



## Article/Artigo

# Phlebotomine sandflies (Diptera: Psychodidae) in São Vicente Férrer, a sympatric area to cutaneous and visceral leishmaniasis in the State of Pernambuco, Brazil

Flebotomíneos (Diptera: Psychodidae) em São Vicente Férrer, uma área simpátrica para leishmaniose tegumentar e visceral, no Estado de Pernambuco, Brasil

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### ABSTRACT

**Introduction:** In the last decades, a considerable geographic expansion of the leishmaniasis in all regions of Brazil has been observed. The present study was carried out to identify the composition of the phlebotomine sandfly fauna and verify the seasonal variation of the main species after environmental changes occurred in São Vicente Férrer Municipality, State of Pernambuco, Brazil. **Methods:** Captures were carried out during four consecutive nights of each month using Centers for Disease Control and Prevention light traps from September 2009 to September 2010. The correlation between the number of phlebotomine sandflies captured and climatic factors (temperature and rainfall) was evaluated. **Results:** A total of 13,872 specimens belonging to 20 species were captured, of which, 6,247 (45%) were females, and 7,625 (55%) were males. *Lutzomyia migonei* was the most abundant species with 9,964 (71.8%) specimens, being predominant in the intradomicile and peridomicile areas with 108 (86.4%) and 9,746 (97%), respectively. In the forest remnants, *Lutzomyia complexa* 2,395 (65%) and *Lutzomyia sordellii* 770 (20.8%) predominated. The correlation analysis between the total number of sandflies captured and climatic factors did not show a significant influence on population density. **Conclusions:** The high abundance of *Lutzomyia migonei* and *Lutzomyia complexa* indicates the possibility of new cases of cutaneous leishmaniasis (CL).

**Keywords:** Leishmaniasis. Sandflies. São Vicente Férrer.

### RESUMO

**Introdução:** Nas últimas décadas tem se observado uma considerável expansão geográfica das leishmanioses em todas as regiões do Brasil. O presente estudo foi realizado para identificar a fauna de flebotomíneos e verificar a sazonalidade das principais espécies após mudanças ambientais ocorridas no município de São Vicente Férrer, Estado de Pernambuco, Brasil. **Métodos:** As capturas foram realizadas durante quatro noites consecutivas por mês usando armadilhas luminosas CDC, no período de setembro de 2009 a setembro de 2010. A correlação entre o número de flebotomíneos capturados e fatores climáticos (temperatura e umidade) foi avaliada. **Resultados:** Foi capturado um total de 13.872 espécimes pertencentes a 20 espécies, sendo 6.247 (45%) fêmeas e 7.625 (55%) machos. *Lutzomyia migonei* foi a espécie mais abundante com 9.964 (71,8%) espécimes, sendo predominante no intradomicílio 108 (86,4%) e peridomicílio 9.746 (97%). Nos resquícios de mata primária, predominaram *Lutzomyia complexa* 2.395 (65%) e *Lutzomyia sordellii* 770 (20,8%). A análise de correlação entre o número total de flebotomíneos capturados e os fatores climáticos não mostrou uma influência significativa na densidade da população. **Conclusões:** A elevada abundância de *Lutzomyia migonei* e *Lutzomyia complexa* indica a possibilidade de novos casos de leishmaniose cutânea (LC).

**Palavras-chaves:** Leishmanioses. Flebotomíneos. São Vicente Férrer.

### INTRODUCTION

The leishmaniasis are diseases caused by *Leishmania* protozoans that are considered by the World Health Organization as one of most important group of neglected tropical diseases worldwide<sup>1</sup>. There are two main clinical forms of leishmaniasis: cutaneous leishmaniasis (CL) and visceral leishmaniasis (VL). The importance of these diseases for public health is due not only to their high incidence and prevalence but also to their widespread geographical distribution and the possibility of developing severe forms, with significant mortality rates in untreated patients with VL and high morbidity in cases of CL<sup>2</sup>.

Because of environmental changes resulting from deforestation and urbanization processes, the leishmaniasis have significantly increased their importance in public health<sup>3,4</sup>. These environmental changes observed in several areas of Brazil have allowed some sylvatic phlebotomine sandfly species to establish in human dwellings and animal shelters around houses, demonstrating their process of adaptation to the anthropic environment<sup>5</sup>.

