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Sand Flies (Diptera: Psychodidae) in a Cerrado Area of the Maranhão State, Brazil

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ABSTRACT - The present paper aims to increase the knowledge on the sand fly fauna in the cerrado areas of Maranhão state in urban, rural and forest environments. The research was carried out from October 2007 to September 2008, between 18:00h and 06:00h, in the municipality of Chapadinha, northeast Maranhão. For insect sampling, CDC light traps were set up in peridomicile and domicile areas of urban and rural zones as well as in Cerrado and Gallery forests. The total of 1,401 specimens belonging to 17 species were sampled, all within the genus *Lutzomyia*. *Lutzomyia longipalpis* (Lutz & Neiva) (52.5%), *Lu. evandroi* (Costa Lima & Antunes) (18.3%), *Lu. whitmani* (Antunes & Coutinho) (12.1%), *Lu. lenti* (Mangabeira) (4.7%) and *Lu. termitophila* (Martins, Falcão & Silva) (4.0%) were the most frequently collected. From an epidemiological viewpoint, five from all of the collected species are vectors of leishmaniasis: *Lu. longipalpis*, *Lu. whitmani*, *Lu. flaviscutellata* (Mangabeira), *Lu. gomezi* (Nitzulescu) and *Lu. chagasi* (Costa Lima). *Lutzomyia chagasi* was registered for the first time in Maranhão state and *Lu. saulensis* (Floch & Abonnenc), *Lu. monstrosa* (Floch & Abonnenc) and *Lu. gomezi* were found for the first time in the eastern part of the state, since they had been reported only in the Amazonian region of Maranhão. Regarding to the studied environments, the urban chicken house had the highest number of specimens collected (801), while the Gallery Forest was the most diverse (15 species). This study demonstrates that the northeast cerrado exhibits a mixed sand fly fauna characterized by an extremely important species vectors assortment involved in the epidemiological cycle of leishmaniasis in Maranhão state.

KEY WORDS: Leishmaniasis, *Lutzomyia*, insect vector

Phlebotomine sand flies constitute a very important group of dipteran insects due to the species diversity and the association with leishmanial parasites throughout the world (Rangel & Lainson 2003). In Brazil, phlebotomine sand flies are well-known vectors of visceral and cutaneous leishmaniasis, with nearly 220 species identified from sylvatic and rural studies undertaken in several phytogeographic regions (Aguar & Medeiros 2003). In the state of Maranhão, northeast Brazil, around 60 sand fly species have already been found by the National Health Foundation technical team in urban and rural peridomiciliary areas in more than 40 municipalities.

The wide-ranging and systematical studies included Amazonian humid forest, ombrophila forest and mixed vegetation composed of Cerrado, "Cocal" and Caatinga sites (Rebêlo *et al* 1999a, Rebêlo *et al* 2000a, Leonardo & Rebêlo 2004, Marinho *et al* 2008). However, studies focusing on the overall sand fly species diversity of the Cerrado biome in Maranhão state were only carried out in a restricted region,

mostly at the municipality of Santa Quitéria (Martin & Rebêlo 2006) in the low Parnaíba River zone.

Therefore, because of the great importance of this transitional area for biodiversity studies in Maranhão state, this work proposes to survey the sand fly fauna associated with the sylvatic and anthropogenic environments as well as the species richness and their monthly and seasonal frequency, making a contribution to the understanding of the phlebotomine sand flies distribution and to the epidemiological profile of leishmaniasis in the state.

Material and Methods

Study area. The municipality of Chapadinha is situated between 3° 44' 17" LS and 43° 20' 29" LW and has a surface area of 3,265 km². The municipality has 60,646 inhabitants, where 23,427 reside in the rural zone and 37,219 live in the urban zone. Chapadinha municipality is situated in the Maranhão cerrado region, an area of 10 million ha

accounting for 30% of the total state area, comprising 33 municipalities. It occupies a large transitional area positioned between three Brazilian regions: north, northern and central west. In the cerrado domain, several other distinct biomes can be found, such as “veredas”, caatinga sites interfluvial mesophytic forest and gallery forest (Oliveira-Filho & Ratter 2002). The studied area is at approximately 100 m a.s.l. and the climate is hot and tropical sub-humid, with annual mean temperature ranging from 28°C to 30°C, with an annual mean precipitation between 1,600 mm and 2,000 mm. The region undergoes a 6-month dry season, from July to December, and a 6-month rainy season, from January to June.

