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Research

Phlebotomines (Diptera: Psychodidae) from a Urban Park of Belém, Pará State, Northern Brazil and Potential Implications in the Transmission of American Cutaneous Leishmaniasis

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Abstract

In urban ecotourism parks, the life cycle of American cutaneous leishmaniasis (ACL) agents can remain established, where phlebotomines may comprise potential risks for visitors. The present study aimed to survey the phlebotomine fauna of a forest park 'Bosque Rodriques Alves-Jardim Botânico da Amazônia' (BRAJBA), in the urban area of Belém, Brazil. The park was monthly surveyed in 2018 using CDC light traps placed in ground and canopy strata. *Leishmania* spp. isolated from dissected females were characterized by polymerase chain reaction-restriction fragment length polymorphism analysis (PCR-RFLP) analysis. Fluctuations in specimen capture were correlated with rainfall. *Nyssomyia antunesi* (Coutinho, 1939) was predominant for all surveyed ecotopes and capture methods in both areas. Females of *Ny. antunesi* resting on tree bases were observed attempting to bite researchers during early morning. One *Bichromomyia flaviscutellata* (Mangabeira, 1942) and one *Trichophoromyia brachipyga* (Mangabeira, 1942) were found naturally infected by flagellates. Only the strain from *Th. brachipyga* was isolated and characterized as *Leishmania* (*Viannia*) *lainsoni* Silveira, Shaw, Braga and Ishikawa, 1987. Monthly fluctuations of the three most abundant species, *Ny. antunesi*, *Trichophoromyia ubiquitalis* (Mangabeira, 1942) and *Th. brachypiga*, had statistically significant negative correlations with rainfall. The present study provided further information to better understand ACL ecology in the Belém urban area, where the urban parks surveyed appeared to offer potential risk of contracting the disease, thus requiring environmental management. These observations highlighted the need for including *Ny. antunesi*, *Bi. flaviscutellata*, *Th. ubiquitalis*, and *Th. brachypiga* in the priority list for continuous entomological surveillance.

Key words: Phlebotomine, American cutaneous leishmaniasis, Urban park, Belém - Pará - Brazil.

Background

Phlebotomines comprise a group of medically important insects due their vector role in the transmission of several pathogens, particularly leishmaniasis agents (Ready 2013). In nature, a complex mosaic between mammals acting as reservoir hosts and phlebotomines is responsible for the maintenance of enzootic *Leishmania* parasites (Lainson and Shaw 2010). Affecting man, some species of *Leishmania* parasites may give rise to American cutaneous leishmaniasis (ACL), which, in some cases, comprises a clinically worrisome disease, with a spectrum ranging from self-limiting to mutilating forms (Silveira et al. 2013, 2018).

In Brazil, ACL presents three epidemiological transmission patterns: (i) sylvatic, (ii) occupational/recreational, and (iii) rural/peri-urban in colonization areas (Brasil 2017). Of these, pattern (ii) has an important impact on ecotourism. Forest parks opened to the public may offer conditions conducive to *Leishmania* spp. life cycles and consequently potential risk for ACL infection, requiring

specialized ecological management and entomological surveillance in these areas.

In the Belém Metropolitan Region (BMR), in Pará State, northern Brazil, ACL epidemiology assumes an occasional characteristic attributed to four *Leishmania* species: *L.* (*Viannia*) *braziliensis* Vianna 1911, *L.* (*Leishmania*) *amazonensis* Lainson and Shaw 1972, *L.* (*V.*) *lainsoni* Silveira, Shaw, Braga and Ishikawa, 1987 and *L.* (*V.*) *lindenbergi* Silveira, Ishikawa, Souza and Lainson 2002 (P.K.S. Ramos, unpublished data). Infection *foci* are mainly associated with forest fragments, where phlebotomines, reservoir hosts, and synanthropic fauna can survive environmental pressures and are able to establish ecological niches for *Leishmania* spp. maintenance (Ferreira et al. 2014).

In this sense, in the center of the urban area of Belém, the capital of Pará State, there are important urban parks that are worthy of attention as potential sources of ACL transmission because they are located in forest fragments with over populated captive mammal and free/semifree fauna. In the context of ACL eco-epidemiology, however, these parks are likely understudied.

Thus, the present work aimed to investigate, in an urban park of Belém, the phlebotomine sand fly fauna, associated or not associated with *Leishmania* spp. infections, spatiotemporal fluctuation patterns (monthly frequency and vertical stratification), and potential implications of these findings in the transmission of ACL agents.

