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Short Communication

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Sand Fly Fauna, Spatial Distribution of *Lutzomyia longipalpis* (Diptera: Psychodidae), and Climate Factors in Dourados, Brazil

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Abstract

Studies of the geographic distribution of sand flies and the factors associated with their occurrence are necessary to understand the risk of leishmaniasis transmission. The objective of this study was to characterize the sand fly fauna, particularly the spatial distribution of *Lutzomyia longipalpis* (Lutz & Neiva), and correlate these with climate factors in the Dourados municipality, Brazil. The collection of sand flies was carried out with CDC LightTraps over two periods: at six sites for three consecutive nights each month from August 2012 to July 2013; and at four other sites for two consecutive nights each month from April 2017 to February 2018. We collected 591 sand flies in the first period and 121 in the second period for a total of 712 sand flies; 697 of the total collected were *Lu. longipalpis*. The minimum and maximum sand fly infestation rate (sites with vector presence) was 11.1% and 83.33% in the first period, and 0% and 50.0% in the second period. No sand flies with *Leishmania* were identified via PCR. *Lu. longipalpis* presented an aggregate disposition with excellent adjustment. Rainfall and relative humidity were the abiotic factors that influenced the vector infestation level. The aggregate distribution for this species was predicted by the environmental factors that favor the proliferation of *Lu. longipalpis*. The results of this study should assist in devising measures to control sand flies in Dourados, Brazil.

Key words: distribution model, sand fly vector, negative binomial distribution

Leishmaniasis is vectored by certain species of sand flies of which there are 20 vector species that inhabit Brazil (Ready 2013). Currently, the species *Lutzomyia longipalpis* (Lutz & Neiva) is considered the main vector of *Leishmania infantum* in Latin America and has a wide distribution in Brazil (Aguiar and Medeiros 2003, MS 2014). *Lutzomyia cruzi* (Mangabeira) is also a vector of visceral leishmaniasis (VL) and inhabits Mato Grosso do Sul area (dos Santos et al. 1998) along with *Lu. longipalpis*. Of the 79 municipalities in the state, *Lu. longipalpis* has been identified in 56% of the municipalities and *Lu. cruzi* in 5% (Almeida et al. 2010b, 2013, 2015).

Increases in the population density of sand flies are related to environmental factors, such as vegetation cover (Oliveira et al. 2012) and the end of the rainy season (Oliveira et al. 2003, Almeida et al. 2010a), in addition to factors relating to socioeconomic conditions (Botelho and Natal 2009). In the municipality of Dourados, State of Mato Grosso do Sul, located in the Midwest region of Brazil, sand flies inhabit the urban and rural areas where autochthonous cases of VL and cutaneous leishmaniasis (CL) have been reported. This study aimed to characterize the sand fly fauna and identify the ecological factors that determine the spatial distribution of *Lu. longipalpis* in Dourados in order to understand the risk of leishmaniasis

transmission and to provide support for decision-making on the monitoring and control of these vectors.

Methods

The Dourados municipality is located in Mato Grosso do Sul State at 22′13″16° S and 54′48″20° W, altitude of 430 m. The municipality has an area of 4,062.236 km2, and has a population of 225,495 people (https://www.ibge.gov.br/cidades-e-estados/ms/dourados.html) (Fig. 1). Collections of sand flies were carried out with automatic CDC Light Traps, that were set from 6 pm to 6 am (at areas where cases of VL were recorded) over two periods. In the first period, traps were set on three consecutive nights each month during August 2012 to July 2013. Six peridomicile locations were selected: Vila Caiuás (in a chicken coop near the riparian forest of the Laranja Doce stream), Vila Índio (in a kennel), Vila Planalto (in a kennel), Chácara Objetivo (in a corral next to the riparian forest of the Laranja Doce stream), Santo André (in a chicken coop), and Parque Coqueiros (in a chicken coop near the riparian forest of the Água Boa stream). The second collection period was carried out from April 2017 to February 2018, on two consecutive nights at a home (balcony), peridomicile, and forest in four locations: Marista (in the 11 mo of collection), Água Boa (in the 11 mo of collection), Canaã III (in the first month of collection), and Colibri (in the last 10 mo of collection). Each period had a total of 18 mo of collections. In Marist, there were dogs and forest residues with the presence of wild animals (non-human primates, marsupials, and rodents). In Canaã III, there were chickens and forest residues with the presence of wild animals (marsupials and rodents) near the Água Boa stream. In Colibri and Água Boa, there were dogs and forest residues with the presence of wild animals (marsupials and rodents) near the Água Boa stream (Fig. 1). All collected sand flies were identified in the Regional Laboratory of Entomology of the State Health Department, following the nomenclature of Galati (2003).

