Vertical stratification and development aspects of phlebotomine sand flies (Diptera: Psychodidae) in an area of Atlantic Forest tree species in a metropolitan region in northeastern Brazil

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ABSTRACT: In the state of Rio Grande do Norte in northeast Brazil, cases of visceral leishmaniasis (VL) occur mainly in the periurban areas of the city of Natal. *Lutzomyia longipalpis* Lutz & Neiva 1912 (Diptera: Psychodidae), a vector of *Leishmania chagasi* (Protozoa: Trypanosomatidae) to humans, is found throughout the state. Flora and fauna influence the distribution of sand fly species, whose horizontal or vertical stratification can be used as a parameter for identifying potential vectors, considering the presence of vertebrate hosts in the area. The purpose of this study was to obtain information about the vertical stratification of phlebotomine sand flies in an endemic area of leishmaniasis in Rio Grande do Norte, and associate it with the presence of other animals in the peridomiciliary environment as well as to analyze, under laboratory conditions, aspects of *L. longipalpis* reproduction in wild females. The sand flies were captured with light traps hung at different heights in species of Atlantic Forest trees and in a peridomiciliary environment in animal shelters. The traps were placed between 17:30 and 6:00 of the following day, in a peridomiciliary and extradomiciliary area of a forest fragment in both dry and rainy months. In the extradomiciliary environment, the traps were installed at 1, 3 and 5 m above the ground. The biological cycle of *L. longipalpis* was followed from the eggs of 200 wild females. Specimens of *L. lenti*, *L. walkeri,* and *L. migonei* were captured. The comparison and statistical analysis showed that *L. longipalpis* is more abundant at a height of 3 m and *L. evandroi* at 1 m. In the animal shelters (chickens, horses, and armadillos), we captured mainly specimens of *L. longipalpis* and *L. evandroi*. The duration of the biological cycle of *L. longipalpis* was approximately 38 days at a temperature of 28° C. ***Journal of Vector Ecology* 32 (2): 336-341. 2007.**

Keyword Index: Vertical stratification, *Lutzomyia longipalpis,* visceral leishmaniasis.

INTRODUCTION

In Brazil, visceral leishmaniasis is an endemic disease in four out of the five regions of the country, with epidemic outbreaks occurring in some areas. Information from the Brazilian Ministry of Health shows that the incidence coefficient of the disease has reached 20.4 cases/100,000 inhabitants in some localities of the northeast (MS 2005).

In the northeastern state of Rio Grande do Norte, the first urban outbreak occurred between 1990 and 1992, and again in 1997 when a new increase in the number of reported cases took place (Jerônimo et al. 1994, 2004). The cases of visceral leishmaniasis in the state occur mainly in the periurban areas of metropolitan Natal (capital of the state). *Lutzomyia longipalpis* Lutz & Neiva 1912 (Diptera: Psychodidae), a species involved in transmitting leishmanias to humans, is distributed throughout the state and represents 85% of the sand flies captured in Rio Grande do Norte (Ximenes et al. 2000).

The infection is determined by interactions among the insect vector, the parasite, and animal reservoir hosts. The main animal reservoir hosts found for *Leishmania* species responsible for the cutaneous or visceral forms vary from region to region, sometimes involving domestic animals, and sometimes wild animals, which act as feeding sources for the hematophagous females.

Sand flies are found in diversified forest ecosystems, whose strata extend from the soil to the tree crown. The flora and fauna of the regions influence species distribution. Horizontal or vertical species stratification can be used as a parameter in identifying potential vectors (Chianotis et al. 1971). Studies conducted in the Brazilian Amazonia (Arias and Freitas 1982, Genaro et al. 1986, Lainson 1983, Cabanillas and Castellón 1999) show the importance of these interrelations in understanding the wild epidemiological cycles of tegumentary leishmaniasis in the activity period of *Lutzomyia umbratilis* and *L. flaviscutellata* in a forest environment and interactions with other animals (Lainson et al. 1983, Dias-Lima et al. 2002).

In Brazil, a number of studies have been developed to assess the vertical stratification of phlebotomine sand flies. Most of these have been conducted in Amazonia, an important endemic area of tegumentary leishmaniasis. In northeast Brazil, the region of highest incidence of VL cases,

there is no reference to vertical sand fly stratification.

