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Major Article

Prevalence of Infection of *Biomphalaria glabrata* by *Schistosoma mansoni* and the risk of urban Schistosomiasis mansoni in Salvador, Bahia, Brazil

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Abstract

Introduction: *Biomphalaria glabrata* is considered to be responsible for the incidence of schistosomiasis in Brazil. Therefore, surveillance of areas where schistosomiasis is prevalent is fundamental for public health planning. This study was aimed to evaluate *B. glabrata* populations in water bodies of the city of Salvador, determine their distribution, estimate the prevalence of *Schistosoma mansoni* infections, characterize shed cercariae, and identify transmission foci. **Methods:** Malacological surveys were carried out in 17 water collections from Salvador. Snail species were identified based on shell and mantle characteristics. Snails were evaluated for *S. mansoni* infection by exposure to light and via real time polymerase chain reaction (qPCR) using *S. mansoni*-18S rRNA subunit specific primers. **Results:** 1,403 *B. glabrata* were collected. Classical cercarial shedding indicated that 5 snails (0.4%) were positive for *S. mansoni*. A higher prevalence of infections was found in Horta de Saramandaia (5.5%) and Lagoa do IAT (1.9%). Non-*Schistosoma* larvae, such as Xiphidiocercaria, Strigeidae, Spirorchiidae and Clinostomidae, were observed in 3.2% of the snails. *S. mansoni* DNA was detected in 6.2% snails via qPCR. **Conclusions:** *B. glabrata* is widely distributed in Salvador, as indicated by 7 water collections associated with a risk of schistosomiasis transmission. To our knowledge, this is the first study to identify *B. glabrata* eliminating cercariae of Clinostomidae, Strigeidae, and Spirorchiidae in Salvador. We propose that qPCR may be employed in combination with classical cercarial shedding. Estimating *S. mansoni* prevalence in snails by only considering the results of light exposure method classical into account may underestimate the problem.

Keywords: Schistosomiasis. *Biomphalaria glabrata*. Prevalence. cercarial types.

INTRODUCTION

Schistosomiasis, a water-transmitted tropical disease (NTDs)—caused by trematode parasites of the genus *Schistosoma*—that remains largely neglected. Several parasites of this genus, such as *S. haematobium*, *S. japonicum*, *S. intercalatum*, *S. mekongi*, and *S. mansoni* are epidemiologically relevant and are capable of parasitizing humans. In Brazil, human schistosomiasis is caused by *S. mansoni,* which is responsible for the intestinal and hepatic forms of this disease[1,2].

Schistosomiasis mansoni, which affects some 240 million individuals worldwide, causes a parasitic disease considered as the third most important socioeconomic and public health issue. In Brazil, schistosomiasis remains an important public health issue due to its prevalence throughout the national territory. According to the "National Survey of Prevalence of Schistosomiasis mansoni and geohelminthosis", conducted among schoolchildren in Brazil, 14 states were found to be endemic for schistosomiasis[3]. Among the Brazilian states endemic for schistosomiais, Bahia has the second highest prevalence with the largest endemic area, which included 251 out of 417 municipalities, including the city of Salvador[4].

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The life cycle of *S. mansoni* is complex, and involves definitive hosts (vertebrates) and intermediate hosts (snails). Previously, transmission of schistosomiasis was found primarily in rural areas. However, intense migratory flows of people from rural endemic areas to urban areas and rapid urbanization contributes to the spreading of parasitic diseases to urban areas. Furthermore, large-scale distribution of the intermediate host—snails of the genus *Biomphalaria*—favors territorial expansion of this disease via the establishment of schistosomiasis transmission foci. In Brazil, 11 species and 1 subspecies of *Biomphalaria* have been described, of which, the following 3 are considered natural hosts of *S. mansoni*; *B. glabrata*, *B. tenagophila*, and *B. straminea*[5].

The distribution of the snail vector is directly correlated with the distribution of schistosomiasis cases[6]. Since the snails are necessary for transmission, updated information on the distribution and characterization of the snail population is essential and contributes directly to the orientation, planning and development of surveillance as well as to the adoption of proper control measures for schistosomiasis. This study was aimed at evaluating *B. glabrata* populations in the water collections from the city of Salvador in order to determine their distribution, identify foci of schistosomiasis transmission to determine the prevalence of *S. mansoni* infection and to characterize shed cercariae.

