## **Major Article**



## Frequency and diversity of phlebotomine sand flies (Diptera: Psychodidae) in Sinop, State of Mato Grosso, Brazil

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#### Abstract

**Introduction:** Understanding the diversity of sand flies is important for the epidemiology and control of leishmaniasis. This study aimed to understand the frequency, diversity, and seasonality of medically important sand flies in the municipality of Sinop, State of Mato Grosso, Brazil. **Methods:** The study was conducted in an urban area, including four ecotypes with different levels of urbanization. The sand flies were collected using light traps for three nights per month, from May 2014 to April 2015. **Results:** A total of 62,745 sand flies was collected, 52.34% of which were female. The frequency and diversity of sand flies was the highest in areas of permanent preservation (APPs) (96.85%), and was lower in more urbanized areas. *Lutzomyia dasypodogeton* was the most frequent species in the APPs. *Lutzomyia antunesi* was the most frequent in neighborhoods with forest fragments and neighborhoods around APPs, and *L. aragaoi* was the most frequent in completely urbanized neighborhoods. A higher frequency and diversity of sand flies was observed in the rainy season (87.92%) than in the dry season (12.08%). Eight medically important species were captured, and *Lutzomyia antunesi*, which is associated with American cutaneous leishmaniasis and visceral leishmaniasis, was observed in all ecotypes throughout the year. **Conclusions:** We observed a high frequency and diversity of sand flies in all urban areas, and some species collected were major vectors of leishmaniasis. These results support the need for further studies of the natural rates of infection of these insects and the circulation of the disease in hosts and vectors.

Keywords: Lutzomyia. American cutaneous leishmaniasis. Visceral leishmaniasis. Leishmania.

#### INTRODUCTION

Leishmaniasis comprises a spectrum of diseases, classified as cutaneous leishmaniasis (CL) or visceral leishmaniasis (VL), that are found in tropical and subtropical regions throughout the world. In Brazil, American cutaneous leishmaniasis (ACL) is a serious public health problem and has been registered in all Brazilian states<sup>(1)</sup>. The central-west region has the third highest number of cases and the highest increasing rates of ACL. Between 2007 and 2014, the State of Mato Grosso (MT) registered 20,818 cases of the disease, all municipalities recorded autochthonous cases, and 1,103 cases were reported in the municipality of Sinop<sup>(2)</sup>. Until August 2015, 985 ACL human cases were reported in the State of MT, and 55 of these cases were reported in the municipality of Sinop<sup>(1)</sup>.

Visceral leishmaniasis is a severe and potentially fatal disease that reemerged in various locations in Brazil in the 1980s and has since spread to new areas, including the central west region. Between 2007 and 2014, the State of MT registered 348 cases of the disease, with a mortality rate of 15.20 in 2013<sup>(2)</sup>. The disease is currently recorded in urban and rural areas in the North, South-Central, and Southeast regions of the State of MT<sup>(3)</sup>. There was only one suspected case of VL reported in humans in the municipality of Sinop<sup>(2)</sup>.

The causative agent of leishmaniasis is a protozoan parasite of the genus *Leishmania*, which is transmitted to mammals through the bite of infected phlebotomine sand flies (Diptera: Psychodidae)<sup>(4) (5)</sup>. Transmission of leishmaniasis was primarily associated with rural and wild areas. However, detrimental environmental changes, such as intensive agriculture, animal

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husbandry, logging, and human services, have disrupted these natural ecosystems, changing the ecology of some species of phlebotomine, and, consequently, the ecoepidemiology of leishmaniasis<sup>(6)</sup>.

Over the last decade, the forested environments in Sinop, the fourth most economically influential municipality in the State of MT, have been undergoing similar detrimental changes to expand urbanization and agriculture. Sinop has the highest number of ACL cases in the State of MT<sup>(2)</sup>, however, the transmission potential of leishmaniasis in the region is unknown. Studies of the sand fly fauna allow a better comprehension of leishmaniasis transmission, which is essential to establish prevention measures and control these diseases. The aim of this study was to understand the frequency, diversity, and seasonality of medically important sand flies in different urban area ecotypes.

