## **ORIGINAL ARTICLE**

# THE SAND FLY SPECIES (DIPTERA: PSYCHODIDAE) IN AN URBAN ENVIRONMENT OF MATO GROSSO DO SUL,

## BRAZIL

Paulo Silva de Almeida<sup>1</sup>, Talita Moreira Silva<sup>1</sup>, Rosilene Francisca Moreira<sup>1</sup>, Vanessa Fukuda Mariano<sup>1</sup>, Miguel Prudencio Oliveira Neto<sup>1</sup>, Dioelen Virgínia Borges Souza de Aquino Coelho<sup>2</sup>, Herintha Coeto Neitzke-Abreu<sup>2,3</sup>, Gustavo Mayr de Lima Carvalho<sup>4</sup> and José Dilermando Andrade Filho<sup>4</sup>

### ABSTRACT

The purpose of this study was to search for *Lutzomyia longipalpis* and *Lutzomyia cruzi* in the urban area of the Água Clara Municipality, Mato Grosso do Sul State, Brazil. Two CDC traps were placed in the intradomicile and the peridomicile areas in four residences once a month, for three consecutive nights during 24 months. Were collected 11 species of sand flies totaling 3,418 specimens, 2,762 males and 656 females. *Lu. longipalpis* was the dominant species and there was no statistical difference between the peridomicile and intradomicile areas. *Lu. cruzi* was not found. The high capture rate for *Lu. longipalpis* in the urban area of Água Clara is cause for concern regarding necessary preventive measures to avoid future cases of visceral leishmaniasis.

KEY WORDS: Leishmaniasis; Lutzomyia longipalpis; Phlebotominae; calazar.

### INTRODUCTION

The sand fly fauna of Mato Grosso do Sul State (MS) consists of 61 species (Almeida et al., 2015). The species *Lutzomyia longipalpis* (Lutz & Neiva, 1912) is considered the main vector of *Leishmania infantun*, the etiologic agent of visceral leishmaniasis (VL) in Latin America and is widely distributed in Brazil (Souza et al., 2009; Santos et al., 2012). It

Received for publication: 30/10/2018. Reviewed: 2/7/2019. Accepted: 30/9/2019.

<sup>1.</sup> Laboratório Regional de Entomologia, Núcleo Regional de Saúde, Secretaria de Estado de Saúde, Dourados, MS, Brazil.

<sup>2.</sup> Programa de Pós-Graduação em Ciências da Saúde, Universidade da Grande Dourados (UFGD), Dourados, MS, Brazil.

<sup>3.</sup> Faculdade de Ciências da Saúde, UFGD, Dourados, MS, Brazil.

<sup>4.</sup> Grupo de Estudos em Leishmanioses, Instituto René Rachou/Fiocruz Minas, Belo Horizonte, MG, Brazil.

Corresponding author: Paulo Silva de Almeida. Laboratório Regional de Saúde/NRS/SES. Rua Hilda Bergo Duarte 940, Centro, CEP 79806-020, Dourados, MS, Brazil. E-mail: psilvadealmeida@yahoo.com.br

has been identified in 40 of the 79 municipalities of MS (Almeida et al., 2010; Almeida et al., 2015), while *Lutzomyia cruzi* (Mangabeira, 1938), the species involved in the transmission of *Le. infantun* in some areas of MS, has been recorded in nine municipalities (Almeida et al., 2015).

Based on reports provided by SINAN (Sistema de Informações de Agravos de Notificação - Epidemiological Surveillance Service in the Health Department of Mato Grosso do Sul), 2,037 cases of VL were confirmed in MS, including in 50 (64%) of its municipalities, and 14 cases were recorded in the Água Clara Municipality from 2004 to 2015.

Recent information on the abundance of these sand flies may provide important aid regarding control measures of these zoonoses. According to the Ministry of Health, *Lu. longipalpis* and *Lu. cruzi* should be researched in municipalities with sporadic, moderate or intense transmission rates and which lack previous entomological investigations (Brazil, 2014). Although transmission is sporadic in the Água Clara Municipality, it is located between areas of intense transmission where cases of VL have been reported but there is no information on the presence of sand flies. Thus, the aim of this study was to search for *Lu. longipalpis* and *Lu. cruzi* in the urban area of the Água Clara Municipality, due to the distribution and epidemiology of VL in MS.