METHODS

Study site and selection of collection points

The study was conducted in Salvador, capital of the State of Bahia, Northeastern region of Brazil (-12.9704; -38.5124); **(Figure 1A)**. Salvador is divided into 12 regions, termed Sanitary Districts, for the purpose of public health administration. Samples were collected from 17 lentic or lotic water collection points distributed in 8 Sanitary Districts of Salvador, namely Boca do Rio, Brotas, Cabula/Beiru, Centro Histórico, Itapuã, Pau da Lima, São Caetano/Valéria and Subúrbio Ferroviário **(Table 1; Figure 1B)**. Five of the sites were in the process of undergoing urban renewal or major construction with little community contact and were therefore eliminated from the assessment. All sites had permanent collections of water throughout the year and were at, or near, points where the human population had significant contact with the water.

Malacological survey and mollusk maintenance

The malacological surveys were conducted between June and December of 2017, in accordance with the technique described by Oliver and Schneiderman[7]. The density of collected snails was made by dividing the number of planorbids collected at each point by the number of collectors that collected in the 10-minute period. The snails were transported to the Gonçalo Moniz Institute (IGM-FIOCRUZ) and kept in glass aquaria with dechlorinated water. The snails were fed on alternate days with thoroughly washed fresh lettuce.

Natural infection survey and snail identification

Snails were placed individually in jars containing 4 mL of filtered, dechlorinated tap water. Screening for *S. mansoni* cercariae and other larval trematodes was carried out via weekly exposure to light (60W/4 hours) over a period of 4 weeks. Snails remaining negative at the end of this period, were analyzed for another 10 d. Positive snails were examined using a stereoscopic microscope, and live cercariae were stained with 5% lugol. Cercarial types were identified according to the criteria established by Alves Pinto and Lane de Melo[8]. Shell crushing was not perfomed because the soft body portion of *Biomphalaria glabrata* was required for morphological identification of the species. All snails were morphologically identified according to Paraense[9].

PCR analysis

The prevalence of *S. mansoni* infection in a randomly selected sub-group of snails was evaluated via real time polymerase chain reaction (qPCR). DNA extraction from snails was conducted using a DNeasy® Blood and Tissue Kit (QIAGEN®, Germany), following the manufacturer's instructions. *S. mansoni*-specific primers were used to amplify the 18S rRNA subunit as follows: Schfo 111 (5' - CGATCAGGACCAGTGTTCAGC - 3') and Schre 111 (5' - GACAGGTCAACAAGACGAACTCG - 3'), as described by Gomes[10] and qPCR was carried out on an ABI PRISM 7000 system (Applied Biosystem, CA, US). The total qPCR reaction volume of 25 µL consisted of 7.5 µL H_2O, 12.5 µL Syber Green, 2 µL of the two amplification primer, 1 µL ROX and 2 µL of template DNA. PCR was performed under the following cycles: 50ºC for 2 min, followed by 40 cycles of amplification (95ºC for 2 min, 95ºC for 15 s, and 60ºC for 30 s). Negative controls were used for each reaction, and a standard curve was constructed using a sample of *S. mansoni* DNA isolated from worms. All reactions were performed in duplicate. ABI PRISM software (version 1.1) was used for the analysis and interpretation of results.

Results

General distribution of *Biomphalaria glabrata*

A total of 1,403 *B. glabrata*, the only vector species found in this study, were collected from 12 water collections **(Table 2)**. Of these, 730 snails survived at the end of 40 d of malacological analyzes, representing a survival rate of 52%. These snails were morphologically identified and submitted for DNA extraction. The highest snail survival rate, that of 69% survivors, was observed in the water collections of Dique do Cabrito and Lagoa do Urubu **(Table 2)**.

B. glabrata was found to be distributed in 8 Sanitary Districts of the city of Salvador. In the Sanitary District of Cabula/Beiru, in particular, where the Horta de Saramandaia is located, 410 snails were collected. In the Boca do Rio Sanitary District, where Parque Pituaçu is located, a total of 294 snails were collected, and in the São Caetano/Valéria Sanitary District, 289 snails were collected in Horta de São Bartolomeu.

Larvae of trematodes found in *B. glabrata*

Of the 730 snails that survived parasitological analyses, 5 snails shed *S. mansoni* cercariae **(Figure 2A)**. Notably, 25 snails shed only non-*Schistosoma* larvae as follows: Xihphidioceraria **(Figure 2B1-3);** Strigeidae **(Figure 2E)**, Spirorchiidae **(Figure 2D)**, and Clinostomatoide **(Figure 2C)**.