The sand flies obtained from the second stage of collection were submitted to polymerase chain reaction (PCR). DNA was obtained

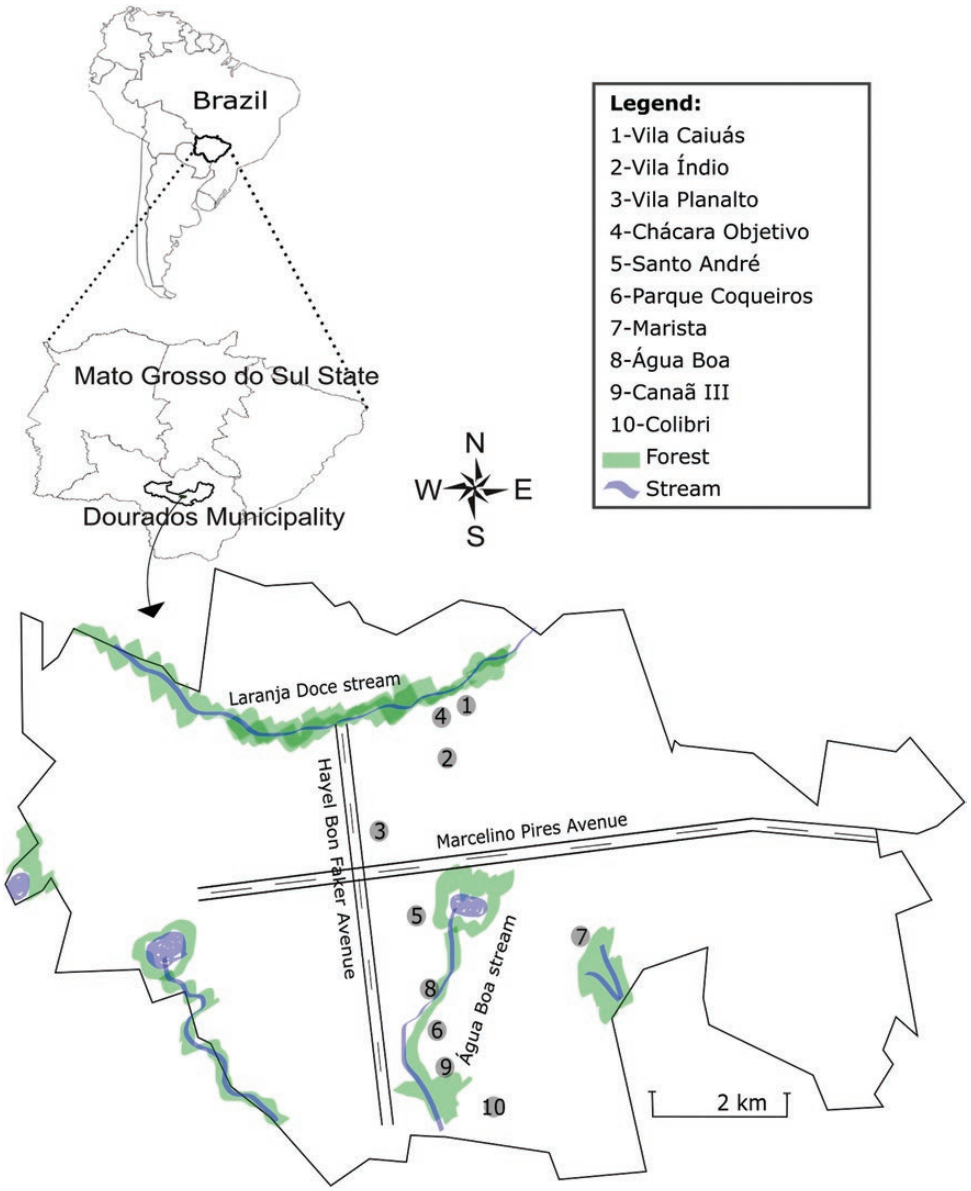

Fig. 1. Geographic location of sand fly collections, in the municipality of Dourados, State of Mato Grosso do Sul, Brazil.

with Chelex 5% solution (Loxdale and Lushai 1998) and then subjected to PCR using primers 13A and 13B detecting a 120-bp fragment of the kDNA minicircle (Rodgers et al. 1990) for *Leishmania* spp. research, according to the protocol described by Silveira et al. (2018).

The spatial distribution of *Lu. longipalpis* was assessed by the Morisita aggregation index (Morisita 1959) with the value of the X^2 distribution calculated and adjusted to the theoretical frequency distribution of the negative binomial type. The infestation rate was calculated as a percentage of the total number of sites with the presence of the *Lu. longipalpis*/total sites assessed (MS 2014). The association of climate factors with *Lu. longipalpis* was determined through Pearson's coefficient. Monthly data on climatic factors were obtained of the Brazilian Agricultural Research Corporation.

Results and Discussion

In the first collection period, 591 sand flies were collected, consisting of 458 males (77.5%) and 133 females (22.5%) of the following species: *Lu. longipalpis* (578 specimens) (97.8%), *Lutzomyia (Nyssomyia) whitmani* (Antunes & Coutinho) (7), *Evandromyia cortelezzii* (Brethes) (4), and *Brumptomyia brumpti* (Larousse) (2). In the second collection period, 121 sand flies were collected, consisting of 39 males and 82 females, of the following species: *Lu. longipalpis* (119) (98.3%), *Psathyromyia paragoai* (Costa-Lima) (1), and *Lu. whitmani* (1).