Methods

Study Area

Belém is the capital and the biggest city of the Brazilian State of Pará, with an estimated population of 1,485.732 inhabitants. Climate is equatorial (average of 85–95% of humidity and 3,084 mm of yearly precipitation), directly influenced by the Amazon rainforest. In the urban area, ACL epidemiology is considered occasional, with only seven autochthonous cases registered in the last 5 yr (2014–2018). Taking into consideration the possibility of ecotourism parks currently open for visitation comprise risk areas for transmission of ACL agents, the following site was selected in the urban area of Belém (Fig. 1) as follows:

Bosque Rodriques Alves-Jardim Botânico da Amazônia (BRAJBA) 1° 25′ 48.2″ S; 48° 27′ 24.9″ W. A 15 ha area of remaining preserved primary forest (80% of green area), with an estimated flora of 10,000 trees from 300 species and a fauna of 345 animals, with 29 species living in captivity and 29 others in free/semifree conditions.

Captures of Phlebotomines

Captures were performed in forested sites of the park described above, with the presence of trees with an average of 20 m height, to sample the canopy environment. Both sampling strata were systematically surveyed from January to December 2018 using Center of Diseases Control (CDC) light traps, placed in ground (n = 2 CDC at 1.5 m above ground level) and in canopy strata (n = 2 CDC at 20 m a.g.l), operating from 06:00 p.m. to 06:00 a.m., during four nights *per* month.

As an effort to make certain that there were not species present that did not respond to the light traps, Shannon captures, with manual aspiration made by two professionals were also performed from 06:00 p.m. to 08:00 p.m. during three intercalated nights of November 2018. Meanwhile, aspirations were performed on tree bases, with aid of an electric aspirator (an adapted CDC trap), from 06:00 a.m. to 08:00 a.m. during three intercalated mornings of October 2018. In both methods, specimens captured while attempting to bite researchers were preserved individually for identification.

Processing and Identification of Phlebotomines

Captured specimens were transported and immediately processed in Ralph Lainson's Leishmaniasis Laboratory, Instituto Evandro Chagas (IEC). Females were prioritized for dissection under sterile conditions for species identification and to find parasites, following the method of Ryan et al. (1987). In the case of flagellate infection, parasitosis was semiquantified according to Freitas et al. (2002), and the pattern of parasite development was classified with the methods of Lainson and Shaw (1987). The gut contents of infected females were triturated, suspended in 0.2 ml of dissection solution, and inoculated in Novy, McNeal, and Nicolle culture media to attempt isolation. Males were identified under fresh conditions and/or stored in 70% ethanol to be later processed for mounting in Berlese or Canada balsam. Species identification followed the taxonomic criteria of Galati (2018).

Environmental Assessment

Climatic data were assessed to find correlations with phlebotomine fluctuations. The data were obtained from the 'Instituto Nacional

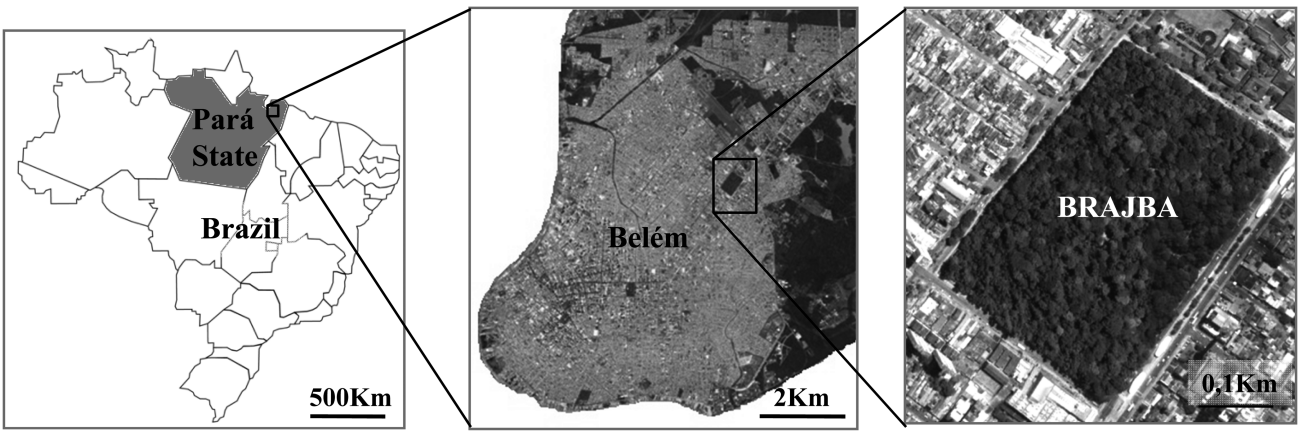

Fig. 1. Study area. The Bosque Rodrigues Alves - Jardim Botânico da Amazônia (BRAJBA) forest park in the urban area of Belém municipality, Pará State, Northern Brazil.

de Metereologia-INMET' database of the Belém Automatic Weather Station (code: 82191) (www.inmet.gov.br).

