

Diversity of sand flies in domiciliary environment of Santarém, state of Pará, Brazil: species composition and abundance patterns in rural and urban areas

Marlisson Augusto Costa FEITOSA¹, Genimar Rebouças JULIÃO², Manoel Djalma Pereira COSTA³, Braulio BELÉM³, Felipe Arley Costa PESSOA²

ABSTRACT

This paper aimed to evaluate the richness, abundance and frequency of sand fly occurrence in rural and urban areas. American visceral leishmaniasis -AVL is endemic in the study area of Santarém municipality, Pará state. Sand flies were collected during 1995-2000, using CDC light traps placed in neighborhoods and rural areas of the municipality. A total of 53.454 individuals and 26 species of sand flies were collected. The most abundant species in both urban and rural environments was *Lutzomyia longipalpis*, vector of AVL in the area. The highest species richness by capture was in rural area. In all years sampled, the largest number of species of sand fly collected was always in rural areas. The species of sand flies in urban and rural area were similar in 11 species. In the rural area other 11 species were found, a total of 22 species. Shannon-Wiener index ranged from 0.12 to 0.84 at rural areas and 0.08 to 0.34 at urban ones. In general, rural localities showed higher diversity (*H'*) of phlebotomines than urban ones. Individual-based rarefaction curves for each area demonstrated that urban localities had the lowest expected number of phlebotomine species and the richest rural ones reach higher expected values with lower amount of individuals than urban sites. The most frequent species were *Lutzomyia longipalpis*, *Evandromyia carmelinoi* and *Bichromomyia flaviscutellata*.

KEYWORDS: leishmaniasis, anthropic impact, Amazon

Diversidade de flebotomíneos no ambiente domiciliar de Santarém, estado do Pará, Brazil: composição de espécies e padrões de abundância em áreas urbanas e rurais

RESUMO.

Com o objetivo de comparar a riqueza, abundância e frequência de ocorrência de flebotomíneos em sítios rurais e urbanos numa área endêmica de leishmaniose visceral no município de Santarém (PA) foi realizado no período entre 1995-2000, capturas de flebotomíneos por meio de armadilhas luminosas CDC montadas no ambiente domiciliar em diferentes bairros e localidades rurais do município. Foram capturados 53.454 indivíduos, de 26 espécies e dentre elas, a mais abundante, tanto em ambiente urbano e rural, foi *Lutzomyia longipalpis*, vetor da leishmaniose visceral americana na região. Em todos os anos de amostragem, a maior riqueza de espécies por captura foi obtida em áreas rurais. As áreas rurais e urbanas compartilharam onze espécies de flebotomíneos; enquanto este mesmo número de espécies foi exclusivo em áreas rurais. Os índices de Shannon-Wiener variaram de 0,12 a 0,84 nas localidades rurais e de 0,08 a 0,34 nas áreas urbanas. Em geral, as áreas rurais apresentaram maior diversidade de flebotomíneos comparado às áreas urbanas. As curvas de rarefação baseadas em indivíduos revelaram um rápido aumento no número esperado de espécies em localidades rurais, indicando que amostras com menor quantidade de indivíduos nestas áreas atingem maior riqueza de espécies. As espécies mais frequentes durante o período de amostragem foram *Lutzomyia longipalpis*, *Evandromyia carmelinoi* e *Bichromomyia flaviscutellata*.

PALAVRAS-CHAVE: leishmanioses, antropização, Amazônia

¹ Instituto de Ciências e Tecnologia das Águas, Universidade Federal do Oeste do Pará Av. Vera Paz, s/nº, Salé, CEP 68135-110 – Santarém – PA. marlisson.feitosa@gmail.com

² Programa de Pesquisa em Ecologia de Doenças Transmissíveis na Amazônia PPETA; Instituto Leônidas e Maria Deane, Fiocruz, Rua Terezina, 476. Adrianópolis. CEP: 69.057-070 Manaus, AM. facpessoa@amazonia.fiocruz.br

³ 9ºCentro Regional de Saúde; Divisão de Endemias - Avenida Marechal Rondon, Nº 1638, Bairro Santa Clara - CEP: 68005-120 - Santarém – PA. manoldjalma@yahoo.com.br