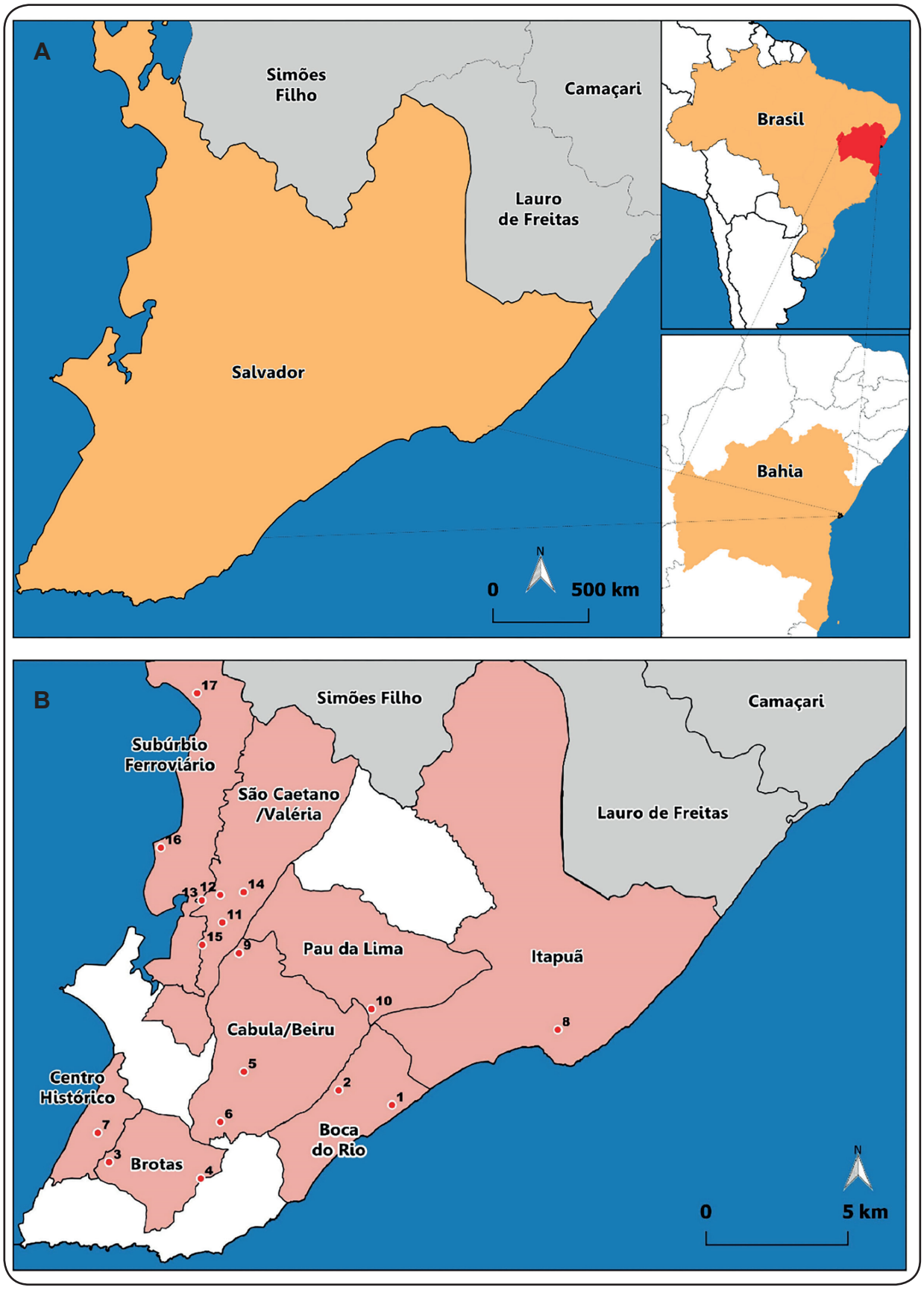

FIGURE 1: (A): study site and **(B):** distribution of water collection in the Sanitary District of the city of Salvador.

TABLE 1: Characterization of collection points in the Sanitary District study sites, type of water collection and presence of vegetation.