Data Analysis

The sampling effort was calculated by multiplying the number of traps installed by the number of hours and by the number of nights of capture for each park. To correct differences in sample effort, we calculated the average number of specimens captured *per* trap/hour (Rebêlo et al. 2009, Vasconcelos dos Santos et al. 2018). The Shannon-Wiener (H) diversity index was estimated and evaluated using the *t* test for Shannon diversity. The Wilcoxon test was used to evaluate differences between the vertical strata. Rarefaction curves were constructed and species richness was estimated with the nonparametric estimators, Chao 2, Jackknife 1, Jackknife 2, and Bootstrap. using Past software version 1.64 (Hammer et al. 2001). Confidence interval was set at 95%. Additionally, to evaluate differences between abundance and richness, the Mann–Whitney *U* test using the BioEstat 5.3 program was used. The species infection rate (SIR) was calculated as follows: SIR = number of infected females of a given species divided by the number of dissected females of a given species and multiplied by 100 (Paiva et al. 2007). To define the association between the monthly frequency of captured phlebotomines and rainfall, a univariate Pearson (*r*) correlation was performed with the aid of Past software (Hammer et al. 2001). Thus, the calculated value indicates the following: > 0.7 to 1 is a strong correlation; 0.3–0.7 is a moderate correlation; 0.1–0.3 is a weak correlation; and < 0.1 is an insignificant correlation.

Leishmania spp. Characterization

Leishmania spp. were characterized by polymerase chain reaction-restriction fragment length polymorphism analysis (PCR-RFLP) following previously established and validated methodology routinely applied in our lab (P.K.S. Ramos, unpublished data) (Simon et al. 2010). Digestion profiles of the amplicons were compared with those of the WHO *Leishmania* reference strains.

Environmental Issues

Following Brazilian legislation, procedures for capturing and processing of sylvatic invertebrate fauna for the present work were authorized by the 'Sistema de Autorização e Informação em Biodiversidade/Instituto Chico Mendes/ Ministério do Meio Ambiente' under protocol number 61923. In addition, permissions were requested and granted within the park.

Results

With 2,304 h of sampling with CDC trap, a total of 25,594 phlebotomines were captured and identified, belonging to 19 species; the most frequent species included *Nyssomyia antunesi* (Coutinho, 1939) (16,516; 64.56%), *Trichophoromyia ubiquitalis* (Mangabeira, 1942) (3,789; 14,76%) and *Th. brachipyga* (Mangabeira, 1942) (2,669; 10.40%), accounting for 89.72% of the total specimens captured (Table 1). Females (15,705) predominated over males (9,889) (ratio 1: 1.6).

With respect to vertical stratification, the most predominant species in the canopy was *Ny. antunesi,* representing 98% (5,817 ♀♀, 2,090 ♂♂) of the abundance, whereas on the ground, the predominant species were *Ny. antunesi* 49% (5,551 ♀♀, 3,058 ♂♂), *Th. ubiquitalis* 21% (1,852 ♀♀, 1,919 ♂♂), and *Th. brachipyga* 15% (1,427 ♀♀, 1,223 ♂♂) (Table 1).

Rarefaction curves for phlebotomines captured in canopy and ground level showed stabilization of species richness observed, considering the sample effort applied, and were in accordance with the values of all nonparametric estimators tested (Fig. 2).

Table 1. Species composition and vertical stratification of phlebotomines captured in the Bosque Rodrigues Alves - Jardim Botânico da Amazônia with CDC light traps, Belém, Brazil (Jan. to Dec. 2018)

Species	Vertical strata						Total	%
	Ground			Canopy				
	♀♀	♂♂	♀♀+♂♂	♀♀	♂♂	♀♀+♂♂		
Nyssomyia antunesi	5,551	3,058	8,609	5,817	2,090	7,907	16,516	64.5
Thichophoromyia ubiquitalis	1,852	1,919	3,771	10	8	18	3,789	14.8
Thichophoromyia brachipyga	1,427(1)	1,446	2,650	13	6	19	2,669	10.4
Pressatia choti	166	823	989	5	5	10	999	3.9
Bichromomyia flaviscutellata	424(1)	326	750	17	24	41	791	3.1
Brumptomyia avellari	300	323	623	8	5	13	636	2.5
Viannamyia furcarta	34	4	38	17	22	39	77	0.3
Sciopemyia sordellii	16	12	28	-	-	-	28	0.1
Evandromyia brachyphalla	10	16	26	-	-	-	26	0.1
Evandromyia monstruosa	9	9	18	-	-	-	18	0.1
Psathyromyia bigeniculata	7	7	14	-	-	-	14	0.1
Viannamyia tuberculata	6	1	7	-	-	-	7	0.0
Evandromyia infraspinosa	1	5	6	-	-	-	6	0.0
Lutzomyia gomezi	5	-	5	-	-	-	5	0.0
Psathyromyia barrettoi barrettoi	3	2	5	-	-	-	5	0.0
Micropygomyia rorotaensis	3	-	3	-	-	-	3	0.0
Psathyromyia aragaoi	1	1	2	-	-	-	2	0.0
Micropygomyia trinidadensis	2	-	2	-	-	-	2	0.0
Psychodopygus ayrozai	-	-	-	1	-	1	1	0.0
Total	**9,817**	**7,729**	**17,546**	**5,888**	**2,160**	**8,048**	**25,594**	

(n): number of specimens found infected by flagellates; ♀♀: females; ♂♂: males.
The Shannon diversity index was: canopy $H = 0.1174$, ground $H = 1.4402$ ($t = 119.13$, $P = 0$).

