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Ecology of *Lutzomyia longipalpis* in an area of visceral leishmaniasis transmission in north-eastern Brazil

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ABSTRACT

Visceral leishmaniasis is a major public health issue in South America, where the disease is rapidly spreading. Changes in ecology and distribution of the principal vector, *Lutzomyia longipalpis* are among the factors accounting for the increasing incidence of the disease in this region. However, information about the ecology of *L. longipalpis* is still incipient, which may directly impair the implementation of effective control programs. Herein, the ecology of *L. longipalpis* was studied in a focus of visceral leishmaniasis in north-eastern Brazil. From August 2009 to August 2010, phlebotomine sand flies were monthly collected in four localities using CDC light traps (~37 per month) and a lantern-baited Shannon trap with mouth aspirators. A total of 24,226 phlebotomine sand flies were collected with light traps and 375 with mouth aspirators. The most abundant species was *L. longipalpis*, representing 97.9% of the specimens collected with light traps and 91.5% with the mouth aspirator. Other species (*Lutzomyia evandroi*, *Lutzomyia lenti* and *Lutzomyia sallesi*) were found in low numbers. Most phlebotomine sand flies (94.6%) were collected at chicken coops and corrals. No significant correlation was found between the monthly abundance of phlebotomine sand flies and the monthly averages of temperature, relative humidity or rainfall. However, interestingly enough, 82.4% of *L. longipalpis* specimens were collected in months when relative humidity surpassed 75%. This study points out that this vector is well adapted to live in different habitats and to different climate conditions. It also suggests that some north-eastern populations of *L. longipalpis* may be more xerotolerant than southern populations. Further studies to assess the relationship between microclimate and *L. longipalpis* density in different Brazilian regions are advised.

1. Introduction

Visceral leishmaniais (VL) is a life-threatening disease caused by intracellular protozoa of the genus *Leishmania*, which are transmitted by phlebotomine sand flies. The incidence of the disease has increased significantly in several endemic regions, including in the Americas, where the control of the disease is impaired by the lack of political commitment, gaps in scientific knowledge, and the weakness of case management and surveillance systems (Romero and Baelaert, 2010; Dantas-Torres et al., 2012).

In the Americas, zoonotic VL is caused by *Leishmania infantum* (syn. *Leishmania chagasi*), which is primarily transmitted by *Lutzomyia longipalpis* (Romero and Baelaert, 2010). Other phlebotomine sand fly species have been regarded as putative vectors of *L. infantum*, including *Lutzomyia cruzi*, *Lutzomyia evansi*, *Lutzomyia forattinii*, and *Lutzomyia migonei* (Santos et al., 1998; Montoya-Lerma et al., 2003; Pita-Pereira et al., 2008; Carvalho et al., 2010; Salomón et al., 2010), although definitive scientific evidence on their vector competence is currently lacking.

Understanding the epidemiology of VL is pivotal for controlling the disease. However, there is limited information about several aspects of the disease epidemiology, including *L. longipalpis* ecology (e.g., host preferences, population dynamics). Moreover, phlebotomine sand flies currently identified as *L. longipalpis* might actually represent a complex of cryptic species (Bauzer et al., 2007), thus further complicating the epidemiological picture of leishmaniasis in the New World, mainly in the absence of ecological data for each population or sibling species.

Studies on habitat preference and seasonality of phlebotomine sand flies have been conducted in leishmaniasis-endemic areas in Brazil. For instance, data on habitat and seasonality of *Lutzomyia wellcomei* contributed to incriminate this species as a vector of *Leishmania braziliensis* in Amazônia and north-eastern Brazil (Ready

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Table 1
Phlebotomine sand flies collected by CDC light traps in a focus of visceral leishmaniasis north-eastern Brazil, from August 2009 to August 2010.