### MATERIAL AND METHODS

### Study area

The Água Clara Municipality is located in the eastern region of Mato Grosso do Sul State (21° 53' 10" S, 54° 9' 21" W) (Figure 1). It encompasses an estimated area of 11,031.07 km<sup>2</sup>, totaling 3.1% of the land area of the State, with an estimated population of 14,474 inhabitants and around 6,600 residences. The economy is mainly based on livestock, plantations and the extraction of wood for the paper and charcoal industries. The vegetation in the municipality resembles the Cerrado biome. The climate is mildly tropical. The average temperature in the coldest month oscillates between 18°C and 20°C. Annual rainfall varies from 1,200 to 1,500 mm, with the heaviest rains occurring between November and February, and a dry period extending for four to five months after the rainy period (March to July) (IBGE, 2017).



Figure 1. Geographical location of the Água Clara Municipality, Mato Grosso do Sul State.

## Collection of sand flies

Two light CDC traps (developed by the Disease Control Center and modified by Gomes et al., 1985) were placed in each of four selected residences, one in the intradomicile and the other in the peridomicile areas. The residences are in different neighborhoods (Centro Velho, Vila dos Ferroviários, Centro and Jardim Nova Água Clara) and were chosen according to previous characteristics indicating they were suitable for finding sand flies, such as the presence of vegetation and domestic animals, especially chickens and dogs. The traps were set for three consecutive nights, from 4 pm to 7 am the following morning, once a month from May 2005 to April 2007, totaling 576 collections over 24 months. The specimens collected were fixed in 70% alcohol and stored, at -20 °C (Silva et al., 2007). For species identification, the sand flies were mounted on slides in Berlese medium and the classification proposed by Galati (2003) was followed.

## Statistical analysis

Faunal analysis of the number of male and female sand flies caught in the traps was based on frequency (F), constancy (C), abundance (A), and dominance (D) (Almeida et al., 2010). Since the assumptions of normality, homogeneity of variance and independence within and among the studied variables were not met, the nonparametric Mann-Whitney test (Zar, 2009) was used to compare the hypotheses of equality between pairs of means with a significance level of  $\alpha = 5\%$ . Relative abundance was calculated by dividing the number of *Lu. longipalpis* collected in each area by the total number of residences surveyed. Frequency of positive residences was calculated as the number of residences positive for *Lu. longipalpis* divided by the total number of residences surveyed multiplied by 100.

## RESULTS

A total of 3,418 individuals of 11 sand flies species (2,762 males and 656 females) were identified. The average number of males caught per month (115.08  $\pm$  105.97) was significantly higher (Z = 3.03, p < 0.003) than females (27.33  $\pm$  28.28). Despite the greater abundance of males, females were found to have greater species richness (Table).

Faunal analysis revealed that *Lu. longipalpis* was dominant (s), and was classified as very frequent (vf), constant (w) and very abundant (va). *Lu. cruzi* was not found. The other species encountered were not dominant (nd) and classified as less frequent (lf), accidental (z) and rare incidence (r) or dispersed (d): *Brumptomyia avellari* (Costa Lima, 1932), *Brumptomyia brumpti* (Larrousse, 1920), *Brumptomyia pintoi* (Costa Lima, 1932), *Evandromyia lenti* (Mangabeira, 1938), *Evandromyia walkeri* (Newstead, 1914), *Evandromyia sallesi* (Galvão & Coutinho, 1939), *Micropygomyia quinquefer* (Dyar, 1929), *Nyssomyia neivai* (Pinto, 1926), *Nyssomyia whitmani* (Antunes & Coutinho, 1939) and *Psathyromyia hermanlenti* (Martins, Silva & Falcão, 1970) (Table).