In the State of Pernambuco, an increase in the number of cases of leishmaniasis has been recorded in all regions of the state, where a progressive expansion was observed in the last 20 years<sup>6</sup>. In the rainforest zone (*Zona da Mata*), the infection caused by *Leishmania (Viannia) braziliensis* is the predominant form of the disease<sup>7</sup>. Cases of VL are concentrated in the cities of the semiarid region (*Sertão*). However, since the 1990s, there has been a dissemination of the disease to the middle scrub (*Agreste*), and rainforest zone has been recorded, as determined by the increased number of cases reported<sup>8</sup>. São Vicente Férrer records autochthonous cases of both forms of leishmaniasis, including reported cases of canine VL<sup>9</sup>. Between 1999 and

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2010, 157 autochthonous cases of CL and 15 cases of VL were reported in this area, being considered as an endemic area for CL and a sporadic transmission area for VL form of the disease.

In this context, the present study was carried out to determine the composition of the phlebotomine sandfly fauna in different environments and verify the seasonal variation of the main species after environmental changes occurred in the study area.

## METHODS

### Study area

São Vicente Férrer (07° 35' 28"W, 35° 29' 29"S, and 419m above sea level) is located in the northern rainforest area of Pernambuco, 130.5km away from Recife, the state's capital. It has an area of 110,489km<sup>2</sup> and a resident population of 16,598 inhabitants, and the climate is tropical, with a mean average annual temperature of 23°C. The entomological survey was conducted in Mundo Novo, a rural community where the incidence of both forms of the disease is observed, as well as an intense process of deforestation. In the area, the primary vegetation has largely been substituted by banana tree plantation, and human dwellings and animal shelters (chicken house and stables) are in proximity to remnants of Atlantic rainforest.

### Sandfly captures

Phlebotomine sandflies were captured from September 2009 to September 2010 with Centers for Disease Control and Prevention (CDC) light traps, from 6pm to 6am, during four consecutive nights of each month, distributed in the intradomicile and peridomicile (chicken house and stables) and forest remnants. The number of

CDC light traps ranged from 20 to 51. The density of phlebotomine sandflies was calculated based on the number of specimens caught per hour of capture (number of traps used per month × 12h).

The specimens captured were brought to the laboratory for taxonomic identification according to Young and Duncan<sup>10</sup>.

### Climatic data

The daily climate data (temperature and rainfall) were provided by the *Instituto de Tecnologia de Pernambuco*. Average monthly values were used in our study.

### Statistical analysis

Male/female ratio and distribution of sandflies in the studied environments were analyzed using chi-square ( $\chi^2$ ) test. Spearman (*r*<sub>s</sub>) correlation coefficient was used to assess the relationship between the number of sandflies captured and climate variables (temperature and rainfall; mean recorded during 10 days preceding the fourth night of capture of each month). Statistical analyses were performed using the R 2.10.0 and BioEstat 5.0 programs. A *p* < 0.05 was considered statistically significant.

## RESULTS

A total of 13,872 phlebotomine sandflies belonging to 20 species were captured, of which, 7,625 (55%) were males, and 6,247 (45%) were females. The number of males was statistically higher than of females ( $\chi^2 = 2,733.75$ , *df* = 1, *p* < 0.0001). *Lutzomyia migonei* was the predominant species with 9,964 (71.8%) specimens, followed by *L. complexa* with 2,447 (17.6%) specimens and *L. sordellii* with 848 (6.1%). Other species represented less than 1% of the captures (Table 1).

TABLE 1 - Distribution of phlebotomine sandflies species by sex and capture sites in Mundo Novo, São Vicente Férrer Municipality, State of Pernambuco, Brazil, from September 2009 to September 2010.

