni*. Nevertheless, the accurate diagnosis of *S. mansoni* circulation in former endemic areas is still necessary.

**Keywords:** Schistosomiasis; malacological survey; cercariae; Brazilian semiarid region.

## INTRODUCTION

According to estimates by the World Health Organization (WHO, 2019), schistosomiasis - a neglected tropical disease caused by *Schistosoma* trematodes - affects about 290 million people in 78 countries, occurring mainly in Sub-Saharan Africa, Asia and South America (Massara *et al.*, 2016; Dubeux *et al.*, 2019). Schistosomiasis is endemic in some Brazilian regions and associated with poverty and lack of sanitation (Campos *et al.*, 2019). Brazil has the highest number of cases among western countries (Saucha *et al.*, 2015) with estimates of 2.5 to 8 million affected by the disease. The Northeast and Southeast regions are the most affected, with the states of Pernambuco, Bahia, Alagoas and Sergipe presenting the highest prevalence rates (Rocha *et al.*, 2016; Pereira *et al.*, 2017).

*Schistosoma mansoni* is the only species causing schistosomiasis in Brazil. Transmission occurs when infected individuals lay their faeces with parasite eggs in water bodies infested by snails, which become infected by miracidia that hatch from the eggs. Infected snails release cercariae that can penetrate the skin of humans, evolving to

schistosomules that will develop to adult worms, which copulate and lay eggs in the mesenteric venous plexus (Barbosa *et al.*, 2018; Carvalho *et al.*, 2018).

Planorbid snails belonging to the *Biomphalaria* genus are the intermediate hosts of *S. mansoni* (Barbosa *et al.*, 2017; Simões *et al.*, 2017). In Brazil *B. glabrata*, *B. straminea* and *B. tenagophila* have been detected naturally infected with *S. mansoni* (Fernandez *et al.*, 2018; Palasio *et al.*, 2019). These snails are present in extensive areas in Brazil and can be found in freshwater collections with speeds below 30 cm/s, streams, ponds, swamps, river backwaters, reservoir margins or artificial collections (irrigation and drainage ditches, water tanks and even small dams) (Ohlweiler *et al.*, 2013). *B. straminea* originated in Brazil but expanded its habitats to surrounding countries, demonstrating the capacity to resist to drought and great fertility (Meier-Brook, 1974; Paraense & Corrêa, 1987). It was introduced in China as an invasive species in 1974, possibly through tropical aquarium plants or fish trade, adapting well to the continent, dispersing considerably and becoming a health concern in Asia (Meier-Brook, 1974; Attwood *et al.*, 2015; Yang *et al.*, 2018). The occurrence of floating or vertical vegetation serves as shelter,

food and support for the spawning of these animals (Paraense, 1961; Camargo et al., 2016). Several species of parasitic trematodes of fish, birds, amphibians, and mammals use *B. straminea* to generate cercariae.

In the state of Piauí, in northeastern Brazil, schistosomiasis transmission occurs in a focal pattern and is virtually restricted to the municipality of Picos where the prevalence of the disease has been maintained below 1% (Brasil, 2011). *B. straminea* is the only snail species found in the region (Silva et al., 2019) it is present in the Guaribas River, which runs through several urban and rural neighbourhoods. This snail species is the most successful and adapted to climate variation, it can be found in almost all Brazilian hydrographic basins (Terra et al., 2018). Despite being less susceptible to *S. mansoni* infection than *B. glabrata* (Bezerra et al., 2018), *B. straminea* has a wide distribution and is responsible for maintaining human infection rates above 50% in some locations in northeastern Brazil (Quinino et al., 2010; Neto et al. 2012; Costa et al., 2017). *B. straminea* has previously been identified in 24 Brazilian states and usually inhabits temporary and permanent water collections, being more adjusted to the dry climate of the Northeast (Paula-Andrade et al., 2019; Ohlweiler et al., 2020).

Studies on schistosomiasis vectors in the state of Piauí are yet to be performed and are necessary to improve control strategies. The present study aimed to assess snail species and larval trematodes shed by them in water bodies within the urban perimeter of the municipality of Picos, state of Piauí.

