Major Article

Revista da Sociedade Brasileira de Medicina Tropical 45(6):696-700, Nov-Dec, 2012

Ecology of phlebotomines (Diptera, Psychodidae) in rural foci of leishmaniasis in tropical Brazil

Clara Maria Lima Silva[1], Leandro Santos Moraes[2], Gustavo Almeida Brito[2], Ciro Libio Caldas dos Santos[2] and José Manuel Macário Rebêlo[2]

[1]. Programa de Pós-Graduação em Biodiversidade e Conservação, Universidade Federal do Maranhão, São Luis, MA. [2]. Departamento de Biologia, Universidade Federal do Maranhão, São Luis, MA.

ABSTRACT

Introduction: This work aimed to study the community structure of sandflies, with regard to the richness, constancy, abundance, and monthly frequency of the species with a focus on the transmission of leishmaniasis. **Methods:** The study was conducted in the rural villages of Bom Jardim and Santa Maria, situated on the edge of a tropical rain forest in the municipality of São Jose de Ribamar, Maranhão, Brazil. The phlebotomines were captured in the intradomiciles and peridomiciles of each village, with Centers for Disease Control (CDC) light traps set in 10 homes in each village, for 1 year, once a month, from 18h to 6h. **Results:** We collected 1,378 individuals of 16 sandfly species. The capture success rate was higher in Bom Jardim (0.61 specimens/hour/trap) than that of Santa Maria (0.35/specimens/hour/trap). The sandflies were more abundant in the peridomiciles (86.1%) and in the rainy season (77%). Five species were considered constants (occurring in more than 50% of samples), 5 accessory (25%-50%), and 6 accidental (<25%). The most abundant species were *Lutzomyia longipalpis* (59.7%) and *L whitmani* (28%). The permutation analysis showed differences between the species composition of the villages and no separation between the intradomicile and peridomicile of each village. The species that most contributed to the dissimilarity between the light traps of the 2 villages were *L. longipalpis*, *L. whitmani*, and *L. evandroi*, contributing to 80.8% of the variation among groups. **Conclusions:** The high level of richness and abundance of species and the presence of competent vectors throughout the year and around houses justify the occurrence of leishmaniasis cases reported in the area.

Keywords: Sandflies. Vector insects. Leishmaniasis. Peridomicile. Amazonia. Northeast.

INTRODUCTION

Phlebotomines are small hematophagous dipterans of the family Psychodidae that contain vector species of leishmaniasis, diseases caused by flagellate protozoa of the genus *Leishmania* (Trypanosomatidae)[1]. These psychodids are diversified in tropical rain forests[2], although many species have adapted to peridomicile environments of rural and urban areas in different endemic areas of leishmaniasis in Brazil[3].

In the State of Maranhão, previous studies performed in rural villages and the outskirts of urban areas have detected the presence of numerous phlebotomines, including species known to transmit leishmaniasis in these environments[4-7]. Poor quality human housing associated with animal husbandry, lack of minimum conditions of sanitation, and low nutrition levels of residents are common conditions that allow the simultaneous occurrence of vectors, susceptibility, and synanthropic/household reservoirs[8-10]. Consequently, the outbreak of canine and human leishmaniasis is imminent.

Two rural villages, with such features, situated on the edge of a tropical rain forest have reported several leishmaniasis cases in recent years, including death records. This fact motivated the development of a study of the phlebotomine fauna, as more than 20 species had been found in the adjacent forest itself[11]. In this sense, the entomological survey was performed in these 2 villages in order to study the diversity, constancy, relative abundance, and monthly frequency of species in the domicile environment, and to expand the knowledge of sandfly ecology.

***Address to*:** Prof. José Manuel Macário Rebêlo**.** Lab. Entomologia e Vetores/Deptº de Patologia/UFMA. Praça Madre Deus 2, 65025-560 São Luis, MA, Brasil.
Phone: 55 98 3248-2929
e-mail: macariorebelo@uol.com.br
Received in 11/10/2011
Accepted in 20/03/2012

METHODS

Study area

The study was conducted in the rural villages of Santa Maria (02°39'19"S and 44°10'39"W) and Bom Jardim (02°38'08"S and 44º 09'54"W), both located in county of São José de Ribamar, in the southeastern portion of the island of São Luis, capital of the State of Maranhão. The distance between the 2 sites is approximately 5km.

