

Phlebotomine sand flies (Diptera: Psychodidae) in forest fragments of Belém metropolitan area, Pará State, Brazil, with considerations on vectors of American cutaneous leishmaniasis agents

Flebotomíneos (Diptera: Psychodidae) em fragmentos florestais da região metropolitana de Belém, Estado do Pará, Brasil, com considerações sobre vetores da leishmaniose tegumentar americana

Flebótomos (Diptera: Psychodidae) en fragmentos forestales de la región metropolitana de Belém, Estado de Pará, Brasil, con consideraciones sobre vectores de la leishmaniasis tegumentaria americana

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ABSTRACT

In order to identify vectors of American cutaneous leishmaniasis (ACL) agents in urban forest fragments of Belém metropolitan area, Pará State, Brazil, an entomological study was conducted between 2009 and 2011 in seven ecologically isolated areas using CDC light traps. A total of 1,119 phlebotomine sand flies were collected and out of 22 species identified, six presented epidemiological importance due to their proven and/or putative implications in the transmission of ACL agents in the Brazilian Amazon. These results reveal the need of entomological surveillance in such areas.

Keywords: Psychodidae; Insect Vectors; Leishmaniasis, Cutaneous.

INTRODUCTION

Phlebotomine sand flies (Diptera: Psychodidae: Phlebotominae) are group of insects of medical importance due to the fact that they are considered natural vectors of *Leishmania* Ross, 1903 species (Euglenozoa: Trypanosomatidae), etiological agents of leishmaniasis^{1,2}. Of the total of 900 sand fly species described, approximately 70 have been implicated in the epidemiology of this disease^{3,4}.

In Brazil, American visceral leishmaniasis (AVL), whose transmission only takes place by a proven phlebotomine sand fly species^{5,6}, differs from American cutaneous leishmaniasis (ACL) that is caused by a variety of dermatropic agents and involves a high diversity of phlebotomine sand fly species with zoophilic behavior in the environment, which bite mammalian hosts. Consequently the disease presents several different enzootic transmission cycles⁷.

In the last ten years, an increase of ACL cases has been reported in several states of the Amazon Region⁸. In Pará State, the disease has been spreading in several mesoregions, including the Northeast region of Pará, where the capital Belém is located. The urbanization of ACL is unusual because of the sylvatic habits of the vectors involved¹. However, the increasing adaptation of some phlebotomine species to artificial ecotopes and peridomiliar conditions, as occurs in southeastern Brazil⁹, suggests that forest fragments with eco-epidemiological conditions in the center of big cities could be potential sources of human infection. In Belém, this fact was highlighted when an outbreak of ACL caused by, until that moment, an unknown parasite described as *Leishmania* (*Viannia*) *lindenbergi* (Silveira et al., 2002) occurred in a small forest fragment surrounded by an urban area¹⁰.

Belém metropolitan area is composed by the Municipalities of Ananindeua, Belém, Marituba, Benevides, Santa Isabel do Pará and Santa Bárbara do Pará, has a total of 154 forest fragments and four urban parks with areas varying from 1 to 1,200 hectares¹¹. Moreover, previous and recent data have been demonstrated that some of these fragments can support enzootic transmission cycles evolving at least four

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Leishmania species as follows: *Leishmania (Leishmania) amazonensis* Lainson & Shaw, 1972, *L. (V.) lainsoni* (Silveira et al., 1987), *L. (V.) naiffi* (Lainson & Shaw, 1989) and *L. (V.) lindenbergi*^{12,13,14,15}.

Regarding that fact, from 1940's to 1980's several entomological studies were carried out in forest areas of Belém and surrounding areas, such as Utinga (Belém), Aurá (Ananindeua) and Pirelli (Marituba) providing an extensive knowledge on the distribution of 62 Phlebotominae species, emphasizing the records of incriminated vectors of ACL, *Lutzomyia (Nyssomyia) flaviscutellata* (Mangabeira, 1942), *Lu. (Trichophoromyia) ubiquitousalis* (Mangabeira, 1942) and the suspected vectors *Lu. (N.) antunesi* (Coutinho, 1939) and *Lu. (Psychodopygus) davisii* (Root, 1934). However, owing to the progressive urbanization and environmental changes over the years, these data seem to be outdated^{16,17}.