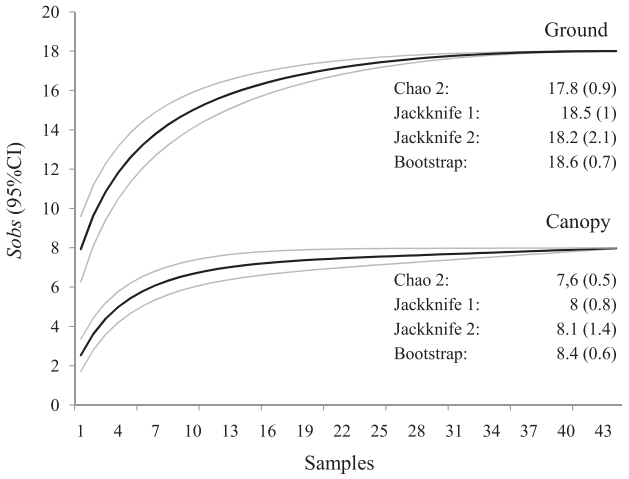

Fig. 2. Rarefaction curves representing species richness observed - *Sobs* of phlebotomines captured the Bosque Rodrigues Alves - Jardim Botânico da Amazônia, Belém, Brazil, from January to December 2018, at ground and canopy levels (black lines), and 95% confidence interval (gray lines), considering equal sample size, with values of their respective species richness estimated - *Sest,* and standard deviations (in brackets).

In relation to the phlebotomines captured *per* hour, with a total sampling effort of 2,316 h, an average of 15.1 specimens/hour, were obtained with CDC ground sampling (1,152 h of sampling), 6.96 specimens/h with CDC canopy sampling (1,152 h), 34.17 specimens/h with Shannon traps (6 h), and 616.17 specimens/h with aspiration on tree bases. *Nyssomyia antunesi* was the species with the highest average specimens/hour for all capture methods: CDC ground (4.82♀♀/h; 2.65♂♂/h), CDC canopy (5.05♀♀/h; 1.81♂♂/h), Shannon trap (12.33♀♀/h; 11.50♂♂/h), and aspiration on tree bases (238.67♀♀/h; 376.50 ♂♂/h). The other species that showed a high capture frequency were *Th. ubiquitalis* (1.61♀♀/h; 1.67 ♂♂/h) and *Th. brachipyga* (1.24 ♀♀/h; 1.06 ♂♂/h) for the CDC ground sampling. Specimens of *Ny. antunesi* were captured attempting to bite the professionals during aspiration on the tree bases (Table 2).

Monthly fluctuations of the three most frequent species, *Ny antunesi, Th. ubiquitalis*, and *Th. brachipyga*, were tested against precipitation. For all these phlebotomines, moderate but statistically significant negative correlations were observed (Fig. 3).

Parasite infections (gregarines and flagellates) were observed on four occasions for specimens captured in CDC ground. Two females of *Bichromomyia flaviscutellata* (Mangabeira, 1942) were found with gregarines and a female of *Bi. flaviscutellata* out of 441 dissected females (SIR: 0.22) and a female of *Th. brachipyga* out of 1,440 dissected females (SIR: 0.06) were found harboring flagellates, with estimated parasitosis and development patterns of (+++/suprapylarian) and (++/peripylarian), respectively. Only the parasite of *Th. brachipyga* was successfully isolated in culture, being cryopreserved in the cryobank of Ralph Lainson's Leishmaniasis Laboratory under the WHO code IBRA/BR/2018/M33013. DNA characterization showed a PCR-RFLP profile identical to that of the *L.* (*V.*) *lainsoni* WHO reference strain (Fig. 4).