Species	Total	Male		Female		Sex ratio
		n	%	*n*	%	
Lutzomyia longipalpis (Lutz and Neiva, 1912)	23,716	15,856	66.9	7860	33.1	2.0
Lutzomyia evandroi (Costa Lima and Antunes, 1936)	392	219	55.9	173	44.1	1.3
Lutzomyia lenti (Mangabeira, 1938)	95	48	50.5	47	49.5	1.0
Lutzomyia sallesi (Galvão and Coutinho, 1939)	23	6	26.1	17	73.9	0.4
Total	24,226	16,129	66.6	8097	33.4	2.0

et al., 1984). A study also demonstrated a strong positive correlation between *L. longipalpis* population density and cases of canine VL in south-eastern Brazil (França-Silva et al., 2005).

Most studies focused on *L. longipalpis* were conducted southern and south-eastern Brazil (e.g., Resende et al., 2006; Saraiva et al., 2011; Oliveira et al., 2012; Pinto et al., 2012). Indeed, limited information is available for other regions, particularly north-eastern Brazil (Ximenes et al., 2006; Amóra et al., 2010a,b), where VL is still a major public health threat (Dantas-Torres and Brandão-Filho, 2006). In addition, data on the seasonal dynamics of *L. longipalpis* might be useful also to further understand the epidemiology of VL but also to ascertain the most favorable period for adopting specific control measures on animals and in the environment. The aim of this study was to investigate the habitat preference and seasonal pattern of *L. longipalpis* in an area highly endemic for VL in north-eastern Brazil.

2. Materials and methods

2.1. Study area

The study was conducted in the municipality of Passira (07°59′42″S; 35°34′51″W, 176 m above the sea level), scrub zone (Agreste) of Pernambuco (north-eastern Brazil). The municipality encompasses an area of 329.75 km^2 and is located 115 km far from Recife, the state's capital. With a population of 28,589 inhabitants (density 86.7 inhabitants/km^2), Passira has its economic activities based in commerce, agribusiness (e.g., tomatoes, corn and beans) and livestock rearing (cattle, goats, sheep and horses). Dogs are commonly kept as companions and most of them are constantly exposed to *L. infantum* (unpublished data). The climate is semi-arid, with annual rainfall of 720.7 mm and average annual temperature of 24 °C. The *caatinga* (xerophytic vegetation and deciduous thorn scrub) represents the predominant vegetation in this region.

2.2. Phlebotomine sand fly collections

From August 2009 to August 2010, phlebotomine sand flies were collected in four localities (namely, Apara, Poço do Pau, Sítio Borba and Varjada de Cima) using CDC light traps and a lantern-baited Shannon trap with mouth aspirators. The traps (~37 traps per month) were installed monthly, for four consecutive nights, between dusk and dawn, from 5:00 pm to 6:00 am, for a total effort of 5844 h of trapping. Trapping with the Shannon trap was performed on an irregular basis, but usually from 6:00 pm to 8:00 pm.

Light traps were placed at ca. 1 m above the ground in different habitats, including chicken coops, corrals, pigpen, plantations, and indoors. Captured specimens were processed and identified based on morphology as described elsewhere (Young and Duncan, 1994).

2.3. Meteorological data

Monthly mean temperature (°C), humidity (%) and rainfall (mm^3) recorded during the study period were provided by the Instituto de Tecnologia de Pernambuco (ITEP).

2.4. Data analysis

Sex ratio and abundance of collected phlebotomine sand flies were calculated according to species, month of collection and habitat. Data was initially assessed for normality using Lilliefors (*k* samples) and eventually log-transformed (log 10) before analysis. Data comparisons were done using Student's *t*-test and Kruskal–Wallis. The correlation between phlebotomine sand flies and climate data assessed using Pearson's (*r*) correlation coefficient. Differences were considered significant if $P < 0.05$. Statistical analysis was carried out using BioEstat (version 5.0; Mamirauá/CNPq, Belém, PA, Brazil).

3. Results

A total of 24,226 phlebotomine sand flies belonging to four species (i.e., *L. longipalpis*, *Lutzomyia evandroi*, *Lutzomyia lenti* and *Lutzomyia sallesi*) were identified (Table 1). The monthly abundance of phlebotomine sand flies varied significantly according to species (Kruskal–Wallis, $P < 0.001$). Indeed, *L. longipalpis* represented 97.9% of the collected specimens, followed by, in descending order, *L. evandroi*, *L. lenti* and *L. sallesi*.