#### **METHODS**

#### Study area

The study was conducted in Sinop (11° 51′51″ S, 55° 30′09″ W), a municipality located in the North of the State of MT, in the Central-West region of Brazil. Sinop has a population of approximately 129,916 inhabitants, with a land area of 3,942.231km<sup>2</sup>. Sinop is considered an important service center for health and education, providing services to approximately 23 municipalities located in a 300km radius from the center of Sinop. The municipality has an equatorial climate with months of rainy season (from October to April), annual precipitation levels of 2,000mm, and average temperatures of 24°C. The vegetation is characterized as tropical rainforest.

#### Sand fly collection and identification

The study was conducted in an urban area, including 3 areas of permanent preservation (APPs): Sinop Forest Park, Unemat Forest, and Horto Botanical Garden. Sand flies were collected using 24 Centers for Disease Control (CDC) light traps distributed at 4 ecotypes (a total of 2 traps per site): I. neighborhoods located around the APPs (sites 1-6); II. neighborhoods close to forest fragments, considered as new neighborhoods (sites 7-12); III. neighborhoods located in a completely urbanized area, considered as old neighborhoods (sites 13-18); and IV. APPs (sites 19-24) (**Figure 1**).

The CDC traps were placed in peridomiciliary areas (near a chicken coop, pig pen, or kennel where available) and in APPs (1 trap in an area with human activity and 1 trap inside the forest). All traps were installed 1.5m above the ground. Sampling was performed monthly from May 2014 to April 2015, on 3 consecutive nights in the first week of each month, from 18:00 to 07:00, totaling 10,368 hours of sampling effort.

Trapped males were preserved in 70% ethanol and slidemounted with Berlese fluid, according to the Langeron technique<sup>(7)</sup>. Trapped females were preserved in 6% dimethyl sulfoxide (DMSO) at -20°C. The head and last 3 abdominal segments of each female sand fly were dissected for identification. Sand flies of both genders were morphologically identified according to the taxonomic criteria proposed by Young and Duncan<sup>(8)</sup>. In addition, females were dissected and stored at -20°C in the collection of the Entomology Laboratory of the State Health Department of MT for future studies of natural infection with *Leishmania*.

#### Data analysis

The data were processed using Microsoft Excel 2010 and statistically analyzed using the Statistical Package for Social Sciences (SPSS) version 17. The Shapiro-Wilk test was used to verify the normality of the data. We used the nonparametric Spearman's rank correlation coefficient to examine the relationship between the number of sand flies and climate variables. The Mann-Whitney test was used to examine the sex ratio of sand flies. We used the test of two proportions to analyze the difference between the ecotypes and the frequency of medically important sand flies. The Kruskal-Wallis test was used to compare sand fly richness across ecotypes. The significance level was set at 5%. To evaluate the influence of climate variables on the populations of sand flies, we used the averages of all of the collection days of temperature (°C), relative humidity (%), precipitation (mm), and wind speed (km/h). Climate data were obtained from Sinop automatic station (National Institute of Meteorology)<sup>(8)</sup>.

#### **Ethical considerations**

This study was approved by the Research Ethics Committee of the State Health Department of Mato Grosso (Protocol no. 481162/2013).

#### RESULTS

A total of 62,745 sand flies was collected, including 32,840 (52.34%) females and 29,905 (47.66%) males. There was a statistically significant difference between genders in the numbers of flies trapped (p = 0.01) (**Table 1**). Thirty-seven phlebotomine sand fly species were identified, including 1 species belonging to the genus *Brumptomyia* (*B. avellari*), and 36 species belonging to the genus *Lutzomyia*. *Lutzomyia dasypodogeton* was the most frequent species (87.87%), followed by *L. aragaoi* (4.51%), *L. hermanlenti* (2.30%), and *L. antunesi* (2.01%). Specimens that could not be identified due to the damage and/or absence of essential morphological characters were classified to genus level as *Lutzomyia* spp. (**Table 1**).

