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Schistosomiasis Mansoni in the Amazon Region: Malacological Surveys of Intermediate Hosts for the Identification of Disease Transmission Areas in Belém, Pará, Brazil

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Abstract: In Brazil, schistosomiasis occurrence is related to the presence of the etiological agent *Schistosoma mansoni*. The presence of mollusks for *Biomphalaria* genus is a necessary condition for development of the parasite cycle. In Pará state, the species *B. schrammi*, *B. kuhniana*, *B. straminea* and *B. glabrata* have already been identified, with reports of the last two occurring in Belém. The aim of this study was to determine the spatial distribution of *Biomphalaria* species in Belém, Pará, Brazil, identifying risk areas for schistosomiasis transmission. Biannual malacological surveys were carried out between September 2013 and October 2017 in 35 neighborhoods of Belém. The collection points were georeferenced using a Global Positioning System (GPS) receiver and the Kernel Density Estimation (KDE) was used to the spatial analysis. The mollusks, after measurement, were exposed to light to verify the presence of *S. mansoni* cercariae. Subsequently, mollusks were sacrificed and fixed for dissection and morphological identification of species. The surveys resulted in a total of 10,803 mollusks collected, of which 9,367 (86.70%) specimens were examined. Among the analyzed mollusks, 5,820 (62.14%) were identified as *B. glabrata* and 3,547 (37.86%) as *B. straminea*. The presence of the parasite was observed in 208 (2.22%) specimens, all *B. glabrata*. Positive mollusks were found in six neighborhoods: Guamá (51 mollusks/24.5%), Sacramenta (47 mollusks/22.6%), Telégrafo (47 mollusks/22.6%), Terra Firme (36 mollusks/17.3%), Condor (20 mollusks/9.6%) and Barreiro (7 mollusks/3.4%). This study confirms that *B. glabrata* species actively participates as intermediate host in the disease maintenance in Belém. The KDE allowed to analyze the spatial distribution of collection sites and consequently to determine possible risk areas of schistosomiasis transmission in Belém. The development of maps identifying sites with schistosomiasis positive snails may support efforts of this municipality by directing activities related to endemic disease control.

Key words: *Biomphalaria* spp., *Schistosoma mansoni*, intermediate hosts, schistosomiasis, spatial analysis.

1. Introduction

Schistosomiasis is a disease transmitted by trematodes of *Schistosoma* genus (Trematoda: Schistosomatidae), being one of the most prevalent parasitic diseases in the world and one of the most important tropical diseases in terms of public health [1].

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In Brazil, this condition, considered chronic or acute infectious-parasitic, is caused by the etiological agent *Schistosoma mansoni* (Sambon, 1907). The disease can be found in 19 federative units and occurs in areas with poor basic sanitation conditions and low human development index [2, 3].

The presence of mollusks belonging to *Biomphalaria* genus is a necessary condition for the development of the parasite cycle. In Brazil, among 11 species and one subspecies, three are known as intermediate hosts of *S. mansoni*, namely *B. glabrata* (Say, 1818), *B. tenagophila* (Orbigny, 1835) and *B. straminea* (Dunker, 1848) [4]. In the state of Pará, the species *B. schrammi* (Crosse, 1864), *B. kuhniana* (Clessin, 1883), *B. straminea* and *B. glabrata* have already been identified, with reports of the last two occurring in Belém [5-8].

The first autochthonous case of this disease in the Amazon region was registered in the state of Pará in 1951, in the locality of Fordlândia (Aveiro municipality), with confirmation of positive cases among children and young residents. The collected snails were described as *B. straminea*, found in streams and drainage ditches at the region [9].

Galvão [10] reported autochthonous cases of schistosomiasis in Belém, more specifically in Reduto neighborhood, related to the intermediate host specie *B. straminea*, but none of the specimens presented natural infection with *S. mansoni*. Paraense et al. [11] examined 364 specimens of snails, collected in Telégrafo neighborhood, detecting the first focus of *S. mansoni* transmission involving *B. glabrata* snails in Belém. The infection rate of the examined specimens was 7.62%. The first strain of *S. mansoni* in Belém from an autochthonous case was isolated by Rodrigues and Barros in 1987 [12].