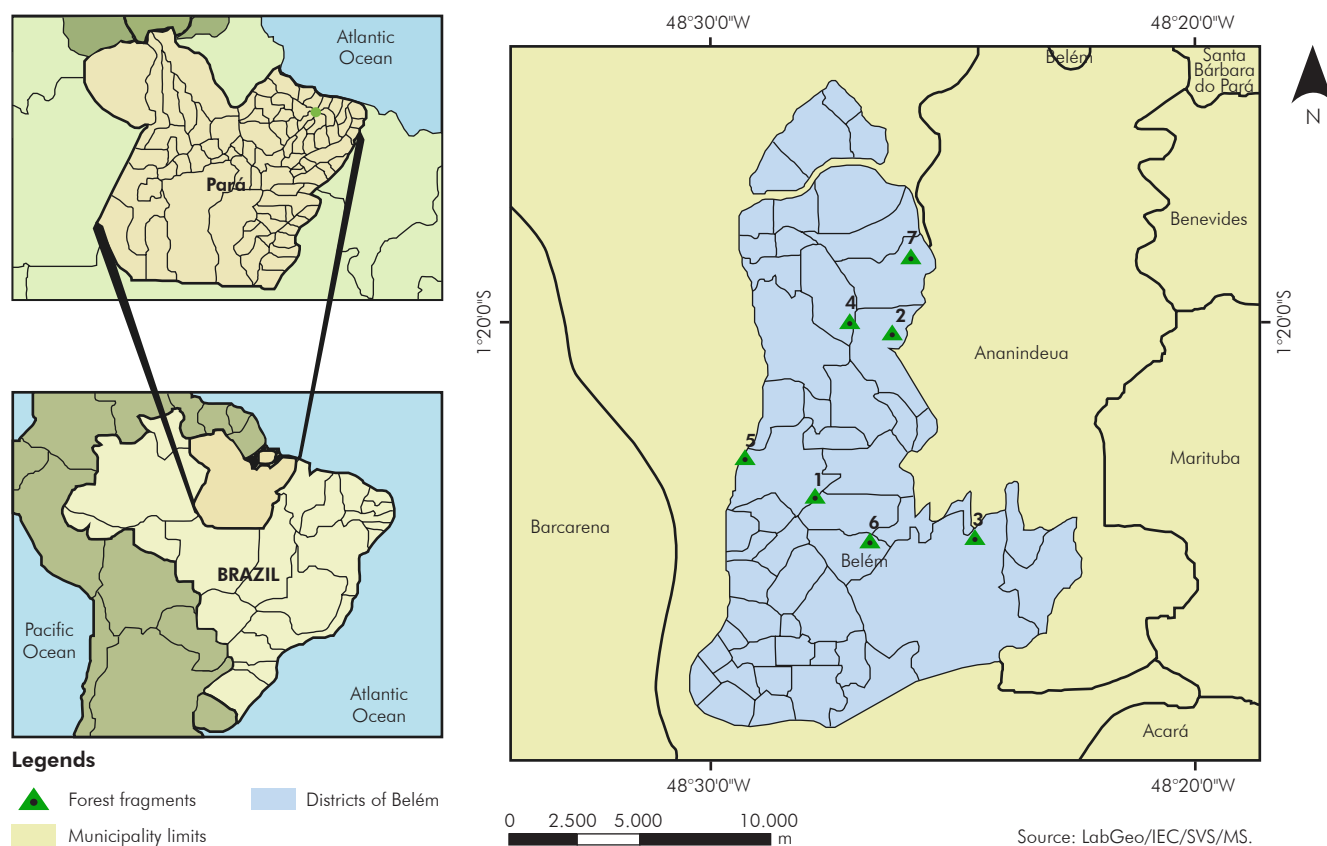
Due to these previous described facts, this study aimed to investigate the Phlebotominae in seven forest fragments in Belém metropolitan area in order to update their fauna and identify potential vectors of cutaneous leishmaniasis.