The frequency of sand flies was statistically higher in the APPs (60,766; 96.85%), especially in the Unemat Forest segment (45,259; 72.13%). The next highest frequency was in neighborhoods with forest fragments (1,914; 3.05%), followed by neighborhoods around APPs (36; 0.06%), and completely urbanized neighborhoods (27; 0.04%) (p < 0.01). There was no statistically significant difference in sand fly frequency between these last two ecotypes (p = 0.45) (**Table 1** and **Figure 1**).

Higher species diversity was observed in the APPs (33 species), followed by the neighborhoods with forest fragments (25 species) and by neighborhoods around APPs and completely urbanized neighborhoods (7 species) (Table 1). There was a statistically significant difference in species diversity between the APPs and other ecotypes (p < 0.01),

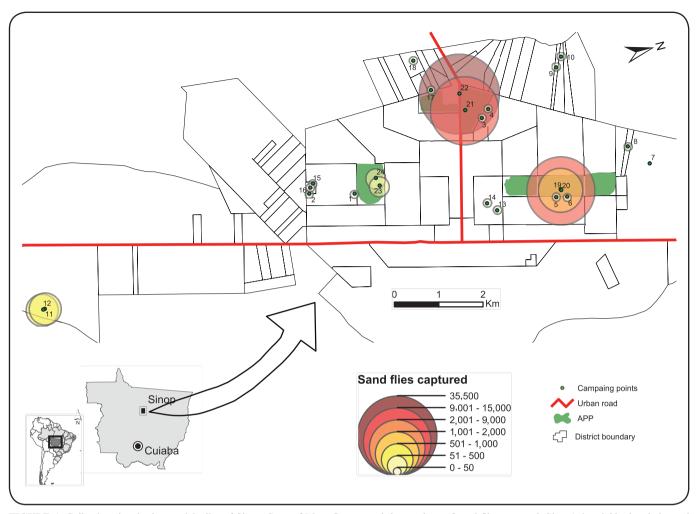


FIGURE 1. Collection sites in the municipality of Sinop, State of Mato Grosso, and the numbers of sand flies captured. Sites 1-6: neighborhoods located around the APPs; sites 7-12: neighborhoods close to forest fragments; sites 13-18: neighborhoods located in a completely urbanized area; sites 19-24: APPs areas. \*APPs: areas of permanent preservation.

and between neighborhoods with forest fragments and neighborhoods around the APPs (p < 0.01). However, there was no statistically significant difference between neighborhoods around the APPs and completely urbanized neighborhoods (p = 1.00). *Lutzomyia dasypodogeton* was the most frequent species in the APPs, *L. antunesi* in neighborhoods with forest fragments and neighborhoods around APPs, and *L. aragaoi* in the completely urbanized neighborhoods (**Table 1**).

Sand fly frequency was higher in the rainy season (87.92%), between November 2014 and April 2015, than in the dry season (12.08%), between May and October 2014 (p < 0.01) (**Table 2** and **Figure 2**). The highest numbers of sand flies were collected in April, March, and February, with 15,528 (24.75%), 13,389 (21.34%), and 13,187 (21.02%) individuals, respectively. In contrast, the lowest number of sand flies [260 (12.41%) individuals] was captured in August. A positive correlation between the total number of sand flies collected and precipitation (r = 0.093, p < 0.01) was observed, but no significant correlations were found for temperature (r = -0.445, p = 0.17), relative humidity (r = 0.198, p = 0.41), or wind speed (r = 0.420, p = 0.17). The highest diversity of species was collected in the rainy season (32 species). *Lutzomyia dasypodogeton*, *L. aragaoi*, *L. hermanlenti*, and *L. antunesi* were the most frequently collected species over the study period (Table 2).

Eight medically important species were captured, including *L. antunesi*, *L. ayrozai*, *L. carrerai carrerai*, *L. umbratilis*, *L. davisi*, *L. flaviscutellata*, *L. whitmani*, and *L. yuilli yuilli*. *L. antunesi* was the most frequent (2.01%) and was found in all ecotypes throughout the study period. The other species were less frequent (less than 1%) and were collected throughout the study period, especially in ecotypes associated with forests (Table 1 and Table 2).