In some areas of Rio Grande do Norte, the proximity to residences of remnant Atlantic Forest fragments, a natural sand fly habitat, associated with the raising of animals in a peridomiciliary environment, provide the necessary conditions for the *L. chagasi* vector species to establish itself and possibly transmit the parasite to humans and other animals (Ximenes et al. 1999).

The purpose of this study was to obtain information about the vertical stratification of phlebotomine sand flies in the metropolitan region of Natal, an important endemic area of visceral leishmaniasis in Rio Grande do Norte, as well as analyze, under laboratory conditions, reproduction aspects of *L. longipalpis*.

MATERIALS AND METHODS

Study area

The study was conducted in Nísia Floresta, one of the municipalities belonging to the metropolitan region of Natal, a homogenous zone on the east coast of Rio Grande do Norte. The municipality covers an area of 313.6 km^2, equivalent to 0.59% of the total area of the state. The climate is sub-humid, with a mean annual rainfall of 1,300 mm. Although the rainy season is irregular, it generally occurs between March and August, with a mean annual temperature of 27° C and mean annual relative humidity of 76%.

The vegetal formation of the area is composed of sub-perennial forest, with evergreen trees with relatively thin trunks and soil covered by a layer of humus and sand-fixing native vegetation. In the more humid areas, Cyperaceae and grass species are found.

In the study area we found pioneer trees and fruit tree species such as the mango tree (*Mangifera indica*), Anacardiaceae, the cashew (*Anacardium occidentale* L.) -Anacardiaceae, the yellow mombin (*Spondias lutea* L.) -Anacardiaceae, the purple mombin (*Spondias purpurea* L.) -Anacardiaceae, the "mangaba" (*Hancornia speciosa* Gomez) -Sapindaceae, the "pitomba" (*Talisia esculenta* St. Hil. Radi) -Sapindaceae, banana plants (*Musa sp.*) -Musaceae, and lemon trees (*Citrus sp.*) –Rutaceae, in addition to native plants known as "murici" (*Byrsonima gardneriana*) – Malpighiaceae and "lixeira" (*Curatella sp*) – Dilleniaceae.

Sand fly capture

Weekly captures were performed in an area located 2 km from Bonfim Lake, 06°01'18.9" S and 35°13'29.2" W at an altitude of 86 m above seal level.

CDC light traps (Haushers Machine Works, NJ, U.S.A.) were installed in the peridomiciliary area, in a chicken coop, and in an armadillo *(Euphractes sexcintus*) brick masonry shelter located 10 and 2 m from the house, respectively, and in the extradomiciliary area in a forest fragment containing the species described in the previous item, approximately 200 m from the residence. The traps were put in place at 17:30 and collected the next morning at 6:00. This was done in the rainy season (May, June, and July) and the dry season (September, October, and November).

Stratification of the captured sand flies

Two traps were installed 1 m above the ground in a "cupiuba" tree (*Tapirira guianensis*) and at 1, 3, and 5 m in an embauba tree (*Cecropia sp*) – Cecropiaceae, with captures performed weekly and always in the same trees.

On the morning after capture, the traps were taken to the laboratory in sealed plastic bags containing moist cotton wadding to maintain cage humidity. In the laboratory, the insects were sorted and the live sand flies transferred to wire cages with nylon screening using a manual aspirator.

