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Sandfly fauna (Diptera: Psychodidae) in an urban area, Central-West of Brazil

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ABSTRACT

Biological and ecological relations among vectors and their pathogens are important to understand the epidemiology of vector-borne diseases. *Camapuã* is an endemic area for visceral and tegumentary leishmaniasis. The aim of this study was to characterize the sandfly fauna present in *Camapuã*, MS, Brazil. Sand flies were collected every fortnight from May 2014 to April 2015 using automatic light traps in the domicile and peridomicile of twelve neighborhoods and forest. The collected specimens were identified based on morphology according to the valid identification keys. In total, 2005 sandflies of five genera and nine species were collected. *Nyssomyia whitmani* and *Lutzomyia cruzi* were the most abundant species. Males were more abundant, with a male-to-female ratio of 2.14. The highest diversity was observed in peripheral neighborhood, with abundant plant cover. The peridomicile presented greater abundance of sandflies, with the predominance of *Ny. whitmani*. No significant correlation between the absolute frequencies of the most abundant species and the precipitation variable was observed; however, there was a predominance of *Lu. cruzi* in the rainy season. We observed a high frequency of sandflies in urban area, especially vector species. The presence of *Nyssomyia whitmani* and *Lutzomyia cruzi* indicate the necessity for health surveillance in the municipality. Additional method of collection such as sticky trap is also recommended for appropriate faunestic study.

KEYWORDS: Camapuã. *Nyssomyia whitmani. Lutzomyia cruzi.* Leishmaniases.

INTRODUCTION

Different sandfly species with wild habits have been adapted to urban areas, where they find suitable sites for their development[1]. Vector-borne diseases are related to the environment and changes in the ecosystem, such as urbanization, with increased density of vector insects, favoring the emergence and reemergence of diseases like Leishmaniasis[2,3].

The leishmaniasis is a group of parasitic diseases caused by protozoa of the *Leishmania* genus (Ross 1903) transmitted through infected sandflies, affecting wild (rodents, marsupials, edentulous) and domestic animals (dogs) as well as the man[4]. The notification and increase on incidence of the leishmaniasis in non-endemic areas indicate the necessity to study local sandfly fauna in order to know the dominance, diversity and dispersion of the species and to identify probable vectors[5].

Between 2007 and 2015, Mato Grosso do Sul notified 1985 cases of VL and 1232 of TL, of which 12 and 11, respectively, were reported in *Camapuã* (MS).

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The municipality is classified as a sporadic leishmaniasis transmission area[6,7]. However, a few studies conducted in the region were conducted in rural areas mainly in caves, with description of *Lu. cruzi*[8] incriminated as vector of *Leishmania* (*L.*) *infantum*[9,10] and *Lu. dispar*, a very anthropophilic considered as a potential vector of *Leishmania* sp.[11].

Considering the cases of leishmaniasis in the municipality and the absence of vector information in the area, the aims of this study were to identify the sandfly fauna in the city of *Camapuã*, Mato Grosso do Sul, Brazil, and to analyze the temporal distribution of species of medical importance and their presence in different ecotypes (intradomicile, peridomicile and forest).

METHODS

Study Area

The study was conducted in *Camapuã* (19º31'53''S and 54º02'38''W, 409 m above sea level), located in Mato Grosso do Sul, Center-West region of Brazil (Figure 1). The municipality has a total area of 6229.615 km^2, the city is located 137 km from the State capital and the population is estimated in 13,712 inhabitants[12,13].

The climate is classified according to Köppen as tropical (AW). The average temperature varies between 20 and 24 ºC and rainfall between 1,000 and 1,500 mm per year, with the dry season from April to September and the rainy season from October to March[13].

The municipality is located in the cerrado biome, and has two phytophysiognomies: campo cerrado (formed by undergrowth, trees and shrubs with thick bark and height of 4 to 8 m) and *cerradão* (consisting of grasses, shrubs up to 3 m and trees with height from 10 to 15 m)[14,15]. However, due to the livestock activities, most of the area is covered by pastures[16,17].

The urban area of *Camapuã* is on soils derived from the fine-grained sandstones of *Pirambóia* formation, with presence of clay that allows the soils to be non-friable. In the nearest collection sites of floodplains, the soils are dark due to the presence of organic matter, which helps to support humidity. The main differences between the capture sites were in relation to the degree of shadowing/sunshine and the proximity to remnants of native vegetation and the river.