#### DISCUSSION

This study tested the hypothesis that the frequency and diversity of sand fly species varies according to ecotype (collection site) and environmental variables. We observed a high frequency and diversity of sand fly species in the APPs. In contrast, frequency and diversity decreased in the other collection sites as ecotypes became more urbanized.

#### TABLE 1

Distribution of the number and proportion of sand flies by species, gender, and ecotype in the municipality of Sinop, State of Mato Grosso, from May 2014 to April 2015.

Species	Gender (%) female male		APPs	neighborhoods with forest fragments	neighborhood s around APPs	completely urbanized neighborhoods	Total (%)
Brumptomyia avellari	17 (0.05)	35 (0.12)	51 (0.08)	1 (0.05)	0	0	52 (0.08)
L. abunaensis	0	1 (0.01)	1 (0.01)	0	0	0	1 (0.01)
L. andersoni	1 (0.03)	0	1 (0.01)	0	0	0	1 (0.01)
L. antunesi*	665 (2.03)	599 (2.00)	218 (0.36)	1,026 (53.61)	16 (44.44)	4 (14.81)	1,264 (2.01)
L. aragaoi	1,243 (3.79)	1,586 (5.30)	2,719 (4.48)	98 (5.12)	3 (8.33)	9 (33.33)	2,829 (4.51)
L. ayrozai*	23 (0.07)	7 (0.02)	30 (0.05)	0	0	0	30 (0.05)
L. begonae	10 (0.03)	0	7 (0.01)	3 (0.16)	0	0	10 (0.02)
L. bourrouli	0	1 (0.01)	0	1 (0.05)	0	0	1 (0.01)
L. braziliensis	1 (0.01)	0	0	1 (0.05)	0	0	1 (0.01)
L. caprina	10 (0.03)	2 (0.01)	8 (0.01)	4 (0.21)	0	0	12 (0.02)
L. c. carrerai*	0	4 (0.01)	4 (0.01)	0	0	0	4 (0.01)
L. christenseni	26 (0.08)	72 (0.24)	98 (0.16)	0	0	0	98 (0.16)
L. claustrei	3 (0.01)	13 (0.04)	3 (0.01)	13 (0.68)	0	0	16 (0.03)
L. complexa	68 (0.21)	53 (0.18)	119 (0.20)	1 (0.05)	0	1 (3.70)	121 (0.19)
L. dasypodogeton	28,859 (87.88)	26,276 (87.87)	54,751 (90.10)	369 (19.28)	7 (19.14)	8 (29.63)	55,135 (87.87)
L. davisi*	87 (0.26)	77 (0.26)	149 (0.24)	14 (0.73)	1 (2.78)	0	164 (0.26)
L. evandroi	0	1 (0.01)	1 (0.01)	0	0	0	1 (0.01)
L. flaviscutellata*	7 (0.02)	10 (0.03)	8 (0.02)	9 (0.47)	0	0	17 (0.03)
L. hermanlenti	513 (1.56)	758 (2.54)	1,186 (1.95)	82 (4.28)	0	3 (11.11)	1,271 (2.03)
L. llanosmartinsi	10 (0.03)	17 (0.06)	25 (0.04)	2 (0.10)	0	0	27 (0.04)
L. longipennis	62 (0.19)	3 (0.01)	63 (0.10)	2 (0.10)	0	0	65 (0.10)
L. longispina	267 (0.81)	2 (0.01)	268 (0.44)	1 (0.05)	0	0	269 (0.43)
L. lutziana	94 (0.29)	91 (0.30)	161 (0.26)	24 (1.25)	0	0	185 (0.29)
L. microps	0	2 (0.01)	2 (0.01)	0	0	0	2 (0.01)
L. monstruosa	4 (0.01)	1 (0.01)	2 (0.01)	0	0	0	5 (0.01)
L. punctigeniculata	0	3 (0.01)	3 (0.01)	0	0	0	3 (0.01)
L. rondoniensis	0	1 (0.01)	1 (0.01)	0	0	0	1 (0.01)
L. runoides	1 (0.01)	3 (0.01)	3 (0.01)	1 (0.05)	0	0	4 (0.01)
L. saulensis	5 (0.02)	1 (0.01)	4 (0.01)	2 (0.10)	0	0	6 (0.01)
L. shannoni	0	1 (0.01)	1 (0.01)	0	0	0	1 (0.01)
L. shawi	2 (0.01)	1 (0.01)	2 (0.01)	1 (0.05)	0	0	3 (0.01)
L. sherlocki	489 (1.49)	62 (0.21)	503 (0.83)	47 (2.46)	1 (2.78)	0	551 (0.88)
L. sordellii	125 (0.38)	64 (0.21)	124 (0.20)	58 (3.03)	6 (16.67)	1 (3.70)	189 (0.30)
Lutzomyia spp.	1 (0.01)	4 (0.01)	2 (0.01)	2 (0.10)	1 (2.78)	0	5 (0.01)
L. umbratilis*	26 (0.08)	8 (0.03)	34 (0.06)	0	0	0	34 (0.05)
L. walker	220 (0.67)	144 (0.48)	213 (0.35)	149 (7.78)	1 (2.78)	1 (3.70)	364 (0.58)
L. whitmani*	0	2 (0.01)	0	2 (0.10)	0	0	2 (0.01)
L. yuilli yuilli*	1 (0.01)	0	0	1 (0.05)	0	0	1 (0.01)
Total	32,840 (52.34)	29,905 (47.66)	60,766 (96.85)	1,914 (3.05)	36 (0.06)	27 (0.04)	62,745
Species (n)	28	34	33	25	7	7	37