MATERIALS AND METHODS

STUDY AREA

Size, geometry and the distance between vegetation fragments can influence many ecological processes, such as death, migration and colonizations of plants and animals¹⁸, so it was selected seven different and

ecologically isolated areas (Figure 1): 1- Parque Ecológico do Município de Belém (1°23'47.11"S; 48°27'56.62"W), with 212 hectares, is a unit of environmental conservation under the responsibility of Secretaria Municipal de Meio Ambiente (SEMMA). It is destined to fauna and flora preservation, and scientific studies and researches, as well as promoting education for integration between man and nature; 2- Complexo Ecológico Parque dos Igarapés (1°20'15.36"S; 48°26'19.14"W) is a private area to provide recreation and ecological tourism in a place of environmental sustainability and preservation of the remaining forest in the urban area; 3- Parque Estadual do Utinga (1°24'39.55"S; 48°24'37.27"W), with 1,200 hectares, is considered the biggest urban park of Belém and with the best ecological conditions due to their size, form and the degree of isolation of forest fragments; 4- Mata da SEDUC (1°19'56.87"S; 48°27'13.38"W), a forest fragment that belongs to Secretaria de Estado de Educação, presenting some depredated areas of rainforest; 5- Porto Fluvial Brucutu (1°22'58.47"S; 48°29'22.16"W), a small forest fragment that serves as air force operational area at Val-de-cães air base; 6- Mata do 2° BIS (1°24'45.15"S; 48°26'47.82"W), a forest operational area of Army's Infantry; 7- Bioparque Amazônia - Crocodilo Safari (1°18'33.58"S; 48°25'52.80"W), a private ecological park with approximately 80 hectares providing contact with four different ecosystems by tracking and ecotourism far away 15 km from the town.



1: Parque Ecológico de Belém; 2: Complexo Ecológico Parque dos Igarapés; 3: Parque Estadual do Utinga; 4: Mata da SEDUC; 5: Porto Fluvial Brucutu; 6: Mata do 2° BIS; 7: Bioparque Amazônia - Crocodilo Safari.

Figure 1 – Map of Belém metropolitan area showing forest fragments studied

CAPTURING

During the rainy seasons from 2009 to 2011, 11 capture campaigns (three days each) were performed in each area described previously: 2009 (March, April, May and June), 2010 (January, February, March, April and May) and 2011 (January and February). Phlebotomine sand flies captures were undertaken using six battery-operated CDC miniature light traps installed at 1.5 m from ground and 10 m distant from each other in a horizontal transect started on the edge of vegetation, from 6:00 pm to 6:00 am providing a total of 12 h capture per trap-night. The sampling effort was calculated, in hours per trap, multiplying the trap exposure time (12 h) with the total days of campaign (33). The phlebotomine sand flies were triaged, killed in ethyl acetate, washed in a detergent solution (neutral soap 5%), saline solution (NaCl 0.9%) and then stored in alcohol 70% until mounting.

MOUNTING AND IDENTIFICATION

The specimens were mounted in microscope slides individually using berlese's fluid (GBI labs, USA). Damaged specimens could not be identified and therefore they were excluded from the analysis. The identification and nomenclature were carried out in accordance with Young and Duncan⁴ taxonomic criteria,

assisted with a regional pictorial key¹⁷. Abbreviations of sand fly names were written with two letters for genus and three for subgenera to avoid confusion to *Leishmania* abbreviation terms (just one letter for both genus and subgenera). A part of sampled material was deposited in the Phlebotomine Sand fly Collection of the Instituto Evandro Chagas (Coleção de Flebotomíneos do Instituto Evandro Chagas – ColFleb IEC), Ananindeua, Pará, Brazil.