Sandfly collection

Sand flies were collected fortnightly from May 2014 to April 2015, using modified *Falcão* light traps. The collection sites were defined according to the report of human cases of leishmaniasis in nearby areas, presence of animal shelters (chicken coop, stable, kennel) and organic matter per domicile. The traps were installed between 06:00 p.m. and 06:00 a.m. in the peridomicile and intradomicile of twelve residences and a trap in the forest. Table 1 shows

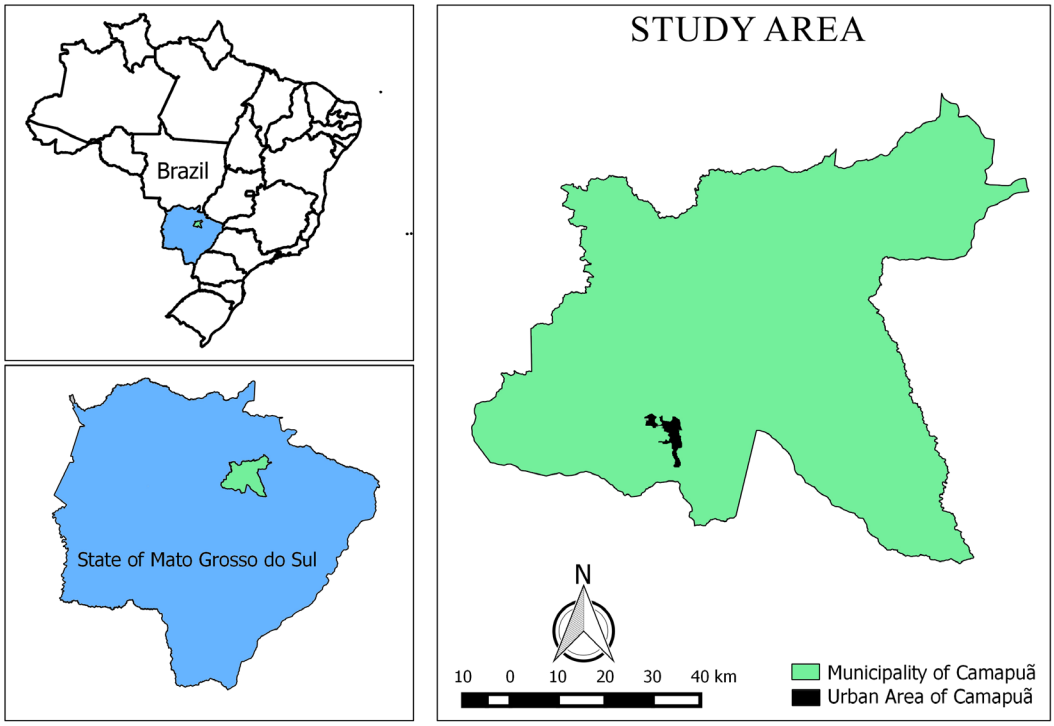

Figure 1 - Geographical location of the study area: municipality of *Camapuã*, State of Mato Grosso do Sul, Brazil

Table 1 - Characteristics of capture sites, *Camapuã*, MS, Brazil

Neighborhood	Characteristics	Presence of animals
Industrial	• Located in the periphery, 3km from the city; Presence of organic matter in the peridomicile, fruit trees and shade;	Dogs (3) Hens (>15) Ducks (4)
Coophavale	• Located in the periphery, on the edge of a cerradão (50m); Organic tree litter (about 5cm), shading;	Hens (>15) Horses (2)
Chácara Pérola	• Located in the periphery, proximity to escarpment (250m); Peridomicile with little shading;	Hens (3) Sheeps (2) Horses (4)
Pedro Luiz Amorim	• Located in central urban area; Peridomicile without shading and organic matter;	–
Diamantina	• Located in central urban area; Peridomicile with shading, presence of fruit trees, organic matter;	Dogs (2) Hens (>15)
Vale do Sol	• Located in central urban area; Peridomicile without shading, presence of grasses;	Dogs (2) Hens (13)
Isolina	• Located in the periphery, near the break of relief (50m) and cerradão (100m); Presence of trees, organic matter;	Dogs (3) Hens (>15)
Olídia	• Located in the periphery, unpaved streets and basic sanitation; Shaded peridomicile, presence of fruit trees, organic matter;	Hens (7) Pig (1)
Alto	• Located in the periphery, on the side of a cliff; Abundant organic tree litter in the peridomicile, fruit trees;	Dog (1) Hens (4) Horse (2)
João Leite	• Located in the outskirts, near the Camapuã River (100m); Presence of organic matter, fruit trees, grain plantation;	Dogs (2) Hens (>15) Goose (4) Peacock (3)
Centro	• Commercial area in the city center; Presence of organic tree litter, banana plantation;	Hens (3)
São Bento	• Located in the periphery, close proximity to the Cerrado (50m); Presence of organic matter, shading, fruit trees;	Hens (>15) Bovine (3)

the characteristics of the environment of each residence.