Sanitary District	Point	Study Site	Classification
Boca do Rio	1	Parque Pituaçu	Stream
	2	Bate Facho	Stream
Brotas	3	Dique do Tororó	Dike
	4	Avenida ACM	Ditch
Cabula/Beiru	5	Alameda Flamengo	Lagoon
	6	Horta Saramandaia	Vegetable garden channel
Centro Histórico	7	Rua Nossa Senhora de Lourdes	Stream
Itapuã	8	Lagoa do Abaeté	Lagoon
Pau da Lima	9	Lagoa do Urubu	Lagoon
	10	Lagoa do IAT	Lagoon
São Caetano/Valéria	11	Rua São Rafael	NA
	12	Horta São Bartolomeu	Vegetable garden channel
	13	Rua das Fontes	NA
	14	Rio do Cobre	River
	15	Dique do Cabrito	Dike
Subúrbio Ferroviário	16	Rua Gevársio Cerqueira	Vegetable garden channel
	17	Rua Ray Charles	NA

NA: not applicable.

S. mansoni cercariae were found only in 2 water collections: the Lagoa do IAT, in the Sanitary District of Pau da Lima; and Horta de Saramandaia, located in the Sanitary District of Cabula/Beiru, which were 5.5% and 1.9% positive, respectively. Additionally, in Horta de Saramandaia, 4.3% snails shed Xiphidiocercaria. In Dique do Cabrito, 1 snail shed Clinostomidae cercariae and 1 snail shed Spirorchiidae cercariae. Positivity rate in both cases was 4.3%. The highest positivity was observed in Lagoa do Urubu, with 31% of snails shedding Strigeidae cercariae **(Table 3)**.

Molecular detection of *S. mansoni*

Of the 1403 snails collected, 626 were used for molecular detection of *S. mansoni* via qPCR. Only product amplifications with a melting temperature equal to that of the positive control, $Ct < 35$, and a correlation coefficient (r^2) of 0.99 were considered positive. All negative controls were negative in all experiments. The sensitivity of qPCR for detection of *S. mansoni* infections was 100% while specificity was 94.5% compared with the results of the light exposure method.

Of the 626 samples, 39 were considered positive, representing a positivity of 6.2%. Of these, only 5 (0.8%) were positive by the light exposure method. No snails that had eliminated other cercarial types were found to be positive for *S. mansoni* via qPCR, while none of the snails were found to be infected with 2 species of cercariae.

Among the 12 water collections containing *B. glabrata*, 5 (41.7%) were positive for *S. mansoni* only, via qPCR as follows: Parque Pituaçu, Avenida ACM, Rua Nossa Senhora de Lourdes, Horta de São Bartolomeu and Dique do Cabrito. The highest positivity via qPCR was observed in the water collection of the Dique do Cabrito, followed by Av. ACM, Lagoa do IAT and Rua Nossa Senhora de Lourdes.

Water collections that were previously determined to be positive for *S. mansoni*, via light exposure, were found to be even more positive for *S. mansoni* via qPCR. In Lagoa do IAT, only 5.5% of snails were found to be positive via the light exposure method, whereas 16.6% were found to be positive via qPCR. Similarly, the water collection of Horta de Saramandaia, which indicated a 1.9% positivity via the light exposure method, showed a positivity of 4.8% via qPCR **(Table 4)**.

DISCUSSION

The malacological survey, conducted by the current study, demonstrated that *B. glabrata* was present in 70.6% of the water collections examined. Most snails were present in streams and

TABLE 2: Total *B. glabrata* counts per water collection, amount, and percentage (%) of alive snails after 40 days of laboratory maintenance.

Sanitary District	Collection Site	Collected Snails	Live Snails after 40 days
Boca do Rio	Parque Pituaçu	294	145 (49%)
	Bate Facho	22	13 (59%)
Brotas	Dique do Tororó	3	2 (66%)
	Avenida ACM	84	48 (57%)
Cabula/Beiru	Alameda Flamengo	0	0
	Horta Saramandaia	410	205 (50%)
Centro Histórico	Rua Nossa Senhora de Lourdes	49	27 (55%)
Itapuã	Lagoa do Abaeté	0	0
Pau da Lima	Lagoa do Urubu	42	29 (69%)
	Lagoa do IAT	33	18 (54%)
São Caetano/Valéria	Rua São Rafael	0	0
	Horta São Bartolomeu	289	144 (49%)
	Rua das Fontes	-	-
	Rio do Cobre	58	28 (48%)
	Dique do Cabrito	33	23 (69%)
Subúrbio Ferroviário	Rua Gevársio Cerqueira	86	48 (56%)
	Rua Ray Charles	0	0
Total		**1403**	**730**

TABLE 3: Cercarian types found in specimens of *Biomphalaria glabrata* in the water collections of Salvador.