Discussion

In Belém, although the BRAJBA is an important source of ecological data, with a well-studied inventory of fauna and flora, ACL ecology in this environment seems to be understudied. Thus, the present work provided, for the first time, with an entomological survey,

Table 2. Average of the five most frequently phlebotomines captured *per* hour using different trapping methods to sample the BRAJBA, Belém, Brazil (Jan. to Dec. 2018)

Species	Capture method															
	CDC ground				CDC canopy				Shannon				Tree bases			
	♀		♂		♀		♂		♀		♂		♀		♂	
	N	A	*N*	A	*N*	A	*N*	A	*N*	A	*N*	A	*N*	A	*N*	A
Nyssomyia antunesi	5,551	**4.82**	3,058	**2.65**	5,817	**5.05**	2,090	**1.81**	74	**12.33**	69	**11.50**	1,432	**238.67***	2,259	**376.50**
Thichophoromyia ubiquitalis	1,852	**1.61**	1,919	**1.67**	10	0.01	8	0.01	3	0.50	11	1.83	0	0.00	1	0.17
Thichophoromyia brachipyga	1,427	**1.24**	1,223	**1.06**	13	0.01	6	0.01	4	0.67	2	0.33	2	0.33	1	0.17
Pressatia choti	166	**0.14**	823	0.71	5	0.00	5	0.00	6	1.00	14	2.33	0	0.00	0	0.00
Bichromomyia flaviscutellata	424	0.37	326	0.28	17	0.01	24	0.02	6	1.00	15	2.50	1	0.17	1	0.17
Brumptomyia avellari	300	0.26	323	0.28	8	0.01	5	0.00	0	0.00	1	0.17	0	0.00	0	0.00
Total	9,720	8.44	7,672	6.66	5,870	5.10	2,138	1.86	93	15.50	112	18.67	1,435	239.17	239,17	377.00

N: number of specimens captured of a given sex (♀ or ♂) and species; A: average of specimens of a given sex (♀ or ♂) and species divided by the hour of sampling of a given capture method; bold values: higher individual values (> 0.9); * specimens found trying to bite professionals; ♀: male; ♂: female.

Total sampling effort: 2,316 h; sampling effort of each capture method:1,152 h CDC ground, 1,152 h CDC canopy, 6 h Shannon traps, and 6h aspiration on tree bases.

information on phlebotomines and their potential implications in the transmission of ACL agents.

Past studies accounted for 62 phlebotomine species from Belém and its outskirts (Ryan 1986). However, over the years, vegetal suppression and environmental pressure, mainly due to the urbanization process, contributed to an apparent reduction of local species diversity. A more recent inventory of seven forest fragments accounted for 22 species (Ferreira et al. 2014), numerically proportional to our present results (19 spp.).

Great species diversity, as well abundance, was found in the ground level. Canopy and ground strata can be seen as distinct habitats, with physical components and biological diversity. Canopy is a primary site of flowering and fruiting, a particular ecological subsystem suitable to housing arboreal frugivorous/herbivorous vertebrates (e.g., sloths and primates), while the ground hosts low-flying insects attracted to terrestrial animals, that generally comprise the greatest composition of phlebotomine fauna in the tropical rainforest. The vertical distribution of Phlebotomines is probably a response to physical and biological conditions of microhabitats, such as temperature, relative humidity, light intensity and spectral composition, air movement, and CO_2 (Chaniotis et al. 1971). It should not be neglected that the other trapping methods (Shannon traps × tree bases) were performed in different months (with different environmental conditions, like rainfall and wind), which strongly impacts the comparison between very different results.

Species infection rate for flagellates was estimated in 0.06 for *Th. brachipyga* and 0.22 for *Bi. flaviscutellata*. Dissection-based infection rates are usually lower than those provided by PCR-based methods, but also can vary greatly in the literature, with high values usually biased by captures performed in the dry season (Le Pont and