The study areas were selected from urban and rural zones, as follow: a) urban zone: the collecting sites were represented by (i) a chicken house and (ii) banana trees, 36 m far from each other, in the peridomiciliary area, and (iii) a gallery forest fragment, belonging to the Reservatório Itamaçoca, an area of the Companhia de Águas e Esgoto do Estado do Maranhão (CAEMA) of Chapadinha. The actual collecting point was situated at 926 m from the reservoir entry, adjacent to a nascent of the reservoir pond; b) rural zone: an area far 14.41 km from the municipality center which included the (iv) intradomicile, a (v) chicken roost and a (vi) pigsty. The rural chicken roost was less structured and often much more exposed than the urban one. The extradomicile environment was represented by (vii) a cerrado forest fragment next to the community.

Insect sampling. Phlebotomine sand flies were captured monthly from October 2007 to September 2008, between 18:00h and 06:00h, by means of CDC light traps. One trap was set up at 1.5 m from the ground in each collecting site, totaling seven light traps. Considering that each trap worked 12h a night and 12 months a year, the collecting effort yielded 144h/trap or, concerning the seven traps, 1,008 h/trap. After sampling, the insects were killed in ethyl acetate, stored in 70% ethanol, slide-mounted and identified to species according to Young & Duncan (1994).

Statistical analyses. The species richness was estimated by summing the number of species found in each collecting site. Sand fly diversity was estimated according to the Shannon-Wiener index by DivEs software 2.0 (Rodrigues 2005). For analyzing the species relative abundance and its confidence limits, the Kato *et al* method (Laroca 1995) was applied. One species was considered dominant when its lower confidence limit was inferior to the superior limit of the absent species. Constance of each species was determined with the formula: $C = P \times 100/N$; where, P = number of collections with the considered species and, N = total number of collections. Thus, the species were classified as constant, accessory or accidental (Silveira Neto *et al* 1976).

Results

Richness and relative abundance. A total of 1,401 phlebotomine sand flies distributed in 17 *Lutzomyia* species were found, with *Lutzomyia longipalpis* (Lutz & Neiva) (52.5%), *Lu. evandroi* (Costa Lima & Antunes) (18.3%), *Lu. whitmani* (Antunes & Coutinho) (12.1%), *Lu. lenti*

(Mangabeira) (4.7%) and *Lu. termitophila* (Martins, Falcão & Silva) (4.0%) being the prevailing species (Table 1). The remaining species accounted for 8.4% of the total sample. Females were predominant (60.7%) over males (39.3%), resulting in a 0.65: 1 male/female ratio. This tendency was observed for all species with the exception of *Lu. whitmani* and *Lu. lenti*, in which males prevailed over females (61.2% and 86.4, respectively). An extreme case was *Lu. gomezi* (Nitzulescu), in which only males were collected (Table 1). Specimens collected for *Lu. sp 1* (group Aragaoi), *Lu. sp 2* (subgenus unknown) and *Lu. sp 3* (subgenus *Psychodopygus*) were insufficient for a correct identification and new samples are necessary for each one of these species.

The collecting effort revealed a capture rate of 1.4 individual per hour per trap. Phlebotomine sand flies were more frequent in the urban zone, where 75.0% of the specimens were captured. The urban chicken house was the most representative ecotope accounting for 58.6% of the total number of specimens sampled (Table 2), while 25.0% were captured in the rural zone.

The most abundant species, *Lu. longipalpis*, was predominant over the other species in the urban (chicken house: 63.8%; banana trees: 48.8%) and rural peridomiciliary environments (pigsty: 66.3%; chicken roost: 37.7%), as well as in the rural cerrado forest (39.2%). However, in the gallery forest, the prevailing species was *Lu. saulensis* (Floch & Abonnenc) (25.5%), while *Lu. evandroi* (41.9%) was the most common in the intradomicile.

The Shannon-Wiener diversity index was higher in the gallery forest, followed by the rural chicken roost. The lowest rates were found in the pigsty and in the urban chicken house (Table 2). The species richness was higher in the urban (15 species) than in the rural area (12 species). Among the species found in the urban areas, all of them were present in the gallery forest, where seven were exclusive for this environment, while eight were also recorded in the peri and intradomiciliary ecotopes. In the rural area, seven species were found in all collecting sites, with *Lu. monstrosa* (Floch & Abonnenc) and *Lu. sordellii* (Shannon & Del Ponte) being collected only in the intradomicile, while *Lu. flaviscutellata* (Mangabeira) and *Lu. pilosa* (Damasceno & Causey) were present only in the rural cerrado fragment (Table 2).