Among the intermediate host species of *S. mansoni* found in Belém, *B. glabrata* is the most relevant, mainly due to its high compatibility with the trematode, which facilitates infection and elimination of a large number of cercariae [13, 14]. Besides *B. straminea* is less susceptible than *B. glabrata* to *S. mansoni* infection, it is worth to note that it plays an important role in the transmission of schistosomiasis and its abundance in natural breeding sites compensates the low infection rates [15].

The use of maps showing the location of *Biomphalaria* in Pará state can be observed in several studies [16-19].

The aim of this study was to determine the spatial distribution of *Biomphalaria* species infected with *S. mansoni* in Belém, Pará, Brazil, identifying risk areas for disease transmission applying geoprocessing techniques.

2. Materials and Methods

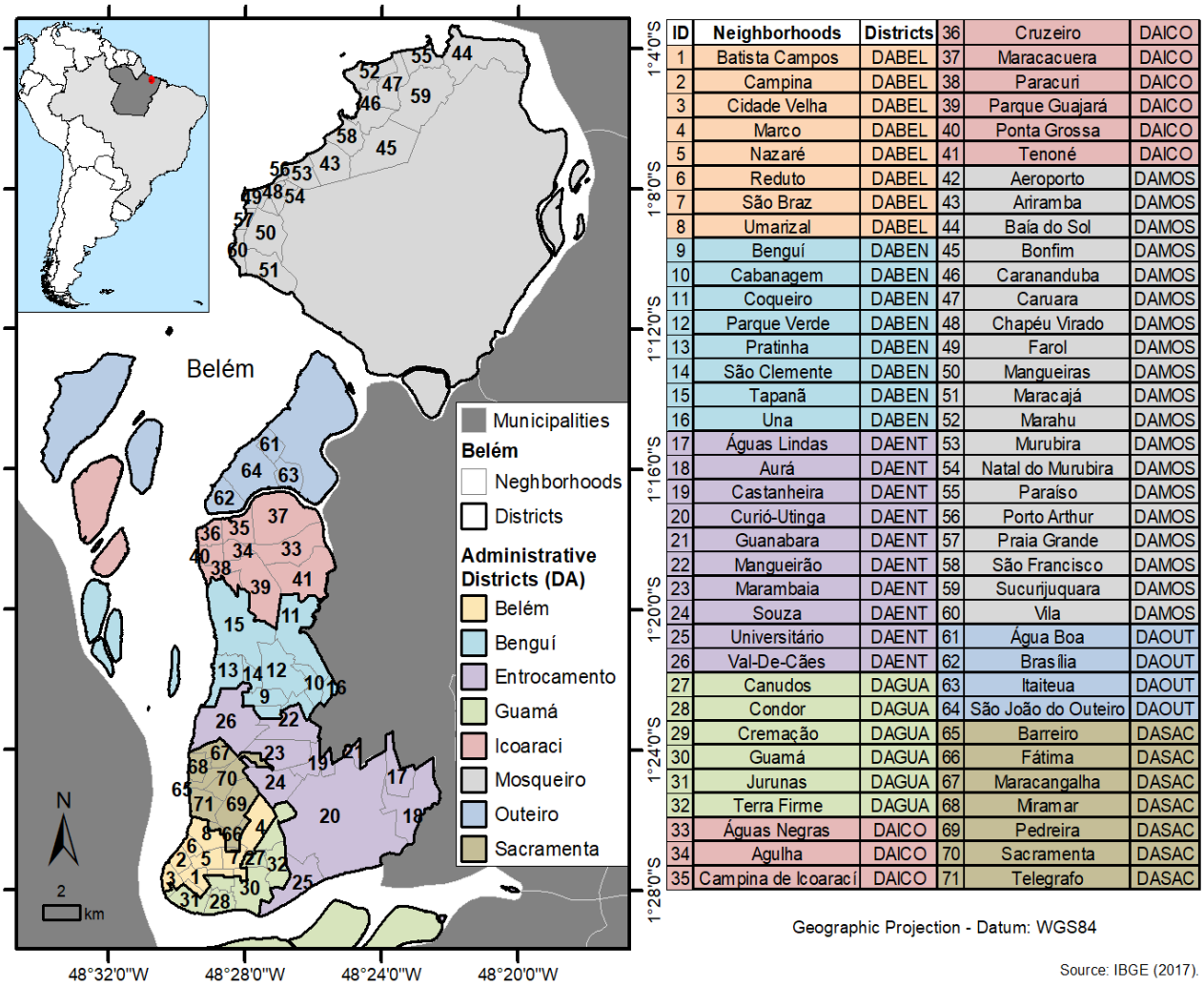
2.1 Study Area

This study was developed in the municipality of Belém, state of Pará, northern Brazil, 01°27′18″ S and 48°30′09″ W, with an estimated population of 1,452,275 inhabitants. Belém is distributed in eight administrative districts: Administrative District of Belém—DABEL, Administrative District of Benguí—DABEN, Administrative District of Entroncamento—DAENT, Administrative District of Guamá—DAGUA, Administrative District of Icoaraci—DAICO, Administrative District of Mosqueiro—DAMOS, Administrative District of Outeiro—DAOUT and Administrative District of Sacramenta—DASAC, comprising 71 neighborhoods (Fig. 1) [20].

2.2 Mollusk Surveys

Biannual malacological surveys were conducted during the rainy season (from December to May) and less rainy season (from June to November) in 35 neighborhoods of Belém between September 2013 and October 2017 [21]. A Garmin GPSMap® 76CSx GPS receiver was used to obtain the geographic coordinates.