Between 2012 and 2018, 1,026 cases of VL and 871 of CL were recorded in the municipalities of Mato Grosso do Sul, the majority of which were in the municipalities of Campo Grande, Corumbá and Três Lagoas, which are currently classified as medium and high risk for VL transmission. During the same period, in Dourados, 23 autochthonous cases of VL and 28 of CL were recorded. The first VL case in Dourados was on the Canaã III. The present study is, to the best of our knowledge, the first description of sand fly fauna in Dourados. The species found here have already been described in several locations in the state (Almeida et al. 2010a,b, 2013, 2015). The presence of *Lu. longipalpis* and *Lu. whitmani*, VL and CL vectors, respectively, indicates potential human exposure to *Leishmania* and thus the need for more efficient vector control. *Lutzomyia whitmani* has been found to be naturally infected with *L. infantum* in other studies (Saraiva et al. 2010, Guimarães-e-Silva et al. 2017). For *Ev. cortelezzi* and *Br. brumpti*, vector competence has not been proven. The male:female ratio in the first collection period was 3.4, and this may be linked to the fact that females hatch after males (Kelly and Dye 1997, Ward et al. 1993). In the second collection period, the male:female ratio was 0.5, which may be due to the presence of hosts and the release of pheromones by the males to attract the females (Casanova 2018), thus resulting in a larger collection of females than the first collection period.