APPs: areas of permanent preservation; *L*.: *Lutzomyia*; *Lutzomyia* spp.: sand flies identified only at genus level: not considered in the count of species. \*Medically important species.

Species Brumptomyia avellari L. abunaensis L. andersoni L. antunesi* L. aragaoi L. ayrozai* L. begonae L. bourrouli L. braziliensis L. caprina L. c. carrerai* L. christenseni L. claustrei L. complexa L. dasypodogeton L. davis*	May 1 0 160 88 0 0 0 1 0 0 4 0 0 4 0 569	2 0 128 82 0 0 0 0 3 0 17	dry s 27 0 0 43 121 0 0 0 0 0 1 0	Aug           season           12           0           31           33           0           0           0           0           0	Sept 0 0 206 60 0 0 0 0 0 0	4 0 91 31 0	0 0 0 66 30 0	0 0 0 36 301	0 0 1 47	1 0 0 61	3 0 0 91	Apr 2 1 0 304	<b>Total</b> 52 1 1
L. c. carrerai* L. christenseni L. claustrei L. complexa L. dasypodogeton	0 0 160 88 0 0 0 1 0 0 4 0 0	0 0 128 82 0 0 0 0 0 3 0 17	0 0 43 121 0 0 0 0 1	0 0 31 33 0 0 0 0	0 0 206 60 0 0	0 0 91 31 0	0 0 66 30	0 0 36	0 1 47	0 0	0 0	1 0	1
L. abunaensis L. andersoni L. antunesi* L. aragaoi L. ayrozai* L. begonae L. bourrouli L. braziliensis L. caprina L. c. carrerai* L. christenseni L. claustrei L. complexa L. davis*	0 160 88 0 0 0 1 0 0 4 0 0	0 128 82 0 0 0 0 3 0 17	0 43 121 0 0 0 0 1	0 31 33 0 0 0 0	0 206 60 0 0	0 91 31 0	0 66 30	0 36	1 47	0	0	0	
L. antunesi* L. aragaoi L. ayrozai* L. begonae L. bourrouli L. braziliensis L. caprina L. c. carrerai* L. christenseni L. claustrei L. complexa L. davis*	160 88 0 0 1 0 0 4 0 0	128 82 0 0 0 0 3 0 17	43 121 0 0 0 0 1	31 33 0 0 0 0	206 60 0 0	91 31 0	66 30	36	47				1
L. aragaoi L. ayrozai* L. begonae L. bourrouli L. braziliensis L. caprina L. c. carrerai* L. christenseni L. claustrei L. complexa L. davis*	88 0 0 1 0 0 4 0 0	82 0 0 0 3 0 17	121 0 0 0 0 1	33 0 0 0 0	60 0 0	31 0	30			61	91	304	
L. aragaoi L. ayrozai* L. begonae L. bourrouli L. braziliensis L. caprina L. c. carrerai* L. christenseni L. claustrei L. claustrei L. complexa L. davis*	0 0 1 0 4 0 0	0 0 0 3 0 17	121 0 0 0 0 1	0 0 0 0	0 0	0		301	5(2)				1,264
L. ayrozai* L. begonae L. bourrouli L. braziliensis L. caprina L. c. carrerai* L. christenseni L. claustrei L. complexa L. dasypodogeton L. davis*	0 0 1 0 4 0 0	0 0 3 0 17	0 0 0 1	0 0 0	0		0	501	563	403	502	615	2,829