Bom Jardim, located approximately 2km away from the forest area, has 220 homes and a total of 880 inhabitants. Santa Maria is located 5km from the forest area, has 60 households, and 240 inhabitants. In these villages, agriculture is the main economic activity.

Houses are generally brick or stucco and have the minimum conditions of sanitation and infrastructure. The peridomicile is characterized by vegetation and wildlife consisting primarily of fruit trees, grasses, and birds. There is no sewer, no garbage collection, and the garbage is burned in most cases. A large amount of waste produced by residents and organic matter such as fallen leaves and fruits are found in the backyards, in addition to captive animals in shelters and/or circling around the houses.

The open tropical rainforest is the main vegetation of the surroundings, followed by flooding vegetation, mangrove, restinga, and secondary forest with 20 years of regeneration[12]. The climate is mesothermal, tropical, and humid, with 2 seasons: a rainy season from January to June, which has an average of 94% of the total annual rainfall, and drought season from July to December, which has only 6% of the total annual rainfall. The total rainfall is high, around 1,900mm annually. Temperatures are high throughout the entire year (average: 26°C), with little annual variation[12].

Methodology

Sandflies were captured from March 2009 to February 2010 using 20 light traps provided by the Centers for Disease Control (CDC) for between 18h and 6h. Ten traps were distributed in 5 houses in the village of Santa Maria and another 10 were set in 5 additional houses in the village of Bom Jardim, with 2 traps placed in each house: 1 in the intradomicile and another in the peridomicile. The households chosen were those containing shelters with domestic animals such as chickens, ducks, pigs, donkeys, dogs, and cats, keeping an average distance of 250m. Each trap operated for 12 h once a month, resulting in 2,880 worked hours.

The insects retained in the traps were killed in ethyl acetate and diaphanized with potassium hydroxide, acetic acid, distilled water, and lactophenol, and mounted in Fora-Berleze liquid between a slide and coverslip. The identification was performed according to the method of Young & Duncan[1] in the Laboratory of Entomology and Vectors of the Federal University of Maranhão.

The Shannon-Wiener index[13] was used to evaluate the diversity, which was estimated through the Jackknife procedure[14], resulting in the construction of a 95% confidence interval. The intervals were compared and those that did not overlap were considered significantly different. The Morisita-Horn index was used for similarity measurement and the Pielou index was used to evaluate evenness.

We calculated the constancy index of the captured sandflies. Species that were present in more than 50% of samples were considered constant, those that were present in between 25% and 50% of samples were considered incidental, and those present in less than 25% of samples were considered accidental[15].

For graphical visualization of species similarity per light trap, we used 2 axes of a non-metric multidimensional scaling (NMDS), held from the dissimilarity matrix and calculated with the Bray-Curtis index of the species relative abundance. It was necessary to exclude a light trap from an intradomicile of Bom Jardim village to perform the analysis, as no sandflies were collected in it. To test the difference between the light trap similarities of the 2 villages, we performed a permutational multivariate analysis of variance (with 999 repetitions, $\alpha = 0.05$, $\beta = 0.2$) using distance matrices[16] with the statistical package vegan from computational environment R[17]. In order to determine which species most contributed to the dissimilarity in species composition between the villages, we used the similarity percentages (SIMPER) analysis.

RESULTS

Species richness and relative abundance

We found 15 phlebotomine species, 1 species of the genus *Brumptomyia* and 14 species of the genus *Lutzomyia.* We captured 1,378 individuals from both villages. The dominant species included *L. longipalpis* (59.7%), *L. whitmani* (28%), *L. evandroi* (3.7%), *L. antunesi* (3%), *L. flaviscutellata* (1.7%), and *L. sordellii* (1.6%) **(Figure 1** and **Table 1)**. Among the species found, 14 were found in Santa Maria and 12 in Bom Jardim. The species *L. termitophila* was only present in Bom Jardim, while *L. olmeca nociva*, *L. servulolimai*, and *L. richardwardi* were found only in Santa Maria. We captured 877 individuals in Bom Jardim. The capture efficiency was 0.61 specimens/hour/trap. The most abundant species were *L. longipalpis* (65.6%) and *L. whitmani* (23.1%). The other species together accounted for 11.3% of the total specimens **(Table 1)**. We collected 501 individuals in Santa Maria, and the capture efficiency was 0.35 individuals/hour/trap. The most abundant species were *L. longipalpis* (49.5%) and *L. whitmani* (36.5%). The other species together accounted for 14% of the total specimens **(Table 1)**.