## MATERIALS AND METHODS

### Setting and collection sites

Collections were performed from July to September 2019 in the municipality of Picos, situated in the semi-arid region of the state of Piauí, 7°04'54" S, 41°28'14" W, with an area of 535,000 km<sup>2</sup> and approximately 78,000 inhabitants (Figure 1). The climate is hot semi-arid tropical, with temperatures ranging from 22°C to 39°C. The average annual rainfall ranges from 800 mm to 1400 mm. The riparian vegetation of the Guaribas River is characterized by a diversity of aquatic macrophytes. The hydrographic basin of the Guaribas River is born in the Serra das Almas, 600 m above sea level and has great importance in relation to water supply for agricultural activities (Viana et al., 2017). The criteria for selecting the collection sites were water bodies that: i) were located in the urban area of Picos, ii) had a record of use by people and iii) were close to areas with some history of confirmed schistosomiasis cases. Seven water bodies were selected: São José, Centro, Canto da Várzea, Passagem das Pedras, Malvas, Ipueiras and Catavento. Each collection site was assessed three times during the study period. In each site, three collection points were selected, totaling 21 points. In the field, an assessment of collection sites was carried out, following an adapted methodology (Guimarães et al., 2012; Bizzo et al., 2014), considering the following characteristics: i) the main type of occupation of the margins, ii) erosion near and/or on

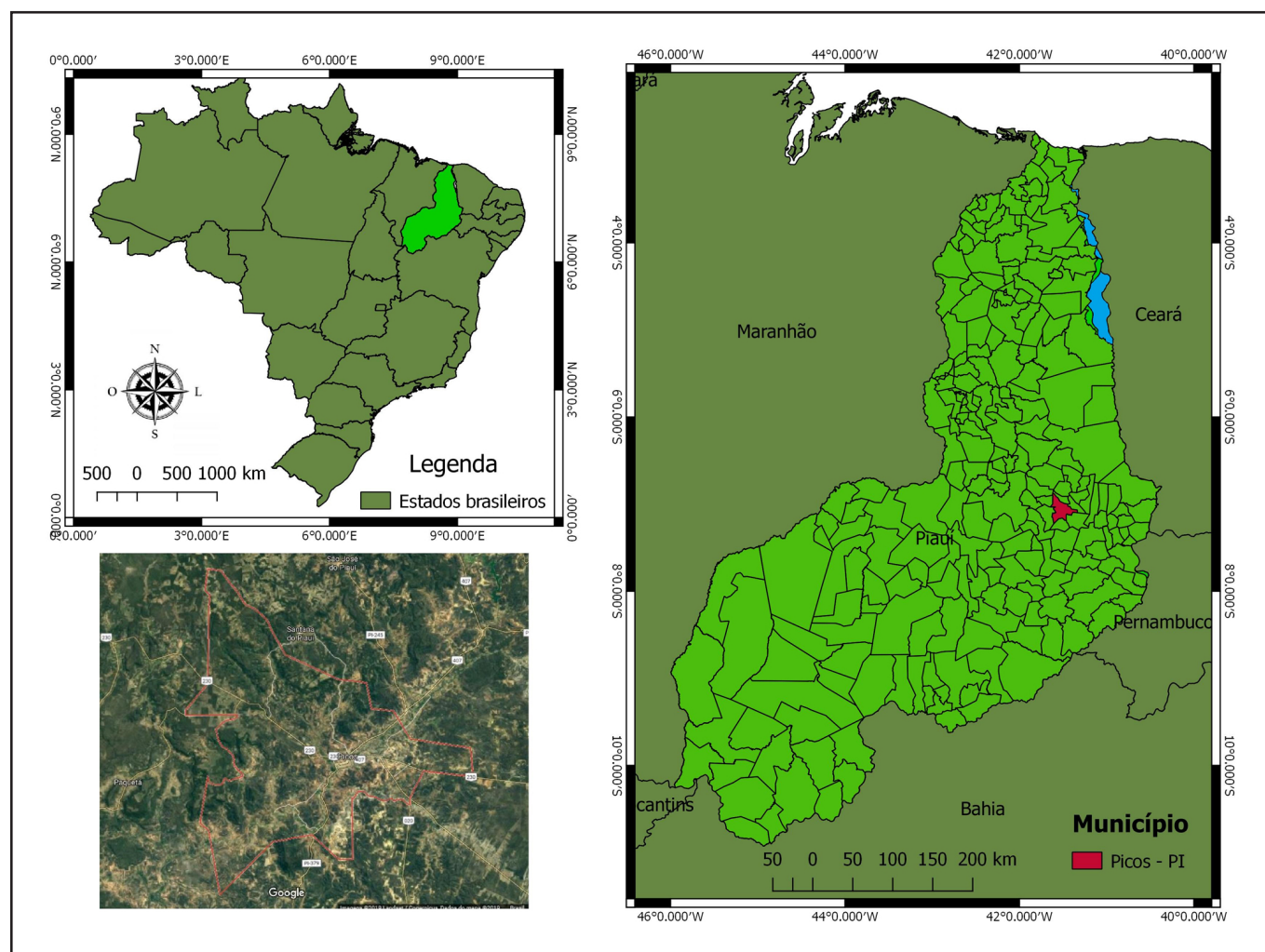


Figure 1. Map depicting the localization of Picos, Piauí, in semi-arid northeastern, Brazil.

the margins and silting, iii) anthropic change in the surroundings, iv) presence and extension of riparian forest, v) presence of human and animal waste, vi) presence of domestic animals, vii) presence of aquatic plants, viii) odour and colour of water, ix) characterization of the bottom, type and odour of the substrate, x) characteristic of the water flow and xi) presence of wild animals. This methodology classifies the conservation status of the collection sites in reduced, moderate or extreme changes.