Most sand fly specimens were collected from homes in Chácara Objetivo (384), followed by Vila Caiuás (56), Parque Coqueiros (49), Santo André (45), Vila Índio (29), and Vila Planalto (28). In the second collection period, most sand flies were collected from Marist (58), followed by Colibri (52), Água Boa (eight), and Canaã (three), with 51 specimens collected from the peridomicile, 50 from the forest, and 20 from the domicile. The sand flies during the second collection period were collected in greater numbers in the forest and peridomicile. The environmental changes caused by deforestation and urbanization can cause significant changes to the ecology of sand flies (Nuorteva 1963, Marzochi and Marzochi 1994) and it is believed that deforestation, forest fragmentation, and an abundance of domestic animals, favor the presence of sand fly breeding sites. In addition, the presence of domestic animals, primarily dogs, and birds, in the peridomicile releases odor and CO_2, which are attractive to sand flies.

In the first collection period, of the 18 monthly sites assessed, the average infestation was 33.78%. More than three infestations per point were found in 13.89%. The average number of *Lu. longipalpis* collected each month was 0.33–8.11 per trap. The Morisita index confirmed that *Lu. longipalpis* spatial distribution was highly aggregated throughout the analyzed period, indicating the existence of favorable conditions for its proliferation. *Lu. longipalpis* showed a negative binomial distribution and the data did not result in an adjustment (Table 1).

In the second collection period, of the 18 monthly sites assessed, the average infestation was 25.76%. More than three infestations per point were found in 4.55%. The average number of *Lu. longipalpis* collected each month was 0–2.22 per trap. The Morisita index showed aggregated *Lu. longipalpis* spatial distribution almost every month. *Lu. longipalpis* showed negative binomial distribution in some months, and in others, did not display the negative binominal distribution (Table 1).

In all locations and all months in which sampling was conducted, *Lu. longipalpis* was the species most collected possibly indicating the species resistance to climatic variations and high capacity for urbanization. The aggregate distribution for *Lu. longipalpis* in an urban environment suggests more human—*Lu. longipalpis* encounters and thus serves as a warning to public health agencies responsible for the control of leishmaniasis. It is common to report *Lu. longipalpis* natural infection by *L. infantum*; infection by *Leishmania braziliensis* (Lana et al. 2015) and *Leishmania amazonensis* (Paiva et al. 2006) has also been described, elevating the importance of this species in public health. The high aggregated distribution is suspected to be also caused by the presence of hosts as domestic animals.

The relative humidity was on average of 70.24% and 88.27%, while the rainfall was 4.13 mm and 9.24 mm, and the temperature was 23.2°C and 28.7°C, in the first and second periods, respectively. For the climatic data, in the first period, there was a significant correlation between the numbers of *Lu. longipalpis* and rainfall ($r = 0.58$), a moderate correlation between relative humidity ($r = 0.41$), and a low correlation between temperature ($r = 0.22$). In the second period, there was a low correlation between the populations of *Lu. longipalpis* and relative humidity ($r = 0.31$), and a negative correlation between rainfall ($r = -0.43$) and temperature ($r = -0.41$) (Fig. 2). In terms of seasonality, there was a greater abundance of *Lu. longipalpis* in the rainy season, as observed in several regions of Brazil (Oliveira et al. 2003; Almeida et al. 2010a, 2013). However, in Campo Grande (Mato Grosso do Sul State), during the cold and dry months, the population decreased and relative abundance increased in the peridomicile (Silva et al. 2008). Abiotic values influence the life cycle of sand flies in various way, including alteration of reproduction (Oliveira et al. 2012). In general, precipitation and relative humidity are favorable to sand flies due to the vegetative growth which increases in the supply of organic matter as well favorable breeding sites (Dias et al. 2007).

No sand flies with *Leishmania* were identified via PCR. The absence of natural infection does not minimize the importance of the results for public health as the study areas have a vector presence and a parasite presence which results in a chronic public health threat. The low socioeconomic level of the population residing in these locations allows the presence of suitable breeding sites in the home environment, which is a favorable for the leishmaniasis cycle (Alvar et al. 2006).