RESULTS

With 360 h/trap of sampling, a total of 1,119 phlebotomine sand fly specimens were collected in the seven studied areas that belong to 22 species/subspecies. All them belonging to the genus *Lutzomyia* (Lu.) França, 1924 and were distributed in nine subgenera: *Psychodopygus* (Psy.) Mangabeira, 1941 - four species; *Trichophoromyia* (Tho.) Barretto, 1962 - three species; *Nyssomyia* (Nys.) Barretto, 1962 - three species; *Evandromyia* (Eva.) Mangabeira, 1941 - three species; *Viannomyia* (Via.) Mangabeira, 1941 - two species; *Sciopemyia* (Sci.) Barretto, 1962 - two species; *Lutzomyia* (Lut.) - one species; *Trichopygomyia* (Thy.) Barretto, 1962 - one species; and *Pressatia* (Pre.) Mangabeira, 1942 - one species; and group *Oswaldoi* Theodor, 1965 - two species (Table 1).

Table 1 – Phlebotomine sandflies captured in seven studied areas at Belém metropolitan region between 2009 and 2011

Species	Sampling places							Total	%
	PEUt	CEPIG	Mata da SEDUC	PEB	Porto Brucutu	Mata do 2º BIS	BACS		
<i>Lu. (Tho.) brachipyga</i>	363						5	368	32.88
<i>Lu. (Psy.) davisii</i>	38	17	201	2		2		260	23.27
<i>Lu. (Tho.) ubiquitousis</i>	253				2		3	258	23.05
<i>Lu. (Nys.) flaviscutellata</i>	21	15	43	3	2	5	2	91	8.14
<i>Lu. (Eva.) infraspinoza</i> (Mangabeira, 1941)	19						8	27	2.41
<i>Lu. (Via.) tuberculata</i> (Mangabeira, 1941)	20	1	2					23	2.05
<i>Lu. rorotaensis</i> (group <i>Oswaldoi</i>) (Floch & Abonnenc, 1944)	16	1	3		1		1	22	1.96
<i>Lu. (Eva.) brachyphalla</i> (Mangabeira, 1941)	10							10	0.89
<i>Lu. (Sci.) sordellii</i> (Shannon & Del Ponte, 1927)	7				1			8	0.71
<i>Lu. (Nys.) antunesi</i>	5	3						8	0.71
<i>Lu. (Psy.) geniculatus</i> (Mangabeira, 1941)	8							8	0.71
<i>Lu. (Tho.) dasypodogeton</i> (Castro, 1939)	7							7	0.62
<i>Lu. (Lut.) gomezi</i> (Nitzulescu, 1931)	2				4			6	0.53
<i>Lu. trinidadensis</i> (group <i>Oswaldoi</i>) (Newstead, 1922)	5		1					6	0.53
<i>Lu. (Psy.) paraensis</i>	3					1		4	0.35
<i>Lu. (Nys.) yuilli yuilli</i> (Young & Porter, 1972)	3							3	0.26
<i>Lu. (Thy.) longispina</i> (Mangabeira, 1942)	3							3	0.26
<i>Lu. (Eva.) monstruosa</i> (Floch & Abonnenc, 1944)	2							2	0.17
<i>Lu. (Via.) furcata</i> (Mangabeira, 1941)	2							2	0.17
<i>Lu. (Psy.) ayrozai</i>	1							1	0.08
<i>Lu. (Sci.) servulolimai</i> (Damasceno & Causey, 1945)	1							1	0.08
<i>Lu. (Pre.) triacantha</i> (Mangabeira, 1942)	1							1	0.08
Species	22	5	5	2	5	3	5	22	
Specimens	790	37	250	5	10	8	19	1119	

PEUt: Parque Estadual do Utinga; CEPIG: Complexo Ecológico Parque dos Igarapés; PEB: Parque Ecológico de Belém; BACS: Bioparque Amazônia- Crocodilo Safari.

The most frequent species were *Lu. (Tho.) brachipyga* (Mangabeira, 1942) (32.88%), *Lu. (Psy.) davis* (Root, 1934) (23.27%), *Lu. (Tho.) ubiquitalis* (23.05%) and *Lu. (Nys.) flaviscutellata* (8.14%). A more diverse and abundant fauna was found at Parque Estadual do Utinga (790 specimens, 22 species), followed by Mata da Seduc (250 specimens, five species) and Complexo Ecológico Parque dos Igarapés (37 specimens, five species). *Lu. (Nys.) flaviscutellata* was the most widely distributed species in the areas, being captured in all the seven capture points sampled and *Lu. (Psy.) davis* and *Lu. rorotaensis* were found in five of them.