The insects were cleaned and assembled according to Forattini[18]. In order to identify the species, the structures of the head, thorax and abdomen were used, with emphasis on the genitalia, following the classification proposed by Galati[19]. The abbreviation of the genera followed Marcondes[20].

Data Analysis

Diversity of species was calculated using the Shannon-Weaver Diversity Index (H')[21] and the measure of dominance/evenness was calculated using the Pielou's Index (J). The evaluation of the most abundant species according to their spatial distribution was performed using the Standardized Index of Species Abundance (SISA)[22]. The frequencies of these species were calculated by the Williams geometric mean[23].

The Wilcoxon test was used for comparisons of the absolute frequencies of total sandflies and of the two most abundant species between the sex and climate seasons (dry and rainy). The association between the accumulated rainfall per month and the absolute frequencies of the most abundant species was evaluated by the Spearman's correlation.

RESULTS

A total of 2,005 specimens was collected and identified among five genera and nine species: *Brumptomyia avellari* (Costa Lima, 1932), *Brumptomyia galindoi* (Fairchild & Hertig, 1947), *Evandromyia lenti* (Mangabeira, 1938), *Evandromyia sallesi* (Galvão & Coutinho, 1940), *Evandromyia teratodes* (Martins, Falcão & Silva, 1964), *Nyssomyia whitmani* (Antunes & Coutinho, 1939), *Psathyromyia aragaoi* (Costa Lima, 1932), *Psathyromyia bigeniculata* (Floch & Abonnenc, 1941) and *Lutzomyia cruzi* (Mangabeira, 1938) (Table 2).

Considering all the capture sites, the predominant species were *Nyssomyia whitmani* (55.3%) and *Lutzomyia*

Table 2 - Distribution of sandflies species by neighborhood, Shannon's Index (H), Pielou's Index (J) and Standardized Index of Species Abundance (SISA), in urban areas of *Camapuã*, MS, 2014 to 2015 (n=2,005)

Species	Capture sites													SISA
	Industrial	Coophavale	*Chácara Pérola*	Pedro Luiz	Diamantina	Vale do Sol	Isolina	*Olídia*	Alto	*João Leite*	Centro	*São Bento*	Mata	
Br. avellari	2	25	3	-	-	1	6	1	1	-	1	1	-	0.74
Br. galindoi	-	1	-	-	-	-	-	-	-	-	-	-	-	0.03
Ev. lenti	1	-	2	-	1	-	-	-	-	1	-	1	2	0.47
Ev. sallesi	-	-	-	-	1	-	1	-	-	-	-	1	1	0.30
Ev. teratodes	-	1	-	-	-	-	-	-	-	-	-	-	-	0.03
Lu. cruzi	3	44	4	1	274	86	178	34	3	48	55	96	1	1.00
Ny. whitmani	49	330	15	2	78	14	296	53	7	7	15	227	17	1.00
Pa. aragaoi	-	-	-	-	-	-	1	1	-	-	-	1	-	0.21
Pa. bigeniculata	-	-	-	-	2	-	2	1	-	1	-	4	-	0.40
Shannon (H)	0,4543	0,6045	-	-	0,5959	0,4563	0,7698	0,8292	-	0,5436	0,5859	0,7403	-	-
Equitability (J)	0,3277	0,3755	-	-	0,3702	0,4153	0,4296	0,5152	-	0,3921	0,5333	0,3804	-	

Br.: Brumptomyia; Ev.: Evandromyia; Lu.: Lutzomyia; Ny.: Nyssomyia; Pa.: Psathyromyia.

cruzi (41.3%). Both were also the most abundant species according to the Standardized Index of Species Abundance (SISA=1.00), present in all the capture sites (Table 2). The males were more frequent (n=1367, 68.2%) than females (n=638, 31.8%), with a male-to-female ratio of 2.14 (W=164860, p=0.002). *Lu. cruzi* showed a higher number of males (4.24, W=170990, p<0.001).

In peridomicile, 1702 (84.9%) specimens were captured, followed by intradomicile with 282 (14.06%), and 21 (1.04%) in the forest. *Nyssomyia whitmani* was predominant in peridomicile and forest, while *Lu. cruzi* was the most frequent in intradomicile.