The monthly relative abundance of *Lu. longipalpis* was higher in September, October and November 2005, and March and May 2006 (Figure 2). There was no significant difference between the monthly relative abundance of traps positive for *Lu. longipalpis* in the peridomicile and intradomicile areas (z = 1,13, P > 0,20). In September and November 2005, March, May and August 2006, and January 2007, *Lu. longipalpis* was detected in four residences.



*Figure 2. Lutzomyia longipalpis* number and relative abundance in four residences in Água Clara Municipality, Mato Grosso do Sul, Brazil, from May 2005 to April 2007.

Mato	
Municipality,	
Clara	
Água	
in	
ound	
s fí	
vironment	
en	
cile	
mi	
perido	
nud J	~
ile â	200
nici	Ē
dor	A
ıtra	5 to
n ii	00
es i	2
fli	ž
and	nu
f Si	£
rs o	azil
ete	B
am	ate
par	S.
nal	Sul
aui	qu
<i>e</i> . F	OSE
Tabl	Gros
	_

Species			Males					Fen	nales						Total			
	u	F%	Ц	C	A	D	u	F%	Ц	C	A	D	u	F%	Ц	C	V	D
3r. avellari		ı	ı	ı	ı	ı	1	0.15	lf	N	r	pu		0.03	lf	N	ч	pu
3r. brumpti	1	0.04	lf	N	r	pu	1	0.15	lf	N	r	pu	7	0.06	lf	N	r	pu
3r. pintoi	1	0.04	lf	N	r	pu	,	ı	·	ı	ı	,	1	0.03	lf	N	r	pu
Ev. lenti	2	0.07	lf	N	r	pu	2	0.30	lf	N	q	pu	4	0.12	lf	N	r	pu
Ev. carmelinoi	,	·		·	ı	·	б	0.46	lf	N	q	pu	б	0.09	If	N	r	pu
Ev. sallesi		ı		'	·	ı	1	0.15	lf	N	r	pu	1	0.03	If	N	r	pu
.u. longipalpis	2,757	99.82	vf	Μ	va	s	642	97.87	vf	Μ	va	s	3,399	99.44	vf	Μ	va	s
Ai. quinquefer		ı		·	·	ı	1	0.15	lf	N	r	pu	1	0.03	If	N	r	pu
Vy. whitmani		ı	ı	ı	ī	ı	1	0.15	lf	N	r	pu	1	0.03	lf	N	r	pu
Vy. neivai	1	0.04	lf	N	г	pu	б	0.46	lf	N	q	pu	4	0.12	lf	N	r	pu
<sup>5</sup> s. hermanlenti		ı	ı	ı	ī		1	0.15	lf	N	r	pu	1	0.03	lf	N	r	pu
[otal	2,762						656						3,418					

Br: Brumptomyia; Ev: Evandromyia; Lu: Lutzomyia; Mi: Micropygomyia; Ny: Nyssomyia; Ps: Psathyromyia; n: number of sand flies captured; F%: relative frequency; F: frequency; If: less frequent; vf: very frequent; C: Constancy; w: constant; y: accessory; z: accidental; A: Abundance; va: very abundant; r: rare; D: Dominance; s: dominant; nd: not dominant.

#### DISCUSSION

The results of the present study revealed a greater abundance of *Lu. longipalpis* compared to the other species in the urban area of Água Clara Municipality. The dominance of *Lu. longipalpis* corroborates other studies conducted in MS (Oliveira et al., 2006; Almeida et al., 2010). The faunal analysis further demonstrates the dominance of *Lu. longipalpis* since it was classified as very frequent, constant and very abundant throughout the study period. These findings are alarming as this species is the main vector of *Le. infantun*, the main etiologic agent of VL. Other species were found in Água Clara but are not important in VL transmission, being also found in other MS municipalities. However, *Ny. whitmani* and *Ny. neivai*, important in the transmission of cutaneous leishmaniasis, were collected and reported in other studies in MS (Almeida et al., 2010; Almeida et al., 2013).