Species	Intradomicile				Peridomicile				Forest remnants				Total			
	♂	♀	n	%	♂	♀	n	%	♂	♀	n	%	♂	♀	n	%
<i>L. migonei</i>	56	52	108	86.4	6,250	3,496	9,746	97.0	62	48	110	3.0	6,368	3,596	9,964	71.8
<i>L. complexa</i>	3	0	3	2.4	3	46	49	0.5	699	1,696	2,395	65.0	705	1742	2447	17.6
<i>L. sordellii</i>	1	2	3	2.4	37	38	75	0.7	258	512	770	20.8	296	552	848	6.1
<i>L. evandroi</i>	11	0	11	8.8	47	50	97	0.96	7	3	10	0.3	65	53	118	0.9
<i>L. naftalekatzi</i>	0	0	0	0.0	22	11	33	0.32	33	42	75	2.0	55	53	108	0.8
<i>L. typynambai</i>	0	0	0	0.0	0	1	1	<0.01	4	76	80	2.2	4	77	81	0.6
<i>L. shannoni</i>	0	0	0	0.0	3	2	5	0.05	26	32	58	1.6	29	34	63	0.5
<i>L. capixaba</i>	0	0	0	0.0	0	1	1	<0.01	24	31	55	1.5	24	32	56	0.4
<i>L. walkeri</i>	0	0	0	0.0	0	0	0	0.0	19	30	49	1.3	19	30	49	0.4
<i>L. ayrozai</i>	0	0	0	0.0	0	0	0	0.0	16	28	44	1.2	16	28	44	0.3
<i>L. whitmani</i>	0	0	0	0.0	22	11	33	0.3	0	0	0	0.0	22	11	33	0.2
<i>L. quinquefer</i>	0	0	0	0.0	7	6	13	0.13	0	4	4	0.1	7	10	17	0.1
<i>L. choti</i>	0	0	0	0.0	0	0	0	0.0	0	15	15	0.4	0	15	15	0.1
<i>L. furcata</i>	0	0	0	0.0	0	0	0	0.0	4	8	12	0.3	4	8	12	0.9
<i>L. fischeri</i>	0	0	0	0.0	0	0	0	0.0	5	0	5	0.13	5	0	5	0.03
<i>L. brasiliensis</i>	0	0	0	0.0	2	2	4	0.03	1	0	1	0.02	3	2	5	0.03
<i>L. viannamartinsi</i>	0	0	0	0.0	0	0	0	0.0	2	1	3	0.08	2	1	3	0.02
<i>L. abonnenci</i>	0	0	0	0.0	0	0	0	0.0	1	1	2	0.05	1	1	2	0.01
<i>L. goiana</i>	0	0	0	0.0	0	0	0	0.0	0	1	1	0.02	0	1	1	<0.01
<i>L. osvaldoei</i>	0	0	0	0.0	0	0	0	0.0	0	1	1	0.02	0	1	1	<0.01
<b>Total</b>	<b>65</b>	<b>60</b>	<b>125</b>	<b>100.0</b>	<b>6,393</b>	<b>3,664</b>	<b>10,057</b>	<b>100.0</b>	<b>1,161</b>	<b>2,529</b>	<b>3,690</b>	<b>100.0</b>	<b>7,625</b>	<b>6,247</b>	<b>13,872</b>	<b>100.0</b>

L.: *Lutzomyia*.

The insects showed preference for the peridomestic environment with 10,057 (72.5%) specimens, followed by the forest remnants with 3,690 (26.6%) and 125 (0.9%) in the intradomicile ( $\chi^2 = 16,424.37$ ,  $df = 2$ ,  $p < 0.0001$ ). *L. migonei* was predominant in the intradomicile and peridomicile (86.4% and 97%, respectively). In forest remnants, the most frequent species were *L. complexa* (65%) and *L. sordellii* (20.8%), whereas other species represented 14.2% for the specimens captured.

*Lutzomyia migonei*, *L. complexa*, *L. sordellii*, and *L. evandroi* were the only species captured in all sites. An interesting aspect was the presence of *L. complexa*, *L. naftalekatzi*, *L. tupynambai*, *L. shannoni*, *L. brasiliensis*, and *L. capixaba* in the peridomestic environment.

Considering the monthly distribution of phlebotomine sandflies (Figure 1), three peaks in the population density of *L. migonei* were observed in February, May, and September 2010, this species being the only one captured in all months. *Lutzomyia complexa* had the highest density in March 2010 and *L. sordellii* in April 2010. The density of these species during the year showed some similarities, as in October 2009 and July 2010, and high divergence from January to May 2010, when an alternated distribution pattern was observed.

During the study period, a mean monthly temperature of 26.1°C (range, 23.3 to 28.3°C) and rainfall of 98.5mm<sup>3</sup> (range, 6.6 to 417.6mm<sup>3</sup>) were recorded (Figure 2). The occurrence of a greater number of phlebotomine sandflies, mainly *L. migonei*, coincided with the periods before and after the raining period. However, no correlation was found between the monthly number of sandflies captured and mean temperature ( $r_s = 0.1592$ ,  $p = 0.6034$ ) or rainfall ( $r_s = 0.1768$ ,  $p = 0.5634$ ) (Figure 3).