Monthly and seasonal distribution. *Lutzomyia longipalpis*, *Lu. evandroi*, *Lu. termitophila*, *Lu. whitmani*, *Lu. lenti* and *Lu. trinidadensis* (Newstead) were defined as constant species as they were sampled in more than 50% of collections. The accessory species, *Lu. sordellii*, *Lutzomyia sp. 1* and *Lu. saulensis* occurred between 25% and 50% of the collections. The remaining species were considered accidental, as they were sampled in less than 25% of collections (Table 3).

Fourteen sand fly species were sampled in the rainy season while thirteen were captured in the dry season. Only ten species were present in both seasons. *Lutzomyia borrouli* (Barreto & Coutinho), *Lu. chagasi* (Costa Lima) and *Lutzomyia sp. 2* were only found in the rainy season, while and *Lu. gomezi*, *Lu. pilosa* and *Lutzomyia sp. 3* only in the dry season (Table 3). The relative abundance of individuals was higher in the rainy season, especially in May (64.2%) (Table 3). The most common species in the rainy season

Table 1 Phlebotomine sand fly species captured in anthropogenic and sylvatic environments in the municipality of Chapadinha, from October 2007 to September 2008.

Species	♂		♀		Total	
	n°	%	n°	%	n°	%
<i>Lutzomyia longipalpis</i>	231	41.9	504	59.3	735	52.5
<i>Lu. evandroi</i>	127	23.0	130	15.3	257	18.3
<i>Lu. whitmani</i>	104	18.9	66	7.8	170	12.1
<i>Lu. lenti</i>	57	10.3	9	1.1	66	4.7
<i>Lu. termitophila</i>	12	2.2	44	5.2	56	4.0
<i>Lu. trinidadensis</i>	9	1.6	30	3.5	39	2.8
<i>Lu. saulensis</i>	1	0.2	26	3.0	27	1.9
<i>Lu. sordellii</i>	3	0.5	20	2.4	23	1.6
<i>Lutzomyia</i> sp. 1	-		11	1.3	11	0.8
<i>Lu. gomezi</i>	5	0.9	-		5	0.4
<i>Lu. flaviscutellata</i>	1	0.2	2	0.2	3	0.2
<i>Lu. chagasi</i>	-		2	0.2	2	0.1
<i>Lu. monstrosa</i>	-		2	0.2	2	0.1
<i>Lutzomyia</i> sp. 2	-		2	0.2	2	0.1
<i>Lu. bourrouli</i>	-		1	0.1	1	0.1
<i>Lu. pilosa</i>	1	0.2	-		1	0.1
<i>Lutzomyia</i> sp. 3	-		1	0.1	1	0.1
Individuals	551	100.0	850	100.0	1,401	100.0
Percentage	39.3		60.7		100.0	
Species	11		15		17	

were *Lu. longipalpis*, *Lu. evandroi*, *Lu. saulensis* and *Lu. sordellii*.

Discussion

The species richness found in the cerrado region of Chapadinha was relatively high, comparable to that obtained from forested areas of the state capital São Luís (Marinho *et al* 2008) and from Buriticupu, Amazonian part of the state (Rebêlo *et al* 2000a,b) and higher from the average 10 species inventoried for other municipalities of Maranhão (Rebêlo *et al* 1999a,b), Araújo *et al* 2000, Barros *et al* 2000, Carvalho *et al* 2000, Martin & Rebêlo 2006).

Several species reported herein have already been found in other ecological areas of Maranhão. *Lutzomyia saulensis*, *Lu. monstrosa* and *Lu. gomezi* are poorly registered in the western Amazonian areas of the state (Rebêlo *et al* 2000a,b), being the presence of these species first recorded in the eastern section of Maranhão. These species also occur in other regions of the Brazilian legal Amazonia (Biancardi *et al* 1982, Castellón *et al* 1994). *Lutzomyia saulensis* was already reported to the cerrado region of Tocantins state (Andrade Filho *et al* 2001). Our report also extends the limits of distribution of *Lu. chagasi* to the far east of the

Amazonian basin, including Maranhão. The remaining species are commonly found in the São Luís Island and in other eastern areas of Maranhão (Rebêlo *et al* 1999a, Martin & Rebêlo 2006, Marinho *et al* 2008).