INTRODUCTION

Sand flies are insects of medical and veterinary importance once some species are able to transmit several pathogens such as *Bartonella* spp., *Phlebotomus* spp., and several *trypanosomatids*, as well-known, *Leishmania* spp. There is a higher richness pattern of sand flies in the Amazon region (Young and Duncan 1994; Barrett et al. 1996). Nevertheless, studies of Amazonian sand fly species generally are restrict to some few descriptive studies related to epidemiological surveys or vertical and horizontal stratification. In Latin America, leishmaniasis represents a great threat to human health, since this disease has been pointed out as an emergent disease due to environmental changes mainly caused by road construction and timber extraction (Patz et al. 2004). In Brazil populations exposed to leishmaniasis are not only inhabitants of rural areas but also miners, hunters, and some periurban populations of some cities (Barrett 1993). Sand fly species domiciliation can result in the occurrence of urban leishmaniasis, as noticed by cases record in the periphery of some Brazilian metropolis (Teodoro et al. 1999; Rêbello 2001; Carvalho et al. 2010; Saraiva et al. 2011).

As several cities in northern Brazil, Santarém municipality, state of Pará, has grown in area and population, with a disordered expansion during the last twenty years (Furtado and Macedo 2006). The first record of American visceral leishmaniasis (AVL) in Santarém was reported by Alencar et al. (1962) who found three autochthon cases of AVL in human and 19 cases in dogs. In the 1990's were recorded 58 cases of human AVL (Pereira et al. 2000) and 177 cases were reported in the past ten years (2001-2010), demonstrating that the problem increased. Concerning the American cutaneous leishmaniasis (ACL), the Zoonosis Control Center of Santarém reported a monthly average of 52 cases of ACL (data base from 1998 to 2003) in the total amount, at least 17 cases were autochthonous from the peripheral neighborhoods of Santarém (Feitosa and Castellón 2009).

In concluding remarks of the Brazilian ACL and AVL Control Program, Lacerda (1994) pointed out the need of more precise data on vector biology to elaborate effective control measures. Currently, risk estimates of leishmaniasis transmission are still very poor due to the lack of parameters based on vector presence, its abundance, and infestation level in a given habitat (Maia-Elkhoury et al. 2008). However, the evaluation of species abundance patterns should allow inferences about some ecological aspects as local population size, rarity, and dominance of species, endemicity, and habitat specialization (Magurran 2004). In addition to relative abundance index, the frequency of occurrence (percentage of samples in which species are present) constitutes an easy and feasible tool to understand alterations in vector (and

other organisms) populations due to man-made environment perturbation (Pessoa et al. 2007; Odum and Barrett 2007).

The present study aimed to answer the following questions: (i) sand fly richness and abundance *per* capture event differ between urban and rural areas?; (ii) diversity, dominance and estimated richness of sand flies differ between urban and rural areas? How similar is the phlebotomine fauna comparing one area to another? (iii) the frequency of occurrence (along sampling period) of sand fly species are the same to these areas? Some implications of sand fly abundance patterns were discussed in the light of leishmaniasis transmission.

MATERIALS AND METHOD

Study area

The study was carried out by the Epidemiological Surveillance team of the Department of Entomology, State Public Health Secretary of Pará (SESPA, 9^o Regional de Endemias), in the period among 1995-2000, in Municipality of Santarém (02°26'S and 54°42'W), state of Pará, Brazil (Figure 1).

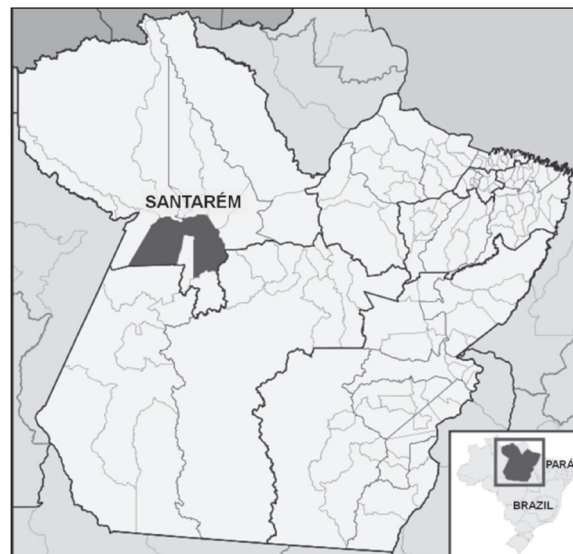


Figure 1 - Location of Santarém municipality, state of Pará, Brazil (Adapted from Campos 2006).