#### Collection and morphological identification of snails

The snail collections were conducted in 2019, at the seven selected water bodies. The snails were collected with a metal catching shell, with holes around 2 mm and bottom of 15 cm, coupled to a wooden rod of 1.5 m in length with the aid of tweezers with fine tips, which was dipped until it reached the surface portion of the river bottom. Up to 10 snails were put in each plastic bottle. The snails were sent to the Laboratory of Parasitology, Ecology and Neglected Diseases of the Federal Institute of Education and Technology of Piauí (IFPI), in Picos, for quantification. Subsequently, the collected specimens were transferred to plastic bottles with dechlorinated water, identified and protected by a plastic lid. The snails were fed weekly, on alternate days, with fresh lettuce properly cleaned. About 10% of the specimens collected were submersed in water at 70°C. The soft parts were removed from the shells, fixed in Railliet-Henry liquid and dissected in a stereomicroscope (Deslandes, 1951). In addition to the collection of snails, environmental data such as temperature, pH and water depth were collected at each site.

#### Identification of trematodes larvae shed by snails

To verify the infection by trematodes, specimens were exposed to an incandescent light spot for 4 hours. The water in the containers was examined through a stereomicroscope to visualize trematodes cercariae (Brasil, 2014). Water samples of cercariae-positive bottles were removed and stained with Lugol (5%) for morphological identification. A specific identification key was used for this process (Pinto & Melo, 2013).

#### Geospatial and statistical analyses

Maps were made in the QGIS program version 3.10, using shapes available on the IBGE (*Instituto Brasileiro de Geografia e Estatística*) website. They were produced using the reference system SIRGAS 2000 / UTM zone 23S and the georeferencing points were marked using a portable GPS following the UTM system. Linear regression was performed to assess the correlation between abiotic parameters of collection sites and the number of snails collected.

#### Ethical Approval

The research protocol (number 60423-5) was approved and submitted by the Biodiversity Authorization and Information System (SISBio) to legal authorization for cataloguing / collecting wild animals, according to the legislation in the country.

## RESULTS

#### Morphological identification of snails

*Biomphalaria* specimens (n=1, 224) were obtained from all seven collection sites in the municipality of Picos. Through morphological analysis, a conspicuous vaginal wrinkling was detected in the reproductive system of all the snails; therefore, they were identified as *B. straminea*.

#### Spatial distribution of snails and correlation with conservation status and abiotic characteristics of collection sites

Table 1 and Figure 2 present the distribution of snails by collection site. Regarding the distribution, the presence of *B. straminea* was greater in the collections of São José and Centro, where 667 and 273 snails were found, respectively. The Catavento and Ipueiras collections had the lowest numbers of specimens, 25 and 17 in total, respectively. As shown in Figure 3, no correlation was observed between the abundance of *B. straminea* specimens and the abiotic characteristics of the water at the collection sites, including pH, temperature, and depth.

#### Trematode larvae identified in *Biomphalaria straminea* specimens

Four cercariae types were shed by *B. straminea* caught in six water collections: São José, Centro, Canto da Várzea, Passagem das Pedras, Malvas and Ipueiras. These cercariae types were classified into two main groups: i) the group of cercariae with single tail, in which one type of cercariae was identified (*Echinostoma* cercariae), and ii) the group of cercariae with bifurcated tail, in which three cercariae types were identified (brevifurcate apharyngeate distome, brevifurcate pharyngeate distome, and longifurcate pharyngeate distome [strigeocercaria]). Brevifurcate apharyngeate distome were further examined following the key proposed by Pinto and Melo (2013) to differentiate Schistosomatidae and Spirorchiiidae cercariae. The presence of spikes in swimming membranes enabled the identification of Spirorchiiidae cercariae in all individuals (Figure 4). Figure 5 presents the spatial distribution of distinct cercariae types in each collection site. Among the four cercariae types identified, brevifurcate apharyngeate distome presented the widest spatial distribution, present in six of the seven sites, followed by *Echinostoma*, present in three collections, brevifurcate pharyngeate distome, present in two collections and longifurcate pharyngeate distome, present in only one water body.

