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Major Article

Freshwater gastropods of the Baixada Maranhense Microregion, an endemic area for schistosomiasis in the State of Maranhão, Brazil: I - qualitative study

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ABSTRACT

Introduction: The Baixada Maranhense Microregion currently has the highest prevalence of schistosomiasis in the State of Maranhão, likely because this parasitosis is characterized as an occupational disease, and increased contact with water increases the risk of infection by *Schistosoma mansoni*. This paper reports the results of the first comprehensive freshwater malacological survey performed in the Baixada Maranhense Microregion. **Methods:** Freshwater mollusks were collected from the twenty-one municipalities of the Baixada Maranhense Microregion and from Bacurituba and Cajapió and were evaluated for infection by trematodes. **Results:** A total of 9,129 mollusks were collected (sixteen species), which included the first records of six species in the State of Maranhão: *Gundlachia radiata*, *G. ticaga*, *Hebetancylus moricandi*, *Plesiophysa guadeloupensis*, *Pomacea bridgesii diffusa* and *Omalonyx* sp. *Biomphalaria glabrata* was found in five municipalities, whereas *B. straminea* was found in nine. *Biomphalaria glabrata* and *B. straminea* were observed in syntopy in Pinheiro and São Bento. Of the 990 specimens of *B. glabrata* and the 2,109 specimens of *B. straminea* that were exposed to and/or analyzed for the presence of larval trematodes, only a single specimen of *B. glabrata* (0.1%) from São Bento shed *S. mansoni* . Other larval trematodes were first observed in mollusks from the State of Maranhão. **Conclusions:** These results indicate that the study area is epidemiologically important due to the presence of two natural vectors of schistosomiasis and the active transmission of schistosomiasis, which was confirmed in the infected specimen that was collected in this study.

Keywords: Freshwater snails. *Schistosoma mansoni.* Vectors. Baixada Maranhense Microregion. Brazil. Cercaria.

INTRODUCTION

Of the snail-transmitted diseases, schistosomiasis is undoubtedly the most important to public health. In Brazil, schistosomiasis occurs in 19 states, which represents a large endemic area along the coast from the State of Maranhão to Espírito Santo and Minas Gerais. Schistosomiasis also occurs in isolated foci within the States of Piauí, Pará, Goiás, Distrito Federal, São Paulo, Rio de Janeiro, Paraná, Santa Catarina and Rio Grande do Sul[1,2]. The disease has been recorded in Maranhão since 1920[3], its distribution is endemic in 20 of 217 municipalities and 29 municipalities have reported foci[4]. The two snail vectors *Biomphalaria glabrata* (Say, 1818) and *Biomphalaria straminea* (Dunker, 1848) have been reported in 30 and 40 municipalities, respectively[5].

The Baixada Maranhense Microregion, which is one of the seven ecological regions of Maranhão, represents the main group of lacustrine basins in the northeast and is a complex ecological system that consists of temporary, shallow, marginal and permanent lakes that are commonly known as flooded grasslands. Despite being an interesting ecological system, the Baixada Maranhense Microregion is not protected from environmental damage, such as extensive-breeding buffalo farming, irrigation projects and dam construction[6]. Currently, the Baixada Maranhense Microregion shows the highest prevalence of schistosomiasis in the state[4]. This parasitosis is characterized as an occupational disease because activities such as fishing, hunting and farming, which result in increased contact with water, also increase the risk of infection by *Schistosoma mansoni* (Sambon, 1907)[7].

Therefore, this paper reports the results of the first comprehensive freshwater malacological survey performed in the Baixada Maranhense Microregion. This study investigated the distribution of snail vectors of *S. mansoni* and contribute to a better understanding of schistosomiasis transmission within this Microregion.