The records collected from these surveys were used to fill out the field form, containing information such as: registration number, date and location of collection,



ID	Neighborhoods	Districts	36	Cruzeiro	DAICO
1	Batista Campos	DABEL	37	Maracacuera	DAICO
2	Campina	DABEL	38	Paracuri	DAICO
3	Cidade Velha	DABEL	39	Parque Guajará	DAICO
4	Marco	DABEL	40	Ponta Grossa	DAICO
5	Nazaré	DABEL	41	Tenoné	DAICO
6	Reduto	DABEL	42	Aeroporto	DAMOS
7	São Braz	DABEL	43	Ariramba	DAMOS
8	Umarizal	DABEL	44	Baía do Sol	DAMOS
9	Benguí	DABEN	45	Bonfim	DAMOS
10	Cabanagem	DABEN	46	Caranandubа	DAMOS
11	Coqueiro	DABEN	47	Caruara	DAMOS
12	Parque Verde	DABEN	48	Chapéu Virado	DAMOS
13	Pratinha	DABEN	49	Farol	DAMOS
14	São Clemente	DABEN	50	Mangueiras	DAMOS
15	Tapanã	DABEN	51	Maracajá	DAMOS
16	Una	DABEN	52	Marahu	DAMOS
17	Águas Lindas	DAENT	53	Murubira	DAMOS
18	Aurá	DAENT	54	Natal do Murubira	DAMOS
19	Castanheira	DAENT	55	Paraíso	DAMOS
20	Curió-Utinga	DAENT	56	Porto Arthur	DAMOS
21	Guanabara	DAENT	57	Praia Grande	DAMOS
22	Mangueirão	DAENT	58	São Francisco	DAMOS
23	Marambaia	DAENT	59	Sucurijuquara	DAMOS
24	Souza	DAENT	60	Vila	DAMOS
25	Universitário	DAENT	61	Água Boa	DAOUT
26	Val-De-Cães	DAENT	62	Brasília	DAOUT
27	Canudos	DAGUA	63	Itaiteua	DAOUT
28	Condor	DAGUA	64	São João do Outeiro	DAOUT
29	Cremação	DAGUA	65	Barreiro	DASAC
30	Guamá	DAGUA	66	Fátima	DASAC
31	Jurunas	DAGUA	67	Maracangalha	DASAC
32	Terra Firme	DAGUA	68	Miramar	DASAC
33	Águas Negras	DAICO	69	Pedreira	DASAC
34	Agulha	DAICO	70	Sacramenta	DASAC
35	Campina de Icoarací	DAICO	71	Telegrafo	DASAC

Fig. 1 Administrative districts and neighborhoods of Belém, state of Pará, Brazil.

municipality, Global Positioning System (GPS) device number, geographical coordinates obtained by tablet or smartphone, presence of snails, additional observations, a photo of the breeding site and the identification of the technician responsible for the information.

