ADADE BRASILER A

Major Article

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

Sirlei Franck Thies^{[1],[2]}, Roberta Vieira de Morais Bronzoni^[3], Mariano Martinez Espinosa^[4], Cladson de Oliveira Souza^[2], Ana Lucia Maria Ribeiro^[5], Emerson Soares dos Santos^[6], Edelberto Santos Dias^[7] and Amílcar Sabino Damazo^[1]

[1]. Programa de Pós-Graduação em Ciências da Saúde, Faculdade de Medicina, Universidade Federal de Mato Grosso, Cuiabá, Mato Grosso, Brasil. [2]. Escritório Regional de Saúde de Sinop, Secretaria de Estado de Saúde de Mato Grosso, Cuiabá, Mato Grosso, Brasil. [3]. Instituto de Ciências da Saúde, Universidade Federal de Mato Grosso, Sinop, Mato Grosso, Brasil. [4]. Departamento de Estatística, Instituto de Ciências Exatas e da Terra, Instituto de Saúde Coletiva, Universidade Federal de Mato Grosso, Cuiabá, Mato Grosso, Brasil. [5]. Instituto de Biologia, Universidade Federal de Mato Grosso, Cuiabá, Mato Grosso, Brasil. [6]. Departamento de Geogra¿ a, Universidade Federal de Mato Grosso, Cuiabá, Mato Grosso, Brasil. [7]. Laboratório de Leishmanioses, Centro de Pesquisas René Rachou, Fundação Oswaldo Cruz, Belo Horizonte, Minas Gerais, Brasil.

Abstract

Introduction: Understanding the diversity of sand Àies is important for the epidemiology and control of leishmaniasis. This study aimed to understand the frequency, diversity, and seasonality of medically important sand Àies 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 Àies were collected using light traps for three nights per month, from May 2014 to April 2015. Results: A total of 62,745 sand Àies was collected, 52.34% of which were female. The frequency and diversity of sand Àies 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 Àies 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 Àies 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, classi; ed 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⁽¹⁾. 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⁽²⁾. Until August 2015,

Corresponding author: Dra. Sirlei Franck Thies. e-mail: sfthies@hotmail.com Received 20 June 2016 Accepted 11 August 2016 985 ACL human cases were reported in the State of MT, and 55 of these cases were reported in the municipality of Sinop⁽¹⁾.

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⁽²⁾. The disease is currently recorded in urban and rural areas in the North, South-Central, and Southeast regions of the State of MT⁽³⁾. There was only one suspected case of VL reported in humans in the municipality of Sinop⁽²⁾.

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 Àies (Diptera: Psychodidae)^{(4) (5)}. Transmission of leishmaniasis was primarily associated with rural and wild areas. However, detrimental environmental changes, such as intensive agriculture, animal

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

Over the last decade, the forested environments in Sinop, the fourth most economically in Auential 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⁽²⁾, however, the transmission potential of leishmaniasis in the region is unknown. Studies of the sand Ay 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 Aies in different urban area ecotypes.

M ETHODS

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 3,942.231km². 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 Ay collection and identi; cation

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 Àies 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 ¿rst 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 slide-mounted with Berlese fluid, according to the Langeron technique⁽⁷⁾. 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 identi, cation. Sand Aies of both genders were morphologically identi, ed according to the taxonomic criteria proposed by Young

and Duncan⁽⁸⁾. 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 coef; cient to examine the relationship between the number of sand Aies and climate variables. The Mann-Whitney test was used to examine the sex ratio of sand Aies. We used the test of two proportions to analyze the difference between the ecotypes and the frequency of medically important sand Aies. The Kruskal-Wallis test was used to compare sand Ay richness across ecotypes. The signi; cance level was set at 5%. To evaluate the inAuence of climate variables on the populations of sand Aies, 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)⁽⁸⁾.

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 Àies was collected, including 32,840 (52.34%) females and 29,905 (47.66%) males. There was a statistically signi¿ cant difference between genders in the numbers of Àies 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 identi¿ ed due to the damage and/or absence of essential morphological characters were classi¿ ed to genus level as Lutzomyia spp. (Table 1).