TABLE 1 - Number of sandfly individuals collected at the intradomiciles and peridomiciles, villages of Santa Maria and Bom Jardim, municipality of São José de Ribamar, Maranhão, Brazil, from March 2009 to February 2010.

Localities	Santa Maria						Bom Jardim							
Environments	intradomicile		peridomicile		subtotal		intradomicile		peridomicile		subtotal		Total	
Species	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Lutzomyia longipalpis	5	18.0	243	51.4	248	49.5	102	62.2	473	66.3	575	65.6	823	59.7
Lutzomyia whitmani	8	28.6	175	37.0	183	36.5	25	15.2	177	25.0	202	23.1	385	28.0
Lutzomyia antunesi	5	17.8	12	2.5	17	3.4	5	3.0	17	2.4	22	2.5	39	3.0
Lutzomyia flaviscutellata	—	—	12	2.5	12	2.4	—	—	12	1.7	12	1.4	24	1.7
Lutzomyia evandroi	2	7.1	9	1.9	11	2.2	26	16.0	15	2.1	41	4.7	52	3.7
Lutzomyia sordellii	2	7.1	8	1.7	10	2.0	—	—	12	1.7	12	1.4	22	1.6
Brumptomyia avellari	2	7.1	3	0.6	5	1.0	—	—	1	0.1	1	0.1	6	0.4
Lutzomyia richardwardi	2	7.1	2	0.4	4	0.8	—	—	—	—	—	—	4	0.3
Lutzomyia brasiliensis	1	3.6	1	0.2	2	0.4	1	0.6	1	0.1	2	0.2	4	0.3
Lutzomyia wellcomei	—	—	2	0.4	2	0.4	—	—	1	0.1	1	0.1	3	0.2
Lutzomyia olmeca nociva	—	—	2	0.4	2	0.4	—	—	—	—	—	—	2	0.1
Lutzomyia infraspinosa	1	3.6	1	0.2	2	0.4	2	1.2	2	0.3	4	0.4	6	0.4
Lutzomyia davisi	—	—	2	0.4	2	0.4	—	—	1	0.1	1	0.1	3	0.2
Lutzomyia termitophila	—	—	—	—	—	—	3	1.8	1	0.1	4	0.4	4	0.3
Lutzomyia servulolimai	—	—	1	0.2	1	0.2	—	—	—	—	—	—	1	0.1
Number of individuals	**28**	**5.6**	**473**	**94.4**	**501**	**36.4**	**164**	**18.7**	**713**	**81.3**	**877**	**63.6**	**1,378**	**100.0**

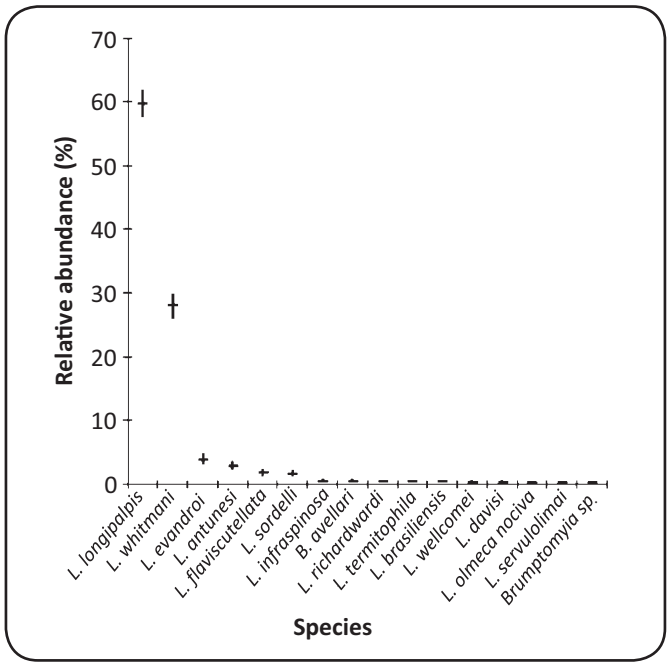

FIGURE 1 - Relative abundance (%) and its confidence limits of sandflies captured in intradomiciles and peridomiciles, villages of Santa Maria and Bom Jardim, São José de Ribamar, Maranhão, Brazil, from March 2009 to February 2010. L: *Lutzomyia*; **B:** *Brumptomyia*.