The collected snails were wrapped in gauze soaked in water, inserted in properly identified plastic bags and stored in a rigid container [8], in which they were transported to the Laboratory of Intestinal Parasites, Schistosomiasis and Malacology (LPIEM) of Parasitology Section (SAPAR) from Evandro Chagas Institute (IEC) where the mollusks were kept in plastic containers with dechlorinated water and fed with fresh lettuce.

2.3 Analysis of Cercarial Shedding of Biomphalaria Snails

The mollusks, after measurement, were individually placed in glass containers with dechlorinated water and exposed to artificial light at a distance of 30 centimeters for a 4 hours period to stimulate the elimination of *S. mansoni* cercariae or any other trematode of medical or veterinary importance. After this step, the water from the containers with snails was analyzed under stereoscopic microscope.

2.4 Identification of Biomphalaria Snails

Subsequently, from the collected snails, five specimens from each collection site were randomly

chosen, sacrificed and fixed in Raillet-Henry solution for dissection and morphological identification of the species, comparing characteristics of the shell and the male and female reproductive organs as described by Deslandes and Paraense [22-26].

2.5 Spatial Analysis

Field form data were exported to an Excel spreadsheet, combining the results of *Biomphalaria* species identification and positivity for infection with *S. mansoni*. The presence of *S. mansoni* infected snails at a defined location was considered as a possible schistosomiasis transmission area.

The cartographic bases were obtained from the Brazilian Institute of Geography and Statistics (IBGE—http://www.ibge.gov.br/).

Spatial data were stored and analyzed in ArcGis 10 software (https://www.arcgis.com) for manipulation and connection of descriptive attributes to the graphical form, as well as for the elaboration of thematic maps.

The Kernel Density Estimator (KDE) is a non-parametric interpolation technique and was used to define the visual identification of risk transmission areas for *S. mansoni* using the georeferenced points of snails [27]. We evaluated the KDE using an adaptive bandwidth in the TerraView software (http://www.dpi.inpe.br/terralib5/wiki/doku.php).

3. Results

In this study, 35 neighborhoods of the municipality of Belém were evaluated: Aeroporto, Água Boa, Águas Negras, Agulhas, Ariramba, Barreiro, Benguí, Cabanagem, Chapéu Virado, Condor, Coqueiro, Cruzeiro, Curió-Utinga, Guamá, Jurunas, Mangueiras, Maracacuera, Maracajá, Maracangalha, Marambaia, Marco, Miramar, Murubira, Parque Verde, Ponta Grossa, Praia Grande, Sacramenta, São Clemente, São Francisco, São João do Outeiro, Tapanã, Telégrafo, Tenoné, Terra Firme and Universitário, between September 2013 and October 2017.

Malacological surveys resulted in the collection of 10,803 *Biomphalaria* snails, and of these, 9,367 specimens examined for the presence of *S. mansoni* which 5,820 (62.14%) were identified as *B. glabrata* and 3,547 (37.86%) as *B. straminea* (Fig. 2).

The species *B. glabrata* was observed in the neighborhoods of Barreiro, Condor, Coqueiro, Guamá, Marco, Sacramenta, Telégrafo, Terra Firme and Universitário, while *B. straminea* mollusks were identified in the neighborhoods of Água Boa, Águas Negras, Ariramba, Barreiro, Cabanagem, Chapéu Virado, Coqueiro, Cruzeiro, Jurunas, Mangueiras, Maracacuera, Maracajá, Marambaia, Marco, Murubira, Parque Verde, Praia Grande, Sacramenta, São Clemente, São Francisco, São João de Outeiro, Tenoné and Terra Firme.

The presence of the parasite was observed in 208 (2.22%) specimens, all *B. glabrata*, as summarized in Table 1.