Regional climate is Am and Amw type by the Köppen classification, a rainy climate with precipitation above 2,000 mm and short dry season (Furtado and Macedo 2006). The mean annual temperature ranges from 25 °C to 28 °C, with rainy season between December and May, maximum precipitation levels usually are observed in February. A tropical rain forest is the predominant vegetation in Santarém, except for Amazonian savannas found in the northwestern area of the municipality.

Sand fly capture

Insect sampling collections were carried out between 1995 and 2000, as routinely activities of endemic AVL Control Program conducted by SESPA in the Santarém municipality. Captures of sand flies were performed weekly, in all localities/districts which registered visceral leishmaniasis cases in human or dog population. At each locality/district, ten houses were randomly chosen and a CDC light trap was placed inside the dormitory room and another one in peridomiliar area (near to domestic animal shelters) in each house. Traps were kept on during two consecutive nights. Sand fly specimens were identified according to Ryan (1986) and nomenclature was adopted according to Galati (2003).

Data analyses

In the present study, species richness and abundance of individuals were counted by the number of sand fly species and total number of individuals captured in a given area (urban or rural) or year, respectively. Since sampling efforts varied among years and areas, richness and abundance were divided by the total number of capture events (traps) carried out at one area/year. Mann-Whitney test was used to verify possible differences between urban and rural areas with respect to sand fly abundance and richness *per* capture event. Shannon-Wiener diversity index (H'), Berger-Parker dominance index (d), estimated richness of phlebotomines ($S_{\text{Chao 1}}$ bias-corrected

and their lower and upper confidence limits (95%), and Sørensen similarity index (C_s) were computed to each sampling year (Magurran 2004, Colwell 2009). Individual-based rarefaction curves (with 95% confidence intervals) were employed to estimate and compare the expected number of species in urban and rural areas (Gotelli and Colwell 2001). The variable frequency of occurrence (species presence along sampling period) was based on the ratio between the number of years that a given sand fly species occurred and total period sampling (six years) and expressed as percentage. Species captured in only one or two years along the study (0-25% frequency class) were defined as rare; moderately common species (hereafter, only common species) were sampled in three or four years of the study (26-75% frequency class) and very common species were collected in five years of study or all years (76-100% frequency class) (Gray *et al.* 2005).

RESULTS

It was collected 53,454 sand fly specimens in a six-year long study, belonging to 26 species. In urban areas, 38,832 individuals were caught, which represented about 2.5 fold of sand fly individuals captured in the rural localities (14,622 individuals) of Santarém. The most numerous and frequent species were *Lutzomyia longipalpis* and *Evandromyia carmelinoi*, comprising together \approx 99% of total individuals sampled in both areas (Table 1).

Table 1 - Number of sand fly individuals captured along six consecutive years in houses at urban and rural areas of Santarém municipality, state of Pará, Brazil.

