

Distribution of phlebotomine fauna (Diptera: Psychodidae) across an urban-rural gradient in an area of endemic visceral leishmaniasis in northern Brazil

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The number of visceral leishmaniasis (VL) cases has increased over the past 10 years in Brazil, especially in the North and Northeast Regions of the country. The aim of this study was to evaluate the urbanisation of VL vectors in Barcarena, Pará, an area in northern Brazil where VL is endemic. Sandflies were captured using Centers for Disease Control (CDC) light traps along an urban-rural gradient. The CDC traps were installed inside hen houses at a height of 150 cm. A total of 5,089 sandflies were collected and 11 species were identified. The predominant species was Lutzomyia longipalpis (rate of 95.15%), which suggests its participation in the transmission of VL. A total of 1,451 Lu. longipalpis females were dissected and no Leishmania infections were detected. Most of the sandflies were captured at the border of a forest (88.25%) and no flies were captured in the urban area, which suggests that transmission is still restricted to rural sites. However, the fact that a specimen was collected in an intermediate area indicates that urbanisation is a real possibility and that vector monitoring is important.

Key words: Phlebotominae - vectors - visceral leishmaniasis - endemic area

Visceral leishmaniasis (VL) is a chronic disease that primarily affects children. The etiological agent in South America is the flagellate protozoan *Leishmania (Leishmania) infantum chagasi* (Lainson & Rangel 2005). The majority of human VL cases in Brazil occur in the northeast regions (MS 2010). In the northern regions, the increase in VL has been most extensive in the states of Pará (PA) and Tocantins (TO) (MS 2010). Although VL is considered to be a rural disease, it has become urbanised in several large and small Brazilian municipalities, including Santarém (PA), Belo Horizonte [Minas Gerais (MG)], Araçatuba (São Paulo), Palmas (TO), Campo Grande [Mato Grosso do Sul (MS)] and Cuiabá [Mato Grosso (MT)] (Oliveira et al. 2003, 2006, Rangel & Lainson 2003, Dujardim 2006, De Paula et al. 2008). The VL urbanisation process is associated with deforestation and disordered urbanisation (Ximenes et al. 2007).

The principal vector of *Lu. chagasi* in Brazil is the phlebotomine sandfly *Lutzomyia longipalpis* s.l. (Lainson & Rangel 2003), which is considered a species complex due to strong evidence involving morphological and physiological differences (Lainson & Rangel 2005, Bau-

zer et al. 2007). *Lu. longipalpis* is distributed among all neotropical regions from Mexico-Argentina and occurs in all Brazilian regions (Souza et al. 2009). In addition to *Lu. longipalpis*, there are other species related to VL transmission in Brazil, including *Lutzomyia cruzi* Mangabeira, 1938, which has been associated with transmission in MT and MS (Santos et al. 1998). *Lutzomyia forattinii*, an anthropophilic species, has been determined to be naturally infected with *Lu. chagasi* (Pita-Pereira et al. 2008) and *Lutzomyia evansi* naturally infected with *Lu. chagasi* has been detected in endemic areas of Colombia (Rangel & Lainson 2003).

No study has been published regarding the relationship between anthroponotic transmission and the increasing cases of VL in PA. In addition, no study monitoring the possible urbanisation process of *Lu. longipalpis* has been performed. Deforestation, which is a consequence of increasing agriculture, cattle and urbanisation, plays an important role in the ecologic niche changes to thousands of phlebotomine species (Lainson & Rangel 2005). Therefore, a study to investigate the presence of *Lu. longipalpis* in environments altered by man is a requirement for the better control of VL in endemic areas. Thus, the present study investigated phlebotomine fauna across an urban-rural gradient and the probable urbanisation of sandflies, mainly *Lu. longipalpis*, which is the putative vector of *L. (L.) infantum chagasi* in Barcarena (PA).

MATERIALS AND METHODS

Study area - Barcarena (01°30'24"S 48°37'12"W) is located northeast of PA and belongs to the metropolitan mesoregion of Belém (Figure). The municipality occupies an area of 1,310 km² and has 94,641 inhabitants.

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Until 2002, the diverse vegetation of Barcarena was mostly flood forest, upland forest (mature and secondary) and swamp forest (Amaral et al. 2002). However, as a result of deforestation, the forests have given way to secondary forests in different stages of development (Pacheco et al. 2007). This region was selected because VL is endemic and the occurrence of VL has increased from 2000–2008 in this region. A total of 301 cases were reported between 2001–2008 according to the Health Department of the municipality of Barcarena.