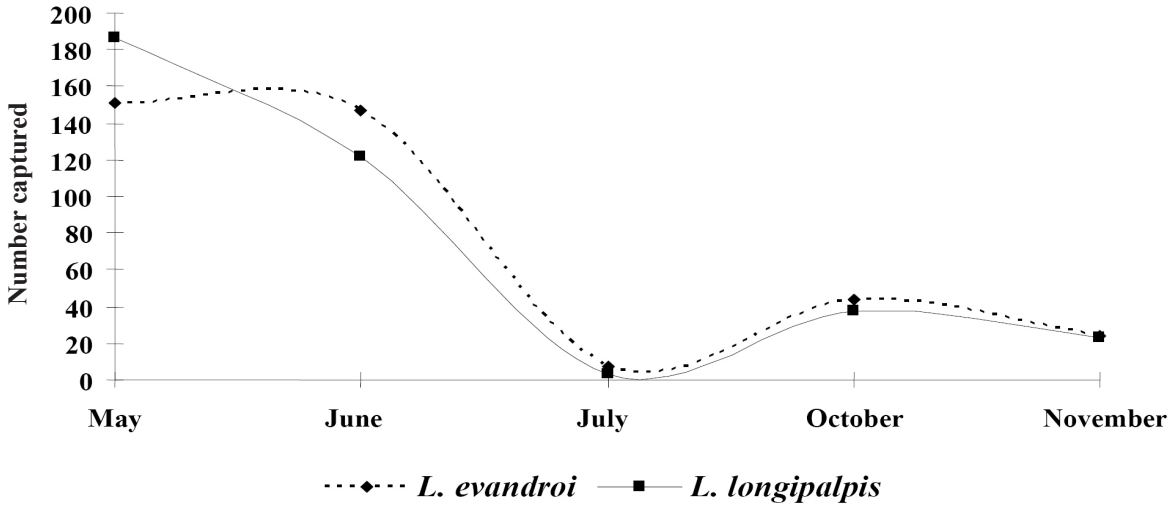

Figure 1. Distribution of *Lutzomyia longipalpis* and *L. evandroi* captured in the rainy and dry seasons.

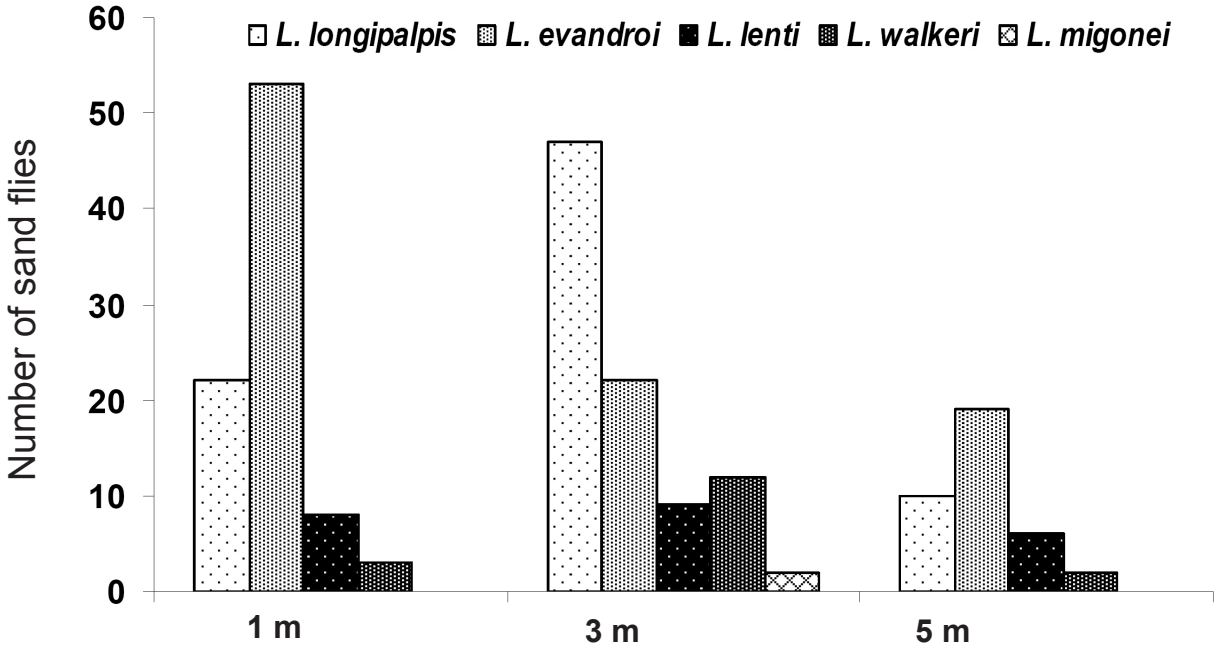

Figure. 2. Number of sand flies captured with light traps in an extradomiciliary environment at 1, 3, and 5 m.

Biological cycle in the laboratory

Two hundred wild females captured in the peridomiciliary environment were followed and after 24 h placed individually for egg laying in plaster-lined plastic pots, which were kept in closed rectangular plastic boxes in an incubation chamber (FANEM – Mod. 347) at a temperature of 28° C and relative humidity between 80 and 90%. A 10% glucose solution in a piece of cotton was offered *ad libitum* (Killick-Kendrick 1977). After egg-laying, dead females were removed and stored in 70% alcohol, and later identified using the keys of Young and Duncan (1994). The eggs were counted and kept in plaster pots for larval eclosion, and observed daily until the emergence of sand fly adults. The larvae were fed with an organic mixture containing feces, rabbit ration, and fish ration.