The frequency of sand Àies 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 signi; cant difference in sand Ày 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 signi \dot{c} cant difference in species diversity between the APPs and other ecotypes (p < 0.01),

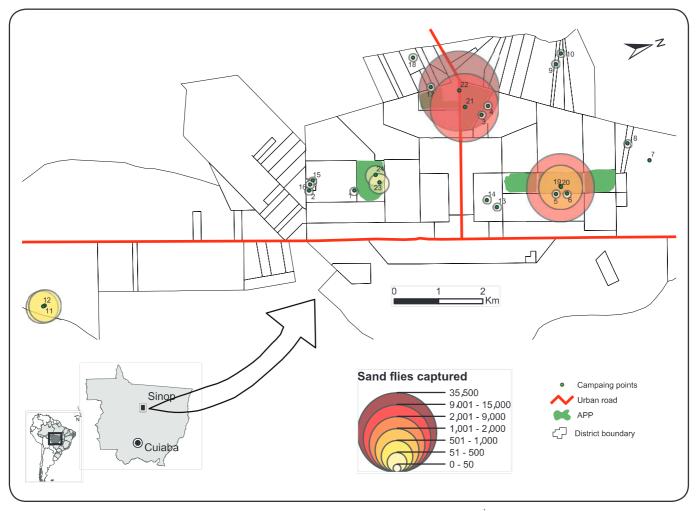


FIGURE 1. Collection sites in the municipality of Sinop, State of Mato Grosso, and the numbers of sand Aies captured. Sites 1-6: neighborhoods located around the APPs; sites 7-12: neighborhoods close to forest fragments; sites 13-18: neighborhoods located in a completely urbanized area; sites 19-24: 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 signi; cant 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 Ày 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 Àies 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 Àies [260 (12.41%) individuals] was captured in August. A positive correlation between the total number of sand Àies collected and precipitation (r=0.093, p<0,01) was observed, but no signi¿ cant 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. Àaviscutellata, 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 Àies by species, gender, and ecotype in the municipality of Sinop, State of Mato Grosso, from May 2014 to April 2015.

