

Species diversity of sandflies (Diptera: Psychodidae) during different seasons and in different environments in the district of Taquaruçú, state of Tocantins, Brazil

Tâmara Oliveira Machado¹, Marcos Antônio Lima Bragança²,
Muzenilha Lima Carvalho³, José Dilermando Andrade Filho^{4/+}

¹Setor de Controle Vetorial, Gerência de Vigilância em Saúde, Secretaria Municipal de Saúde, Palmas, TO, Brasil
²Curso de Ciências Biológicas, Universidade Federal do Tocantins, Porto Nacional, TO, Brasil ³Núcleo de Entomologia, Secretaria Estadual de Saúde, Palmas, TO, Brasil ⁴Coleção de Flebotomíneos, Centro de Referência Nacional e Internacional para Flebotomíneos, Centro de Pesquisas René Rachou-Fiocruz, Belo Horizonte, MG, Brasil

Phlebotomine sandflies are the vectors for the protozoan parasites that cause leishmaniasis. The present study investigated the species composition of sandfly fauna in the rural district of Taquaruçú, municipality of Palmas, state of Tocantins, Brazil and compared the diversity of species among intradomicile, peridomicile and forest environments during the dry and rainy seasons. Sandflies were collected using CDC light traps over the course of three months during the dry and rainy seasons. A total of 767 specimens were captured, belonging to different 32 species. The most abundant species were Micropygomyia goiana (Martins, Falcão & Silva), Sciopemyia sordellii (Shannon & Del Ponte), Evandromyia carmelinoi (Ryan Fraiha, Lainson & Shaw), Evandromyia termitophila (Martins, Falcão & Silva), Nyssomyia whitmani (Antunes & Coutinho) and Lutzomyia longipalpis (Lutz & Neiva). The highest species diversity (30) and the greatest percentage of specimens (78.3%) were obtained during the rainy season. During the dry season, the species richness and abundance were greater in domestic environments. However, during the rainy season, the forest displayed the highest species richness and the domestic environment exhibited the greatest species abundance. Several important vector species are reported in this study.

Key words: *Evandromyia* - leishmaniasis - Phlebotominae - *Psathyromyia* - *Psychodopygus*

The phlebotomine sandflies belong to the subfamily Phlebotominae of the order Diptera. The females of most species are hematophagous and are responsible for the transmission of the protozoan parasites that cause visceral leishmaniasis (VL) and American cutaneous leishmaniasis (ACL) (Forattini 1973).

In 1988, the state of Tocantins (TO) was created by the Federal Constitution of Brazil. The creation of this state caused rapid socio-economic growth in this region that was often unplanned. Consequently, this rapid growth impacted the environment and may have led to public health problems in the state. For example, leishmaniasis has become a problem because sandflies adapt relatively easily to environmental changes and their presence is now common in urban and rural areas of Brazil (Leonardo & Rebêlo 2004, Nunes et al. 2008).

It is important to understand the species diversity and behaviour of sandflies in affected areas and during different seasons. For instance, if conditions are favourable for sandflies, they can initiate a new cycle of leishmaniasis that endangers the residents in these regions. For these reasons and because there is little information

about sandflies in TO, particularly in the rural district of Taquaruçú, this study aimed to identify the species composition of sandflies in this locality. In addition, the diversity of these insects was compared among intradomicile, peridomicile and forest environments during the dry and rainy seasons. These data will help support future actions to control leishmaniasis in this area.

The district of Taquaruçú is located in the Environmental Protection Area of Serra do Lajeado between the coordinates 10°18'S 48°09'W, 32 km from Palmas. The average annual rainfall in this region is 1.600 mm, with a distinct dry season occurring from April-September and a separate wet season from October-March (AM-ATP 2000). Since 2004, there has been no record of VL in this region and only three cases of ACL have been confirmed (SINAN 2012). Sampling sites located 4.4 km apart (site 1 and site 2) were selected for this study. At each sampling site, sandflies were collected in the following three environments: intradomicile (bedrooms), peridomicile (chicken coops) and extradomicile (forest). For sampling site 1, the edge of the forest was 20 m from the house and for sampling site 2 the edge of the forest was 10 m from the house.