Similarity and diversity

The 2 NMDS axes accounted for 93% of the dissimilarity in species composition between the light traps, and each village formed distinct clusters **(Figure 2)**. However, there was no clear separation between the traps in the intradomiciles and peridomiciles within each village cluster. The permutation analysis demonstrated the difference between the species composition of the villages (df = 1, F = 3.46, p < 0.01), while there was no separation between the intradomiciles and peridomiciles of each village. The species that most contributed to the dissimilarity between the light traps in the 2 villages were *L. longipalpis*, *L. whitmani*, and *L. evandroi*, contributing 80.8% of the variation between the groups **(Table 2)**.

Diversity was higher in the Santa Maria village (H' = 1.30), where the 95% confidence interval ranged from 1.19−1.41, while diversity was lower in Bom Jardim (H' = 1.09, confidence interval: 1.01−1.17). Evenness was higher in the Santa Maria village (0.48) than that of Bom Jardim (0.42) and the percentage of dissimilarity between both villages was high (86%). Proportions of similarity between the intra- and peridomestic environments were 96% in Bom Jardim and 71% in Santa Maria. Evenness was higher in the intradomiciles than in the peridomiciles in both Bom Jardim (intradomiciles = 0.58, peridomiciles = 0.39) and Santa Maria (intradomiciles = 0.89, peridomiciles = 0.45).

Association with environments

The sandflies were more abundant in the peridomiciles (86.1%) than in the intradomiciles (13.9%) **(Table 1)**. The dominant species found in the intradomiciles were *L. longipalpis* (55.7%), *L. whitmani* (17.2%), *L evandroi* (14.6%), *and L antunesi* (5.2%). In the peridomiciles, the dominant species were *L. longipalpis* (60.4%) and *L. whitmani* (29.7%).

In Bom Jardim, all 12 species frequented the peridomiciles and 7 were collected from inside the homes. The abundance of species

TABLE 2 - Mean species abundance per light trap and cumulative percentages of species contribution to the dissimilarity between the villages of Santa Maria and Bom Jardim, São José de Ribamar, Maranhão, Brazil.

Species	Santa Maria	Bom Jardim	Cumulative (%)
Lutzomyia longipalpis	24.8	64.0	47.7
Lutzomyia whitmani	18.0	22.4	73.2
Lutzomyia evandroi	1.1	4.6	80.8
Lutzomyia antunesi	1.5	2.4	86.7
Lutzomyia sordellii	1.1	1.2	89.7
Lutzomyia flaviscutellata	1.2	1.3	91.9

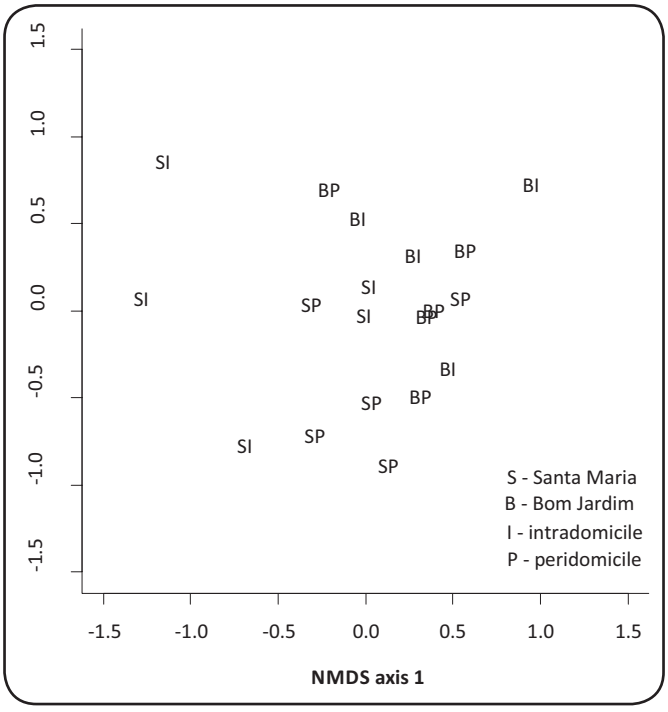

FIGURE 2 - Non-metric multidimensional scaling (NMDS) derived from the Bray-Curtis dissimilarity matrix, created with relative abundance of sandfly species collected in Santa Maria and Bom Jardim villages, São José de Ribamar, Maranhão, Brazil.