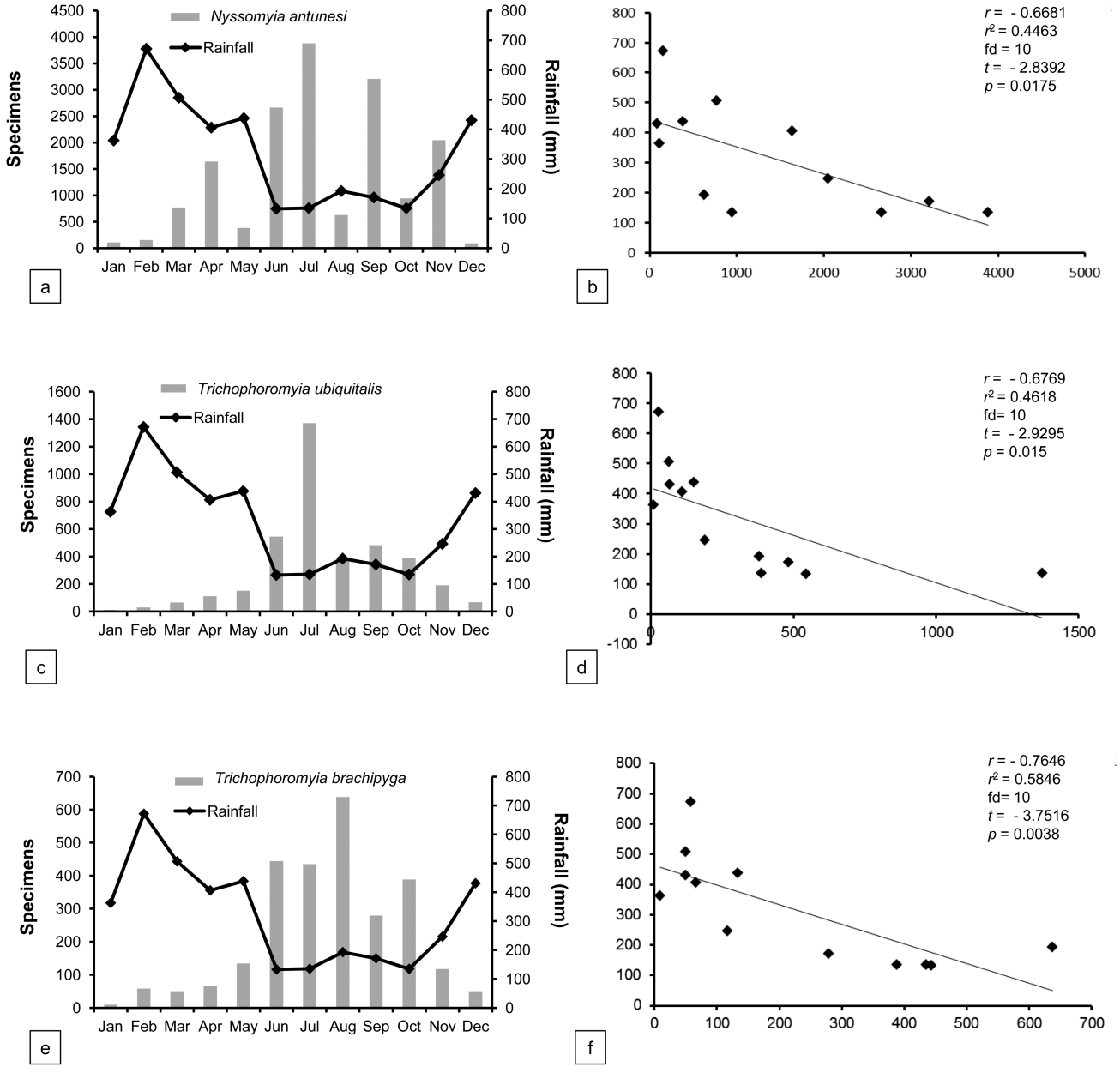

Fig. 3. Monthly fluctuation of *Nyssomyia antunesi* (a), *Trichophoromyia ubiquitalis* (c), and *Trichophoromyia brachipyga* (e) captured in the Bosque Rodrigues Alves - Jardim Botânico da Amazônia, Belém, Brazil, from January to December 2018, and their respective correlations (b, d, f) with rainfall.

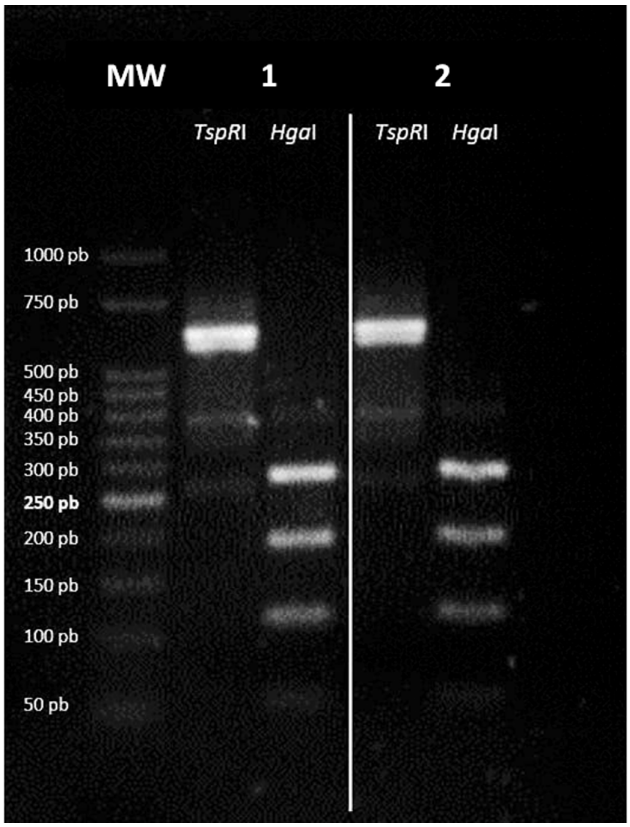

Fig. 4. PCR-RFLP profile (RPOF2/RPOR2 primers, TspRI/HgaI endonucleases) of a strain isolated from a naturally infected *Trichophoromyia brachipyga*, captured in the Bosque Rodrigues Alves - Jardim Botânico da Amazônia, Belém, Brazil, compared with that of the *Leishmania (Viannia) lainsoni* WHO reference strain. MW: molecular weight marker; 1: *Th. brachipyga* strain (IBRA/BR/2018/M33013); 2: *Leishmania (Viannia) lainsoni* WHO reference strain (MHOM/BR/1981/M6426).