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METHODS

The Baixada Maranhense Microregion is 17,909km^2 and corresponds to approximately 5.4% of the total area of Maranhão, which includes 21 municipalities[8]. Freshwater mollusks were collected from each of the municipalities (Anajatuba, Arari, Bela Vista do Maranhão, Cajari, Conceição do Lago Açu, Igarapé do Meio, Matinha, Monção, Olinda Nova, Palmeirândia, Pedro do Rosário, Penalva, Peri-Mirim, Pinheiro, Presidente Sarney, Santa Helena, São Bento, São João Batista, São Vicente Férrer, Viana and Vitória do Mearim) and from Bacurituba and Cajapió, which are two neighboring municipalities with geographic characteristics that are similar to those of the Baixada Maranhense Microregion. The specimens were obtained from different biotopes (streams, rivers, marsh areas, drainage and sewage ditches, ponds, flooded areas and irrigation canals) in November 2011 and in March, July and October 2012. Four collectors performed 10-minute searches in each biotope, and all collection sites were georeferenced.

In the *Laboratório de Malacologia* of *Instituto Oswaldo Cruz*/FIOCRUZ, Rio de Janeiro-RJ, specimens were placed in aquaria containing dechlorinated water, substrate and food (fresh lettuce leaves). To search for larvae, the samples were processed following the protocol of Mattos et al.[9]. Except for *Omalonyx* sp., all of the snails were individually exposed to artificial light to determine if they were infected with *S. mansoni* larvae or any other trematode of medical or veterinary importance, and the trematode larvae were identified using key specifics according to Schell[10]. The mollusks were examined using a stereomicroscope, and negative mollusks were crushed to confirm the absence of larval trematodes[11]. Live cercarial stages were obtained by exposure under an incandescent light, and other larval stages were determined during crushing or anatomical dissections. Ten specimens of each sample were anesthetized in Hypnol 0.05% solution for five hours, killed by hot water (70°C) and fixed in Railliet-Henry solution for morphological analysis[11]. Mollusk samples were deposited at the Coleção de Moluscos of Instituto Oswaldo Cruz (CMIOC).

RESULTS

Mollusks were confirmed in 176 of the 230 biotopes that were surveyed **(Table 1)**, including *S. mansoni* vectors **(Figure 1)**. Some species were recorded for the first time, as shown in **Figure 2**.

Five gastropod families were obtained: Ampullariidae, Ancylidae, Planorbidae, Physidae and Succineidae. A total of 9,129 individuals were collected and identified, including 990 specimens of *Biomphalaria glabrata* (Say, 1818); 122 specimens of *Biomphalaria schrammi* (Crosse, 1864); 2,106 specimens of *Biomphalaria straminea* (Dunker, 1848); 275 specimens of *Drepanotrema anatinum* (d'Orbigny, 1835); 56 specimens of *Drepanotrema cimex* (Moricand, 1839); 273 specimens of *Drepanotrema depressissimum* (Moricand, 1839); 1,811 specimens of *Drepanotrema lucidum* (Pfeiffer, 1839); 9 specimens of *Gundlachia radiata* (Guilding, 1828); 9 specimens of *Gundlachia ticaga* (Marcus & Marcus, 1962); 17 specimens of *Hebetancylus moricandi* (d'Orbigny, 1837); 1,047 specimens of *Physa marmorata* Guilding, 1828; 31 specimens of *Plesiophysa guadeloupensis* (Crosse & Fischer *in* Mazé, 1883); 1,424 specimens of *Pomacea maculata* Perry, 1810 and 12 specimens of *Pomacea bridgesii diffusa* (Blume, 1957). In addition, shells or juveniles of Ancylidae (24 shells), *Biomphalaria* sp. (863 shells) and *Omalonyx* sp. (57 juveniles) were obtained.

The highest diversity of mollusks species was observed in the municipality of São Bento, where 12 species were reported. In contrast, the municipality of Cajari had the lowest species diversity (only *P. maculata*).

Of the species of medical and veterinary importance, *B. glabrata* was identified in five municipalities, whereas *B. straminea* was found in nine. These species were observed in syntopy in two biotopes of Pinheiro and in four biotopes of São Bento. Of the 990 specimens of *B. glabrata* and 2,109 specimens of *B. straminea* that were exposed and/or analyzed for the presence of larval trematodes, only a single specimen of *B. glabrata* (0.1%) from the municipality of São Bento shed *S. mansoni* cercariae.