L. begonae L. bourrouli L. braziliensis L. caprina L. c. carrerai* L. christenseni L. claustrei L. complexa L. dasypodogeton L. davis*	0 0 1 0 4 0 0	0 0 3 0 17	0 0 0 1	0 0 0	0		U	1	2	12	3	12	30
L. bourrouli L. braziliensis L. caprina L. c. carrerai* L. christenseni L. claustrei L. complexa L. dasypodogeton L. davis*	0 1 0 4 0	0 0 3 0 17	0 0 1	0 0		0	1	6	1	2	0	0	10
L. braziliensis L. caprina L. c. carrerai* L. christenseni L. claustrei L. complexa L. dasypodogeton L. davis*	1 0 4 0 0	0 3 0 17	0 1	0	U	0	0	0	0	0	0	1	1
L. caprina L. c. carrerai* L. christenseni L. claustrei L. complexa L. dasypodogeton L. davis*	0 0 4 0 0	3 0 17	1		0	0	0	0	0	0	0	0	1
L. c. carrerai* L. christenseni L. claustrei L. complexa L. dasypodogeton L. davis*	0 4 0 0	0 17		0	0	2	0	3	1	0	2	0	12
L. christenseni L. claustrei L. complexa L. dasypodogeton L. davis*	4 0 0	17		0	0	-	0	1	0	2	0	0	4
L. claustrei L. complexa L. dasypodogeton L. davis*	0 0		7	27	0	33	4	3	0	3	0	0	98
L. complexa L. dasypodogeton L. davis*	0	0	0	0	0	0	5	9	0	1	0	1	16
L. dasypodogeton L. davis*		0	0	0	0	0	7	10	22	13	16	53	121
L. davis*	507	1,646	1,485	98	849	1,083	1,202	4,107	5,234	12,132	12,538	14,192	55,135
	3	2	0	1	0	3	1,202	18	65	9	12,556	40	164
	1	0	0	0	0	0	0	0	0	0	0	40	104
L. flaviscutellata*	2	0	0	0	0	0	0	3	4	3	5	0	17
L. hermanlenti	2 39	27	15	4	19	18	13	238	4 315	200	137		
												246	1,271 27
L. llanosmartinsi	2	0	0	0	0	0	1	0	8	1	2	13	
L. longipennis	0	0	1	0	1	2	0	51	10	0	0	0	65 2(0
L. longispina	1	0	0	0	1	0	0	2	0	261	4	0	269
L. lutziana	9	5	4	6	3	2	1	17	40	28	42	28	185
L. microps	0	0	0	0	0	0	2	0	0	0	0	0	2
L. monstruosa	0	0	0	0	0	1	0	4	0	0	0	0	5
L. punctigeniculata	0	0	1	1	0	1	0	0	0	0	0	0	3
L. rondoniensis	0	0	0	0	0	0	0	0	0	0	1	0	1
L. runoides	1	0	1	0	0	0	1	0	1	0	0	0	4
L. saulensis	1	0	0	1	0	0	0	1	0	2	1	0	6
L. shannoni	0	0	0	0	0	0	0	0	1	0	0	0	1
L. shawi	0	1	0	0	0	0	0	0	0	0	2	0	3
L. sherlocki	4	77	37	22	35	42	36	179	98	21	0	0	551
L. sordellii	7	5	15	17	25	15	28	39	21	6	6	5	189
Lutzomyia spp.	3	0	0	0	0	1	0	1	0	0	0	0	5
L. umbratilis*	27	6	0	0	0	0	0	1	0	0	0	0	34
L. walker	17	0	4	7	35	49	23	73	93	26	22	15	364
L. whitmani*	0	0	0	0	2	0	0	0	0	0	0	0	2
L. yuilli yuilli*	0	0	0	0	0	0	0	0	0	0	1	0	1
Total	940	2,001	1,762	260	1,236	1,379	1,432	5,104	6,527	13,187	13,389	15,528	62 745
%	1.5	3.19	2.81	0.41	1.97	2.20	2.28	8.13	10.40	21.02	21.34	24.75	62,745