## DISCUSSION

Considering the intermediate host species of schistosomiasis, only *B. straminea* was present in the water collections assessed in Picos, Piauí. The abundance of this snail species in the studied communities supports the data stating that it is adapted to the Brazilian semiarid region (Carvalho et al., 2008a). Although *B. straminea* is resistant to the release of cercariae when infected, it has high population densities, compensating for its lower susceptibility to *S. mansoni* (Gomes et al., 2016). The greatest abundance of *B. straminea* was observed in the São José and Centro collection sites. This result may be explained by the larger collection area in these sites and by the presence of lentic water, vegetation and organic (muddy) substrates, which provide favourable conditions for the distribution of snails (Carvalho et al., 2008b).

In the present study, four types of digenetic trematodes larvae were shed by *B. straminea* specimens found in the water collections analysed. Longifurcate pharyngeate distome (Strigeocercaria) may belong to the Diplostomidae and Strigeidae families. They are characterized by having a developed ventral sucker and by being intestinal parasites of birds and mammals. Their metacercariae are formed in annelids, mollusks, fish or amphibians. Considering the Northeast Region of Brazil, Rodrigues et al. (2017) report the occurrence of this cercariae in *B. straminea* and *B. glabrata* snails in a malacological survey carried out in the municipalities of São Bento and São Luis, in the State of

**Table 1.** Number of *Biomphalaria straminea* specimens obtained, conservation status, abiotic characteristics and types of cercariae identified by light exposure of snails at different collection sites in Picos, Piauí, Brazil, 2019

Collection site	Snails collected	Conservation status*	Mean number of snails collected $\pm$ SD in each collection	Mean pH $\pm$ SD	Mean temperature $\pm$ SD (°C)	Mean deep $\pm$ SD (meters)	Cercariae types identified in snails
São José	667	Reduced/moderate alterations	222 $\pm$ 88	7.86 $\pm$ 0.32	30 $\pm$ 1.4	17.7 $\pm$ 2.5	Distoma brevifurcate apharingeate + Distoma longifurcate pharingeate
Centro	273	Moderate/extreme alterations	91 $\pm$ 62.1	7.70 $\pm$ 0.00	33.1 $\pm$ 2.9	7.3 $\pm$ 1.5	Distoma brevifurcate apharingeate + <i>Echinostoma</i>
Canto da Várzea	108	Reduced alterations	36 $\pm$ 27.8	7.53 $\pm$ 0.23	30.7 $\pm$ 0.7	23.7 $\pm$ 8.0	Distoma brevifurcate apharingeate + <i>Echinostoma</i>
Passagem das Pedras	102	Moderate alterations	34 $\pm$ 22.3	7.66 $\pm$ 0.21	31 $\pm$ 3.1	15.6 $\pm$ 1.2	Distoma brevifurcate apharingeate
Malva	32	Moderate alterations	10.6 $\pm$ 5.5	7.50 $\pm$ 0.20	31.9 $\pm$ 1.2	13.7 $\pm$ 2.3	Distoma brevifurcate apharingeate
Ipueiras	17	Moderate alterations	5.6 $\pm$ 1.2	8.13 $\pm$ 0.50	30.3 $\pm$ 3.9	16.6 $\pm$ 5.8	Distoma brevifurcate apharingeate, Distoma brevifurcate pharingeate + <i>Echinostoma</i>
Catavento	25	Extreme alterations	8.3 $\pm$ 10.4	8.40 $\pm$ 0.30	29.3 $\pm$ 2.5	10 $\pm$ 2.0	Distoma brevifurcate pharingeate

\*Evaluated through the score of points generated by the following indicators: main type of occupation of the margins, erosion near and/or on the margins and silting up, anthropic change in the surroundings, presence and extension of riparian forest, presence of human and animal waste, presence of domestic animals, presence of aquatic plants, odor and color of water, characterization of the bottom, type and odor of the substrate, characteristic of the water flow, and presence of wild animals (Guimarães et al., 2012; Bizzo et al., 2014).