Positive mollusks for *S. mansoni* were found in six neighborhoods: Guamá (51 mollusks/24.5%), Sacramenta (47 mollusks/22.6%), Telégrafo (47 mollusks/22.6%), Terra Firme (36 mollusks/17.3%), Condor (20 mollusks/9.6%) and Barreiro (7 mollusks/3.4%). Among the 208 snails positive for *S. mansoni*, 102 were collected during the rainy season and 106 were obtained in the less rainy season (Table 2).

Using the KDE, it was possible to obtain clusters according to color representations: green (low risk), yellow (medium risk), orange (high risk) and red (very high risk) (Fig. 3).

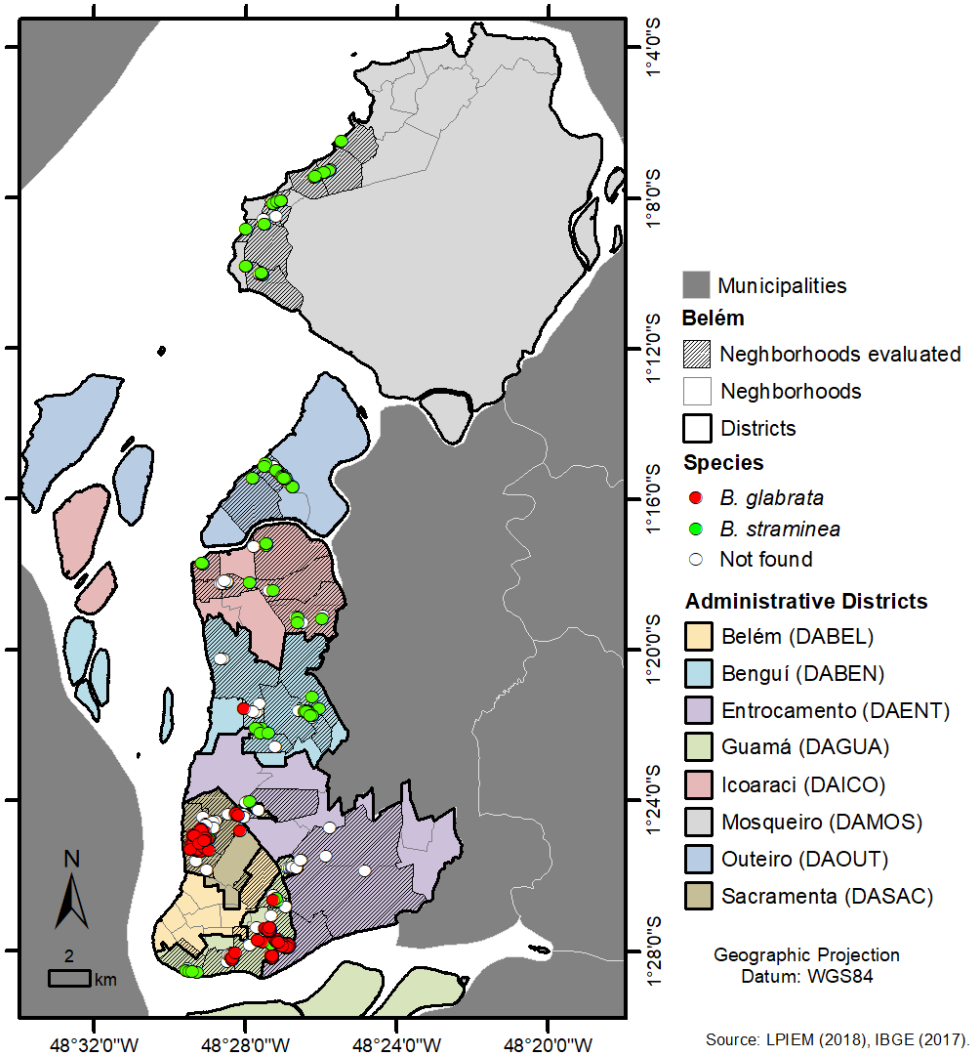


Fig. 2 Spatial location of the *Biomphalaria* species distributed among the administrative districts of Belém, state of Pará, Brazil.

Table 1 Geographical coordinates of *Biomphalaria* snails, positive for *Schistosoma mansoni* infection in Belém, State of Pará, Brazil, from September/2013 to October/2017.