# TABLE 2 Distribution of sand fly species according to the month and year of capture in the municipality of Sinop, State of Mato Grosso, from May 2014 to April 2015.

L: Lutzomyia; Lutzomyia spp.: sand flies identified only at genus level: not considered in the species count. \*Medically important species.

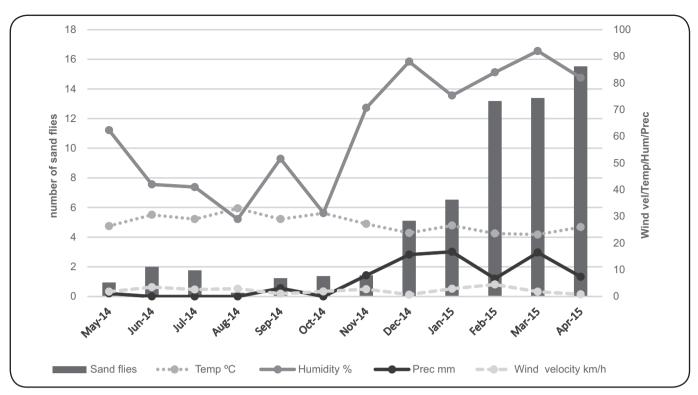


FIGURE 2. Monthly dynamics of sand flies and the correlation with wind velocity (km/h), temperature (°C), relative humidity (%), and precipitation (mm) in the municipality of Sinop, State of Mato Grosso, from May 2014 to April 2015.

The presence of vegetation, moist soil, decaying organic matter, and shaded areas, which were found in abundance in the APPs, are favorable to the development of sand flies in all stages of their lifecycle<sup>(9)</sup>. In addition, we observed a diversity of small mammals, such as armadillo, fox, anteater, paca, agouti, and capybara (unpublished data) in the forest areas. These animals serve as a food source for sand flies<sup>(10)</sup>.

Although such frequencies and diversities of sand fly species have been reported previously in urban, rural, and/or forest areas in the state of  $MT^{(11)(12)(13)(14)}$ , this study is the first to observe *B. avellari, L. abunaensis, L. bourrouli, L. caprina, L. monstruosa,* and *L. rondoniensis* in the municipality of Sinop.

Despite previous suggestions that light traps attract higher numbers of male sand flies<sup>(15)</sup>, the number of female sand flies was significantly higher in this study, which concurs with a study in the State of Amazonas<sup>(16)</sup>. Generally, when the number of female sand flies observed at a particular location is large, the growth of the sand fly population is greater and, thus, the risk of leishmaniasis transmission is also greater.

The species *L. dasypodogeton* was the most frequently observed in this study, and was found in all ecotypes, and more commonly in the APPs and neighborhoods with forest fragments. A recent study shows that this particular species is found in abundance in the Northern region of  $MT^{(17)}$ . This may be due to the presence of armadillos in the region, which are considered a reservoir for *Leishmania*<sup>(18)</sup>. We observed the presence of armadillo burrows in the APPs during the installation of traps, which could have contributed to the high frequency of this species

in these areas. *Lutzomyia dasypodogeton* may also have high dispersal distance since it was observed in all ecotypes.