With regard to sex, males (66.6%) predominated over females (33.4%) (sex ratio, 2:1). The monthly number of males was generally higher than females, except in August 2010 when females prevailed (Fig. 1A). In spite of these differences, no significant variation was found in relation to the number of males and females collected monthly (Student's *t*-test, $P = 0.0875$). Indeed, there was a significant positive correlation between the number of males and females collected monthly ($r = 0.929$, $P < 0.0001$) (Fig. 1B).

As far as habitat of collection, most phlebotomine sand flies were collected in all collection sites, including indoors. The highest numbers of phlebotomine sand flies were collected near chicken coops and corrals. In fact, there was a strong positive correlation in the monthly abundance of phlebotomine sand flies collected in chicken coops and corrals (Fig. 1C).

No significant correlation was found between the monthly abundance of phlebotomine sand flies and the monthly averages of temperature ($r = 0.168$, $P = 0.58$), relative humidity ($r = 0.485$, $P = 0.09$) or rainfall ($r = 0.104$, $P = 0.74$). Interestingly, 82.4% of the specimens of *L. longipalpis* were collected in months with relative humidity >75% (Fig. 2).

A total of 375 phlebotomine sand flies were collected using mouth aspirators in Shannon traps, of which 91.5% were identified as *L. longipalpis*; *L. lenti* and *L. sallesi* were also collected, though in low numbers. Some females of *L. longipalpis* collected from Shannon traps were dissected and examined under light microscopy, but no flagellates were observed.

4. Discussion

This study provides data on the ecology of phlebotomine sand flies in north-eastern Brazil *L. longipalpis*, which was most abundant species found in the study area throughout the observation period and in different habitats. This phlebotomine sand fly is the

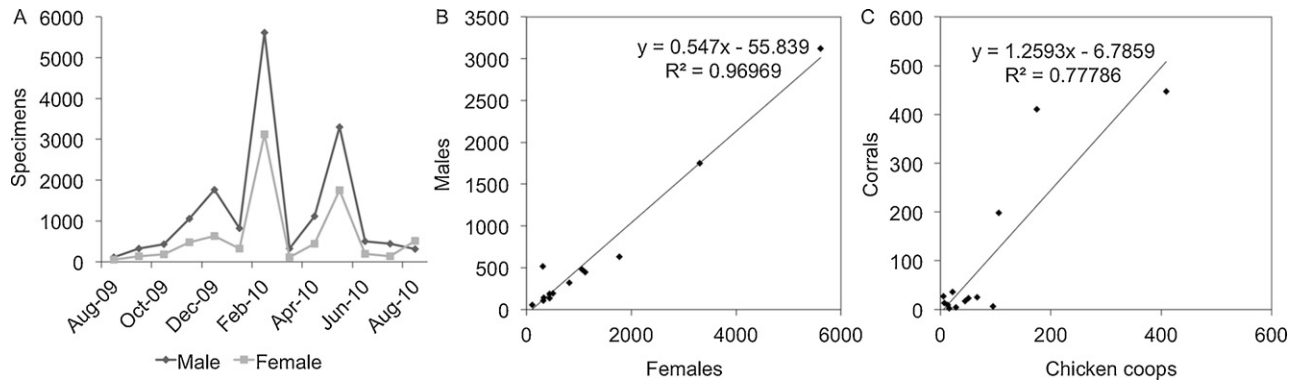

Fig. 1. Monthly number of males and females of phlebotomine sand flies (A), correlation between the monthly number of males and females (B), and correlation between the monthly abundance of phlebotomine sand flies collected in corrals and chicken coops (C).

principal vector of *L. infantum* in the New World (Dantas-Torres et al., 2012) and its predominance over other species in areas where VL is endemic has been reported in different Brazilian regions (Resende et al., 2006; Silva et al., 2007; Nunes et al., 2008; Almeida et al., 2010; Pinto et al., 2010).