Neighborhoods	Geographical coordinates (S)	Geographical coordinates (W)	Collection date	*Biomphalaria* spp. collected mollusks	Positive mollusks for *S. mansoni* infection	Identification (species)
Barreiro	’01°24’46.7”	’48°29’10.4”	March/2014	201	7	*B. glabrata*
	’01°24’56.5”	’48°29’20.4”	September/2014			
	’01°24,849’	’48°29,137’	April/2016			
	’01°24’56,9”	’48°29’22,6”	March/2017			
	’01°24’56,3”	’48°29’20,3”	August/2017			
Condor	’01°28’11.6”	’48°28’20.6”	October/2013	180	20	*B. glabrata*
	’01°28’11.4”	’48°28’19.5”	April/2014			
	’01°28’11.9”	’48°28’20.6”	April/2014			
	’01°28’11.3”	’48°28’19.5”	August/2014			
	’01°28’11.8”	’48°28’20.5”	August/2014			
	’01°28,194’	’48°28,341’	March/2016			

(Table 1 continued)

Neighborhoods	Geographical coordinates (S)	Geographical coordinates (W)	Collection date	*Biomphalaria* spp. collected mollusks	Positive mollusks for *S. mansoni* infection	Identification (species)
Guamá	’01°28’12.3”	’48°27’16.7”	October/2013	648	51	*B. glabrata*
	’01°28’09.2”	’48°27’18.2”	October/2013			
	’01°28’08.3”	’48°27’18.6”	October/2013			
	’01°27’25.4”	’48°27’27.1”	April/2014			
	’01°28’10.8”	’48°27’16.9”	August/2014			
	’01°28’09.5”	’48°27’18.0”	August/2014			
	’01°28’08.3”	’48°27’16.1”	August/2014			
	’01°27’24.9”	’48°27’19.5”	August/2014			
	’01°27’27.4”	’48°27’22.4”	August/2014			
	’01°27’27.6”	’48°27’22.8”	August/2014			
	’01°27’25.5”	’48°27’26.9”	August/2014			
	’01°28’09.4”	’48°27’17.9”	September/2015			
	’01°27’27.4”	’48°27’22.4”	September/2015			
	’01°27’24,3”	’48°27’20,8”	August/2016			
	’01°28’10,4”	’48°27’18,3”	February/2017			
	’01°28’11,1”	’48°27’16,2”	September/2017			
	’01°28’09,2”	’48°27’18,1”	September /2017			
Sacramenta	’01°25’05.0”	’48°29’04.4”	October/2013	335	47	*B. glabrata*
	’01°25’02.7”	’48°29’00.9”	October/2013			
	’01°25’04.1”	’48°29’04.0”	March/2014			
	’01°25’02.7”	’48°29’00.9”	March/2014			
	’01°25’04.1”	’48°29’04.0”	September/2014			
	’01°25’02.5”	’48°29’01.2”	September/2014			
	’01°25’02.6”	’48°29’01.0”	May/2015			
	’01°25,049’	’48°28,994’	April/2016			
	’01°25,070”	’48°29,063”	April/2016			
	’01°25’04,2”	’48°29’03,9”	March/2017			
	’01°22’02,4”	’48°29’00,6”	August/2017			
	’01°25’04,1”	’48°29’03,8”	August/2017			
Telégrafo	’01°25’19.8”	’48°29’16.8”	October/2013	241	47	*B. glabrata*
	’01°25’15.3”	’48°29’17.6”	March/2014			
	’01°25’12.5”	’48°29’15.4”	March/2014			
	’01°25’09.1”	’48°29’13.7”	March/2014			
	’01°25’20.0”	’48°29’17.0”	March /2014			
	’01°25’20.8”	’48°29’16.7”	June/2014			
	’01°25’15.5”	’48°29’17.4”	September/2014			
	’01°25’15.4”	’48°29’17.6”	May/2015			
	’01°25’09.2”	’48°29’13.8”	May/2015			
Terra Firme	’01°27’47.4”	’48°27’07.4”	April/2014	280	36	*B. glabrata*
	’01°27’53.5”	’48°27’00.7”	August/2014			
	’01°27’47.5”	’48°27’07.3”	August/2014			
	’01°27’54.7”	’48°26’58.4”	May/2015			
	’01°27,792’	’48°27,122’	March/2016			
	’01°27’47,5”	’48°27’07,3”	February/2017			
	’01°27’49,3”	’48°26’52,1”	September/2017			
	’01°27’50,3”	’48°26’59,9”	September/2017			
	’01°27’53,5”	’48°27’00,9”	September/2017			
Total	-	-	-	1,885	208	-

Table 2 Number of mollusks positive for *S. mansoni* collected during the rainy season and less rainy season in six neighborhoods of Belém, between September 2013 to October 2017.