DISCUSSION

The Phlebotominae fauna of Belém metropolitan area has been studied since the 1940s¹⁶, but few recent data regards the distribution on forest fragments remaining to the progressive vegetal suppression have been reported. As like other recent studies of the sand flies of the Amazon biome, known as having the most diverse fauna¹⁹, the present report shows a high diversity of species (22 species) distributed in ten supraspecific taxa (nine subgenera and one species group). However, despite this high diversity, is noteworthy its reduction, since in accordance with Ryan¹⁷ the sand fly fauna of Belém consisted of 62 species. On the other side these present results are in accordance with a recent survey carried out in Ananindeua municipality, where a diversity of 23 species in a forest fragment surrounded by urban area was found¹⁵.

In fact the present study does not seem to have an extensive sampling effort when compared with the previous ones^{16,17}. Anyway, it is the first time that ecologically isolated areas are analyzed and give us relevant eco-epidemiological information.

The sand flies captured in this study provided an updated picture of the phlebotomine fauna in forested areas of the Belém metropolitan region and also indicates, with previous data, that some species could be potentially involved in the transmission of ACL. Besides 22 species, six of them presented epidemiological importance due to their proven and/or putative implications in the transmission of ACL in Brazilian Amazon as follows: *Lu. (Tho.) ubiquitalis*, *Lu. (Psy.) davis*, *Lu. (Nys.) flaviscutellata*, *Lu. (Nys.) antunesi*, *Lu. (Psy.) paraensis* (Costa Lima, 1941) and *Lu. (Psy.) ayrozai* (Barretto & Coutinho, 1940). Entomoepidemiological aspects of the most frequent potential vectors discussed below.

Lu. (Psy.) davis, the second most numerous species (26.27%) and the first one in epidemiological importance is considered as other species of *Psychodopygus* with a strong anthropophilic tendency²⁰. Moreover, several previous studies about frequency and infection rates have shown that this species should be a potential vector of zoonotic cutaneous leishmaniasis since some specimens have been recorded naturally infected with *L. (V.) braziliensis* and *L. (V.) naiffi* in Brazilian Amazon^{19,21,22,23}.

Lu. (Tho.) ubiquitalis was the third numerous species found in this study (23.05%) and the second most epidemiologically important. In previous studies at Belém metropolitan area, in the Municipality of Benevides, this phlebotomine was found to be infected with *L. (V.) lainsoni*¹⁴ and latter incriminated as the main vector in the transmission of this parasite on the basis of its antropophilic behavior under laboratory conditions²⁴.

Despite it was not used a more specific trap (Disney) for catching *Lu. (Nys.) flaviscutellata*, this species was found in all seven areas studied, with frequencies varying from 2.7% to 62.5%, indicating a irregular distribution in the metropolitan area of Belém. Moreover, it's known that this species is also distributed in other areas of this region: contiguous areas to the Parque Estadual do Utinga, as Mocambo, Utinga, Catu and Água Preta forests²⁵, and others far away as Environmental Protected Area of Pirelli and Bosque Rodrigues Alves (unpublished data). These data have considered the possibility of *Lu. (Nys.) flaviscutellata* is being adapted to different environments along over Belém reinforcing its epidemiological importance since this species is so far recognized as the main vector of *L. (V.) amazonensis*^{7,12,26}.

Lu. (Nys.) antunesi was rarely found (0.71%) and in only two areas sampled (Parque Estadual do Utinga and Complexo Ecológico Parque dos Igarapés). This fact was unexpected, mainly for the absence of specimens in the samples of the Mata do 2º BIS. A entomological survey was done to describe an outbreak of *L. (V.) lindenbergi* during 1996 in this same area, although no female of *Lu. (Nys.) antunesi* was found infected for this parasite, it remains in the list of possible vectors, at that moment, the most frequent (50.7%) among the species captured and predominant (83.7%) within the antropophilic ones captured¹⁰.