Sand fly Species	Urban Area	Rural Area
<i>Lutzomyia longipalpis</i> (Lutz and Neiva) ‡	37,389	13,583
<i>Evandromyia carmelinoi</i> Ryan, Fraiha, Lainson and Shaw	1,320	643
<i>Nyssomyia whitmani</i> (Antunes and Coutinho) †	56	107
<i>Bichromomyia flaviscutellata</i> (Mangabeira) †	39	65
<i>Micropygomyia rorotaensis</i> (Floch and Abonnenc)	9	41
<i>Nyssomyia antunesi</i> (Coutinho)	5	19
<i>Brumptomyia avellari</i> (Costa Lima)	3	24
<i>Nyssomyia umbratilis</i> (Ward and Fraiha) †	3	4
<i>Lutzomyia gomezi</i> (Nitzulescu)	2	8
<i>Psychodopygus davisii</i> (Root) †	1	8
<i>Micropygomyia trinidadensis</i> (Newstead)	1	3
<i>Nyssomyia shawi</i> Fraiha, Ward and Ready	1	0
<i>Trichopygomyia trichopyga</i> (Floch and Abonnenc)	1	0
<i>Micropygomyia oswaldoi</i> (Mangabeira)	1	0
<i>Brumptomyia travassosi</i> (Mangabeira)	1	0
<i>Trichophoromyia castanheirai</i> (Damasceno, Causey and Arouck)	0	76
<i>Psathyromyia aragaii</i> (Costa Lima)	0	21
<i>Viannamyia tuberculata</i> (Mangabeira)	0	5

Table 1 - Continuation

Sand fly Species	Urban Area	Rural Area
<i>Evandromyia evandroi</i> (Costa Lima and Antunes)	0	4
<i>Trichopygomyia longispina</i> (Mangabeira)	0	3
<i>Trichopygomyia dasypodogeton</i> (Castro)	0	3
<i>Viannamyia furcata</i> (Mangabeira)	0	1
<i>Psychodopygus wellcomei</i> (Fraiha, Shaw and Lainson) †	0	1
<i>Trichophoromyia ubiquitalis</i> (Mangabeira) †	0	1
<i>Evandromyia pinotti</i> (Damasceno and Arouck)	0	1
<i>Psychodopygus ayrozai</i> (Barretto and Coutinho)	0	1
Total	38,832	14,622

† Main or putative vectors of American cutaneous leishmaniasis (ACL) in northern Brazil.
‡ Main vector of American visceral leishmaniasis (AVL) in northern Brazil.

Urban x rural areas

Despite higher number of individuals in urban sites, rural areas showed more sand fly species (22 versus 15 species) and a higher richness per capture event ($U=0.0$, $p=0.004$, Figure 2a). Although no significantly difference was observed between the areas in relation to the abundance per capture event ($U=8.0$, $p=0.109$), a higher number of specimens was captured in each event in rural areas compared to urban ones, in the majority of sampled years (Figure 2b).

Diversity, dominance, observed and estimated richness, and similarity

The Shannon-Wiener index ranged from 0.12 to 0.84 at rural areas and 0.08 to 0.34 at urban ones. In general, rural localities showed higher diversity (H) of phlebotomines than urban ones, except by the year 1998. Inverse pattern

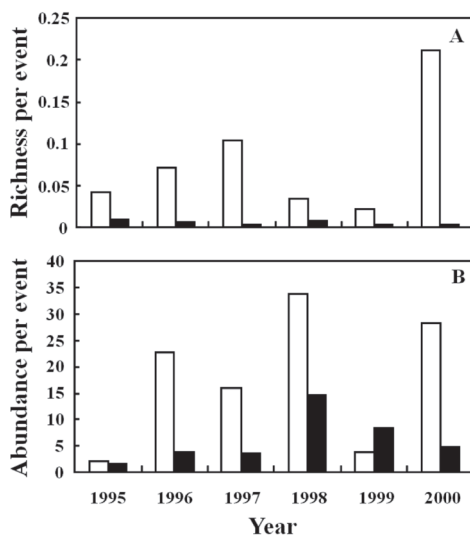


Figure 2 - Sand fly richness (a) and abundance (b) per capture event in rural (white bars) and urban (black bars) in the six-year sampling, at Santarém municipality, state of Pará, Brazil.

was observed to Berger-Parker index (d) in rural areas, which usually presented lower dominance of the most abundant species (*L. longipalpis*) than urban localities. We also observed differences between observed and estimated richness (Chao1, bias corrected) due to presence of singletons (species represented by one individual) and doubletons (species represented by two individuals) in some years; however these differences occurred with the same frequency in urban and rural areas. Similarities in the species incidence at urban and rural areas varied from 0.38 to 0.60; however almost all years had values around 0.50, except for 1998 (Table 2). Confidence intervals of individual-based rarefaction curves for each area clearly demonstrated that urban localities had the lowest expected number of phlebotomine species and the richest rural ones reach higher expected values with lower amount of individuals than urban sites (Figure 3).