Neighborhood	Rainy season	Less rainy season	Total
Guamá	16	35	51
Sacramenta	17	30	47
Telégrafo	37	10	47
Terra Firme	18	18	36
Condor	9	11	20
Barreiro	5	2	7
Total	102	106	208

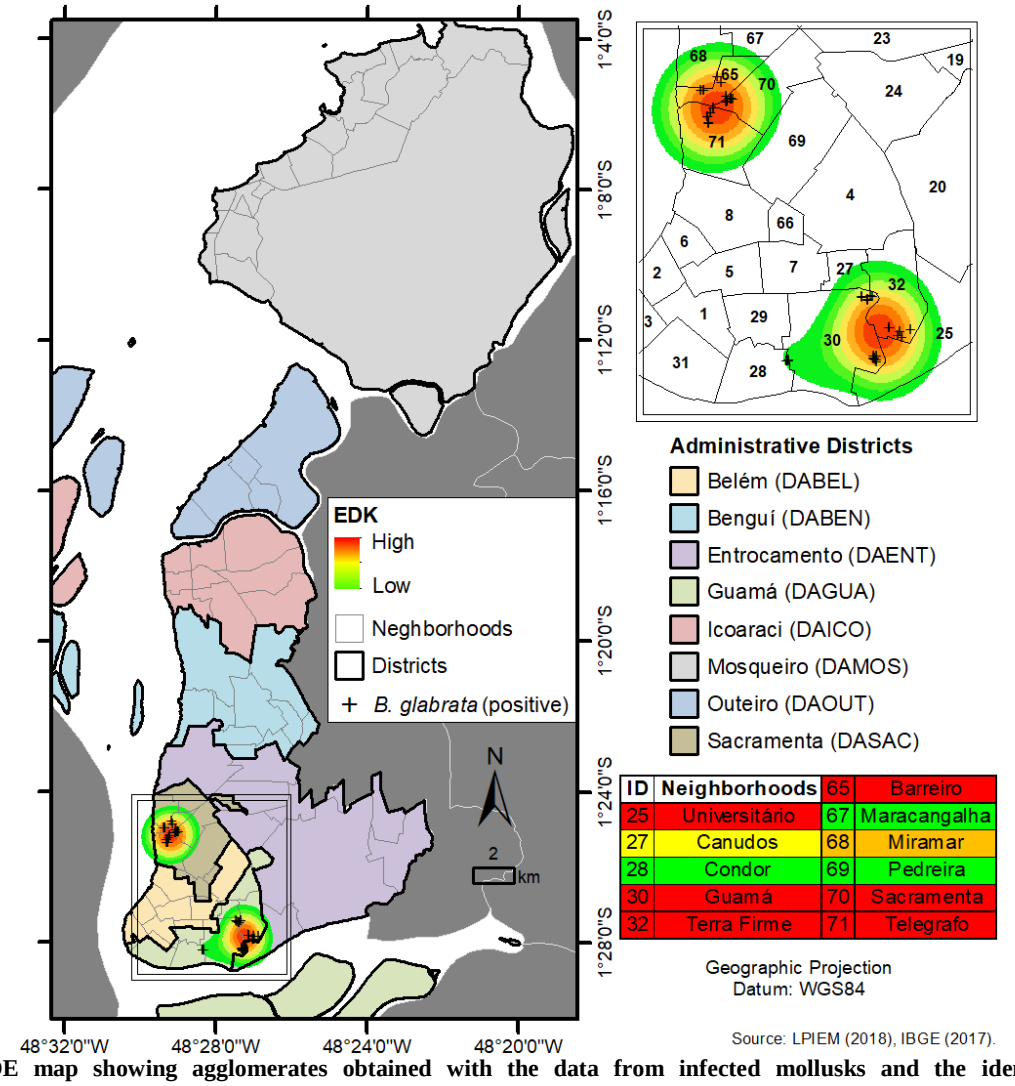


Fig. 3 KDE map showing agglomerates obtained with the data from infected mollusks and the identification of schistosomiasis transmission risk areas.