Sanitary District	Water Collection	Positive Snails	Cercarial Types	Positivity (%)
Cabula/Beiru	Horta de Saramandaia	4/205	*S. mansoni*	1.9%
		9/205	Xiphidiocercaria	4.3%
Pau da Lima	Lagoa do Urubu	9/29	Strigeidae	31%
	Lagoa do IAT	1/18	*S. mansoni*	5.5%
São Caetano/Valéria	Dique do Cabrito	1/23	Spirorchiidae	4.3%
		1/23	Clinostomidae	4.3%

ditches, which together represented 50% of the water collections sampled.

The highest concentration of *B. glabrata* was observed in the water collections of Horta de Saramandaia and Horta de São Bartolomeu. "Horta is Portuguese for "garden", which in Salvador often implies a large area under cultivation for local and commercial production. Although *Biomphalaria* snails are commonly found in natural water collections, highest population densities are usually observed in artificial breeding sites such as drainage and irrigation ditches associated with human activity[11]. Constant irrigation of vegetable gardens provides ideal breeding grounds for *Biomphalaria* spp[12].

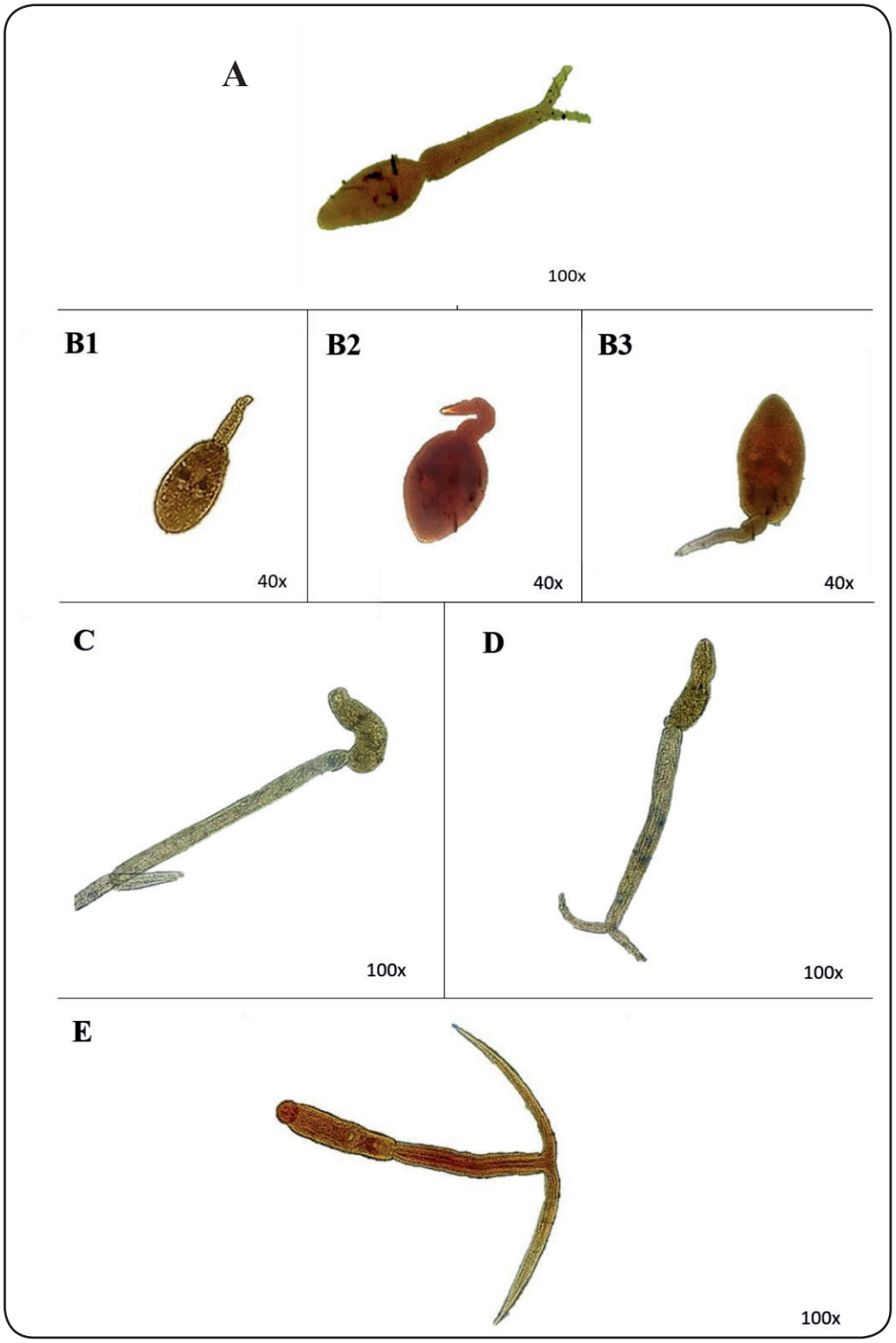

FIGURE 2: Larvae of trematodes found in *B. glabrata*. **(A):** *S. mansoni* Cercariae; **(B):** Xiphidiocercaria: **(B1):** Lutzi Cercariae; **(B2,3):** Santense Cercariae; **(C):** Clinostomidae, **(D):** Spirorchiidae **(E):** Strigeidae.

TABLE 4: Infection Rates obtained via qPCR and light exposure methods in surviving *B. glabrata* samples.

Sanitary District	Collection Site	*B. glabrata*	Infection Rate		
			Light Exposure Method		qPCR (+)
			S. mansoni	Outros	
Boca do Rio	Parque Pituaçu	100	–	–	5 (5%)
	Bate Facho	13	–	–	–
Brotas	Dique do Tororó	2	–	–	–
	Av. ACM	48	–	–	9 (18,7%)
Cabula/Beiru	Horta Saramandaia	145	4 (1,9%)	9 (4,3%)	7 (4,8%)
Centro Histórico	R. N. Senhora de Lourdes	28	–	–	3 (10,7%)
Pau da Lima	Lagoa do Urubu	29	–	9 (31%)	–
	Lagoa do IAT	18	1 (5,5%)	–	3 (16,6%)
São Caetano/Valéria	Horta São Bartolomeu	100	–	–	2 (2%)
	Rio do Cobre	28	–	–	–
	Dique do Cabrito	23	–	2 (8,6%)	10 (43,4%)
Subúrbio Ferroviário	R. Gevársio Cerqueira	48	–	–	–
Total		**626**	**5**	**20**	**39**