The highest Shannon's Diversity Index was reported in *Olídia* neighborhood (H=0.82) where five species were collected. São Bento neighborhood showed a lower diversity (H=0.74), but presented the greatest dominance of species, with seven of the nine species collected in the city. The index was not calculated for *Chácara Pérola*, Pedro Luiz Amorim, Alto and Mata due to the low density of collected insects (Table 2).

There was no significant correlation between rainfall and frequency of *Lu. cruzi* (r=0.224; p=0.485) and *Ny. whitmani* (r=-0.335; p=0.287). The Williams' average and monthly precipitation (mm^3) are shown in Figure 2. We observed a predominance of *Lu. cruzi* in the rainy season, but not of *Ny. whitmani*. Table 3 shows the absolute frequency of sandflies between dry and rainy season.

DISCUSSION

In Mato Grosso do Sul (MS), a total of 66 species of

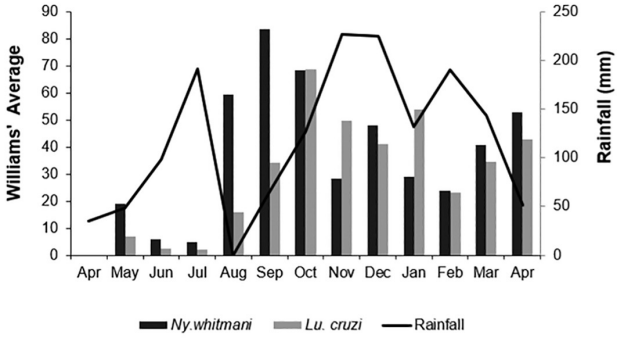

Figure 2 - Monthly Williams' average of the most frequent species according to SISA and rainfall (mm), in urban areas of *Camapuã*, MS, Brazil, 2014 to 2015

Table 3 - Absolute frequency of total collected sandflies and most abundant species according to SISA and climatic seasons, in urban areas of *Camapuã*, MS, 2014 to 2015

Species	Climatic seasons		*p**
	Dry	Rainy	
Lu. cruzi			
Male	144	525	0,002
Female	37	121	0,050
Total	181	646	0,001
Ny. whitmani			
Male	296	364	0,815
Female	153	297	0,163
Total	449	661	0,340
Total of specimens			
Male	446	920	0,014
Female	196	443	0,014
Total	642	1363	<0,001

*Wilcoxon test.

phlebotomine was identified[9,24-30]. The species recorded in *Camapuã* were already reported in MS and the composition is similar to that of other urban areas in the State[24,26,28].

The most frequent species were *Ny. whitmani* and *Lu. cruzi*, important in the epidemiology of leishmaniasis. *Ny. whitmani* has been implicated in the transmission of *Leishmania* sp. in Northeastern, Southeastern, Central-West, Southern and recently in South of Brazil[31,34], while *Lu. cruzi* has been incriminated in the transmission of *L.* (*L.*) *infantum* in the Central-West of Brazil[9,10]. *Ny. whitmani* was collected in all the capture sites. The species shows different behavior in Brazil and is suspected to be a cryptic species complex with wild populations and some adapted populations to ecological changes and involved in the epidemiological chain of TL[32-35]. It is probable that the species is involved in the transmission of *Leishmania* sp. in the studied area, considering the involvement in other regions[34], infection capacity[31], density, distribution and notification of cases in the municipality.

Although *Ny. whitmani* shows adaptation to anthropic environments, the species is still found in forest areas[35]. In *Camapuã,* the species was more frequent in residences with abundant vegetation, mainly in peripheral neighborhoods. These sites offer ideal conditions for the development of sandflies[36].

The second most frequent species was *Lu. cruzi*. Its importance was already reported in *Corumbá* (MS)[9] and Jaciara, Mato Grosso (MT)[10], where the species was found naturally infected by *Leishmania*[37-39], reinforcing its role as the vector of this agent. *Lu. cruzi* was described and redescribed from insects captured in *Camapuã*[8,40]. It is commonly found in areas of savannah and wetland[41], and already identified in six other municipalities of MS[9,24,26,42].

The species was collected in all the capture sites, mainly in central areas, demonstrating its predilection for anthropic environments, as noted in *Corumbá* (MS)[9,26] and Jaciara (MT)[10,37]. *Lu. cruzi* shows similar behavior to *Lu. longipalpis* in relation to habitat, occupying urbanized areas[43-45], especially with presence of abundant vegetation[46,47].