followed the general pattern, i.e., 81.3% of specimens were collected in the peridomiciles, versus 18.7% collected from inside homes **(Table 1)**. In Santa Maria, the 14 species present also were found in the peridomiciles and 9 were collected from inside the homes. The abundance of species also followed the general pattern, i.e., 94.4% of specimens were collected in the peridomiciles, while only 5.6% were collected from inside homes **(Table 1)**.

Monthly and seasonal frequencies

The presence of the various species of sandflies was recorded throughout the year, although the highest concentration of species was collected in the rainy months **(Table 3)**. The abundance of the specimens was also higher (77%) in the rainy season compared to the dry season (23%). Five species were considered constant, being present in more than 50% of collections, as *L. longipalpis* and *L. whitmani* species were present continuously throughout the year, with the equivalent constancy index of C = 100.0 **(Table 3)**. Five species were classified as accessory, as they were present in 25%-50% of collections. The remaining species were accidental since they were found in less than 25% of collections.

TABLE 3 - Number of sandfly individuals collected from March 2009 to February 2010 in the villages of Santa Maria and Bom Jardim, São José de Ribamar, Maranhão, Brazil.

Season	Rainy						Dry						
Species/Months	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**CI**
Lutzomyia longipalpis	141	442	123	12	19	1	28	4	11	—	7	35	100.0
Lutzomyia whitmani	62	135	10	3	12	6	62	21	40	6	4	24	91.7
Lutzomyia evandroi	11	31	—	—	—	1	—	—	1	2	1	5	58.3
Lutzomyia antunesi	3	—	1	—	—	1	8	3	10	2	4	7	75.0
Lutzomyia flaviscutellata	1	2	1	—	—	5	11	2	1	—	—	1	66.7
Lutzomyia sordellii	3	11	3	—	—	1	—	3	—	—	—	1	50.0
Lutzomyia infraspinosa	3	1	—	—	—	—	—	—	—	—	—	2	25.0
Brumptomyia avellari	—	—	—	—	—	—	—	—	4	—	—	2	16.7
Lutzomyia richardwardi	1	—	—	2	—	—	1	—	—	—	—	—	25.0
Lutzomyia termitophila	1	3	—	—	—	—	-	—	—	—	—	—	16.7
Lutzomyia brasiliensis	—	—	—	1	1	1	1	—	—	—	—	—	33.3
Lutzomyia wellcomei	—	1	—	1	—	—	1	—	—	—	—	—	25.0
Lutzomyia davisi	—	3	—	—	—	—	—	—	—	—	—	—	8.3
Lutzomyia olmeca nociva	—	—	—	—	—	2	—	—	—	—	—	—	8.3
Lutzomyia servulolimai	—	1	—	—	—	—	—	—	—	—	—	—	8.3
Total of individuals	**226**	**630**	**138**	**19**	**32**	**18**	**112**	**33**	**67**	**10**	**16**	**77**	
Total of species	**9**	**10**	**5**	**5**	**3**	**8**	**7**	**5**	**6**	**3**	**4**	**8**	

Jan: January; **Feb:** February; **Mar:** March; **Apr:** April; **Jun:** June; **Jul**: July; **Aug:** August; **Sep:** September; **Oct:** October; **Nov:** November; **Dec:** December; **CI:** constancy index.

DISCUSSION

The number of sandfly species found in this study was fairly representative when compared with those obtained in other rural areas of São Luis Island, such as Raposa[4], Paço do Lumiar[5], and São Jose de Ribamar[6]. This result may be due to the proximity of the tropical rain forest in which a large number of species was found[4]. Such proximity, perhaps, explains the presence of 7 different species in the villages of this study (*B. avellari*, *L. olmeca nociva, L. richardwardi*, *L. sordellii, L. infraspinosa, L. termithophila*, and *L. servulolimai*) despite the absence of an occurrence record in rural and urban areas; for this reason, these species have been regarded as strictly wild species. Beyond these species, *L. whitmani, L. olmeca nociva* and *L. davisi* are also present in the tropical rainforest[11,18] and behave similarly to peridomestic species, leading us to assume that adjacent wild environments can act as maintainers of populations capable of invading nearby established rural settlements, even after vector control actions have been performed.