Only the water collections from Horta de Saramandaia and Lagoa do IAT were found to be positive for *S. mansoni* via the light exposure method after 30 d, with infection rates of 1.9% and 5.5%, respectively. Given the conditions governing cultivation and irrigation in Horta de Saramandaia, the findings from that location were expected. The presence of channels excavated for irrigation of vegetables, compounded by precarious sanitary conditions of the neighborhood and the high population density of *B. glabrata,* provide the necessary environment for maintaining the life cycle of *S. mansoni* at this site. Furthermore, in 2015, the Zoonoses Control Center (CCZ), identified *B. glabrata* specimens which shed *S. mansoni* cercariae. In the Lagoa do IAT region, similar conditions that were favorable for maintaining the *S. mansoni* life cycle, such as residential sewage flushed directly into the water collection and residents living with schistosomiasis, were observed.

The qPCR confirmed that all water collections found to be positive via the light exposure method, were also positive via *S. mansoni* DNA. Furthermore, classical methods combined with PCR were able to detect higher levels of infection prevalence. These findings were corroborated by the results of previous studies. Jannotti-Passos and Souza[13] used LS-PCR in association with light exposure to determine the prevalence of *S. mansoni* infection following 7 and 42 d exposure of *B. straminea* and *B. tenagophila* to miracidia. Although other studies evaluated infections in other species of *Biomphalaria*, using different PCR techniques, their results corroborate those found in the current study, since apparent infection prevalence increased from 20% to 55% in *B. straminea*, and from 45% to 67.6% in *B. tenagophila*.

Positivity for *S. mansoni* seen via PCR and the absence of cercarian elimination may be explained away as being due to snail immune system activity. Non-successful infections, which do not lead to the elimination of cercariae, are detected by PCR, because parasite DNA is not completely degraded[14]. This phenomenon may also be explained by the fact that some primary sporocysts either degenerate or are encapsulated by hemocytes, leading to unsuccessful infections. Thus, sporocysts play a fundamental role in disease progression, since the production levels of cercariae are directly associated with the development and concentration of sporocysts in the snail[15].