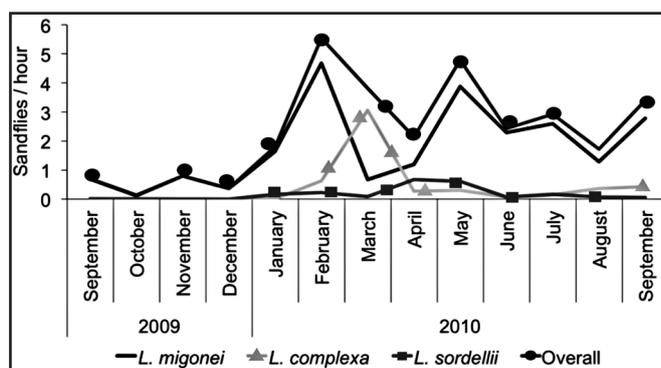


FIGURE 1 – Monthly distribution of phlebotomine sandfly species per hour of capture in Mundo Novo, São Vicente Férrer Municipality, State of Pernambuco, Brazil, from September 2009 to September 2010.

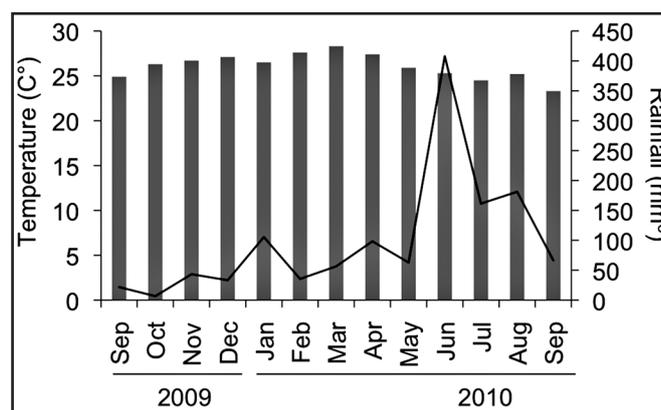


FIGURE 2 – Monthly averages for climate variables (temperature and rainfall) in Mundo Novo, São Vicente Férrer Municipality, State of Pernambuco, Brazil, from September 2009 to September 2010.

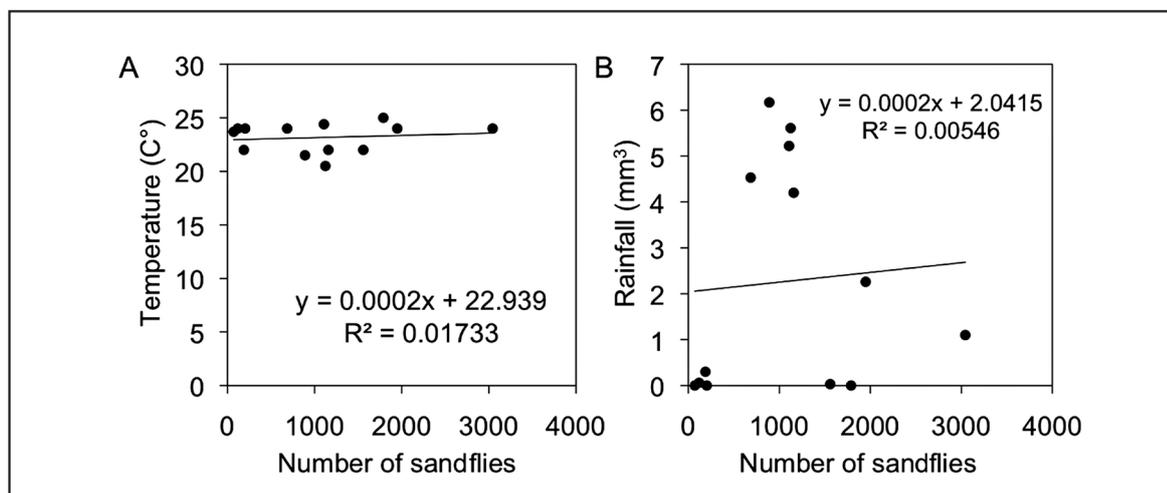


FIGURE 3 – Correlation analysis between population density of phlebotomine sandfly species and temperature (A) and rainfall (B) in Mundo Novo, São Vicente Férrer Municipality, State of Pernambuco, Brazil, from September 2009 to September 2010.