To explain that fact, it would be possible that, once the sampling of this area was undertaken only at rainy season, probably carried out in the beginning of the emergence of adult population. Moreover, recent studies on the seasonal variation of *Lu. (Nys.) antunesi* in Colombia²⁷ and in Ananindeua Municipality¹⁵, near from the present study area, showed that this species had been increased in abundance inversely correlating with the precipitation. Other probable reason is that *Lu. antunesi* is known to be attracted by Shannon trap¹⁰, because of technical conditions it was not possible to be used.

It's necessary to give attention for the most frequent species found, *Lu. (Tho.) brachipyga* (32.88%). Although most of the species from *Trichophoromyia* subgenus does not seem to be very antropophilic and *Lu. (Tho.) ubiquitalis* is, up to now, the only one incriminated in the transmission of ACL^{14,24}. In Bolivia, *Lu. (Tho.) vellascoi* Le Pont & Desjeux, 1992 was found infected by an unidentified Leishmanian parasite in a ACL endemic area where a first human case attributed to *L. (V.) lainsoni* was identified²⁸ and recently in Peru, *Lu. (Tho.) auraensis* (Mangabeira, 1942) was found infected with *L. (V.) lainsoni* and *L. (V.) braziliensis*²⁹. Moreover, two dissected

females of *Lu. (Tho.)* sp. [ambiguous identification of *Lu. (Tho.) brachipyga* or *Lu. (Tho.) adelsonsouzai* (Santos, Silva Barata Andrade & Galati, 2014)] were found housing flagellates potentially compatible to peripylarian *Leishmania* parasites in an area under occurrence of ACL at Brazilian Amazon (TV Santos, unpublished data). All these findings indicate that some *Trichophomyia* species could play an important role in the transmission of leishmaniasis agents and their high frequency in entomological surveys, such as *Lu. (Tho.) brachipyga* in this study may present epidemiological relevancy.

The Parque Estadual do Utinga presented the most richness species (22 species out of 22 total) and abundance of specimens (70.7%) than the other areas analyzed. These data are in accordance with previous studies about this complex of contiguous areas that were indicated as the best ecological conditions to environmental preservation due their size and degree of isolation¹¹, such characteristics also favor to maintain *Leishmania* life cycles^{7,8}.

CONCLUSION

With these present data, it can be concluded that the phlebotomine sand fly fauna of Belém metropolitan area presents 22 species currently, with at least six beings of epidemiological interest in the potential transmission of five leishmaniasis agents, indicating that continuous investigations on the sampled fragments are need for the entomological surveillance.

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Flebotomíneos (Diptera: Psychodidae) em fragmentos florestais da região metropolitana de Belém, Estado do Pará, Brasil, com considerações sobre vetores da leishmaniose tegumentar americana

RESUMO

Com o objetivo de identificar agentes transmissores da leishmaniose tegumentar americana (LTA) em fragmentos florestais na área urbana da região metropolitana de Belém, Estado do Pará, Brasil, foi conduzido um estudo entre 2009 e 2011 em sete áreas ecologicamente isoladas usando armadilhas luminosas CDC. Um total de 1.119 flebotomíneos foi coletado e das 22 espécies identificadas, seis apresentaram importância epidemiológica devido as suas implicações comprovadas e/ou suspeitas na transmissão dos agentes de LTA na Amazônia brasileira. Tais resultados revelam a necessidade de vigilância entomológica nessas áreas.

Palavras-chave: Psychodidae; Insetos Vetores; Leishmaniose Cutânea.

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RESUMEN

Con el objetivo de identificar agentes transmissores de la leishmaniasis tegumentaria americana (LTA) en fragmentos forestales en el área urbana de la región metropolitana de Belém, Estado de Pará, Brasil, se llevó a cabo un estudio entre 2009 y 2011 en siete áreas ecológicamente aisladas usando trampas luminosas CDC. Se colectó un total de 1.119 flebótomos e de las 22 especies identificadas, seis presentaron importancia epidemiológica debido a sus implicaciones comprobadas y/o putativas en la transmisión de los agentes de LTA en la Amazonía brasileña. Tales resultados revelan la necesidad de vigilancia entomológica en esas áreas.

Palabras clave: Psychodidae; Insectos Vectores; Leishmaniasis Cutánea.



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