Other types of cercariae were shedded from six different mollusk species **(Table 2)**: Armatae cercaria, Brevifurcate-apharingeate-cercaria, Echinostome cercaria, Monostome cercaria, Ornatae cercaria, Strigea cercaria, Ubiquita cercaria and Vivax cercaria. In addition, the metacercariae of Echinostomatidae were observed in *B. glabrata* specimens from São Bento, Pinheiro and São Vicente Férrer, whereas one specimen of *P. maculata* from São Vicente Férrer was infected with Echinostomatidae rediae.

Echinostome cercaria were the most frequent cercariae found in the mollusks, and the highest percentage of infected snails was recorded in São João Batista (14.2%), followed by Conceição do Lago Açu (8.8%). *Drepanotrema lucidum* was infected with the highest diversity of cercariae (five different types).

Of the fifteen species that were examined, nine contained no larval trematodes: *B. schrammi, Biomphalaria* sp., *D. anatinum, D. cimex, G. radiata, G. ticaga, H. moricandi, P. guadeloupensis* and *P. bridgesii diffusa.*

DISCUSSION

The Baixada Maranhense Microregion is epidemiologically important because of the presence of two natural vectors of schistosomiasis and the occurrence of active transmission of schistosomiasis, which was confirmed based on the infected specimen that was collected. According to the information available at the Schistosomiasis Information System (SISPCE)[2], the Baixada Maranhense Microregion shows a higher prevalence of schistosomiasis than Peri Mirim (7.2% in 2010; 4.07% in 2011) and São Bento (6.12% in 2010; 6.48% in 2011; and 6.13% in 2012).

This study revealed an extended distribution of two species of medical importance: *B. glabrata* in Bacurituba and

TABLE 1 - Families, species and municipalities where mollusks were found in the Baixada Maranhense Microregion, Maranhão, Brazil.

	Ampullariidae		Ancylidae			Planorbidae									Physidae	Succineidae
Municipality	*Pomacea maculata*	*Pomacea bridgesii diffusa*	*Gundlachia radiata*	*Gundlachia ticaga*	*Hebetancylus moricandi*	*Biomphalaria glabrata*	*Biomphalaria schrammi*	*Biomphalaria straminea*	*Biomphalaria* sp.	*Drepanotrema anatinum*	*Drepanotrema cimex*	*Drepanotrema depressissimum*	*Drepanotrema lucidum*	*Plesiophysa guadeloupensis*	*Physa marmorata*	*Omalonyx* sp.
Anajatuba	+		+							+	+	+	+		+	
Arari	+						+	+	+	+	+	+	+		+	+
Bacurituba	+					+	+			+			+		+	
Bela Vista do Maranhão	+								+	+			+			
Cajapió	+			+									+			
Cajari	+															
Conceição do Lago-Açu	+							+	+	+			+		+	
Igarapé do Meio	+							+	+	+			+		+	
Matinha	+						+			+		+	+		+	
Monção	+		+					+	+				+		+	+
Olinda Nova	+									+			+			
Palmeirândia	+	+			+					+			+		+	+
Pedro do Rosário	+						+	+	+	+			+	+	+	+
Penalva	+							+							+	
Peri-Mirim	+				+	+	+			+			+		+	+
Pinheiro	+					+	+	+	+	+	+	+			+	+
Presidente Sarney	+						+		+	+	+		+			
Santa Helena	+				+		+		+	+	+	+	+	+	+	+
São Bento	+	+	+	+		+	+	+	+	+			+		+	+
São João Batista	+									+					+	
São Vicente Férrer	+	+	+	+		+										
Viana	+		+										+			
Vitória do Mearim	+							+	+	+	+	+	+		+	+

B. straminea in Conceição do Lago Açu, Igarapé do Meio, Monção, Pedro do Rosário, Penalva and Vitória do Mearim.

Within the Baixada Maranhense Microregion, *B. glabrata* has reportedly been found in the municipalities of Anajatuba, Palmeirândia, Peri Mirim, Pinheiro, São Bento, São João Batista, São Vicente Férrer and Viana. Previous reports have found *B. straminea* in Arari, Cajapió, Matinha, Palmeirândia, Peri-Mirim, Pinheiro, Santa Helena, São Bento, São João Batista, São Vicente Férrer and Viana[12]. Our study confirms the existing data regarding the occurrence of *B. glabrata* in Peri Mirim, Pinheiro, São Bento and São Vicente and *B. straminea* in Arari, Pinheiro and São Bento.