Shared and exclusive x rare, common, and very

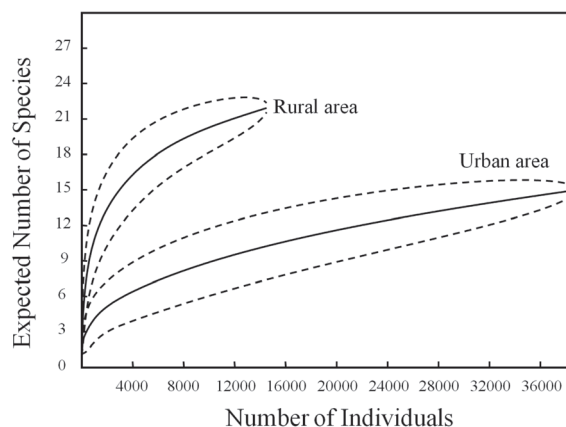


Figure 3 - Individual-based rarefaction curves for phlebotomine species in urban and rural areas at Santarém municipality, state of Pará, Brazil.

Table 2 - Number of sampled localities, number of traps, Shannon-Wiener diversity index (H'), Berger-Parker dominance index (d), observed (S^{obs}) and estimated richness of phlebotomines ($S^{Chao 1}$), lower and upper confidence limits (95%), and Sørensen similarity index (C_s) of species between urban and rural areas of Santarém municipality, state of Pará, Brazil.

Year	Area	Localities	Traps	H'	d	S_{obs}	$S_{Chao 1}$	C_s
1995	Urban	122	607	0.33	0.93	6	6.0 (6.0-6.0)	0.50
	Rural	59	326	0.84	0.82	14	17.0 (14.4-37.0)	
1996	Urban	13	962	0.08	0.99	7	7.3 (7.0-13.0)	0.57
	Rural	12	195	0.26	0.95	14	15.5 (14.1-29.1)	
1997	Urban	19	1700	0.13	0.97	7	13.0 (8.0-44.4)	0.53
	Rural	03	86	0.40	0.92	9	9.0 (9.0-9.0)	
1998	Urban	15	1132	0.16	0.97	10	11.0 (10.1-20.7)	0.38
	Rural	03	177	0.12	0.98	6	6.5 (6.0-14.3)	
1999	Urban	13	797	0.10	0.98	3	3.0 (3.0-3.0)	0.60
	Rural	16	323	0.62	0.82	7	7.0 (7.0-7.0)	
2000	Urban	22	1076	0.34	0.90	4	4.0 (4.0-4.0)	0.55
	Rural	01	33	0.75	0.75	7	7.0 (7.0-7.0)	

common species

Besides higher sand fly richness, rural areas also presented higher number of exclusive species (11 species) while urban sites had only four species. These two habitats shared eleven Phlebotominae species. It was observed that exclusive species (to rural or urban areas), such as *Psychodopygus wellcomei* and *Trichophoromyia ubiquitousis* usually tended to have lower frequency of occurrence and smallest values of relative abundance (Figures 4 and 5). These rare species were sporadically sampled, in only one or two years (0-25% occurrence class) of the study and with scarce number of specimens. Nevertheless, some sand fly species did not fit in this pattern. For instance, *Trichophoromyia castanheirai*, exclusive to rural areas, was a common species, and occurred in four of the six years of study.

Except for the dominant species *L. longipalpis* and *E. carmelinoi*, *T. castanheirai* presented a great relative abundance (Table 1, Figure 4 and 5). In opposite trend, shared species like *L. longipalpis*, *E. carmelinoi* and *Bichromomyia flaviscutellata* were the most frequent, very common species (75-100% of occurrence) in the study, and caught in at least five sampling years. Another general tendency was that all species shared by rural and urban areas were trapped on higher numbers at the former area, like *Nyssomyia whitmani* and *N. antunesi* (Table 1).