Table 1. *Lutzomyia longipalpis* infestation collected with CDC Light Traps, number per sample point, aggregation index and distribution test, in Dourados, Mato Grosso do Sul State, Brazil, from Aug. 2012 to July 2013 and April 2017 to Feb. 2018

Months	*n*				*n* of traps (18)			% of traps		Morisita Index	Negat. Binom. Dist. χ^2	Gl
	Dom	Peri	Forest	Total	Neg	1–3 sandflies	> 3 sandflies	pos	> 3 sandflies			
Aug/12	-	6	-	6	15	2	1	16.67	5.56	7.20^{ag}	0.09^{ad}	1
Sep/12	-	9	-	9	13	4	1	27.78	5.56	5.00^{ag}	1.59^{ad}	1
Oct/12	-	26	-	26	12	4	2	33.33	11.11	4.10^{ag}	1.55^{ad}	3
Nov/12	-	74	-	74	3	10	5	83.33	27.78	2.33^{ag}	8.31^{ad}	7
Dec/12	-	18	-	18	8	9	1	55.56	5.56	1.53^{ag}	1.66^{ad}	3
Jan/13	-	72	-	72	16	0	2	11.11	11.11	9.05^{ag}	0.68^{ad}	1
Feb/13	-	72	-	72	14	2	2	22.22	11.11	8.49^{ag}	1.74^{ad}	3
Mar/13	-	96	-	96	13	2	3	27.78	16.67	5.82^{ag}	4.00^{ad}	4
April/13	-	146	-	146	9	0	9	50.00	50.00	2.47^{ag}	3.48^{ad}	8
May/13	-	40	-	40	11	4	3	38.89	16.,67	4.52^{ag}	1.53^{ad}	3
June/13	-	7	-	7	15	3	0	16.67	0	4.29^{ag}	0.79^{ad}	1
July/13	-	12	-	12	14	3	1	22.22	5.56	6.55^{ag}	0.13^{ad}	2
Average	-	578	-	578	11.92	3.58	2.5	33.78	13.89	-	-	-
April/17	3	4	13	20	9	8	1	50.00	5.56	3.66^{ag}	26.02^{nad}	8
May/17	0	0	0	0	18	0	0	0	0	-	-	-
June/17	1	10	0	11	15	1	1	16.67	11.11	8.18^{ag}	20.04^{nad}	1
July/17	6	16	18	40	9	1	4	50.00	22.22	3.56^{ag}	28.52^{nad}	4
Aug/17	1	14	3	18	14	2	1	22.22	11.11	6.60^{ag}	26.29^{nad}	2
Sep/17	2	2	5	9	10	7	0	44.44	0	1.00^{ra}	-	6
Oct/17	4	2	2	8	12	6	0	33.33	0	1.29^{ag}	2.83^{ad}	5
Nov/17	3	1	6	10	9	6	0	50.00	0	1.67^{ag}	0.78^{ad}	5
Dec/17	0	1	2	3	15	3	0	16.67	0	0	0.018^{ad}	2
Jan/18	0	0	0	0	18	0	0	0	0	-	-	-
Feb/18	0	0	0	0	18	0	0	0	0	-	-	-
Average	20	50	49	119	13.36	3.82	0.82	25.76	4.55	-	-	-

Dom, domicile; peri, peridomicile; neg, negative; pos, positive; ag, aggregate distribution when the Morisita index is significantly > 1; ra, random; ad, population adheres to the Negative Binomial distribution; nad, population does not adhere to the Negative Binomial distribution; Gl, number of degrees of freedom of the χ^2 distribution.