Pajot 1980) or favoring the dissection of fed/gravid females (Freitas et al. 2002).

As expected, *Ny. antunesi* was the most frequently collected species for all environments and capture methods. These findings corroborate our past experience of entomological surveillance while searching presumed ACL infection sites in Belém and outskirts (Vasconcelos dos Santos, personal observation). In addition, based on its high frequency and anthropophilic behavior, this species has drawn attention for the suspected transmission of *L.* (*V.*) *lindenbergi*, a parasite species with geographical distribution apparently restricted to Belém and its outskirts (Silveira et al. 2002), but recently extended to the Brazilian western Amazon region (Cantanhêde et al. 2019). Surprisingly, in the present work, a markedly high number of specimens were captured on tree bases during the morning, including females attempting to bite the professionals, most probably reacting due disturbance on their resting sites. This result highlights the potentially aggressive behavior of this species during a time when visitors may be exposed in these parks. Similar biting behavior on tree bases during early morning is well documented for *Nyssomyia umbratilis* (Ward & Fraiha, 1977) in the Guianan/Amazonian region, where this phlebotomine is recognized as the main vector of *L.* (*V.*) *guyanensis* Floch, 1954. Both *Nyssomyia* species may migrate between ground and canopy, according to the needs of its life cycle. While during the morning, *Ny. umbratilis* can be found on tree bases, over the night it is essentially arboreal, climbing to the treetops to bit sloths and anteaters (Ready et al. 1986). In same circumstances, birds may also serve as alternative blood sources to maintain the population dynamics (Vasconcelos dos Santos et al. 2018).

Still in regard of vertical stratification of *Ny. antunesi*, this species represented 98% and 49% of the phlebotomine community in the canopy and ground strata, respectively. These results contrast with those of Silveira et al. (2002), who surveyed, in the late 1990s, an ACL *focus*, a military forest fragment approximately 2 km from the BRAJBA. In that occasion, *Ny. antunesi* was regarded as a predominantly terrestrial phlebotomine species. Conversely, present results lead us to speculate that there is an attractive feeding source for *Ny. antunesi* in the canopy strata and, if it is the vector of *L.* (*V.*) *lindenbergi* as originally supposed, it is reasonable to suspect of an arboreal mammal, as potential reservoir host. Attempting to fill this eco-epidemiological gap, our future steps in the studied area may include searching for feeding sources of phlebotomines, in particular for that species.

Bichromomyia flaviscutellata has been found to be infected by gregarines and flagellates. Gregarines are relative common in this phlebotomine species, and despite a lack of molecular methods to perform species identification of this protozoan, our experience allows us to speculate that they were *Psychodiella* sp. oocysts (most likely *Psychodiella chagasi* (Adler and Mayrink, 1961) (Vasconcelos dos Santos et al. 2018). With respect to flagellate infection, the suprapylarian behavior and luxuriant development of large promastigotes also allows us to speculate that they belonged to *L.* (*L.*) *amazonensis*. Belém is the type-locality of that leishmanian parasite, and natural infections are well documented in other forest fragments of the region where *Bi. flaviscutellata* is the only known vector on the local scale (Lainson and Shaw 1968).

The medical importance of *Trichophoromyia* has thus far gained attention. In Brazil, there are 23 described species, with some of them potentially implicated in the transmission of ACL agents (Vasconcelos dos Santos et al. 2019). In the present work, two species, *Th. ubiquitalis* and *Th. brachipyga* accounted for 14.76% and 10.40% of the captured specimens, respectively. Based on naturally infected specimens and anthropophilic behavior under lab conditions, *Th. ubiquitalis* has been implicated in the vector transmission of *L.* (*V.*) *lainsoni* (Silveira et al. 1991, Lainson et al. 1992). Conversely, one female of *Th. brachipyga* was found naturally infected with *L.* (*V.*) *lainsoni* in the present work. *Trichophoromyia* specimens captured in an hydroelectric system affected area with sympatric occurrence of indistinguishable females of *Th. brachipyga* and *Th. adelsonsouzai* (Vasconcelos dos Santos, Silva, Barata, Andrade & Galati, 2014) were found housing peripylarian flagellates morphologically suggestive to be metacyclic stages of *L.* (*V.*) *lainsoni* (Ferreira et al. 2014, Vasconcelos dos Santos, personal communication). It is not clear whether these apparently isolated facts may represent a single occasional finding or an underreported alternative vector role of this phlebotomine in ACL epidemiology due to *L.* (*V.*) *lainsoni*. If *Th. brachipyga* truly shares a vector role with *Th. ubiquitalis*, present finding is worrisome because this species is very frequently occurring in some forest environments, such as the Parque Estadual do Utinga, the largest forested park of Belém (Ferreira et al. 2014). Life cycle of *L.* (*V.*) *lainsoni* is quite possible to be maintained in the BRAJBA, since the mammal inventory of that park accounts several specimens of the rodent *Cuniculus paca* (Linnaeus, 1766), the potential reservoir host of that parasite. Also worth of note, both *Trichophoromyia* species were found predominantly at ground level, spatially congruent with the ecotope of *C. paca*.