| Species | Ecotype (%) neighborhoods completely | | | | | | | | | |
|----------------------|---------------------------------------|----------------|----------------|--------------------------|-------------------------------|--|---------------|--|--|--|
| | Gender (%) female male | | APPs | 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) | | | |
| bourrouli | 0 | 1 (0.01) | 0 | 1 (0.05) | 0 | 0 | 1 (0.01) | | | |
| . 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) | | | |
| . c. carrerai* | 0 | 4 (0.01) | 4 (0.01) | 0 | 0 | 0 | 4 (0.01) | | | |
| christenseni | 26 (0.08) | 72 (0.24) | 98 (0.16) | 0 | 0 | 0 | 98 (0.16) | | | |
| claustrei | 3 (0.01) | 13 (0.04) | 3 (0.01) | 13 (0.68) | 0 | 0 | 16 (0.03) | | | |
| complexa | 68 (0.21) | 53 (0.18) | 119 (0.20) | 1 (0.05) | 0 | 1 (3.70) | 121 (0.19) | | | |
| _ 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 | | | |
| . davisi* | 87 (0.26) | 77 (0.26) | 149 (0.24) | 14 (0.73) | 1 (2.78) | 0 | 164 (0.26) | | | |
| evandroi | 0 | 1 (0.01) | 1 (0.01) | 0 | 0 | 0 | 1 (0.01) | | | |
| Àaviscutellata* | 7 (0.02) | 10 (0.03) | 8 (0.02) | 9 (0.47) | 0 | 0 | 17 (0.03) | | | |
| hermanlenti | 513 (1.56) | 758 (2.54) | 1,186 (1.95) | 82 (4.28) | 0 | 3 (11.11) | 1,271 (2.03) | | | |
| . llanosmartinsi | 10 (0.03) | 17 (0.06) | 25 (0.04) | 2 (0.10) | 0 | 0 | 27 (0.04) | | | |
| . longipennis | 62 (0.19) | 3 (0.01) | 63 (0.10) | 2 (0.10) | 0 | 0 | 65 (0.10) | | | |
| . longispina | 267 (0.81) | 2 (0.01) | 268 (0.44) | 1 (0.05) | 0 | 0 | 269 (0.43) | | | |
| lutziana | 94 (0.29) | 91 (0.30) | 161 (0.26) | 24 (1.25) | 0 | 0 | 185 (0.29) | | | |
| microps | 0 | 2 (0.01) | 2 (0.01) | 0 | 0 | 0 | 2 (0.01) | | | |
| monstruosa | 4 (0.01) | 1 (0.01) | 2 (0.01) | 0 | 0 | 0 | 5 (0.01) | | | |
| punctigeniculata | 0 | 3 (0.01) | 3 (0.01) | 0 | 0 | 0 | 3 (0.01) | | | |
| . rondoniensis | 0 | 1 (0.01) | 1 (0.01) | 0 | 0 | 0 | 1 (0.01) | | | |
| . runoides | 1 (0.01) | 3 (0.01) | 3 (0.01) | 1 (0.05) | 0 | 0 | 4 (0.01) | | | |
| . saulensis | 5 (0.02) | 1 (0.01) | 4 (0.01) | 2 (0.10) | 0 | 0 | 6 (0.01) | | | |
| shannoni | 0 | 1 (0.01) | 1 (0.01) | 0 | 0 | 0 | 1 (0.01) | | | |
| . shawi | 2 (0.01) | 1 (0.01) | 2 (0.01) | 1 (0.05) | 0 | 0 | 3 (0.01) | | | |
| sherlocki | 489 (1.49) | 62 (0.21) | 503 (0.83) | 47 (2.46) | 1 (2.78) | 0 | 551 (0.88) | | | |
| sordellii | 125 (0.38) | 64 (0.21) | 124 (0.20) | 58 (3.03) | 6 (16.67) | 1 (3.70) | 189 (0.30) | | | |
| utzomyia spp. | 1 (0.01) | 4 (0.01) | 2 (0.01) | 2 (0.10) | 1 (2.78) | 0 | 5 (0.01) | | | |
| . umbratilis* | 26 (0.08) | 8 (0.03) | 34 (0.06) | 0 | 0 | 0 | 34 (0.05) | | | |
| . walker | 220 (0.67) | 144 (0.48) | 213 (0.35) | 149 (7.78) | 1 (2.78) | 1 (3.70) | 364 (0.58) | | | |
| . whitmani* | 0 | 2 (0.01) | 0 | 2 (0.10) | 0 | 0 | 2 (0.01) | | | |
| 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 Àies identi¿ed only at genus level: not considered in the count of species. *Medically important species.

TABLE 2

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

Number of sand Àies per month

| | Number of sand Aies per month | | | | | | | | | | | | |
|----------------------|---|-------|-------|------|-------|-------|--------------|-------|-------|--------|--------|--------|--------|
| | May | Jun | Jul | Aug | Sept | Oct | Nov | Dec | Jan | Feb | Mar | Apr | Total |
| Species | dry season | | | | | | rainy season | | | | | | Total |
| Brumptomyia avellari | 1 | 2 | 27 | 12 | 0 | 4 | 0 | 0 | 0 | 1 | 3 | 2 | 52 |
| L. abunaensis | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| L. andersoni | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| L. antunesi* | 160 | 128 | 43 | 31 | 206 | 91 | 66 | 36 | 47 | 61 | 91 | 304 | 1,264 |
| L. aragaoi | 88 | 82 | 121 | 33 | 60 | 31 | 30 | 301 | 563 | 403 | 502 | 615 | 2,829 |
| L. ayrozai* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 2 | 12 | 3 | 12 | 30 |
| L. begonae | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 6 | 1 | 2 | 0 | 0 | 10 |
| L. bourrouli | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| L. braziliensis | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| L. caprina | 0 | 3 | 1 | 0 | 0 | 2 | 0 | 3 | 1 | 0 | 2 | 0 | 12 |
| L. c. carrerai* | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 2 | 0 | 0 | 4 |
| L. christenseni | 4 | 17 | 7 | 27 | 0 | 33 | 4 | 3 | 0 | 3 | 0 | 0 | 98 |
| L. claustrei | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 9 | 0 | 1 | 0 | 1 | 16 |
| L. complexa | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 10 | 22 | 13 | 16 | 53 | 121 |
| L. dasypodogeton | 569 | 1,646 | 1,485 | 98 | 849 | 1,083 | 1,202 | 4,107 | 5,234 | 12,132 | 12,538 | 14,192 | 55,135 |
| L. davis* | 3 | 2 | 0 | 1 | 0 | 3 | 12 | 18 | 65 | 9 | 11 | 40 | 164 |
| L. evandroi | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| L. Àaviscutellata* | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 4 | 3 | 5 | 0 | 17 |
| L. hermanlenti | 39 | 27 | 15 | 4 | 19 | 18 | 13 | 238 | 315 | 200 | 137 | 246 | 1,271 |
| L. Ilanosmartinsi | 2 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 8 | 1 | 2 | 13 | 27 |
| L. longipennis | 0 | 0 | 1 | 0 | 1 | 2 | 0 | 51 | 10 | 0 | 0 | 0 | 65 |
| 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 | |
| % | 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 |
| Species (n) | 25 | | | | | | 32 | | | | | | 37 |
| | d in the species count *Medically important species | | | | | | | | | | | | |