In order to implement an effective vector program control, it is crucial to understand the seasonal distribution of sand flies<sup>(19)</sup>, since the control measures must be applied during months with high frequency of sand flies. However, population dynamics of sand flies may vary from region to region, as well as with climatic variables<sup>(20) (21)</sup>. In this study, and others<sup>(22) (23) (24) (25)</sup>, the highest sand fly frequency and diversity occurred during the rainy season. In addition, a positive (albeit weak) correlation between sand fly frequency and precipitation levels was observed, which has also been reported in other studies<sup>(6) (16) (26) (27)</sup>. This suggests that higher precipitation may directly increase the proliferation and survival of sand flies<sup>(28)</sup>. However, unlike other studies<sup>(29) (30) (31)</sup>, no significant correlation between sand fly frequency and relative humidity, temperature, or wind speed was observed.

Considering that the highest frequencies of sand flies were collected during the rainy season and in the APPs (where chemical controls are prohibited), the suggested control measures are related to health education in the communities and schools focused on prevention and health protection, as recommended by the health ministry.

In this study, medically important species of sand flies were mainly found in the APPs. These areas are located in the middle of urban zones and are intended for leisure and tourism, as well as being areas of environmental conservation. There is a large volume of human activity in these areas throughout the year, increasing the risk of contact with the disease vectors. However, these particular sand fly species were also found in urban areas, probably because some were adapted to degraded natural environments<sup>(32)</sup>.

Over the last few decades, extensive areas of Sinop have been deforested for agriculture and urbanization. However, neighborhoods remain close to forest areas and, consequently, there is more contact between wildlife, domestic animals, and humans. Domestic animals may become a new, easy, and relatively safe food source for sandflies, providing ideal conditions for them to adapt to urban areas<sup>(33)</sup> (<sup>34</sup>).

Among the medically important species, *L. antunesi* has been associated with the transmission of ACL and VL in Colombia and Brazil<sup>(35)(36)</sup>. This species is prevalent in the Northern region of Brazil<sup>(37)(38)</sup>, as well as in the Northern region of  $MT^{(39)}$ , where it was found naturally infected with *Leishmania infantum*<sup>(14)</sup>. The frequency of *L. antunesi* in the urban areas of Sinop highlights the importance of new studies focusing on this species in these areas. This is particularly important given the evidence of natural infection<sup>(14)</sup> and the absence of a known main vector of VL (*L. longipalpis*), despite the presence of dogs positive to VL reported in recent studies (unpublished data).

Other species collected in this study are involved in the transmission of ACL in different regions of Brazil and South America, such as L. carrerai carrerai<sup>(10)</sup> (vector of Leishmania braziliensis)<sup>(40)</sup>, L. whitmani (vector of Le. braziliensis<sup>(41) (42)</sup> and Le. shawi: reported in the Amazon basin)(43) (44), L. yuilli yuilli(10) (found experimentally infected with Le. forattinii)(45), L. flaviscutellata (vector of Le. amazonensis)<sup>(46)</sup>, L. avrozai<sup>(10)</sup> (host of Le. naiffi)<sup>(45)</sup>, L. davisi (vector of Le. naiffi<sup>(47)</sup> and found to be infected with Le. braziliensis)(45), and L. umbratilis(37) (vector of Le. amazonensis)<sup>(45) (48) (49)</sup>. Among these, L. whitmani, L. yuilli yuilli, and L. flaviscutellata are species known to have a high capacity to adapt to and reproduce in urban environments<sup>(29)(50)</sup>. This adaptation is most likely related to the flexible eating habits of these species, which feed on any available host in modified environments<sup>(51)</sup>. Conversely, L. flaviscutellata feeds on wild rodents, which circulate among all of the different ecotypes studied, facilitating the adaptation of these insects in non-forest areas<sup>(49)</sup>.

This is the first study of sand fly ecology conducted in the municipality of Sinop that highlight the movement of medically important species of sand flies in all ecotypes, especially during the rainy season. This indicates that sand flies can move from one environment to another, adapting to new environmental conditions and food supplies. Monitoring these insects through systematic collection is essential for planning effective prevention strategies and controlling leishmaniasis in the municipality. Finally, the high sand fly density, especially of known *Leishmania* vector species, indicates the need for further studies on the natural infection rate of these insects and the circulation of the disease in hosts and vectors.

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#### **Conflicts of interest**

The authors declare that there are no conflicts of interest.

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