Phlebotomine capture - Captures were performed in five excursions of 10 days each. The captures occurred in October, November and December of 2007, February of 2008 and January of 2009, totalling 50 days. From November–February, the phlebotomine population density is higher than in other months, most likely because it is the end of the summer period in PA. To evaluate phlebotomine movement from the forest to the urban areas, the captures were performed along two transects. Each transect crossed four areas: (i) forest, an area with dense vegetation and without the presence of human habitation, (ii) edge of forest, an area adjacent to the woods with sparse human habitation and scarce street lighting, (iii) intermediate areas, corresponding to the city periphery with blocks of houses and street lighting, but with less vegetation cover than the edge of forest area, and (iv) urban areas, areas with high population density, night lighting, little vegetation and where most of the buildings in the city are located. Three similar sampling points were selected in each area and totalled 12 sampling points per transect.

The first transect covered an area that has been colonised by humans for over 12 years and the four collection areas were represented by the neighbourhoods of Araticu (forest and edge of forest), Bairro Novo (intermediate area) and City Centre (urban area). The second transect covered an area that has been recently colonised (4 years ago) and the captures were made in the neighbourhoods of Bar-

bolândia (forest and edge of forest), Betânia (intermediate area) and City Centre (urban area), as shown in Figure. The two transects are similar; however, the edge of forest area in the second transect contained human habitation, low vegetation density and intense wind exposure.

Phlebotomines were captured using Centers for Disease Control (CDC) light traps (HP model) installed 1.5 m above ground level at 6:00 pm and removed the next day at 6:00 am. In all areas, except for the forest, the CDC light traps were installed in peridomicile areas or next to or inside hen houses. Phlebotomine species were identified according to Young and Duncan (1994) using a Zeiss optical microscope.

Detection of natural infection - To evaluate the rate of natural infection with *Leishmania* parasites, phlebotomine females captured at the sampling points were taken to the laboratory where they were positioned on a glass slide with a drop of saline solution to remove the digestive tube, and the flies were examined to identify the presence of promastigotes.

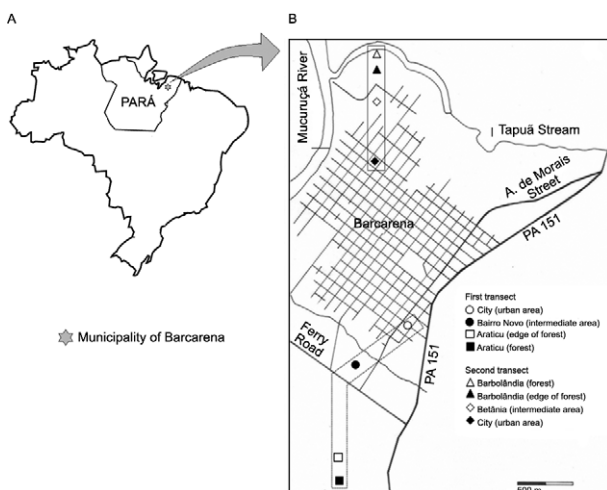
Statistical analysis - Statistical analysis of the differences between male and female phlebotomine numbers were performed using the chi-square test. All *p* values < 0.05 were considered to be statistically significant.

RESULTS

During the experiment, 5,089 phlebotomines, which were comprised of 3,440 males and 1,649 females and belonged to 11 species, were captured (Table I). The predominant species was *Lu. longipalpis* (95.15%), followed by *Lutzomyia (Sciopemyia) sordellii* (2.06%) and *Lutzomyia (Nissomyia) flaviscutellata* (1.76%), and the latter is a proven vector of *Lutzomyia amazonensis*. The number of *Lu. longipalpis* males was significantly higher than the number of *Lu. longipalpis* females ($p < 0.0001$) (Table I). In contrast, among the other species captured, the number of females was equal to or greater than the number of males. Other epidemiologically important species captured in the study areas include *Lutzomyia (Nissomyia) antunesi* and *Lutzomyia (Psychodopigus) paraensis*, which represented less than 1% of the phlebotomine sandflies captured. These species are associated with the transmission of *Leishmania* sp. in PA.

All specimens were collected in the forest area or at the edge of the forest (Table II) except for one specimen of *Lu. sordellii*, which was collected in the intermediate area of the first transect. No sandflies were captured in the urban area during this study. Most of the specimens (90%) were captured in the first transect and only 10% were captured in the second transect. The species richness and abundance patterns differed between the first and second transects. In the first transect (longer human colonisation), *Lu. longipalpis* was the most abundant species at the forest edge and *Lu. flaviscutellata* was the predominant species in the forest area. In the second transect, phlebotomines were only captured in the forest edge area and *Lu. longipalpis* was the most abundant species.

The greatest species richness was observed in the forest edge area of the first transect (10 species) and the species richness was reduced by half (5 species) in the



Study area. A: Brazil map presenting state of Pará and the geographic localization of the municipality of Barcarena; B: map of distribution of transects areas in Barcarena.

forest edge area of the second transect. The forest area of the first transect presented the second highest species richness (7) and no sandflies were captured in the forest area of the second transect.