L.: Lutzomyia; Lutzomyia spp.: sand Àies identi¿ed only at genus level: not considered in the species count. *Medically important species.

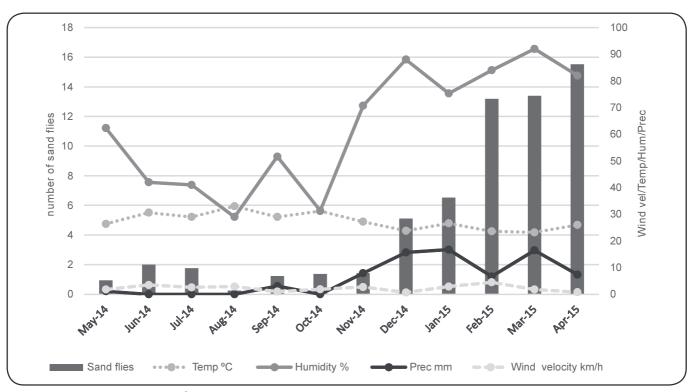


FIGURE 2. Monthly dynamics of sand Àies and the correlation with wind velocity (km/h), temperature (°C), relative humidity (%), and precipitation (mm) in the municipality of Sinop, State of Mato Grosso, from May 2014 to April 2015.

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 Àies in all stages of their lifecycle⁽⁹⁾. 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 Àies⁽¹⁰⁾.

Although such frequencies and diversities of sand Ày 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 ¿rst 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 Àies⁽¹⁵⁾, the number of female sand Àies was signi¿ cantly higher in this study, which concurs with a study in the State of Amazonas⁽¹⁶⁾. Generally, when the number of female sand Àies observed at a particular location is large, the growth of the sand Ày 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⁽¹⁸⁾. 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 Àies⁽¹⁹⁾, since the control measures must be applied during months with high frequency of sand Àies. However, population dynamics of sand Àies may vary from region to region, as well as with climatic variables⁽²⁰⁾ (21). In this study, and others⁽²²⁾ (23) (24) (25)</sup>, the highest sand Ày 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⁽⁶⁾(16)(26)(27)</sup>. This suggests that higher precipitation may directly increase the proliferation and survival of sand Àies⁽²⁸⁾. However, unlike other studies⁽²⁹⁾(30)(31), no signi¿cant correlation between sand Ày 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 Aies 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 Ày species were also found in urban areas, probably because some were adapted to degraded natural environments⁽³²⁾.

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 sandAies, providing ideal conditions for them to adapt to urban areas⁽³³⁾ (³⁴⁾.