Lutzomyia longipalpis was the most prevalent species occurring in all sampling sites all year round. This is a well adapted species to anthropogenic environments, vectoring *Leishmania chagasi* in several Brazilian regions (Soares & Turco 2003, Lainson & Rangel 2005, Nunes *et al* 2008), including Maranhão (Rebêlo *et al* 1999b, Martin & Rebêlo 2006).

The occurrence of *Lu. whitmani* in the peridomiliary area shows that this species is highly adapted to human conditions, a fact already noted in other regions of Maranhão (Rebêlo *et al* 1999a,b, Martin & Rebêlo 2006, Leonardo & Rebêlo 2004). This vector has previously been found naturally infected with *Leishmania* species from the *braziliensis* complex, which is involved in the cutaneous leishmaniasis transmission in Maranhão (Oliveira-Pereira *et al* 2006), and in other Brazilian areas as well (Rangel & Lainson 2003, Costa *et al* 2007).

Although rarely trapped in most entomological inventories carried out in cutaneous leishmaniasis transmission areas in Maranhão, the low abundance of *Lu. flaviscutellata* should not be neglected, since this is a well known vector of *Le.*

Table 2 Number of individuals captured in anthropogenic and sylvatic environments according to the season in the municipality of Chapadinha, from October 2007 to September 2008.

Zone	Urban						Rural						Total								
	Peridomicile			Sylvatic			Peridomicile			Sylvatic											
	Chicken house	Banana trees	Gallery forest	Chicken roost	Pigsty	Intra domicile	Cerrado forest	Room	Room	Cerrado forest	Room	Room									
n°	%	n°	%	n°	%	n°	%	n°	%	n°	%	n°	%	n°	%						
Species	n°	%	n°	%	n°	%	n°	%	n°	%	n°	%	n°	%	n°	%					
<i>Lutzomyia longipalpis</i>	524	63.8	60	48.8	3	2.8	587	55.9	26	37.7	59	66.3	21	24.4	42	39.2	148	42.2	735	52.5	
<i>Lu. evandroi</i>	105	12.8	41	33.3	9	8.5	155	14.8	12	17.4	17	19.0	36	41.9	37	34.6	102	29.1	257	18.3	
<i>Lu. whitmani</i>	141	17.2	1	0.8	4	3.8	146	13.9	14	20.3	6	6.7	4	4.6	8	7.5	24	6.8	170	12.1	
<i>Lu. lenti</i>	28	3.4	11	8.9	5	4.7	44	4.2	1	1.4	3	3.4	10	11.6	8	7.5	22	6.3	66	4.7	
<i>Lu. termitophila</i>	18	2.2	8	6.5	11	10.4	37	3.5	6	8.7	1	1.1	3	3.5	9	8.4	19	5.4	56	4.0	
<i>Lu. trinidadensis</i>	4	0.5			12	11.3	16	1.5	4	5.8	2	2.2	10	11.6	7	6.5	23	6.6	39	2.8	
<i>Lu. saulensis</i>			27	25.5	27	25.5	27	2.6												27	1.9
<i>Lu. sordellii</i>	1	0.1	1	0.8	19	17.9	20	1.9	1	1.4	2	2.3			3	2.8			23	1.6	
<i>Lutzomyia</i> sp. 1	1	0.1	1	0.8	4	3.8	6	0.6	4	5.8			1	0.9	5	4.7			11	0.8	
<i>Lu. gomezi</i>			5	4.7	5	4.7	5	0.5											5	0.4	
<i>Lu. flaviscutellata</i>			2	1.9	2	1.9	2	0.2			1	1.1			1	0.9			3	0.2	
<i>Lu. chagasi</i>															1	0.9			2	0.1	
<i>Lu. monstrosa</i>			1	0.9	1	0.9	1	0.1	1	1.4					1	0.9			2	0.1	
<i>Lutzomyia</i> sp. 2			2	1.9	2	1.9	2	0.2											2	0.1	
<i>Lu. bourrouli</i>			1	0.9	1	0.9	1	0.1											1	0.1	
<i>Lu. pilosa</i>															1	0.9			1	0.1	
<i>Lutzomyia</i> sp 3			1	0.9	1	0.9	1	0.1											1	0.1	
Individuals	821	100	123	100	106	100	1050	100	69	100	89	100	86	100	107	100	351	100.0	1,401	100.0	
Percentage	58.6		8.7		7.6		75.0		4.9		6.3		6.1		7.6		25.0		100.0		
Diversity	0.47		0.53		0.98		0.74		0.46		0.67		0.64								

Table 3 Number of individuals captured in anthropogenic and sylvatic environments according to the season in the municipality of Chapadinha, from October 2007 to September 2008.