When comparing the number of specimens in the different ecotypes, peridomicile was the site with the highest density of sandflies, probably due to environmental ruralization, with abundance of organic matter and presence of animals such as chickens, pigs and dogs. In these places, the specimens find food and microhabitat for the development of their immature forms, increasing the quantity of these Diptera around the residence, which is considered a risk factor for the occurrence of leishmaniasis[48]. In *Camapuã*, chicken coop was present in all residences, with the exception of Pedro Luiz Amorim neighborhood, which may explain the low density of insects in this capture site.

Even if these insects did not develop in the peridomicile, they could reach it easily by forest areas nearby, attracted by the light trap and available food source[1]. The alternation habit between the forest and the anthropic environment favors the circulation of the parasite, since sandflies can feed on infected wild animals inside the forest and transmit the etiological agent to domestic animals present in the peridomicile, changing the classical pattern of TL transmission[49].

In the intradomicile, the most abundant species was *Lu. cruzi*. Inside the residences with the highest number of collected insects were, in addition to the residents, there were dogs. This species is eclectic with regard to food habits[50], which may have favored their collection inside the residences.

The greatest species dominance was found in São Bento neighborhood (n=7), however the Shannon's Index was lower than the *Olídia* neighborhood with five different species collected. This is because the Shannon's Index (H) also considers the species dominance, abundance and balance between them[51]. The sites with the greatest species diversity were neighborhoods located in the periphery with favorable habitat for the presence of insects, like shading and abundance of organic matter.

Males were more abundant than females. The greater capture of this sex may have been favored by the collection methodology used, since the males are more attracted to the light of the trap[52] and are actively seeking hosts to attract females and increase the chances of copulation[53]. Considering that traps were installed near potential breeding sites and the fact that males hatch before females, these may also have facilitated male collection. Besides that, after blood feeding, females seek for safe shelters for digestion and maturation of ovarian follicles, which decreases their chance of being captured[1,26,42,52,53].

Regarding the male-to-female ratio for *Lu. cruzi*, it was observed that the species presented higher male number in relation to the total specimens. *Lu. cruzi* is a sibling species of *Lu longipalpis*, and lekking has been documented for these species. During lekking, males release sex pheromones to attract females to mate, also attracting more males. This phenomenon also seems to occur among *Lu. cruzi*, once the pheromone 9-methyl-germacrene-B of insects from *Corumbá* (MS) was isolated[54]. In this municipality, Casaril *et al*.[26] have also captured more males of *Lu cruzi*.

Regarding the periodicity, the dry and rainy seasons in MS are well defined. A predominance of *Lu. cruzi* was observed in the rainy season, presenting peaks in October,

January and April, similar to the previously reported trimodal behavior[9,26].

Nyssomyia whitmani showed no difference between the dry and rainy periods, and, for the precipitation, a negative but not significant correlation was observed between the species and the variable. The species shows great plasticity in relation to climatic changes and, although it is more frequent in the months of June, July and August[34], in this study, the species was more captured in August, September and October.

In relation to other species found in the city of *Camapuã*, we have *Ev. lenti* which was originally considered refractory to *Leishmania* infection[55], but has already been found naturally infected by *L.* (*V.*) *braziliensis*[56]. *Pa. bigeniculata*, species belonging to Shannon's complex was found infected by flagellates in Bonito (MS)[57]. *Ev. sallesi* was captured in the intradomicile and, although it is not anthropophilic, it is important to note that the species was found naturally infected by *L.* (*L.*) *infantum*[58] and may contribute to the circulation of parasites in the environment.

Br. avellari, Br. galindoi, Ev. teratodes and *Pa. aragaoi* are found mainly in armadillo holes (Dasypodidae) and prefer wild environments[42,59]. These species together accounted for just over 3% of the total captured specimens and may have been attracted to light from residences and the trap.

It is noteworthy that *Lu. longipalpis*, the main vector of *L.* (*L.*) *infantum*, was not collected during the study period, although there is indicative of their occurrence in the area based on geographic distribution models[60]. Another species that has been previously reported in the municipality[11], *Lu. dispar*, was not captured in this study, probably because previous studies were conducted in the forest area and near the caves. Besides that, this species is highly anthropophilic and the appropriate trap was not used.

This study contributes to the knowledge of the sandfly fauna in *Camapuã* and State of MS. The presence of *Lu. cruzi* and *Ny. whitmani* reinforces the necessity for regular health education programs, associated with human and canine community surveys. These measures aim to prevent those cases of leishmaniasis occurring in *Camapuã,* since the increased incidence of these diseases has been observed in the municipality since 2007.

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