Among the medically important species, L. antunesi has been associated with the transmission of ACL and VL in Colombia and Brazil⁽³⁵⁾⁽³⁶⁾. This species is prevalent in the Northern region of Brazil⁽³⁷⁾⁽³⁸⁾, as well as in the Northern region of MT⁽³⁹⁾, where it was found naturally infected with Leishmania infantum⁽¹⁴⁾. 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⁽¹⁴⁾ 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(10) (vector of Leishmania braziliensis)(40), L. whitmani (vector of Le. braziliensis(41) (42) and Le. shawi: reported in the Amazon basin)(43) (44), L. yuilli yuilli(10) (found experimentally infected with Le. forattinii)(45), L. Àaviscutellata (vector of Le. amazonensis)(46), L. ayrozai(10) (host of Le. naif;)(45), L. davisi (vector of Le. naif; 47) and found to be infected with Le. braziliensis)(45), and L. umbratilis(37) (vector of Le. amazonensis)(45) (48) (49). Among these, L. whitmani, L. yuilli yuilli, and L. Aaviscutellata are species known to have a high capacity to adapt to and reproduce in urban environments⁽²⁹⁾⁽⁵⁰⁾. This adaptation is most likely related to the Aexible eating habits of these species, which feed on any available host in modiced environments⁽⁵¹⁾. Conversely, L. Aaviscutellata feeds on wild rodents, which circulate among all of the different ecotypes studied, facilitating the adaptation of these insects in non-forest areas⁽⁴⁹⁾.

This is the ¿rst study of sand Ày 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 Àies 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 Ày 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.

Acknowledgments

The authors thank Pedro Paulo Lopes and José de Oliveira for collaboration in ¿eldwork.

ConAicts of interest

The authors declare that there are no conAicts of interest.

Financial Support

Fundação de Amparo à Pesquisa em Mato Grosso, Project number 226574/2013 Ministério da Saúde, Call number 20/2013.

REFERENCES

- Ministério da Saúde. Sistema de Informação de Agravos De Noti¿ cação (SINAN). Leishmaniose tegumentar americana - Casos con¿ rmados Noti¿ cados no Sistema de Informação de Agravos de Noti¿ cação - SINAN Net. Disponível em: http://dtr2004.saude. gov.br/sinanweb/tabnet/dh?sinannet/lta/bases/ltabrnet.def. Acesso em 14/03/2016.
- Dorval MEMC, Oshiro ET, Cupollilo E, Castro ACC, Alves TP. Ocorrência de leishmaniose tegumentar americana no Estado de Mato Grosso do Sul associada à infecção por Leishmania (Leishmania) amazonensis. Rev Soc Bras Med Trop 2006; 39:43-46.
- Mestre GLC, Fontes CJF. A expansão da epidemia da leishmaniose visceral no Estado de Mato Grosso 1998-2005. Rev Soc Bras Med Trop 2007; 40:42-48.
- Maroli M, Feliciangeli MD, Bichaud L, Charrel RN, Gradoni L. Phlebotomine sand Aies and the spreading of leishmaniases and other diseases of public health concern. Med Vet Entomol 2013; 27:123-147.
- Ready PD. Biology of phlebotomine sand Aies as vectors of disease agents. Annu Rev Entomol 2013; 58:227-250.
- Ximenes MFFM, Silva VPM, Queiroz PVS, Rego MM, Cortez AM, Batista LMM, et al. Flebotomineos (Diptera: Psychodidae) e leishmanioses no Rio Grande do Norte, Nordeste do Brasil - Re\u00e0exes do Ambiente Antropico. Neotrop Entomol 2007; 36:128-137.
- Langeron M. Précis de microscopie. Masson et Cie, Libraires de L'Académie de Medicine, Saint-Germain, Paris. 1949.
- Brazil RP, Brazil BG. Biologia de Flebotomíneos neotropicais.
 In: Rangel EF, Lainson R, editors. Flebotomíneos do Brasil.
 Rio de Janeiro: Editora FIOCRUZ, 2003. p. 257-274.
- Aguiar GM, Medeiros WM. Distribuição regional e habitats das espécies de Aebotomíneos do Brasil. In: Rangel EF, Lainson R, organizadores. Flebotomíneos no Brasil. Rio de Janeiro: Editora FIOCRUZ, 2003. p. 207-255.
- Maciel GBML, Missawa NA. Fauna Àebotomínica (Diptera: Psychodidae) em aldeias indígenas do Estado de Mato Grosso. Rev Soc Bras Med Trop 2009; 42:597-602.
- Amaral AFS, Varjão JR, Silva GB, Arrais-Silva WW. Phlebotomine fauna (Diptera: Psychodidae: Phlebotominae) in a residential area and in a fragment of savanna vegetation in the municipality of Pontal do Araguaia, Mato Grosso, Brazil. Rev Bras Parasitol Vet 2011; 20:165-167.
- Alves GB, Oshiro ET, Leite MC, Melão AV, Ribeiro LM, Mateus NLF, et al. Phlebotomine sandÀies fauna (Diptera: Psychodidae) at rural settlements in the municipality of Cáceres, state of Mato Grosso, Brazil. Rev Soc Bras Med Trop 2012; 45:437-443.