Season Species / months	Rainy						Dry						Total
	J	F	M	A	M	J	J	A	S	O	N	D	
<i>Lutzomyia longipalpis</i>	98	57	80	2	278	1	25	12	12	23	22	125	735
<i>Lu. evandroi</i>	77	24	17	4	2	3	30	15	11	35	12	27	257
<i>Lu. whitmani</i>	2	1	6	1	143		10	2	1	4			170
<i>Lu. lenti</i>	12	5	4				14	3	4	12	8	4	66
<i>Lu. termitophila</i>	6	4	9	3	3	1	4	4		13	4	5	56
<i>Lu. trinidandensis</i>	5	4			2		4	1	3	11	7	2	39
<i>Lu. saulensis</i>	12		7		1						7		27
<i>Lu. sordellii</i>	5		12				2			2	1	1	23
<i>Lu. gomezi</i>										1	4		5
<i>Lu. flaviscutellata</i>					1						2		3
<i>Lu. chagasi</i>			1		1								2
<i>Lu. monstrosa</i>					1		1						2
<i>Lu. bourrouli</i>	1												1
<i>Lu. pilosa</i>										1			1
<i>Lutzomyia</i> sp 1	1	1					1		7		1		11
<i>Lutzomyia</i> sp 2			2										2
<i>Lutzomyia</i> sp. 3											1		1
Total	219	96	138	10	432	5	91	37	38	102	69	164	1,401

amazonensis, the causative agent of diffuse cutaneous leishmaniasis (Shaw *et al* 1972).

Two more species of medical interest concerned in leishmaniasis transmission were found: *Lu. gomezi*, incriminated in transmitting cutaneous leishmaniasis in Ecuador, Panama and Venezuela (Jorquera *et al* 2005, Sharma & Singh 2008), and *Lu. chagasi*, implicated in the transmission cycle of *Le. braziliensis* complex in the Amazonian region (see Rangel & Lainson 2003).

The role of chicken houses in attracting and maintaining sand fly populations has been discussed (Alexander *et al* 2002), possibly indicating that these artificial shelters may be an important reservoir of visceral leishmaniasis focus in human surroundings. Chicken rearing is a very common activity among the inhabitants of Maranhão (Dias *et al* 2003) and the presence of animals in the peridomicile, along with other environmental elements, is thought to be a risk factor for visceral leishmaniasis transmission (Moreno *et al* 2005). The forest area in which a light trap was set up for capturing sand flies presents environmental aspects of gallery forest. It is known that Amazonian and Atlantic gallery forests can be found along the humid valleys in the cerrado biome, and this feature may explain the phlebotomine sand fly fauna and the diversity values found for those environments. The physiognomic characteristics found in the gallery forest seem to work as a refuge for the sand fly species commonly found only in humid forests such as *Lu. gomezi* and *Lu. saulensis*

(Castellón *et al* 1994, Rebêlo *et al* 2000b).

The seasonal profile of phlebotomine sand flies depicted herein corroborates other inventories carried out in Maranhão, where a great amount of individuals have constantly been trapped in the rainy season. The annual occurrence of *Lu. longipalpis* suggests that the visceral leishmaniasis transmission is not dependent on the rainy period, even though an increased sand fly population has been collected during the highest pluviometric index season, as previously reported (Rebêlo *et al* 1999b, Carvalho *et al* 2000, Araújo *et al* 2000). *Lutzomyia whitmani* occurred almost all year round and mostly in the rainy season, as already observed in cerrado region (Rebêlo *et al* 1999b) and in the Amazonian areas of Maranhão (Rebêlo *et al* 2001).

This work contributes for a better understanding of the phlebotomine species occurring in Maranhão, mainly in the northeastern cerrado domain, for which new records of sand fly species distribution were presented. The finding of potential vectors of *Leishmania* parasites in Chapadinha also raises the possibility that autochthonous cases of leishmaniasis may have already been in place.

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