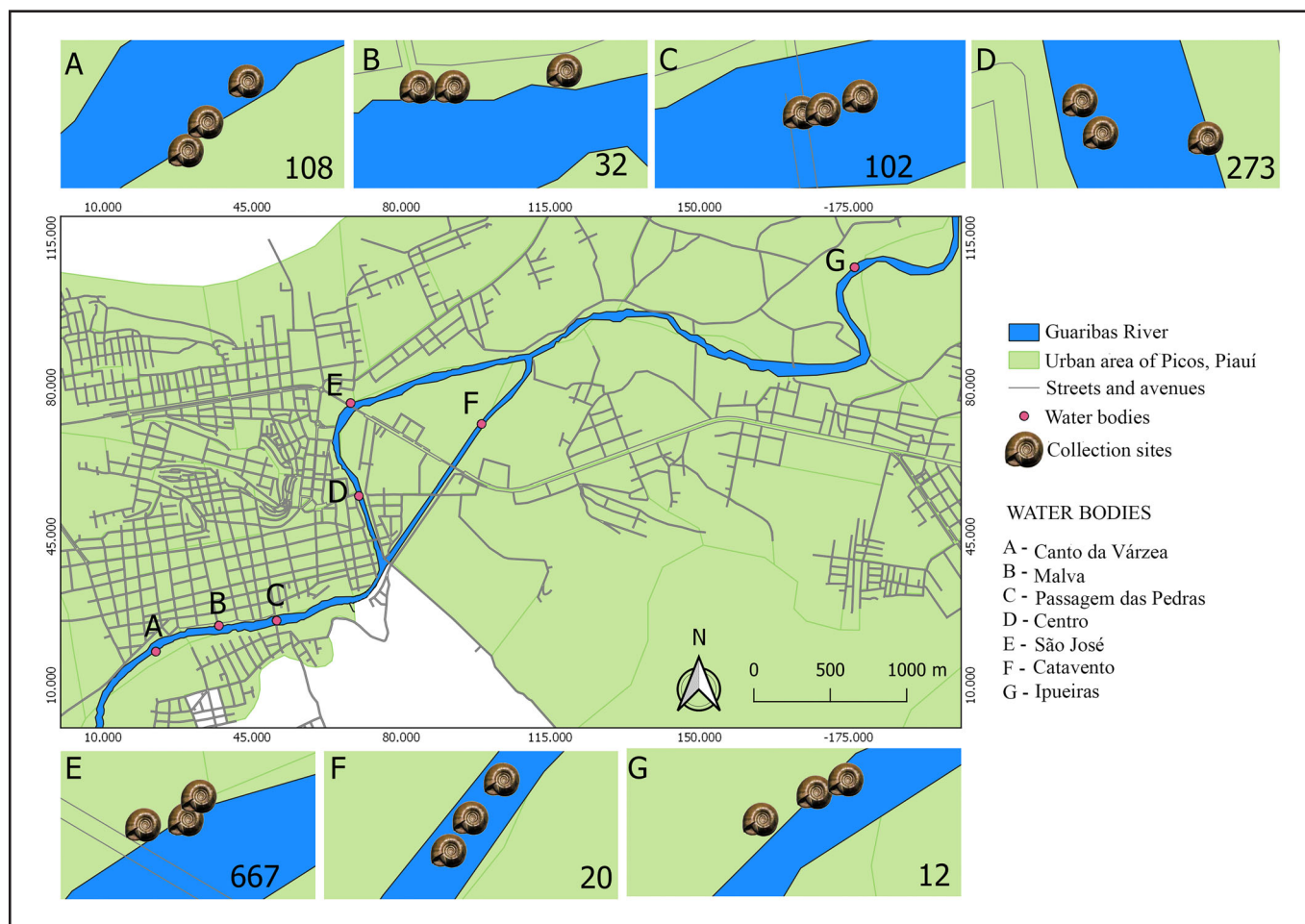


Figure 2. Spatial distribution of *Biomphalaria straminea* by collection site in Picos, Piauí, Brazil.