The present study confirmed the occurrence of the non-vector planorbid species *B. schrammi* and *D. depressissimum* in São Bento and *D. lucidum* in Arari, which coincides with the results of a previous study that was performed in the Amazon Region by Paraense[12]. The most common species of *Drepanotrema* that were observed in this study included

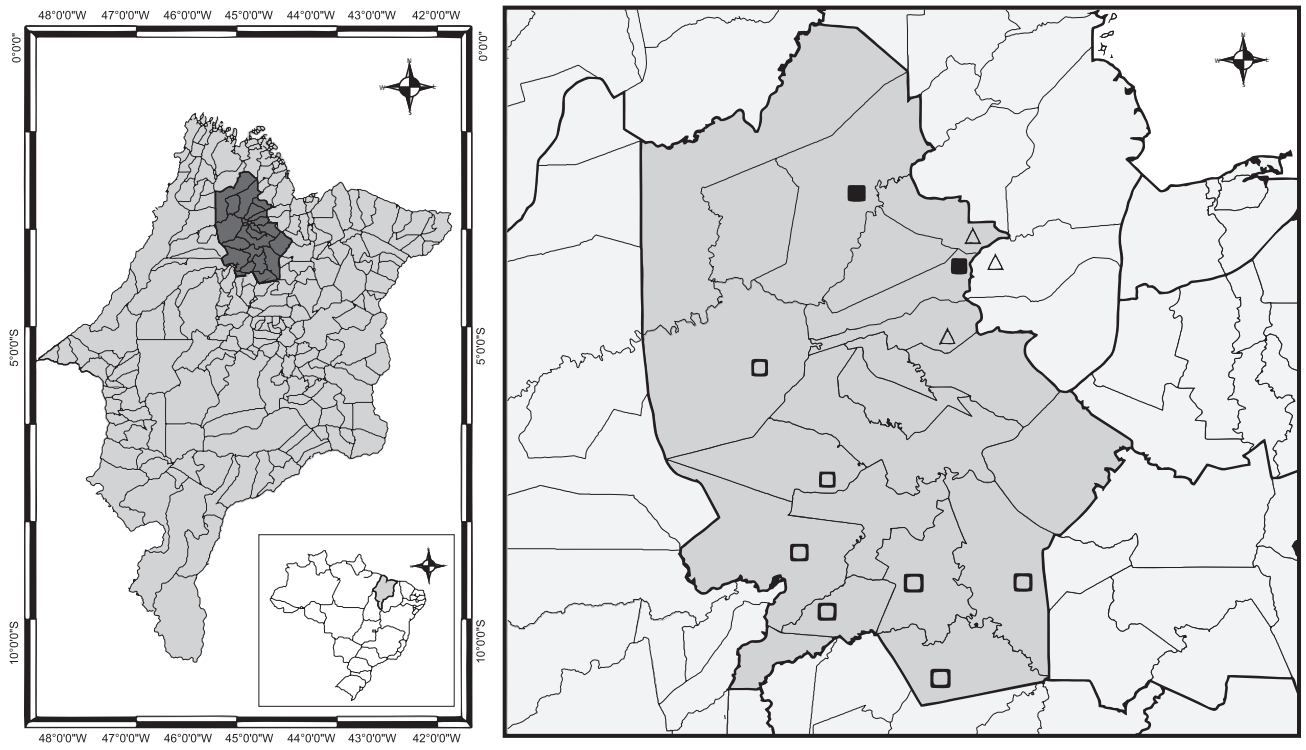

FIGURE 1 - Map of the State of Maranhão and the Baixada Maranhense Microregion (left). Baixada Maranhense Microregion (right) showing the distribution *Schistosoma mansoni* vectors: △ *Biomphalaria glabrata*; □ *Biomphalaria straminea* and ■ *B. glabrata* and *B. straminea.*