The compositions and relative abundances of the species were more similar between homes in both villages, which is justified by the closeness of sampling points. The similarity observed between peridomestic and indoor areas was due to the majority of species living in the peridomicile areas moved into the homes, which was verified in another locality of São José de Ribamar[6].

The fact that some households of different villages have formed groups is justified by the relative similarity in the type of buildings, with poorly established areas around houses and indoors, thus providing shelter and blood meals for sandflies. We call attention to the larger contribution of *L. longipalpis* and *L. whitmani* to the dissimilarity between the traps from the 2 villages.

The first species is a visceral leishmaniasis vector and has been found in high numbers in human dwellings and domestic animal shelters in the City of São José de Ribamar[6,19]. From 2008–2009, 20 human cases of the disease were reported; in the past 8 years, 129 cases were reported, with 1 case leading to a death in the village of Bom Jardim[20] and other fatalities occurred in other areas of northeastern Maranhão[7,21]. The second, a vector of cutaneous leishmaniasis, is more abundant in peridomestic areas in the Amazon of Maranhão[22-25]. The abundance of these species in the rural sector increases the risk of leishmaniasis transmission, as flies become adapted to living with humans and domestic animals in this environment.

The various studies conducted in Maranhão clearly demonstrate that the temperature favors the presence of sandflies throughout the year, due to its high means every month and annual variation of only around 3 degrees only[26]. However, the rain is the environmental factor that often determines the fluctuation in the number of individuals and species over the year. The rains, in addition to moistened soil increasing the relative air humidity, can also cause a greater availability of food resources for the sandflies, which explains the overall prevalence of these insects during the rainy season[26].

The two most abundant species showed antagonistic behavior in the temporal distribution, while *L. longipalpis* followed the behavior of most species, predominating in the rainy season, as observed in other areas of the São Luis Island,[26] while *L. whitmani* adversely attained higher abundance in the dry season. However, such behavior cannot be considered a pattern, as an inverse phenomenon was observed in rural northeastern Maranhão, where Martin and Rebêlo[7] found high frequencies of *L. longipalpis* during the dry period and *L. whitmani* during the rainy season. These observations show that the structure of sandfly communities may have variations in space and time; however, seasonal abundance segregation was also noted in these 2 species, perhaps as a response to competition for resources in this new anthropic environment.What appears to be a pattern in the sandfly community in Maranhão is the largest number of ancillary and incidental species to the detriment of the most constant species, as noted in all studies in Maranhão[4-7,11,18]. This phenomenon may result

from variations in the availability of blood sources, breeding grounds, and shelters, which favor a small number of opportunistic species such as *L. longipalpis* and *L. withmani* that tend to be prevalent in all months of the year; this trend not observed in species considered incidental and ancillary over time.

In summary, sandflies showed a moderate number of species that alternated throughout the year, with dominant species including the competent vectors of visceral (*L. longipalpis*) and cutaneous (*L. whitmani*) leishmaniasis. The infestation of these insects in the rural sector can be favored by the proximity to tropical rain forest, associated with changes imposed by human activities, and the precarious sanitary infrastructure and urban services, which also contribute to the proliferation of these insects, thus increasing the risk of leishmaniasis transmission in these environments. Therefore, it is necessary for monitoring studies to be performed with regard to abundance, blood food supply, and infection rate of vector species in order to aid the health agencies in the prevention and control of leishmaniasis in the villages of Santa Maria and Bom Jardim.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABSTRACT IN PORTUGUESE

Ecologia de flebotomíneos (Diptera, Psychodidae) em foco rural de leishmaniose no Brasil tropical