Males generally predominated over females, as observed in studies conducted other regions of Brazil (Michalsky et al., 2009b; Almeida et al., 2010). For instance, a study conducted in south-eastern Brazil, in an area of active VL transmission, reported an overall male:female ratio of 2.9:1 (Michalsky et al., 2009b), which is close to the overall sex ratio (i.e., 2:1) recorded in the present study.

Most phlebotomine sand flies were collected in animal shelters, including chicken coops and corrals, whereas a low number of specimens were collected inside the houses. Indeed, it is acknowledged that *L. longipalpis* may adapt to different habitats and feed on different animals (Lainson and Rangel, 2005), which partly accounts for its role as a vector of *L. infantum* in Brazil. Domestic animals are among the preferred blood source of phlebotomine sand flies in the peri-domestic environment (Dantas-Torres et al., 2006) and this might be relevant for the epidemiology of VL. For instance, chickens are among the preferred hosts of *L. longipalpis* in some endemic areas and high numbers of specimens have been collected in chicken coops (Ximenes et al., 1999; Saraiva et al., 2011). In this regard, there has been speculation on the role of chickens in the epidemiology of VL (Alexander et al., 2002), even if these animals are definitely not susceptible to *L. infantum* infection (Otranto et al., 2010). Indeed, it has been suggested that chicken coops could act as a barrier for phlebotomine sand flies and also that chickens could act as zooprophylactic agents by killing the parasites present in infected vectors (Alexander et al., 2002). However, the high numbers of *L. longipalps* in chicken coops observed in this study may indicate that their presence near human houses might be a risk indicator for VL transmission. Certainly, further studies are needed to better understand the real effect of chickens and chicken coops near human houses on the transmission of *L. infantum* in endemic areas.

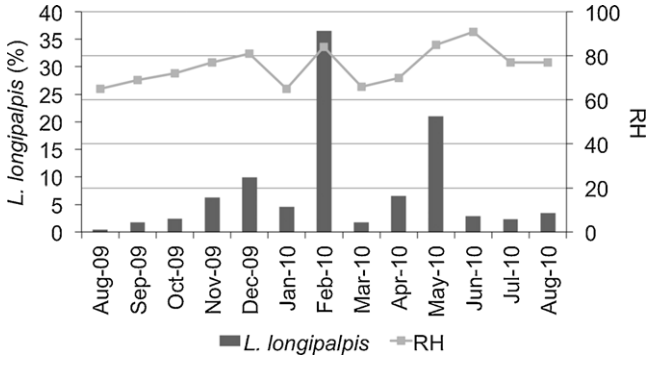

Fig. 2. Monthly percentage of *L. longipalpis* specimens collected in relation to monthly mean relative humidity (RH).

The seasonal dynamics of phlebotomine sand flies may vary widely from region to region and the effect of temperature, rainfall, and relative humidity on these insects may vary as well (Tarallo et al., 2010). For instance, a study conducted in Montes Claros, Minas Gerais, reported a relationship between *L. longipalpis* and some climate variables (cumulative rainfall, average temperature and relative humidity) (Michalsky et al., 2009a). Indeed, this vector is usually more abundant during or immediately after rainy periods (Souza et al., 2004; Michalsky et al., 2009a,b). In the present study, most *L. longipalpis* specimens were collected in months with average relative humidity >75% but there was no significant correlation between monthly abundance of *L. longipalpis* and monthly averages relative humidity or rainfall. Considering the low thermal seasonality (i.e., annual temperature range) typically found in north-eastern Brazil, we speculate that the dynamics of *L. longipalpis* in this region is less dependent on climate variables.

This study provides additional data on the ecology of *L. longipalpis*, the main vector of *L. infantum* in South America. It shows out that *L. longipalpis* is well adapted to live in different habitats and climate conditions in north-eastern Brazil, being active at any time of the year. Our findings also suggest that some north-eastern populations of *L. longipalpis* may be more xerotolerant than southern populations, but further research is needed before drawing any conclusion on this matter.

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