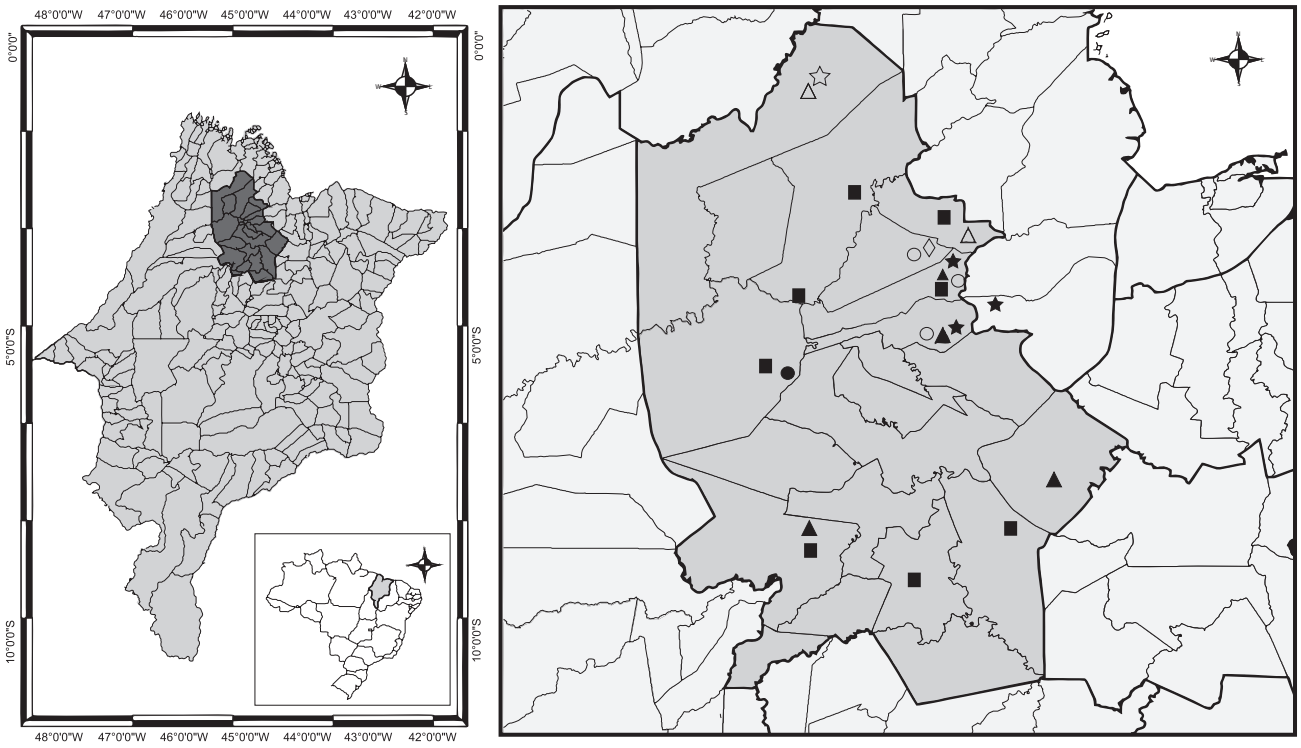

FIGURE 2 - Map of the State of Maranhão and the Baixada Maranhense Microregion (left). Baixada Maranhense Microregion (right) showing the first record of species: ▲ *Gundlachia radiata*;★ *Gundlachia ticaga*; △ *Hebetancylus moricandi*; ● *Plesiophysa guadeloupensis*; ○ *Pomacea bridgesii diffusa*; ■ *Omalonyx* sp.; ◊ *Omalonyx* sp. and *H. moricandi*; ☆ *Omalonyx* sp. and *P. guadeloupensis*.

TABLE 2 - Types of cercariae and the municipalities where they were found in the Baixada Maranhense Microregion, Maranhão, Brazil.