A total of 1,451 *Lu. longipalpis* females were dissected to evaluate the natural infection rate; however, none of the flies were infected with *Leishmania* promastigotes. Among the other species, one *Lu. sordellii* female captured at the edge of the forest in the first transect was infected with unidentified flagellates.

DISCUSSION

This phlebotomine capture study revealed that Barcarena presents phlebotomine species of high richness and abundance in areas under anthropic impact. Similar results showing phlebotomine fauna richness have been obtained in other Brazilian cities (Andrade-Filho et al. 2001, Silva et al. 2007, 2008). Oliveira et al. (2003) captured 1,245 phlebotomines belonging to 28 species in Campo Grande (MS) and *Lu. longipalpis* was captured in an urban area.

In Barcarena, 98% of the specimens were collected at the edge of the forest. This finding is similar to results reported by Andrade-Filho et al. (2001), where 2,677 phlebotomines were captured in TO and 89.17% were captured at the edge of the forest with high species richness. Feliciangeli et al. (2006) captured *Lutzomyia pseudolongipalpis* in high abundance (98.16%) in four houses at the forest edge in El Brasil, a rural community of Venezuela. The authors also observed that the abundance of sandflies decreased with distance from the forest edge. Quintana et al. (2010) also observed a higher abundance of phlebotomines in modified areas compared to primary vegetation areas.

Lu. longipalpis represented more than 93% of all specimens and it was the most abundant species captured at the edge of the forest in both transects. The adapta-

tion of *Lu. longipalpis* to an anthroponotic environment has been observed in several studies (Oliveira et al. 2003, Ribeiro et al. 2007, Missawa et al. 2008, Rangel & Vilela 2008). This species has been found in shelters for domestic animals, such as birds, dogs and pigs, and it has also been found indoors, which demonstrates its attraction to humans (Rangel & Vilela 2008, Michalsky et al. 2009).

It is important to highlight the abundance of *Lu. longipalpis* at the edge of the forest in the second transect, which is where 500 *Lu. longipalpis* specimens were captured and only 13 phlebotomines from other species were captured. The natural vegetation in the area of the second transect was recently removed for the construction of human dwellings; therefore, the human impact is greater in this area than along the first transect. This finding suggests that *Lu. longipalpis* is more adaptable to disturbed areas than the other phlebotomine species captured in this area. These results are similar to those observed by Oliveira et al. (2003) in Campo Grande (MS), where a total of five species were detected in the urban area and *Lu. longipalpis* was the most abundant (49%). Ribeiro et al. (2007) observed that *Lu. longipalpis* presented higher capture rates in occupied areas of land than other captured species.

The number of *Lu. longipalpis* males captured in Barcarena was significantly higher than the number of *Lu. longipalpis* females. This pattern was also observed by Michalsky et al. (2009), who captured 78.72% *Lu. longipalpis* males in Janaúba (MG) and by Silva et al. (2007), who captured 79% males in Campo Grande (MS). One hypothesis to explain this pattern is that *Lu. longipalpis* males form groups at blood meal sites for mating purposes (Kelly & Dye 1997). According to previous studies, chemical mediators produced by hosts and vectors for finding a sexual partner may be related to the larger number of males observed compared to females for some species of sandflies (Kelly & Dye 1997).

TABLE I
Total number of phlebotomine species captured in the municipality of Barcarena, state of Pará

Species	Males (n)	Females (n)	Total n (%)
<i>Lutzomyia longipalpis</i>	3,391	1,451	4,842 (95.15)
<i>Lutzomyia sordellii</i>	22	83	105 (2.06)
<i>Lutzomyia flaviscutellata</i>	12	78	90 (1.76)
<i>Lutzomyia (Helcorcytomyia) trinidadensis</i>	12	12	24 (0.47)
<i>Lutzomyia (Lutzomyia) gomezi</i>	0	7	7 (0.14)
<i>Lutzomyia antunesi</i>	3	4	7 (0.14)
<i>Lutzomyia (Viannamyia) furcata</i>	0	4	4 (0.08)
<i>Lutzomyia (Psychodopigus) davisi</i>	0	4	4 (0.08)
<i>Lutzomyia paraensis</i>	0	4	4 (0.08)
<i>Lutzomyia (Pintomyia) damascenoi</i>	0	1	1 (0.02)
<i>Lutzomyia (Helcorcytomyia) pusilla</i>	0	1	1 (0.02)
Total	3,440	1,649	5,089 (100)