Introdução: Este trabalho teve como objetivo estudar a estrutura da comunidade de flebotomíneos, dando a conhecer a riqueza, constância, abundância e a frequência mensal das espécies em foco de transmissão de leishmaniose. **Métodos:** O estudo foi realizado nas localidades rurais de Bom Jardim e Santa Maria, situadas às margens de uma mata ombrófila infestada por esses insetos, no município de São José de Ribamar, Maranhão, Brasil. Os flebotomíneos foram capturados no intra e peridomicílio de cada vila, com 10 armadilhas luminosas tipo CDC em cada vila, durante um ano, uma vez por mês, das 18h às 6h. **Resultados:** Foram capturados 1.378 indivíduos de dezesseis espécies e o esforço de captura foi maior em Bom Jardim (0,61 indivíduos/hora/armadilha) que em Santa Maria (0,35). Os flebotomíneos prevaleceram no peridomicílio (86,1%) e na estação chuvosa (77%). Cinco espécies foram consideradas constantes (ocorreram em mais de 50% das coletas), cinco acessórias (25% e 50%) e seis acidentais (< 25%). As espécies mais abundantes foram *Lutzomyia longipalpis* (59,7%) e *Lutzomyia whitmani* (28%). Estatisticamente, detectou-se uma diferença na composição das espécies entre os povoados. As espécies que mais contribuíram para a dissimilaridade entre as armadilhas das duas vilas foram *Lutzomyia longipalpis*, *Lutzomyia whitmani* e *Lutzomyia evandroi*, com 80,8% de variação entre os grupos. **Conclusões:** A elevada riqueza e abundância de flebotomíneos e a presença de vetores competentes ao longo do ano, justificam a ocorrência de casos de leishmanioses relatados na área.

Palavras-chaves: Flebotomíneos. Insetos vetores. Leishmanioses. Peridomicílio. Amazônia. Nordeste.