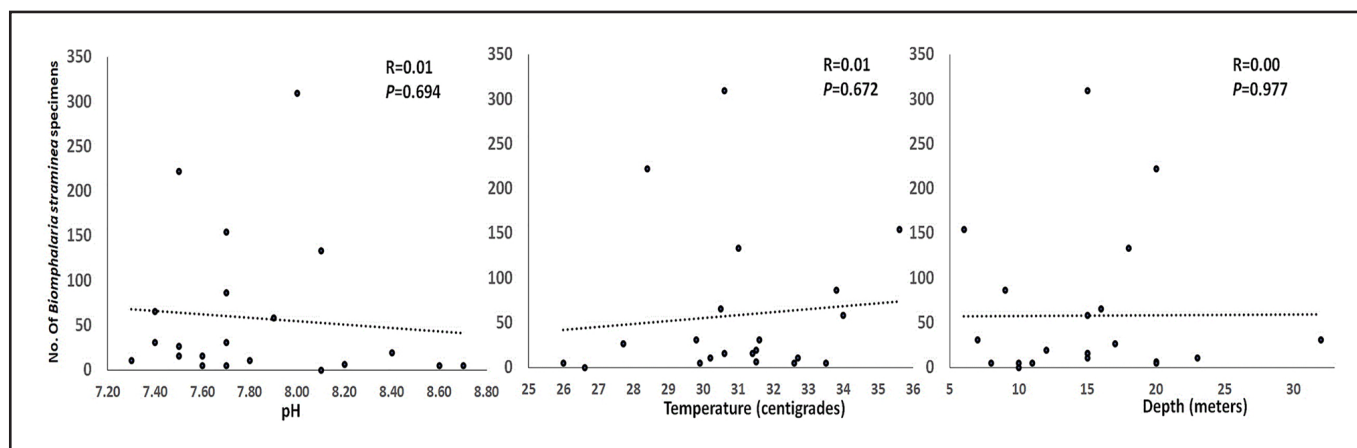
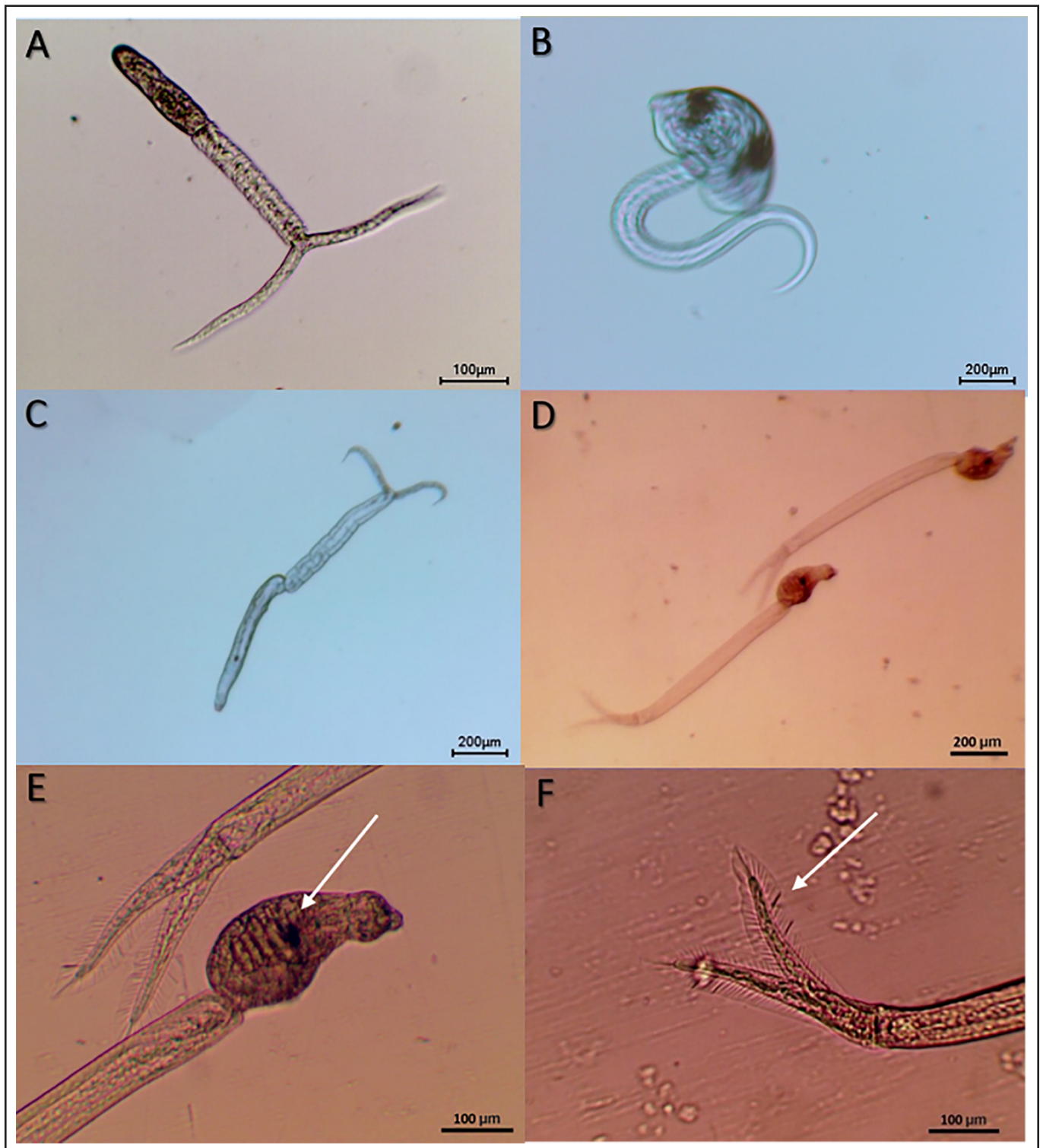


Figure 3. Correlation between abundance of *Biomphalaria straminea* specimens and the abiotic characteristics of the water at the collection sites, Picos, Piauí, as assessed through linear regression analysis.