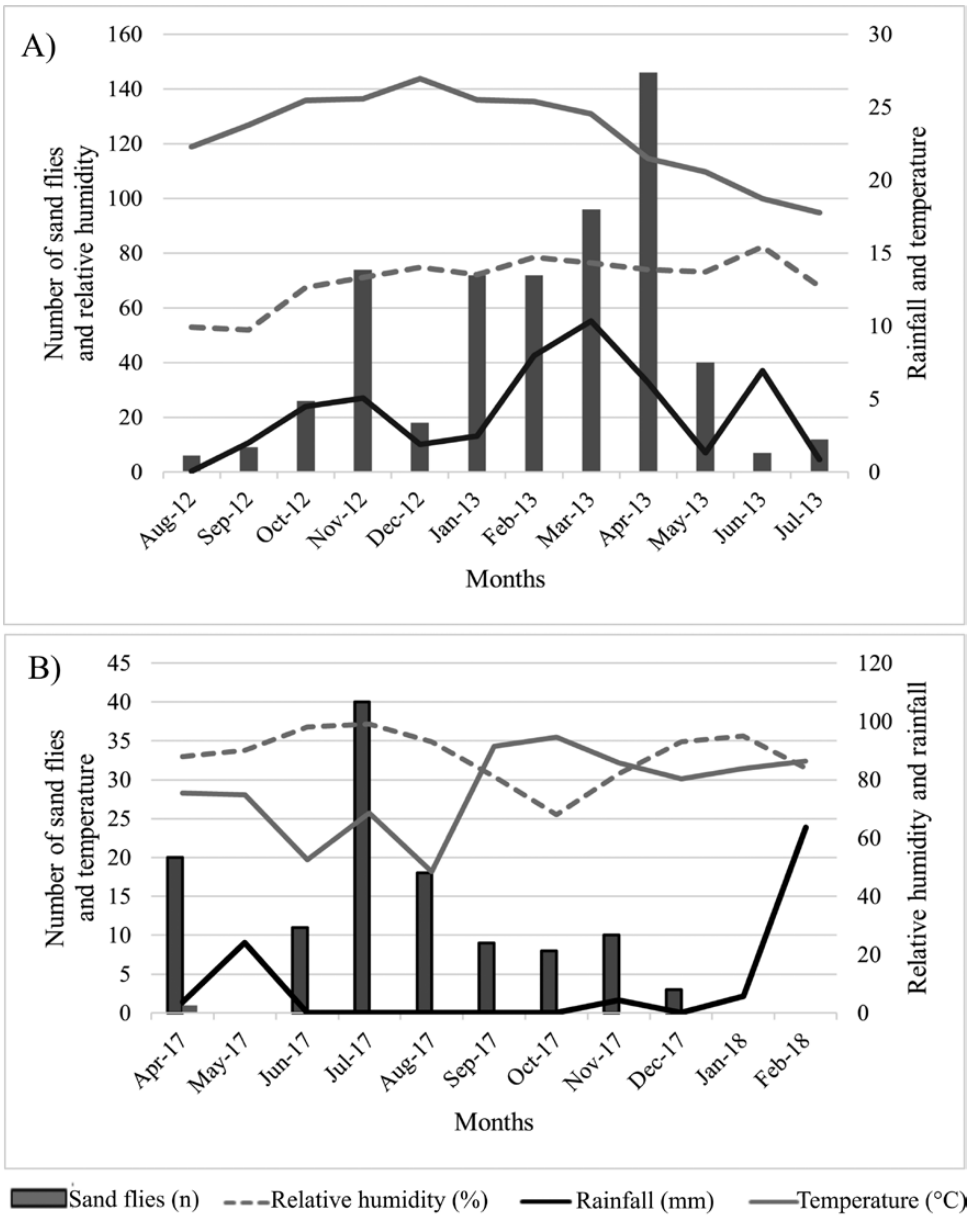

Fig. 2. Monthly distribution of *Lutzomyia longipalpis* collected with CDC Light Traps and data of temperature (°C), average rainfall (mm) and relative humidity (%), in Dourados, Mato Grosso do Sul State, Brazil, in periods from (A) August 2012 to July 2013, and (B) April 2017 to February 2018.

Lu. longipalpis is a medically important species that inhabits anthropic environments (Oliveira et al. 2006, Almeida et al. 2010b) and has great dispersion capacity. The occurrence of aggregate distribution for this species is confirmed in this study. Association with verdant native vegetation, decomposing organic matter, presence of livestock, high rainfall, and relative humidity may all contribute to the proliferation of this vector. Research involving new methodologies for surveying fauna over the years with determining associations with climatic and environmental factors should be encouraged.

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