During this study, evidence of *Lu. longipalpis* urbanisation in the Barcarena was not observed. However, the urbanisation of this species has been reported in many Brazilian municipalities, including Campo Grande (MS) (Oliveira et al. 2003, 2006), Uberlândia (MG) (De Paula et al. 2008), Teresina (Piauí) and São Borja (Rio Grande do Sul) (Costa et al. 2007, Souza et al. 2009). In Natal (Rio Grande do Norte), the presence of *Lu. longipalpis* was detected in urban and peri-urban areas (Ximenes et al. 2007). In addition, in Belo Horizonte (MG), the VL urbanisation process was found to be rapid and 29 out of the 30 reports of VL in the metropolitan area of MG in 1994 were from Belo Horizonte (Da Luz et al. 2001). More recently, 1,550 *Lu. longipalpis* females, six of which were infected with *Lu. chagasi*, were captured in the Janaúba, which is an area of intense transmission of VL. Janaúba is similar to Barcarena with respect to environmental alterations, which can be described by rapid and intense rural population migration to an urban periphery that lacks adequate housing and sanitation infrastructure (Michalsky et al. 2011).

Another phlebotomine species of epidemiological importance captured in the study area was *Lu. flaviscutellata*, a species that has been incriminated as a vector of *L. (Leishmania) amazonensis*, the etiological agent of cutaneous leishmaniasis (Lainson et al. 1994). *Lu. flaviscutellata* was the most abundant species in forest areas (73.33%) and the second most abundant at the edge of the forest (26.67%). Similar results were observed by Rebêlo et al. (1999), where 69.8% were captured in the forest area in São Luiz (Maranhão) and by Oliveira et al. (2003), who verified a higher abundance of *Lu. flaviscutellata* in the forest area (5) compared to the peridomestic area (1) in Campo Grande (MS).

Other species of epidemiological importance captured during this study in Barcarena include *Lu. antunesi* and *Lu. paraensis*. These species are involved in transmitting *Leishmania* sp. in PA (Rangel & Lainson 2003). *Lu. paraensis* naturally infected with *Leishmania (Viannia) naiff* was captured in Benevides (PA) (Rangel & Lainson 2003). Although Barcarena has had no reports of autochthonous cutaneous leishmaniasis, the presence of the vector species in the forest and forest edge areas suggest the possibility of the establishment of a cutaneous leishmaniasis transmission cycle. It is important to note that even though *Lu. sordellii* has not been incriminated in *Leishmania* sp. transmission, it was the only species captured in the intermediate area, which suggests the possibility of its urbanisation. These data emphasise the need for entomological monitoring in Barcarena.

Although Barcarena is considered to be an area where VL is endemic, no natural infections were detected in the sandflies captured in this study. Several studies have reported that the natural infection rate is low among sandflies and is approximately 1%. Nascimento et al. (2007) dissected 81 females and detected only one infected fly. Neitzke et al. (2008) analysed 2,487 females and found an infection rate of 0.04%. Therefore, the negative results from the microscopic analysis performed in this study are probably attributable to the low rates of natural infection and not to a lack of infection in the studied areas, especially considering that 301 cases of human VL were reported between 2000-2008 in Barcarena.

TABLE II

Distribution of the phlebotomine species captured per area in the municipality of Barcarena, state of Pará

Species	Transects (n)								Total
	First				Second				
	FA	EF	IA	UA	FA	EF	IA	UA	
<i>Lutzomyia longipalpis</i>	6	4,336	0	0	0	500	0	0	4,842
<i>Lutzomyia sordellii</i>	2	100	1	0	0	2	0	0	105
<i>Lutzomyia flaviscutellata</i>	66	16	0	0	0	8	0	0	90
<i>Lutzomyia trinidadensis</i>	0	22	0	0	0	2	0	0	24
<i>Lutzomyia gomezi</i>	2	5	0	0	0	0	0	0	7
<i>Lutzomyia antunesi</i>	2	4	0	0	0	1	0	0	7
<i>Lutzomyia furcata</i>	0	4	0	0	0	0	0	0	4
<i>Lutzomyia davisi</i>	2	2	0	0	0	0	0	0	4
<i>Lutzomyia paraensis</i>	4	0	0	0	0	0	0	0	4
<i>Lutzomyia damascenoi</i>	0	1	0	0	0	0	0	0	1
<i>Lutzomyia pusilla</i>	0	1	0	0	0	0	0	0	1
Total	84	4,491	1	0	0	513	0	0	5,089
Total of species	7	10	1	0	0	5	0	0	11

EF: edge of forest; FA: forest area; IA: intermediate area; UA: urban area.

Intensive and constant monitoring of sandflies on the edge of the forest and in intermediate areas is recommended to detect local patterns and periods of higher vector abundance, which will allow for better preventive anti-vector intervention (Salomón et al. 2009).

In conclusion, Barcarena has a rich sandfly fauna and *Lu. longipalpis* was the most abundant species in the forest area and at the forest edge. These data suggest that, among the 11 species captured in Barcarena, *Lu. longipalpis* is the species most adapted to disturbed areas. Thus, this study demonstrates the importance of constant entomological surveillance and monitoring in this area.

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