DISCUSSION

In the present study, we observed an intrinsic abundance pattern to each sand fly species, which is related to aspects of its behavior, ecology and mainly, to differential capacity of adaptation to changes in urban and rural environments.

Higher sand fly richness per capture event in rural areas were not unexpected, once these insects are usually associated to forested areas (Aguiar and Medeiros 2003), and in northern Brazil, rural areas still have contact with extensive areas of primary forests or forest remnants. Conversely, no statistical difference between rural and urban areas was observed in the abundance per capture event, probably related to great variability in the number of sand fly specimens collected in each sample. Even so, the results suggested that more sand fly individuals/trap was caught in the rural sites, except by 1999 sample, which an inverse pattern was obtained. Furthermore, rural localities had a more diverse phlebotomine fauna: Shannon index averaged to each area were 2.5 fold larger in rural areas than urban ones and the expected number of species reached higher values with less individuals (compared to urban areas) in the rarefaction method; the last one, removes the effects of unequal sampling efforts (Gotelli and Colwell 2001).

Few studies compared simultaneously sand fly diversity between Brazilian urban and rural areas in a long time series. Our results showed a moderate overlapping in the species composition at rural and urban areas; the Sørensen index

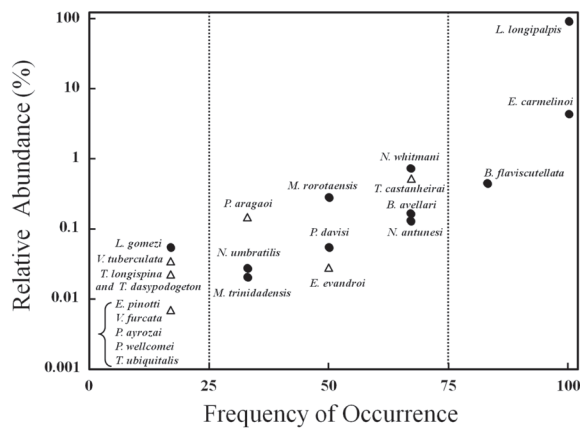


Figure 4 - Relative abundance of sand fly species and their frequency of occurrence along six years sampling (see text for details) in rural sites at Santarém municipality, state of Pará, Brazil. Species exclusive to rural sites and shared species were depicted by triangles and full circles, respectively.

generally indicated some proportionality between the number shared and exclusive species in each year (except for 1998). At least, nine sand fly individuals of four species were captured in urban areas of Ilhéus, state of Bahia, while fourteen sand fly species and 3,375 individuals were trapped in rural areas (Carvalho *et al.* 2010). At Porto Nacional, state of Tocantins, comparisons of Index of Species Abundance (ISA) pointed that *L. longipalpis* was the dominant species in these two areas and more numerous in urban areas (Vilela *et al.* 2008), as also observed in the present study. Aguiar and Medeiros (2003), in their habitat description of Brazilian species of sand flies, mentioned that *E. carmelinoi* is usually found in crops and livestock areas. In the Santarém survey, this sand fly species was very abundant in domiciles of urban areas, although it was the second mostly caught in rural areas. *N. umbratilis* is a species associated to forested areas, but it was captured in urban areas, though in low number of specimens. *N. shawi*, *T. trichopyga* and *B. travassosi* have also been associated to forest habitats such as burrows of armadillo and other wild animals according to Aguiar and Medeiros (2003). In the present study, these sand fly species were sporadically sampled and the scarce number of specimens was trapped in urban areas of Santarém. The authors also pointed that *V. tuberculata* and *E. evandroi* could be found in the inner and external walls of human domiciles; however these species were exclusive to rural areas of Santarém municipality.