RESULTS

A total of 1,180 phlebotomine sand flies were captured in the study area, representing an average of 3.6 males for each captured female.In the extradomiciliary environment we found five species of the genus: *Lutzomyia evandroi* Costa Lima & Antunes was the most prevalent with 45.82% of the sand flies captured, followed by *Lutzomyia longipalpis* Lutz & Neiva with 40.13%, *L. walkeri* newstead with 6.10%, *L. lenti* Mangabeira 5.94%, and *L. migonei* França with 0.17%. Of the sand flies captured, 1.84% could not be identified because the specimens were damaged. *L. longipalpis* and *L. evandroi* were similarly distributed throughout the year, both with peak abundance in May and October (Figure 1).

L. longipalpis, L. evandroi, L. lenti, and *L. walkeri* were captured in vegetation 1 m above the ground and *L. longipalpis, L. evandroi, L. lenti, L. walkeri,* and *L. migonei* were captured at 3 m. At 5 m we captured sand flies of the species *L. longipalpis, L. evandroi, L. lenti,* and *L. walkeri* (Figure 2).

L. longipalpis and *L. evandroi* specimens were present in the three strata while *L. migonei* was captured only at a height of 3 m. The comparison and statistical analysis using Fisher's test showed that *L. longipalis* is more abundant

Table 1. Sand fly density in peridomiciliary and extradomiciliary environments.

Species	Peridomicile (n)	Extradomicile (n)	Total
L. evandroi	463	94	557
L. longipalpis	394	79	473
L. lenti	50	23	73
L. walkeri	57	17	74
L. migonei	1	2	3
Total	965	215	1,180

at 3 m ($P = 0.003$) and *L. evandroi* at 1 m. The remaining differences are not significant ($P > 0.05$).

In the chicken coop, the armadillo shelter, and near a horse, all in the peridomiciliary environment, the abundance of all phlebotomine sand flies was greater than that in the extradomiciliary environment, except *L. migonei* (Table 1).

Biological cycle

The duration of the *L. longipalpis* biological cycle was approximately 38 days at 28° C.The period of time between egg laying and the eclosion of the first larva was around nine days. The mean period of time between larval eclosion and pupal formation was about 18 days. The mean period of time between the pupal phase and the emergence of adults was 11 days. The oviposition rate was approximately 14 eggs per wild female, and the longevity of females born in the laboratory, from emergence until the end of egg laying, was approximately seven days. These females were fed on a sedated hamster (ketamine/xylazine) for 1 h.

Other insects

A total of 2,011 specimens of the following orders were attracted to the light traps: Diptera (1,538 specimens); Orthoptera (147), Lepidoptera (148), Neuroptera (95), Coleoptera (45), Hymenoptera (36), Phasmida (01) and Mantodea (01).

DISCUSSION

Studies conducted in different environments in Brazil and other countries have shown variations in the composition of phlebotomine sand fly fauna dependent on environmental characteristics, seasonality, and capture methods (Morrison et al. 1995; Ximenes et al. 2006).

The spatial distribution of phlebotomine sand flies is little understood; studies in Amazonia and in several countries show different distribution patterns. In the current study, *L. longipalpis*, a transmitting species of *Leishmania chagasi* to humans, was more prevalent in the higher strata than *L. evandroi*. In the state of Parana, a stratification pattern was observed varying from 1 to 15 m, with a reduced number of species and specimens at higher capture altitudes (Chiannotis et al. 1971). The study carried out by Memmot and Sutton (1994) in Costa Rica suggests that the species *L. trapidoi, L. ylephiletor,* and *L. shannoni* have variable stratification whose causes were not established. Interspecific interactions, competition for space, courtship, rest sites, and microclimatic alterations could result in the selection of one or another stratum and the increase or decrease in insect density at a determinate level in the forest. Our study also could not explain the causes that led to the spatial distribution of phlebotomine sand flies. However, it did allow us to identify sand fly stratification in tree species in an endemic area of visceral leishmaniasis, a likely association with the host animals of the peridomiciliary area and the adaptation of phlebotomine sand flies to deforested areas. The physical and biological differences of both Atlantic Forest fragments and Caatinga (semi-arid forest) areas are different from those of Amazonia. The semi-arid climate and vegetal composition of the area influence the distribution of phlebotomine sand fly species and the relations established between vectors and hosts. Geoffroy et al. (1986) and Cabanillas and Castellon (1999) suggested similar relations between vectors and hosts in Amazonia. The smaller number of specimens at 5 m may be related to stronger air currents in the tree crown (Ximenes et al. 2006). At between 1 and 3 m, it is likely that hematophagous females use common marmosets (*Callithrix jacchus*) and opossums (*Didelphis*) as food sources.