4. Discussion

The municipality of Belém has many problems related to public sanitation, such as peri-domiciliary ditches and streams with still or low water flow, macrophytic vegetation and reception of sewage and household waste, which helps the development and maintenance of schistosomiasis cycle [28]. These characteristics also make possible the establishment of *Biomphalaria* mollusks close to house areas, maintaining disease transmission [29].

The study identified *B. glabrata* snails infected with *S. mansoni* in the neighborhoods of Barreiro, Condor, Guamá, Sacramenta, Telégrafo and Terra Firme, indicating transmission risk in these areas. The neighborhoods of Barreiro, Condor, Guamá, Marco, Montese, Sacramenta, Sideral, Telégrafo and Terra Firme also presented *B. glabrata* negative sites for *S. mansoni*.

Breeding sites of *B. straminea* were found in 21 other neighborhoods: Aeroporto, Agulha, Ariramba, Barreiro, Benguí, Cruzeiro, Guamá, Icoaraci, Jurunas, Mangueiras, Maracajá, Marambaia, Marco, Mosqueiro, Outeiro, Parque Verde, Sacramenta, Sideral, Tenoné, Terra Firme and Vila. However, all *B. straminea* specimens were negative for *S. mansoni*. It is noteworthy that sites presenting negative snails for *S. mansoni* infection are not protected from becoming active transmission sites for the disease.

The KDE located very high-risk clusters in the Barreiro, Guamá, Sacramenta, Telégrafo, Terra Firme and Universitário neighborhoods, high-risk in the Miramar neighborhood, medium-risk in the Canudos neighborhood, and low-risk in the Condor, Maracangalha e Pedreira neighborhoods. Proximity of neighborhoods, inadequate sanitation conditions and flooded areas are favorable to the migration of snails through water collections that are interconnected by channels and streams from one neighborhood to another.

The presence of *Biomphalaria* mollusks in Telégrafo area has been observed since 1984, when 561 snails were collected and 480 belonged to *B. glabrata*. Of those, 27 were eliminating *S. mansoni* cercariae and another 81 specimens were identified as *B. straminea* [30]. Santos et al. [31] studied 19 sites in Telégrafo between 1992 and 1994 resulting in 2,095 specimens of *B. glabrata* snails collected, of which 276 (13.1%) were eliminating *S. mansoni* cercariae.

Malacological surveys from 2008 demonstrated the expansion of planorbid distribution in Belém. Among 35 neighborhoods evaluated, only Campina, Comércio and Umarizal did not present intermediate hosts of schistosomiasis, although the predominance of *B. straminea* focus was observed in this study, *B. glabrata* was found in the neighborhoods of Benguí, Cabanagem, Cremação, Guamá, Jurunas, Pratinha, Sacramenta, Telégrafo and Terra Firme [32].

The municipality of Belém is in a region with influence of the fluvial system and tides, limited by an urban floodplain [33]. The precarious sanitary conditions of neighborhoods in Belém and the presence of houses that are under stilts in areas of direct influence of the freshwater tide are determinants factors for the maintenance and dispersion of intermediate hosts of schistosomiasis. The mapping of areas where exist mollusks acting like intermediary hosts for schistosomiasis is important because it allows the determination of risk areas for disease transmission, providing subsidies for health services in control and surveillance activities and allowing the update of the municipality planorbidic chart.

5. Conclusions

In Belém, the species of *B. glabrata* was identified as intermediate host for schistosomiasis, actively participating in the maintenance of the disease.

With the results obtained using the geoprocessing techniques was possible to determine possible risk areas of schistosomiasis transmission. The development of maps identifying sites where schistosomiasis positive snails were found could be a

valuable tool to direct the efforts of the control programs.

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