REFERENCES

1. Young DG, Duncan MA. Guide to the identification and geographic distribution of *Lutzomyia* sand flies in Mexico, the West Indies; Central and South America (Diptera: Psychodidae). Mem Am Ent Inst 1994; 54:1-881.
2. Ward RD, Shaw JJ, Lainson R, Frahia H. Leishmaniasis in Brazil: VIII. Observations on the phlebotomine fauna of area highly endemic of cutaneous leishmaniasis the Serra dos Carajás, Pará State. Trans R Soc Trop Med Hyg 1973; 67:174-183.
3. Rangel E, Lainson R. Ecologia das leishmanioses. *In:* Rangel EF, Lainson R, editors. Flebotomíneos do Brasil. Rio de Janeiro: Fundação Oswaldo Cruz; 2003. p. 291-336.
4. Araújo JC, Rebêlo JMM, Carvalho ML, Barros VLL. Composição dos flebotomíneos (Diptera, Psichodidae) do município da Raposa-MA, Brasil. Área endêmica de Leishmanioses. Entomol y Vect 2000; 7:33-47.
5. Barros VL, Rebêlo JMM, Silva FS. Flebotomíneos (Diptera, Psichodidae) de capoeira do município de Paço do Lumiar, Estado do Maranhão, Brasil. Área endêmica de leishmanioses. Cad Saude Publica 2000; 16:265-270.
6. Carvalho ML, Rebêlo JMM, Araújo JC, Barros VLL. Aspectos ecológicos dos flebotomíneos (Díptera, Psychodidae) do município de São José de Ribamar, MA, Brasil. Área endêmica de leishmanioses. Entomol y Vect 2000; 7:19-32.
7. Martin AMC, Rebêlo JMM. Dinâmica espaço-temporal de flebotomíneos (Diptera, Psychodidae) do município de Santa Quitéria, área de cerrado do Estado do Maranhão, Brasil. Iheringia 2006; 96:273-384.
8. Abreu-Silva AL, Lima TB, Macedo AA, Moraes-Júnior FJ, Dias EL, Batista ZS, et al. Soroprevalência, aspectos clínicos e bioquímicos da infecção por *Leishmania* em cães naturalmente infectados e fauna de flebotomíneos em uma área endêmica na ilha de São Luís, Maranhão, Brasil. Rev Bras Paras Vet 2008; 17 (supl I):197-203.
9. Caldas AJM, Costa JML, Silva AAM, Vinhas V, Barral A. Risk factors associated with asymptomatic infection by *Leishmania chagasi* in north-east Brazil. Trans R Soc Trop Med Hyg 2001; 95:1-5.
10. Dias FOP, Lorosa ES, Rêbelo JMM. Fonte alimentar e peridomiciliação de *Lutzomyia longipalpis* (Lutz & Neiva, 1912) (Psychodidae, Phlebotominae). Cad Saude Publica 2003; 19:1373-1380.
11. Azevedo PCB, Lopes GN, Fonteles RS, Vasconcelos GC, Moraes JLP, Rebêlo JMM. Efeito da fragmentação em comunidades de flebotomíneos (Diptera; Psychodidae) em áreas de floresta ombrófila de São Luís, Maranhão. Neotrop Entomol 2011; 40:271-277.
12. Instituto Brasileiro de Geografia e Estatística (IBGE). Dados sobre vegetação do Brasil [Internet]. Brasília: IBGE; [Cited 2009 May 27] Available from: http://www.ibge.gov.br/.
13. Pielou EC. Ecological diversity. John Wiley & Sons, Inc., 1975.
14. Zahl S. Jackknifing an index of diversity. Ecology 1977; 58:907-913.
15. Silveira Neto S, Nakano O, Barbin D, Villa Nova NA. Manual de ecología dos insetos. São Paulo: Agronômica Ceres; 1976.
16. Anderson MJ. A new method for non-parametric multivariate analysis of variance Austral. Ecology 2001; 26:32-46.
17. R Development Core Team. R: A language and environment for statistical computing [Software]. ISBN 3-900051-07-0, URL. Vienna, Austria: R Foundation for Statistical Computing; 2011. Available from: http://www.R-project.org/.
18. Marinho RM, Fonteles RS, Vasconcelos GC, Azevedo PCB, Moraes JLP, Rebêlo JMM. Flebotomíneos (Diptera, Psychodidae em reservas florestais de áreas metropolitana de São Luís, Maranhão, Brasil. Rev Bras Ent 2008; 52:112-116.
19. Nascimento MDSB, Costa JML, Fiori BIP, Viana GMC, Filho MSG, Alvim AC, et al. Aspectos epidemiológicos determinantes na manutenção da leishmaniose visceral no Estado do Maranhão-Brasil. Rev Soc Bras Med Trop 1996; 29:233-240.
20. Rebêlo JMM, Araújo JC, Carvalho ML, Barros VLL, Silva FS, Oliveira ST. Flebótomos (*Lutzomyia*, Phlebotominae) da ilha de São Luís, zona do Golfão maranhense, Brasil. Rev Soc Bras Med Trop 1999; 32:247-253.
21. Fonteles RS, Vasconcelos GC, Azevêdo PCB, Moraes JLP, Lorosa ES, Lopes GN. Preferência alimentar sanguinea de *Lutzomyia whitmani* (Diptera, Psychodidae) em área de transmissão de leishmaniose cutânea americana no Estado do Maranhão, Brasil. Rev Soc Bras Med Trop 2009; 42:647-650.
22. Pereira YNO, Rebêlo JMM, Moraes JLP, Pereira SRFP. Diagnóstico molecular da taxa de infecção natural de flebotomíneos (Psychodidae, *Lutzomyia*) por *Leishmania* sp. na Amazônia maranhense. Rev Soc Bras Med Trop 2006; 39:540-543.
23. Rebêlo JMM, Oliveira ST, Barros VLL, Silva FS. Flebotomíneos (Diptera, Psychodidae) de Lagoas, município de Buriticupu, Amazônia maranhense. I - Riqueza e abundância relativa das espécies em área de colonização recente. Rev Soc Bras Med Trop 2000; 33:11-19.
24. Rebêlo JMM, Oliveira ST, Barros VLL, Silva FS. Flebótomos da Amazônia Maranhense IV. Riqueza e abundância relativa das espécies em área de colonização antiga. Entomol y Vect 2000b; 7:61-62.
25. Rebêlo JMM. Frequência horária e sazonalidade de *Lutzomyia longipalpis* (Diptera, Psychodidae) na Ilha do Maranhão, Brasil. Cad Saude Publica 2001; 17: 221-227.
26. Sistema de Informação de Agravos de Notificação (SINAN). Sistema Nacional de vigilância em saúde. Relatório de situação. Maranhão. Série C. Projetos, Programas e Relatórios; Brasília: Ministério da Saúde; 2009.