- 14. Thies SF, Ribeiro ALM, Michalsky EM, Myiazaki RD, Fortes-Dias CL, Fontes CJF, et al. Phlebotomine sandAy fauna and natural Leishmania infection rates in a rural area of Cerrado (tropical savannah) in Nova Mutum, State of Mato Grosso in Brazil. Rev Soc Bras Med Trop 2013; 46:293-298.
- Aguiar AM, Soucasaux T. Aspectos da ecologia dos Àebótomos do Parque Nacional da Serra dos Órgãos, Rio de Janeiro. I- Frequência mensal em isca humana (Diptera, Psychodidae, Phlebotominae). Mem Inst Oswaldo Cruz. 1984; 79: 179-209.
- Silva EA, Andreotti R, Honer MR. Comportamento de Lutzomyia longipalpis, vetor principal da leishmaniose visceral americana, em Campo Grande, Estado do Mato Grosso do Sul. Rev Soc Bras Med Trop 2007; 40:420-425.
- de Almeida PS, de Andrade AJ, Sciamarelli A, Raizer J, Menegatti JA, Negreli Moreira Hermes SC, et al. Geographic distribution of phlebotomine sandAy species (Diptera: Psychodidae) in Central-West Brazil. Mem Inst Oswaldo Cruz 2015; 110:551-559.
- Martins AV, Falcão AL, da Silva JE, Miranda Filho R. Nota sobre Lutzomyia (Trichopygomyia) Dasypodogeton (Castro, 1939) com a redescrição do macho e da fêmea (Diptera: Psichodidade, Phlebotominae). Mem Inst Oswaldo Cruz 1983; 78:223-230.
- Saraiva L, Lopes JS, Oliveira GBM, Batista FA, Falcão AL, Andrade Filho JD. Estudo dos Aebotomíneos (Diptera: Psychodidae) em área de leishmaniose tegumentar americana nos municípios de Alto Caparaó e Caparaó, Estado de Minas Gerais. Rev Soc Bras Med Trop 2006; 39:56-63.
- Costa PL, Dantas-Torres F, da Silva FJ, Guimarães VC, Gaudêncio K, Brandão-Filho SP. Ecology of Lutzomyia longipalpis in an area of visceral leishmaniasis transmission in north-eastern Brazil. Acta Trop 2013; 126:99-102.
- Gaglio G, Brianti E, Napoli E, Falsone L, Dantas-Torres F, Tarallo VD, et al. Effect of night time-intervals, height of traps and lunar phases on sand Ay collection in a highly endemic area for canine leishmaniasis. Acta Trop 2014; 133:73-77.
- Resende MC, Camargo MCV, Vieira JRM, Nobi RCA, Porto NMN, Oliveira CDL, et al. Seasonal variation of Lutzomyia longipalpis in Belo Horizonte, State of Minas Gerais. Rev Soc Bras Med Trop 2006; 39:51-55.
- 23. Almeida PS, Minzão ER, Minzão LD, Silva SR, Ferreira AD, Faccenda O, et al. Aspectos ecológicos de Aebotomíneos (Diptera: Psychodidae) em área urbana do município de Ponta Porã, Estado de Mato Grosso do Sul. Rev Soc Bras Med Trop 2010; 43:723-727.
- Barros VL, Rebêlo JMM, Silva FS. Flebotomíneos (Diptera, Psychodidae) de capoeira do município do Paço do Lumiar, estado do Maranhão, Brasil. Área endêmica de leishmanioses. Cad Saude Publica 2000; 16:265-270.
- Martin AMCB, Rebêlo JMM. Dinâmica espaço-temporal de Àebotomíneos (Diptera, Psychodidae) do município de Santa Quitéria, área de cerrado do estado do Maranhão, Brasil. Iheringia Sér Zool 2006; 96:283-288.
- Jeraldo VLS, Góes MAO, Casanova C, de Melo CM, Araújo ED, Brandão Filho SP, et al. SandAy fauna in an area endemic for visceral leishmaniasis in Aracaju, State of Sergipe, Northeast Brazil. Rev Soc Bras Med Trop 2012; 45:318-322.
- 27. Arias JR. Freitas RA. On the vectors of cutaneous leishmaniasis in central amazon of Brasil. 3. Phlebotomine sand Ày strati¿ cacion in a terra ¿rme forest. Acta Amaz 1982; 12:599-603.
- Deane LM, Deane MP. Sobre a biologia do Phlebotomus longipalpis, transmissor da leishmaniose visceral, em uma zona endêmica do Estado do Ceará. I. Distribuição, predominância e variação estacional. Rev Bras Biol 1965; 15:83-95.