Although there are limitations in this study due to the small number of residences analyzed, the results present a high number of sand flies collected, showing the importance of monitoring and management of VL. The relative abundance of Lu. longipalpis was higher in the peridomicile, except in May and September 2005, which is similar to the findings of Oliveira et al. (2006) and Almeida et al. (2010). This is due to the greater availability of food sources in domestic shelters, since sand flies suck blood from several mammals and birds (Carvalho et al., 2017) and feed according to the availability of the food (Membrive et al., 2004). Furthermore, the greatest contribution to the differences observed in abundance between males and females was in Lu. longipalpis (ratio of 4.29:1), a finding similar to Almeida et al. (2010). This factor can be explained in relation to Lu. longipalpis, by its behavior in forming aggregates (Leks), attracting males and females to the site of the host to mate (Dve et al., 1991). Still, the light trap can attract variation in the sex ratio, favoring the capture of males, because the females after blood-sucking return to their shelter (Barretto, 1943; Aguiar et al., 1985). More sand flies were collected in the warmer humid months. There is a correlation between the environment (temperature, relative humidity and rainfall) and the abundance of sand flies (Silva et al., 2007; Almeida et al., 2010).

The distribution of *Lu. longipalpis* and VL in Central and South America reinforces that this is the main vector of the disease as reported by Deane and Deane (1954). In Brazil, the vector was originally found in rural areas, however, since the 1980s, it has also been reported in urban environments, especially in the outskirts of cities. This expansion seems to be determined primarily by the adaptation of the vector to the urban environment (Brazil, 2013). Different populations of sand flies adapt to the environment and do not always depend on the presence of vegetation (Casaril et al., 2014).

The number of traps used is one of the limitations of the study, as is the use of only one type of trap. It is known that some species are more or less collected when using, for example, an automatic light trap and a Shannon trap. Another important factor to be mentioned is the large proportion of males collected compared to females when using a light automatic trap, however, it is important to note that the traps used here are the most appropriate for collecting *Lu. longipalpis* in urban areas.

Currently, VL is a major public health problem in Brazil. Of 55,530 cases of human VL reported in the Americas between 2001 and 2016, 97.0% were from Brazil (PAHO, 2018). Given these data, and the results of the present study, the high capture rate of *Lu. longipalpis*, the main vector of VL, in the urban area of Água Clara causes concern regarding necessary preventive measures such as clearing away organic matter, using screens on doors and windows and promoting public awareness.

#### ACKNOWLEDGEMENTS

The authors would like to thank José Oliveira da Silva and Marcos Antônio Batista Teixeira, technicians from the Ministry of Health, for their assistance with the field research; and professors Luiz Donizethe Minzão, Edima Ramos Minzão and Odival Faccenda for assistance in the statistical analysis. JDAF received a research scholarship from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq # 301421/2013-7).

## REFERENCES

- Aguiar GM, Vilela ML, Schuback PA, Soucasaux T, Azevedo ACR. Aspectos da ecologia dos flebótomos do Parque Nacional da Serra dos Orgãos, Rio de Janeiro: IV. Frequência mensal em armadilhas luminosas (Diptera, Psychodidae, Phlebotominae). *Mem Inst Oswaldo Cruz 80*: 465-482, 1985.
- Almeida PS, Nascimento JC, Ferreira AD, Minzão LD, Portes F, Miranda AM, Faccenda O, Andrade Filho JD. Espécies de flebotomíneos (Diptera: Psychodidae) coletadas em ambiente urbano em municípios com transmissão de Leishmaniose Visceral do Estado de Mato Grosso do Sul, Brasil. *Rev Bras Entomol* 54: 304-310, 2010.
- Almeida PS, Leite JA, Araújo AD, Batista PM, Touro RBS, Araújo VS, Souza EJ, Rodrigues JB, Oliveira GA, Santos JV, Faccenda O, Andrade Filho JD. Fauna of phlebotomine sand flies (Diptera, Psychodidae) in areas with endemic American cutaneous leishmaniasis in the State of Mato Grosso do Sul, Brazil. *Rev Bras Entomol* 57: 105-112, 2013.
- Almeida PS, Sciamarelli A, Raizer J, Menegatti JA, Hermes SCNM, Carvalho MSL, Gurgel-Gonçalves R. Geographic distribution of phlebotomine sandfly species (Diptera: Psychodidae) in Central-West Brazil. *Mem Inst Oswaldo Cruz 110*: 551-559, 2015.
- Carvalho GML, Rego FD, Tanure A, Silva ACP, Dias TA, Paz GF, Andrade Filho JD. Blood meal identification in field-collected sand flies from Casa Branca, Brazil, using the cytochrome b PCR method. *J Med Entomol* 54:1049-1054, 2017.