	Trematode			
Municipality	Types of cercariae	Possible family	Mollusk host	% Positive snails (infected/total)
Anajatuba	Brevifurcate-apharingeate-cercaria	Schistosomatidae or Spirorchiidae	*P. maculata*	0.58 (1/172)
Anajatuba	Echinostome cercaria	Echinostomatidae	*P. marmorata*	1.2 (1/82)
Anajatuba	Strigea cercaria	Strigeidae or Diplostomatidae	*D. lucidum*	1.2 (1/83)
Anajatuba	Xiphidiocercariae (Ornatae cercaria)	Haplometridae or Macroderoididae	*D. lucidum*	1.2 (1/83)
Arari	Echinostome cercaria	Echinostomatidae	*P. marmorata*	1.88 (1/53)
Arari	Strigea cercaria	Strigeidae or Diplostomatidae	*D. depressissimum*	4.76 (1/21)
Arari	Xiphidiocercariae (Ubiquita cercaria)	Microphallidae	*D. lucidum*	0.5 (2/397)
Bacurituba	Xiphidiocercariae (Armatae cercaria)	Plagiorchiidae or Telorchiidae	*P. maculata*	7.14 (1/14)
Conceição do Lago-Açu	Xiphidiocercariae (Armatae cercaria)	Plagiorchiidae or Telorchiidae	*P. maculata*	1.35 (1/74)
Conceição do Lago-Açu	Echinostome cercaria	Echinostomatidae	*B. straminea*	8.8 (11/125)
Conceição do Lago-Açu	Xiphidiocercariae (Ubiquita cercaria)	Microphallidae	*P. maculata*	6.75 (5/74)
Matinha	Echinostome cercaria	Echinostomatidae	*P. maculata*	1.96 (1/51)
Matinha	Vivax cercaria	Cyathocotylidae	*P. marmorata*	7.14 (1/14)
Pedro do Rosário	Strigea cercaria	Strigeidae or Diplostomatidae	*D. lucidum*	0.91 (4/437)
Peri-Mirim	Monostome cercaria	Notocotylidae or Pronocephalidae	*D. lucidum*	8.33 (8/96)
Pinheiro	Echinostome cercaria	Echinostomatidae	*B. glabrata*	1.36 (1/73)
Pinheiro	Echinostome cercaria	Echinostomatidae	*P. maculata*	2.38 (1/42)
Santa Helena	Not identified	----------	*D. lucidum*	0.47 (1/211)
São Bento	Xiphidiocercariae (Armatae cercaria)	Plagiorchiidae or Telorchiidae	*P. marmorata*	0.29 (1/339)
São Bento	Brevifurcate-apharingeate-cercaria*	Schistosomatidae	*B. glabrata*	0.22 (1/444)
São Bento	Brevifurcate-apharingeate-cercaria	Schistosomatidae or Spirorchiidae	*P. maculata*	0.32 (1/305)
São Bento	Echinostome cercaria	Echinostomatidae	*P. marmorata*	0.58 (2/339)
São Bento	Xiphidiocercariae (Ubiquita cercaria)	Microphallidae	*P. maculata*	0.32 (1/305)
São João Batista	Echinostome cercaria	Echinostomatidae	*P. maculata*	14.2 (1/7)
São Vicente Férrer	Xiphidiocercariae (Armatae cercaria)	Plagiorchiidae or Telorchiidae	*B. glabrata*	1.12 (5/445)
São Vicente Férrer	Echinostome cercaria	Echinostomatidae	*B. glabrata*	2.02 (9/445)
São Vicente Férrer	Xiphidiocercariae (Ubiquita cercaria)	Microphallidae	*P. maculata*	0.52 (1/189)
Viana	Xiphidiocercariae (Armatae cercaria)	Plagiorchiidae or Telorchiidae	*P. maculata*	3.33 (1/30)
Viana	Echinostome cercaria	Echinostomatidae	*P. maculata*	3.33 (1/30)
Viana	Xiphidiocercariae (Ubiquita cercaria)	Microphallidae	*P. maculata*	3.33 (1/30)
Vitória do Mearim	Echinostome cercaria	Echinostomatidae	*B. straminea*	1.75 (1/57)

* *Schistosoma mansoni* larvae.