- Souza CM, Pessanha JE, Barata RA, Monteiro EM, Costa DC, Dias ES. Study on Phlebotomine sand Ay (Diptera: Psychodidae) fauna in Belo Horizonte, State of Minas Gerais, Brazil. Mem Inst Oswaldo Cruz 2004; 99:795-803.
- Dias ES, França-Silva JC, Silva JC, Monteiro EM, Paula KM, Gonçalves CM, et al. Flebotomíneos (Diptera: Psichodidae) em um foco de leishmaniose tegumentar no Estado de Minas Gerais. Rev Soc Bras Med Trop 2007; 40:49-52.
- Missawa NA, Dias ES. Phlebotomine sand Àies (Diptera: Psychodidae) in the municipality of Várzea Grande: an area of transmission of visceral leishmaniasis in the State of Mato Grosso, Brazil. Mem Inst Oswaldo Cruz 2007; 102:913-918.
- Peterson AT, Shaw JJ. Lutzomyia vectors for cutaneous leishmaniasis in Southern Brazil: ecological niche models, predicted geographic distributions, and climate change effects. Int J Parasitol 2003; 33:919-931.
- Nascimento JC, Paiva BR, Malforte RS, Fernandes WD, Galati EAB. Natural infection of phlebotomines (Diptera: Psychodidae) in visceral leishmaniasis focus in Mato Grosso do Sul, Brazil. Rev Inst Med Trop Sao Paulo 2007; 49:119-122.
- 34. Barata RA, França-Silva JC, Mayrink W, Silva JC, Prata A, Lorosa ES, et al. Aspectos da ecologia e do comportamento de Aebotomíneos em área endêmica de leishmaniose visceral, Minas Gerais. Rev Soc Bras Med Trop 2005; 38:421-425.
- 35. Vásquez Trujillo A, González Reina AE, Góngora Orjuela A, Prieto Suárez E, Palomares JE, Buitrago Alvarez LS. Seasonal variation and natural infection of Lutzomyia antunesi (Diptera: Psychodidae: Phlebotominae), an endemic species in the Orinoquia region of Colombia. Mem Inst Oswaldo Cruz 2013; 108:463-469.
- 36. Silveira FT, Ishikawa EAY, De Souza AAA, Lainson R. An outbreak of cutaneous leishmaniasis among soldiers in Belém, Pará State, Brazil, caused by leishmania (viannia) lindenbergi n. sp. A new leishmanial parasite of man in the amazon region. Parasite 2002; 9:43-50.
- 37. Azevedo ACR, Souza NA, Menezes CRV, Costa WA, Costa SM, Lima JB, et al. Ecology of sand Àies (Dipetra: Psychodidae: Phlebotominae) in the north of the state of Mato Grosso, Brazil. Mem Inst Oswaldo Cruz 2002; 97:459-464.
- 38. Vilela ML, Pita-Pereira D, Azevedo CG, Godoy RE, Britto C, Rangel ER. The phlebotomine fauna (Diptera: Psychodidae) of Guaraí, state of Tocantins, with an emphasis on the putative vectors of American cutaneous leishmaniasis in rural settlement and periurban áreas. Mem Inst Oswaldo Cruz 2013; 108:578-585.
- Ribeiro ALM, Missawa NA, Zeilhofer P. Distribution of phlebotomine sandAies (Diptera: Psychodidae) of medical importance in Mato Grosso State, Brazil. Rev Inst Med Trop São Paulo 2007; 49: 5:317-321.
- 40. Grimaldi Jr G, Tesh RB, McMahon-Pratt D. A review of the geographic distribution and epidemiology of leishmaniasis in the New World. Am J Trop Med Hyg 1989; 41:687-725.
- 41. Luz E, Membrieve N, Castro EA, Dereure J, Pratlong F, Dedet A, et al. Lutzomyia whitmani (Diptera: Psychodidae) as vector of Leishmania (Viannia) braziliensis in Paraná state, southern Brazil. Ann Trop Med Parasitol 2000; 94:623-631.
- 42. Rangel EF, Lainson R, Souza AA, Ready PD, Azevedo ACR, Papavero N. Lutzomyia (Nyssomyia) whitmani (Antunes & Coutinho, 1939) (Diptera: Psychodidae: Phlebotominae), a vector of cutaneous leishmaniasis in Brazil. Mem Inst Oswaldo Cruz. 1994; 89 (supl I):202.
- 43. Rangel EF, Lainson R. Ecologia das leishmanioses: transmissores de leishmaniose tegumentar Americana. In: Rangel EF, Lainson R,