The interactions between the female sand flies and the host animals are determinants in the distribution of some species in Amazonia. *L. umbratilis* females, a vector of *leishmania guyanensis*, are found in the highest strata feeding on sloths and anteaters, whereas *L. flaviscutellata* is found near the ground feeding on rodents (Genaro et al. 1986).

In our study, *L. longipalpis, L. evandroi, L. lenti,* and *L. walkeri* were captured at 1, 3, and 5 m, whereas *L. migonei* was captured only at 3 m. *L. longipalpis* was captured in an area with specimens of Anacardiaceae, Cecropiaceae, Apocynaceae, Poligonaceae, and Burseraceae, in particular in embauba (*Cecropia sp*) and cupiuba (*Tapirira guianensis*) branches. The *Tapirira guianensis* is one of the most abundant tree species in Atlantic Forest fragments in Nisia Floresta (Oliveira et al. 2001). The cecropias produce sweet substances in the branches and inner bark of the trunk, attracting ants. The cupiubas produce fruit with a sweet juicy pulp. Both are eaten by the common marmosets of the region (Castro 2003) and may also be a source of carbohydrates for phlebotomine sand flies. In addition, the females may feed on the common marmosets present in these trees. Carbohydrates contribute to the migration and morphological transformations of the promastigote forms of leishmanias in the digestive tract of phlebotomine sand flies (Sacks 1989). In arid regions sand flies of both sexes feed on the vegetal tissues of a number of species in the region (Schlein and Jacobson 1999).

The two most abundant species, *L. longipalpis* and *L. evandroi,* are widely distributed in Brazil (Martins et al. 1978, Young and Duncan 1994). The former is the most important vector of visceral leishmaniasis in the Americas. Both were most abundant in May and October. The seasonal dynamics of *L. longipalpis* and the influence of abiotic factors were discussed in a previous study (Ximenes et al. 2006).

In the peridomiciliary environment, the sand fly density was greater than that found in the extradomiciliary environment. Captures were associated with chickens, horses, and armadillo, and show the selective behavior of these insects and their adaptation to the human environment, as was demonstrated in earlier studies (Teodoro et al. 1993, Morrison et al. 1995, Travi et al. 1998). Many of the *L. longipalpis* females captured in the armadillo (*Euphractes sexcintus*) shelter near the residence had ovarioles in the final maturation stage, suggesting that this is likely the resting site used by the females. *Lutzomyia migonei* was found in Nísia Floresta in both extra- and

peridomiciliary environments. This species was found naturally infected by *Leishmania brasiliensis*, an etiologic agent of dermal leishmaniasis (Queiroz et al. 1991). Its susceptibility to developing this parasite was also shown in the laboratory (Nieves and Pimenta 2000). It is therefore a species potentially involved in the transmission of protozoa in endemic areas of dermal leishmaniasis.

Approximately four times as many males as females were captured in this study. The number of males, as previously shown in various studies, is generally higher than that of females (Castellón et al. 1989, Cabanillas and Castellón 1999, Ximenes et al. 2000). The disproportionality between sexes is associated to the behavioral profile of the species and is part of courtship strategies between males and females (Kelly and Dye 1997).

The comparative analysis of the biological cycles of *L. longipalpis* and *L. evandroi* from the egg laying of wild females in laboratory shows that the duration of the biological cycle was shorter. The reduced number of eggs may be related to the amount of blood ingested by the females, given that females fed in laboratory lay more eggs. There was no difference in life expectancy between the two species. In the larval period, *L. longipalpis* and *L. evandroi* lived for 18 and 24 days, respectively. These differences are likely associated to the temperatures to which they were submitted. *L. longipalpis* was raised at 28° C while *L. evandroi* was raised at 26° C (Ximenes et al. 2001). The increased temperature probably favored the faster development of *L. longipalpis* under laboratory conditions.

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