No specimen of *Trichophoromyia*, distinct from *Th. brachipyga* and *Th. ubiquitalis*, was found in the BRAJBA. According to the phlebotomine inventory of Pará State presented in Ryan (1986), *Th.*

auraensis (Mangabeira, 1942) and *Th. castanheirai* (Damasceno, Causey & Arouck, 1945), whose females are indistinguishable from those of *Th. brachipyga*, have been also registered in Belém. Strong modification has been observed in that city over the years, probably compromising the local population dynamics of phlebotomines, such as *Trichophoromyia* species, leading some of them to be rare or even absent, as apparently observed in other recently surveyed forest fragments of Belém, geographically related, but ecologically isolated from the BRAJBA (Ferreira et al. 2014). In others environmentally affected ecotopes, such as those in the western Amazonian Brazil, however, *Th. auraensis*¸ for instance, retain to be present under urbanization pressure (Azevedo et al. 2008, Araujo-Pereira et al. 2014), suggesting that other micro-spatial factors should be influencing the *Trichophoromyia* bionomics, or that some specie's populations may respond differently to that variable.

Trichophoromyia auraensis has gained medically importance in western Amazonian Brazil (Teles et al. 2017) and Peru (Valdivia et al. 2012), in the occasion of some females have been found to be DNA-positive for *Leishmania* spp. In respect of the taxonomy of the presently captured specimens of *Trichophoromyia*, there is no doubt that they belong to *Th. ubiquitalis* and *Th. brachipyga*. Males from these two species, reparably distinguishable from each other were the only *Trichophoromyia* found, taking into account considerable effort performed to ensure sampling sufficiency and avoid pseudo-absences of rare or seasonal species, as clearly observed in the rarefaction curve.

Monthly fluctuations of the three species, *Ny. antunesi, Th. ubiquitalis*, and *Th. brachipyga*, were negatively correlated with precipitation. Conversely, the literature generally positively associates the overall phlebotomine fauna with rainfall, but each species can respond differently to that variable (Furtado et al. 2016). These unexpected results may be explained, in part, by the highly excessive level of rainfall in the Belém metropolitan region, which reached 700 mm in February 2018, most likely providing unsuitable conditions for phlebotomine survival. It is known that equilibrium of ecological/environmental factors, including water availability, is essential for life maintenance (Vasconcelos dos Santos et al. 2018). Still in regard to *Ny. antunesi*, a negative correlation with rainfall is not absolutely unexpected. In Colombia, for instance, a seasonal variation study showed that this species increased in abundance with an inverse correlation to rainfall (Vásquez-Trujillo et al. 2013).

Conclusions

In summary, the present study provided further elements to better understand ACL ecology in Belém urban areas. The urban park surveyed may offer potential risks of disease transmission for which environmental management and continuous entomological surveillance are required. The results also highlighted the medical importance of *Trichophoromyia* species, mainly due to the observations of a possible role of *Th. brachipyga* in the transmission of *L.* (*V.*) *lainsoni*. Present and past data also note *Bi. flaviscutellata* as an important vector in Belém. On the other hand, *Ny. antunesi* remains outstanding among the list of potential *L.* (*V.*) *lindenbergi* vectors, especially with regard to its observed high frequency and potentially aggressive behavior of females resting on tree bases during the early morning. The monthly fluctuations of these species, however, do not seem to be positively correlated with rainfall.

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Availability of Data and Materials

All data supporting the conclusions of this article are included within the article and its additional files. The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Author Contributions

Study design: T.V.S., F.T.S., M.M.P.; data acquisition: Y.d.V.S.U., T.V.S.; resources: F.T.S., M.M.P.; data analysis: Y.d.V.S.U., T.V.S., F.T.S., E.J.M.S., P.K.S.R., M.M.P.; manuscript—original draft: Y.d.V.S.U., T.V.S.; manuscript—final version: Y.d.V.S.U., T.V.S., F.T.S., E.J.M.S., P.K.S.R., M.M.P. All authors read and approved the final version of the manuscript.

Ethics Approval and Consent to Participate

Not applicable.

Consent for Publication

Not applicable.

Competing Interests

The authors declare that they have no competing interests.

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