At Santarém, the most frequent species during the study were *L. longipalpis*, *E. carmelinoi* and *Bichromomyia flaviscutellata*. The last two species presented lower relative abundance in relation to the first one, responsible by the great dominance index values in both rural and urban areas. In spite of epidemic AVL scenario at several cities of Brazil (Gontijo and Melo 2004), the main vector at northern

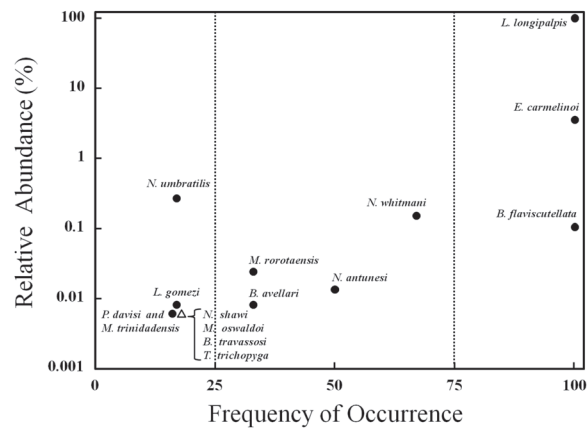


Figure 5 - Relative abundance of sand fly species and their frequency of occurrence along six years sampling (see text for details) in urban sites at Santarém municipality, state of Pará, Brazil. Species exclusive to urban sites and shared species were depicted by triangles and full circles, respectively.

country, *L. longipalpis*, is an inherently sylvatic species, caught also in intact forests with no human domicile nearby (Lainson and Rangel 2005). The transmission cycle of AVL was initially restricted to wild and rural areas. However, nowadays it acquired complexity in urban areas, with a widespread epidemic events in São Luís, Natal, Aracaju, Boa Vista, Santarém, Belo Horizonte, Montes Claros, Cuiabá, and Campo Grande, where the disease has been associated to urbanization process (Gontijo and Melo 2004).

Higher absolute abundances of *L. longipalpis* in rural and urban areas of Santarém seemed to be a constant threat to AVL control. Previous entomological surveys also recorded high density of this species at several peripheral suburbs of Santarém (Senra *et al.* 1985). In these surveys, it was pointed that the species natural infection rate by *Leishmania chagasi* reached approximately seven per cent. In the same districts, AVL positive ratio was 32.3% in dogs, with 119 and 13 human documented cases in urban and rural areas, respectively (Senra *et al.* 1985). Another study conducted in 2002-2003, *L. longipalpis* and *E. carmelinoi* were the most abundant sand fly species (59.7% and 35.5%, respectively) in three outlying neighborhoods of Santarém city (Feitosa and Castellon 2009), where it was verified a higher number of sand fly individuals in the rainy season and at domiciles nearest of the remnant forests.

The main species captured and associated to ACV vectors were *N. whitmani*, *B. flaviscutellata*, *Nyssomyia umbratilis*, *Psychodopygus davisi*, *P. wellcomei*, and *T. ubiquitous*. The first two Phlebotominae species seem to play major role in ACL transmission in Santarém due to their abundances (163 individuals of *N. whitmani* and 104 of *B. flaviscutellata*) compared to the other species. It is an evidence that these species are not becoming synanthropic, as observed in other

regions of Brazil (Teodoro *et al.* 1999; Rêbello 2001; Carvalho *et al.* 2010; Saraiva *et al.* 2011).

Leishmaniasis in Brazilian localities usually has shown a peridomestic transmission pattern, with adaptation of some vector sand fly species to modified environments, which also include domestic animals in the transmission cycle. However, cases of canine and human AVL in typical urban areas are now more frequent, suggesting a modification of the classic model of endemic area (Rêbello *et al.* 1999; Teodoro *et al.* 1993). Higher disease prevalence in women and children and markedly domiciliary and peridomestic presence of vectors reveal a tendency of sand fly adaptation to man-made habitats, where they are responsible for transmission of *Leishmania* spp. (Santos *et al.* 2000; Marzochi and Marzochi 1997). Sand fly captures in henhouse, pigsty, dog kennel, and other animal shelters, showed that such animals act as attraction factor (Rêbello 2001). Furthermore, some evidences also point out that phlebotomine insects have found ideal conditions to breed in these places or nearby them (Feitosa and Castellon 2009). Further studies in northern Brazil may consider the impact of forest removal (to human colonization) in the diversity and natural infection rates of sand flies (Pessoa *et al.* 2007), the differential infection rates in urban and rural areas and possible new breeding and resting habitats of these insects.

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