## DISCUSSION

The diversity of the phlebotomine sandfly fauna of São Vicente Férrer has previously been reported<sup>6</sup>. However, a relevant point in this study is the change in the diversity of the fauna and distribution of some species in the same collection sites after 7 years. The preliminary study in 2002<sup>6</sup> showed the occurrence of 17 species. In the current study, 20 species were observed, including the first report of

*L. ayrozai*, *L. goiana*, and *L. viannamartinsi* in this area. It also was worthy of note the presence of *L. shannoni*, *L. naftalekatzi*, *L. tupynambai*, *L. brasiliensis*, and *L. capixaba* in the peridomestic environment, which was not previously reported. Although captured sporadically, the presence of these species reflects the result of environmental changes that occurred in this area, such as intense deforestation associated with the absence of sanitary conditions in the animal shelters localized in the peridomestic environment, contributing to the possible domiciliation of these species.

The greater abundance and diversity of phlebotomine sandflies in the peridomicile has been observed in several areas of Brazil<sup>11-14</sup>. This behavior increases the risk of transmission of the leishmaniasis, and it is related to the presence of animals in household surroundings, as well as vegetation and soil type, which might favor the establishment of breeding sites for phlebotomine sandflies<sup>15</sup>.

The predominance of *L. migonei* often has been reported in the area of CL associated with *Leishmania (Viannia) braziliensis*<sup>16,17</sup>. The abundance of this species in the intradomicile and peridomicile, as well as the constant presence during the study, reveals their adaptation to the anthropic environment. This behavior also has been observed in several Brazilian States, such as in São Paulo<sup>18</sup>, Paraná<sup>19</sup>, and Minas Gerais<sup>20</sup>. Aguiar et al.<sup>21</sup> highlighted the high anthropophily of *L. migonei*, its adaptability to modified environments, and its low occurrence in wild environments, which also was observed in the study area. Moreover, the vector role of *L. migonei* has been suggested in areas of autochthonous cases of VL where *L. longipalpis*, the classic vector of *Leishmania (Leishmania) infantum*, is not found<sup>6,22,23</sup>.

*Lutzomyia complexa*, the predominant species in forest remnants, which presents considerable distribution in Pernambuco, also has been reported in Recife, Paudalho (northern rainforest area) and Amaraji (southern rainforest area)<sup>24</sup>. The current study corroborates the data presented by Brandão-Filho et al.<sup>25</sup> that reported the preference of this species for forested environments and its relationship with the enzootic cycle of CL. *Lutzomyia complexa* also has an extensive geographical distribution in the Amazon region, in the State of Pará and the south Amazon River, including the Marajó island, where is incriminated in the transmission of *Leishmania (Viannia) braziliensis*<sup>26</sup>, being highly anthropophilic, preferentially biting human rather than dogs or chickens<sup>27</sup>.

*Lutzomyia sordellii*, the third most frequent species in the study area, is recorded in all regions of the country<sup>28</sup>, being reported in peridomestic environments, such as animal shelters (e.g., chicken, pig pens, and corrals), houses, tree trunks, and caves in different areas of Brazil<sup>6,29,30</sup>, but so far, there is no consistent data on its participation in the transmission of CL.

The seasonal pattern of the phlebotomine sandfly fauna showed an irregular distribution, where the increase in population density occurred before or after the rainy period. A similar finding was presented by Gomes<sup>31</sup> during the study of a population of *L. umbratilis* in the Amazon region. Nonetheless, other authors have observed high occurrence of these sandflies also during rainy months<sup>29,32</sup>.

According to Ximenes et al.<sup>33</sup>, the pattern of seasonal variation of phlebotomine sandflies is influenced by several abiotic factors, depending on ecology of species and the geographical peculiarities of the area in which the study is conducted. Thus, because of variation in climatic factors over time, the same species may have different seasonal patterns in the same geographical area.

The lack of correlation between the total number of specimens captured and climatic variables showed that the temperature and rainfall did not affect the sandfly population, as observed by Dias et al.<sup>34</sup> and Souza et al.<sup>35</sup> in the State of Minas Gerais, Brazil, and Missawa and Dias<sup>12</sup> in the State of Mato Grosso, Brazil. Nevertheless, other studies have revealed a significant correlation between the number of phlebotomine sandflies, rainfall, and humidity<sup>32,36</sup>, which favors vegetation growth and accumulation of organic matter in the soil, allowing the emergence of breeding sites<sup>34</sup>.

Studies about the distribution of phlebotomine sandfly species are important to identify areas of active leishmaniasis transmission

that might in turn be related to a particular environment. In this sense, data here presented can support monitoring and surveillance programs and the establishment of effective control measures against the leishmaniasis in São Vicente Férrer.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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