- Barretto MP. Observações sobre a biologia, em condições naturais, dos flebótomos do Estado de São Paulo (Diptera, Psychodidae). São Paulo: Tipografia Rossolillo. 1943. 160p.
- Brazil RP. The dispersion of *Lutzomyia longipalpis* in urban areas. *Rev Soc Bras Med Trop 46*: 263-264, 2013.
- Brazil, Ministry of Health. Manual de Vigilância e Controle da Leishmaniose Visceral. 1a. ed. Editora Ministério da Saúde, Brasilia/DF. 2014. 122p.
- Casaril AE, Monaco NZN, Oliveira EF, Eguchi GU, Paranhos Filho AC, Pereira LE, Oshiro ET, Galati EAB, Mateus NLF, Oliveira AG. Spatiotemporal analysis of sandfly fauna (Diptera: Psychodidae) in an endemic area of visceral leishmaniasis at Pantanal, central South America. *Parasit Vectors* 7: 364, 2014.
- Deane MP, Deane LM. Infeção experimental do Phlebotomus longipalpis em raposa (Lycalopex vetulus) naturalmente parasitada pela Leishmania donovani. O Hospital 46: 651-653, 1954.
- Dye C, Davies CR, Lainson R. Communication among phlebotomine sandflies: A field study of domesticated *Lutzomyia longipalpis* populations in Amazonian Brazil. *Animal Behav* 42: 183-192, 1991.
- Galati EAB. Classificação de Phlebotominae. In: Rangel EF, Lainson R, editors. *Flebotomíneos* do Brasil. 1 ed. Editora Fiocruz, Rio de Janeiro. 2003. p.23-51.
- Gomes AC, Rabello EX, Natal D. Uma nova câmara coletora para armadilha CDC-miniatura. *Rev Saúde Pública 19*: 190-191, 1985.
- IBGE. Conheça Cidades e Estados do Brasil. Brasília, 2017. Available from: https://cidades. ibge.gov.br/brasil/ms/panorama. Access in August 5, 2019.
- Membrive NA, Rodrigues G, Membrive U, Monteiro WM, Neitzke HC, Lonardoni MVC, Silveira TGV, Teodoro U. Flebotomíneos de municípios do Norte do Estado do Paraná, sul do Brasil. *Entomol Vectores 11*: 673-680, 2004.
- 16. Oliveira AG, Galati EAB, Oliveira O, Oliveira GR, Espíndola IAC, Dorval MEC, Brazil RP. Abundance of *Lutzomyia longipalpis* (Diptera: Psychodidae: Phlebotominae) and urban transmission of visceral leishmaniasis in Campo Grande, state of Mato Grosso do Sul, Brazil. *Mem Inst Oswaldo Cruz 101*: 869-874, 2006.
- Pan American Health Organization (PAHO). Leishmaniases: epidemiological report of the Americas. *Rep Leishmaniases* 3: 1-4, 2018.
- Santos DR, Ferreira AC, Bisetto Junior A. The first record of *Lutzomyia longipalpis* (Lutz & Neiva, 1912) (Diptera: Psychodidae: Phlebotominae) in the State of Paraná, Brazil. *Rev Soc Bras Med Trop* 45: 643-645, 2012.
- 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 40*: 420-425, 2007.
- Souza GD, Santos E, Andrade Filho JD. The first report of the main vector of visceral leishmaniasis in America, *Lutzomyia longipalpis* (Lutz & Neiva) (Diptera: Psychodidae: Phlebotominae), in the state of Rio Grande do Sul, Brazil. *Mem Inst Oswaldo Cruz 104*: 1181-1182, 2009.
- 21. Zar JH. Biostatistical Analysis. 5th ed. New Jersey, Prentice Hall, 2009. 960p.