Maranhão. Silva et al. (2021) also identified this type of cercariae infecting *B. straminea* in the Brazilian state of Alagoas.

*Echinostoma* cercariae have also been detected in *B. straminea* from Picos. They represented larval stages of trematodes belonging to the families Echinostomatidae and Psilostomidae. Cercariae of these groups, after being released by snails, penetrate other mollusks, fishes, and amphibians. Adult individuals can parasitize different

classes of vertebrates. Souza et al. (2006) and Souza and Melo (2012), registered the presence of *Echinostoma* cercariae in *B. glabrata*. Souza et al. (2008) registered cercariae from the family Diplostomatidae, Strigeidae, Echinostomatidae and Strigeidae in studies conducted in Pernambuco. Silva and Melo (2013), in a study performed in Ouro Branco, Minas Gerais, identified *Echinostoma* cercariae infecting *B. tenagophila*, being the most frequent cercariae during the survey. Human echinostomiasis is endemic in the Asian

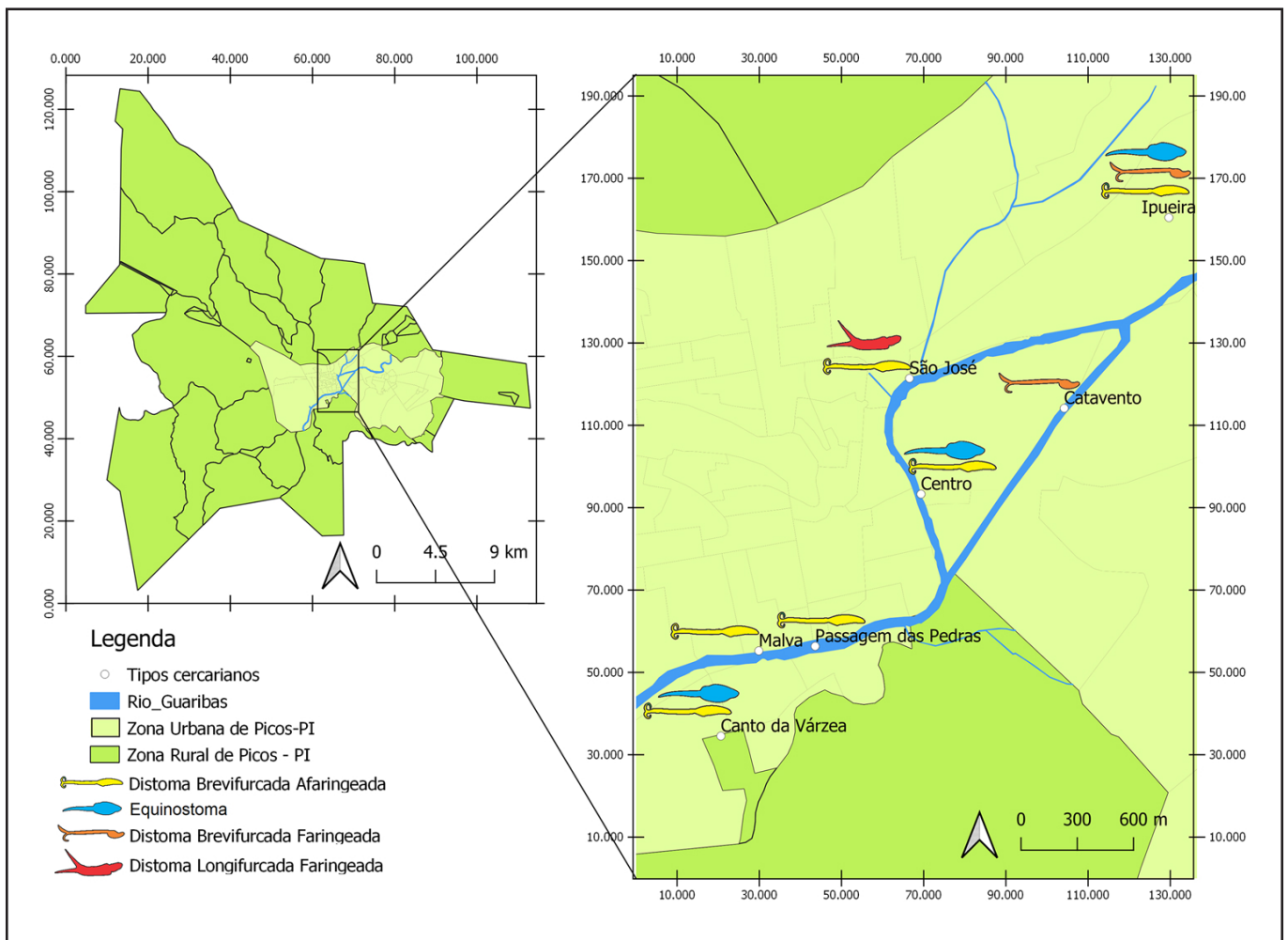


**Figure 4.** Cercariae types found in the different water collections analysed in the municipality of Picos, Piauí, Brazil: Strigeocercaria (A), Echinostoma (B), Brevifurcate apharyngeate distome (C), Brevifurcate pharyngeate distome (D) and the anatomical structures of cercaria: Ocellus (E) and Caudal spikes (F).

continent, but data on morbidity are scarce. Human infections have not been recorded in Brazil but, in paleoparasitology studies, eggs were detected in human coprolites (Sianto *et al.*, 2005; Leles *et al.*, 2014). *Echinostomes* represent a large group of enteric trematodes infecting distinct vertebrate hosts, including humans, which can acquire infection by ingesting fish, snails, crustaceans, and frogs. Although endemic foci of human infections seem to be restricted to

Asia, the susceptible population may be expanding (Toledo & Esteban, 2016).

Larvae classified as brevifurcate apharyngeate distome are produced in sporocysts by trematodes belonging to the Schistosomatidae and Spirorchidae families. The morphological differentiation between specimens from these two families is possible through light microscopy, because in Spirorchidae there are pigmented ocelli



**Figure 5.** Spatial distribution of distinct cercariae types in each collection site, Picos, Piauí, Brazil.

and spikes in natatory structures, which are absent in Schistosomatidae cercariae. These structures must be considered so there are no errors in the identification of individuals, considering that in some schistosomiasis endemic regions there is also the occurrence of trematodes belonging to the Spirorchidae family. These larvae can parasitize the circulatory system of birds and mammals and the blood system of turtles Smith (1997). In the Schistosomatidae family there are species that only infect birds. The studies on this type of cercariae have gained increasing importance, as they may be involved in the occurrence of cercariae dermatitis in humans (Kolářová et al., 2013; Horák et al., 2015; Macháček et al., 2018).