Moreover, late development of the immune response to *S. mansoni* may lead to a delay in cercarian release. Significant tissue changes which occur in infected *Biomphalaria* prevent the elimination of cercariae. Focal and diffuse proliferation of hemocytes accompanied by an expansion of the extracellular matrix in a manner similar to that seen in granulomas, was observed in *B. glabrata*[16]. Lemos and Andrade[17] proposed that these tissue changes may develop gradually in infected snails that had previously eliminated cercariae. However, these tissue changes do not guarantee complete eradication of the infection, since some sporocysts that remain may be able to complete the development cycle of the parasite, whereby cercariae may be released at any time within 9 months following infection[16].

Late release of cercariae may also occur due to reproduction between susceptible and resistant snails, which influences the timing of *S. mansoni* development in the snail. A study of *B. glabrata,* generated by crossing resistant and susceptible species, reported that descending snails exhibited a delayed pre-patent phase, which could last up to 10 months[18]. Additionally, such late releases may also be related to sporocytogenesis[19]. Jourdane and Théron observed that changes that compromise the production of cercariae, such as secondary sporocyst migration

to ectopic regions (cephalopodal region and kidney), may occur during sporocystogenesis[20]. This phenomenon has been observed in partially resistant *B. glabrata*, with delays in the release of cercariae up to 7 months[21].

To our knowledge, this is the first record of other cercarian types, such as Strigeidae, Clinostomidae and Spirorchiidae, in the city of Salvador. Alves Pinto and Lane de Melo reported the presence of Spirorchiidae and Clinostomidae cercariae in the 3 schistosomiasis transmitter species in the state of Minas Gerais[7]. Clinostomidae cercariae are considered to be parasites of the oral cavity of birds, but accidental human infections have been reported[22]. Strigeidae cercariae have also been identified in the States of Maranhão, Minas Gerais and Rio de Janeiro[23-25].

The presence of *B. glabrata* shedding Xifidiocercariae was observed in the water collections of Subúrbio Ferroviário in Salvador by the CCZ in 2017. Previous studies have already evaluated the presence of this cercarian type in *Biomphalaria* spp. from other sites[26,27]. This cercarian type, which has not been found to be responsible for any clinically important disease, has been considered as a source of biological control for mosquito larvae[28].

The absence of coinfection in snails that were observed in this study may be due to cercarian antagonism, which leads to competition between larvae of different trematodes and results in a reduction in the number of parasites able to complete development. However, simultaneous elimination of cercariae during coinfections have been observed in *S. mansoni* and *Cercaria lutzi* coinfections exclusively in *B. tenagophila*[24].

B. glabrata was not found in the water collections of Alameda Flamengo, Lagoa do Abaeté, Rua São Rafael, Rua das Fontes and Rua Ray Charles. Three of these locations were undergoing major public construction work, such as sanitary sewer placement or street paving. Considering that parasitic diseases reflect sanitary conditions as well as hygiene habits of a population, these results demonstrated that effective public interventions is fundamental for improving living conditions as well as for preventing and regulating parasitic diseases[29].

A limitation of this study was the reduction of snail survival rates during weekly malacological analyses that lasted 30 d. This suggests that the duration of the analysis may have influenced *B. glabrata* survival, as it is possible that snails that did not survive were parasitized by *S. mansoni*, may have had different susceptibility profiles or differences in the amount of miracidia penetrated[30].

In the future, we hope to assess more water collections in the city of Salvador, in order to evaluate infections in snails using a combination of conventional and molecular techniques. An additional goal is to evaluate resistance and susceptibility profiles of these snails.

Our results indicate that *B. glabrata* is widely distributed in the city of Salvador, and 7 of its water collections carry a risk of schistosomiasis transmission. In addition, we propose that qPCR may be utilized to evaluate *S. mansoni* infections in *B. glabrata* during the pre-patent phase. It is evident that estimating *S. mansoni* prevalence in snails by taking only the light exposure method classical into account may underestimate the issue. To the best of our knowledge this is the first study of *B. glabrata* eliminating Clinostomidae, Strigeidae, and Spirorchiidae cercariae in Salvador.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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