D. anatinum and *D. lucidum*. Similarly, in the freshwater malacological survey studies that were performed in the state of Rio de Janeiro, *D. anatinum* was the most frequently observed in the four Mesoregions (Metropolitana, Centro Fluminense, Norte and Noroeste Fluminense), whereas *D. lucidum* was the most frequently observed in the Sul Fluminense Mesoregion[13-16].

The diversity of cercarial types shed from *D. lucidum* was also observed in other studies; Echinostome cercaria was the most frequent type[17]. *Drepanotrema lucidum* from the state of Mato Grosso presented the largest variety of larval trematode forms, and Echinostome cercaria were present in most of the gastropod families that were studied in all of the Brazilian regions[17]. Echinostomatidae is the most common trematode family that parasitizes freshwater gastropods in Brazil[9]. The biological interactions between snails and trematodes found in Maranhão state extends the list of cercariae in mollusks from Brazil that was recently related by Pinto and Melo[18].

Brevifurcate-apharingeate-cercaria can cause cercarial dermatitis[19,20]; these types of cercaria were found in specimens of *B. glabrata* from São Bento and *P. maculata* from Anajatuba. Some trematode larvae of Schistosomatidae can cause cercarial dermatitis, which is an effective skin immune response caused by cercarial penetration. The apparent emergence of cercarial dermatitis should be understood as the spread of disease to a new area (e.g., Chile and the United Kingdom) or the increased contact between people and larval trematodes[21]. Although not recorded in Brazil, the intermediate mollusk hosts are widely distributed in the country. These trematodes have also been found in birds[22].

This study provides the first record of the ampullariid *P. bridgesii diffusa* in the state of Maranhão. Currently, this species, which is native to the Amazon Basin, is found in Brazil, Bolivia and Peru[23]. The Baixada Maranhense Microregion is situated in the transition zone between the Amazon and the Northeast [24], which is more precisely located in the Amazonia Maranhense, where *P. bridgesii diffusa* was recorded in a contiguous area that covers three municipalities. The wide distribution of the congeneric species *P. maculata* within the region and the large number of specimens that were collected confirm that the species has a wide range of morphological, behavioral and physiological adaptations that have possibly contributed to the high diversity and distribution of those mollusks in a large population density[25]. *Pornacea maculata* is distributed from the western region of Brazil, the border of Paraguay, to the south and north of the Amazon Basin, and it is an important component of the biodiversity of freshwater mollusks of the Pantanal[26].

Ancylidae that were found in this study are widely distributed in South America[27]. Furthermore, our results are similar to those of previous studies that reported *G. radiata* and *G. ticaga* as the most common species. Indeed, in the state of Rio de Janeiro, *G. ticaga* was the most common species[13-16;28,29], whereas, in the state of Goiás[30], *G. radiata* and *G. ticaga* were among the species that were found.

Three species of the genus *Omalonyx* have been recorded in Brazil: *Omalonyx matheroni* (Pontiez & Michaud, 1835) from Amazonas, Minas Gerais, Pará, Paraná, Pernambuco, Rio de Janeiro and São Paulo[31]; and *O. brasiliensis* (Simroth, 1896) and *O. convexus* (Heynemann, 1868) from the State of Rio Grande do Sul[32,33]. This report is the first account of *Omalonyx* sp. in Maranhão State.

Similarly, this paper reports the first record of *P. guadeloupensis* in Maranhão. In Brazil, *P. guadeloupensis* was previously reported in the States of Bahia, Espírito Santo, Minas Gerais, Paraíba, Pernambuco, Rio de Janeiro, Rio Grande do Norte and Sergipe[34]. In the present study, the specimens were found in two neighboring municipalities, Pedro do Rosário (locality of Três Palmeiras) and Santa Helena (locality of Mangabeira).

This paper is the first extensive malacological survey of freshwater mollusks in the state of Maranhão. Additional malacological surveys with the intention of obtaining quantitative data to contribute to future studies, such as the monitoring of snail vectors of schistosomiasis and the gain of a comprehensive knowledge of freshwater mollusks from that region, are underway. Additionally, further molecular studies on the larval forms of trematodes that infect mollusks will be performed to aid in their identification.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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