The brevifurcate pharyngeate distome cercariae are produced by representatives of the Clinostomidae family, which parasitize the oral cavity of birds and their metacercariae are formed in fish, with cases of accidental infection in humans previously reported (Yamashita, 1938; Kifune et al., 2000; Park et al., 2009; Hara et al., 2014; Lee et al., 2017). These cercariae are differentiated from others of the brevifurcated type by the presence of a pleated membrane in the dorsal region of the body, a bulb-shaped caecum, in addition to requiring a second intermediate host for the life cycle to be completed.

The present study expanded the knowledge on trematode richness in the semiarid Brazil. Up-to-date information on the distribution and characterization of trematode larvae populations is essential and contributes to the elucidation of biological interactions of the parasite-

host relationship. The absence of *S. mansoni* cercariae corroborates schistosomiasis control program data, suggesting interruption of transmission in the urban area of the municipality. Nevertheless, as there are isolated records of cases in some rural areas from Picos, the municipality remains classified as a focal area for schistosomiasis transmission.

The possibility of cercariae misidentification by technicians must be pondered for schistosomiasis control, considering that the methodology performed by malacological surveillance teams consists of crushing the snails. This process makes difficult to visualize anatomic diagnostic structures in trematode larvae. Nonetheless, despite widely used in several studies, the classic light exposure method has limitations, mainly in low parasitic loads, interruption of sporocyst development or death of snails after exposure. Thus, these limitations prevent the assessment of *S. mansoni* circulation in endemic regions and point to the need of molecular strategies to obtain more accurate data.

Therefore, other assessment tools are important to be used to confirm the interruption of the active transmission of schistosomiasis mansoni in the region, such as the periodic performance of parasitological tests of feces in humans, as well as their complementation using serological and molecular tests. Another important tool is malacological surveillance with identification and mapping of areas with the presence of vector mollusks.



Health education actions are another important means of interrupting the disease at school and community levels, through mass dissemination of information and awareness of the importance of carrying out diagnostic test and treatment for the disease.

In summary, the survey of larval trematode fauna infecting snails in water bodies used by communities in the Brazilian semiarid region is complex and requires detailed analysis and identification procedures.

#### ACKNOWLEDGMENTS

The authors would like to thank the professionals involved, in particular, communitarian health agents of Picos, Piauí and the Graduate Program in Tropical Medicine of Oswaldo Cruz Institute, FIOCRUZ.

#### Conflicts of interest

The authors declare that they have no conflict of interest.

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