- editores. Flebotomíneos do Brasil. $1^{\rm st}$ edition. FIOCRUZ. Rio de Janeiro: 2003. p. 291-310.
- 44. da Costa SM, Cechinel M, Bandeira V, Zanuccio JC, Lainson R, Rangel EF. Lutzomyia (Nissomyia) whitmani s.l. (Antunes & Coutinho, 1939) (Diptera: Psychodidade: Phlebotominae): geographical distribuition and the epidemiology of American cutaneous leishmaniasis in Brazil- Mini-review. Mem Inst Oswaldo Cruz 2007; 102:149-153.
- Lainson R. The Neotropical Leishmania species: a brief historical review of their discovery, ecology and taxonomy. Rev Pan-Amaz Saude 2010: 1:13-32.
- 46. Lainson R, Shaw JJ, Silveira FT, de Souza AAA, Braga RR, Ishikawa EAY. The dermal leishmaniases of Brazil, with special reference to the eco-epidemiology of the disease in Amazonia. Mem Inst Oswaldo Cruz 1994; 89: 435-443.
- 47. Gil LHS, Basano AS, Souza AA, Silva MGS, Barata I, Ishikawa EA, et al. Recent observations on the sand Ay (Diptera: Psychodidae) fauna of the State of Rondônia, Western Amazônia, Brazil: the importance of Psychdopygus davisi as a vector of

- zoonotic cutaneous leishmaniasis. Mem Inst Oswaldo Cruz 2003; 98:751-755
- Ready PD, Lainson R, Shaw JJ, Ward RD. The ecology of Lutzomyia umbratilis (Ward & Fraiha, 1977) (Diptera: Psychodiade), the major vector to man of Leishmania braziliensis guyanensis in north-eastern Amazonian Brazil. Bull Entomol Res 1986; 76:21-40.
- Rebêlo JMM, Araújo JAC, Carvalho ML, Barros VLL, Silva FS, Oliveira ST. Flebótomos (Diptera, Phlebotominae) da Ilha de São Luis, zona do Golfão Maranhense, Brasil. Rev Soc Bras Med Trop 1999; 32: 247-253.
- 50. Sandoval CM, Gutiérrez R, Cárdenas R, Ferro C. Especies del gênero Lutzomyia (Psychodidae, Phlebotominae) en áreas de transmisión de leishmaniasis tegumentaria visceral en el departamento de Santander, en la cordillera oriental de los Andes colombianos. Biomédica 2006; 26 (suppl 1):218-227.
- Muniz LHG, Rossi RM, Neitzke HC, Monteiro WM, Teodoro U. Estudo dos hábitos alimentares de Aebotomíneos em área rural no sul do Brasil. Rev Saude Publica 2006; 40:1087-1093.