Three CDC light traps were installed in each environment between 03:00 pm-05:00 pm and removed on the following day between 07:00 am-09:00 am. This collection procedure was performed over three consecutive nights in each month and collections were performed three times during the dry season (June-August 2007) and three times during the rainy season (November

+ Corresponding author: jandrade@cpqrr.fiocruz.br

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2007-January 2008). Identification of the collected sandfly species was performed according to the classification of Galati (2003) and the abbreviation of generic names followed the system of Marcondes (2007).

The statistical Past program version 1.8 (Hammer et al. 2001) was used to analyse the diversity (richness and equitability) and similarity of the collected sandflies. To quantify diversity, the Shannon-Wiener diversity index (H') was calculated as follows:

$$H' = - \sum_{i=1}^S p_i \ln p_i$$

where p = the frequency of species, \ln = natural log and S = species richness. For comparisons of diversity between the dry and rainy seasons, the intradomestic, peridomestic and forest environments and the months of each period we used t tests at a 5% probability. The Pielou evenness index (J) was obtained using the formula $J = H'/\ln S$, with S being the number of species per site. The Jaccard similarity index (c_j) was used for cluster analysis of the Jaccard distance according to the following equation:

$$(c_j) = c/(a + b + c)$$

where a = species found at site "a", b = species found in sample "b" and c = species found in both samples ("a" and "b"). Abundance analysis was performed using the chi-squared test with the Past program version 1.8.

A total of 767 phlebotomine sandflies were collected belonging to 32 different species (Table). The most highly represented subtribe was *Lutzomyia* with 14 species identified, followed by *Psychodopygina*, with 11 species identified, *Sergentomyia*, with six species identified and *Brumptomyia*, with one species identified. One morphospecies specimen could not be identified because only this single female of the morphospecies was found, although it may be either *Psychodopygus wellcomei* (Fraiha, Shaw & Lainson 1971) or *Psychodopygus complexa* (Mangabeira 1941). The most abundant species are shown in Table.

The average value of the H' was 2.54 and that of the J evenness index was 0.73. The species richness was highest in the rainy season; however, 18 species were common to both periods of the year. Two species were exclusive to the dry season and 12 species were found only in the rainy season (Table). The c_j between the rainy and dry seasons was 0.56.

Climate data for the district of Taquaruçu during the months in which the sandflies were captured showed that in the dry season the average rainfall was 0.0 mm, the temperature was 26.7°C and the relative humidity (RH) was 55.4%. In the rainy season, the average rainfall was 47.6 mm, the temperature was 26.8°C and the RH was 70.2%. The abundance of sandflies in the dry season (22%) was lower than in the rainy season (78%) and this difference was significant ($\chi^2 = 245.6$, $p < 0.01$). There was no significant difference in the H' between the two periods of the year ($t = 0.71$, $p = 0.47$). The J for the dry season was 0.81 and for the rainy season was 0.72.

The greatest species richness during the rainy season occurred in the forest (22 species), followed by the peridomestic (20 species) and intradomestic (19 species)

areas. The species distribution by environment is shown in Table. The cluster similarity analysis for both seasons indicated a greater similarity between the peridomestic and forest areas than with the intradomestic area.

The intradomestic areas presented the greatest abundance of sandflies during the dry period (42.8%), followed by the peridomestic (42.2%) and forest (15.1%) locations. During the rainy season, the peridomestic areas showed the highest percentage of sandflies (41.5%), followed by the intradomestic (32%) and forest (26.5%) locations.

All t test comparisons of H' values between the different environments during the dry season were non-significant ($p > 0.05$). However, during the rainy season, this index was higher in the peridomestic than the intradomestic area ($t = -2.75$, $p < 0.05$) and in the forest compared to the intradomestic ($t = -6.22$, $p < 0.05$) and peridomestic areas ($t = -4.15$, $p < 0.05$). The differences in H' values for the same environment between different periods of the year were not significant for the intradomestic and peridomestic areas ($p > 0.05$); however, this difference was significantly higher in the forest during the rainy season ($t = -2.28$, $p < 0.05$).

The values of the J for the dry season were 0.75, 0.87 and 0.94 for the intradomestic, peridomestic and forest environments, respectively. During the rainy season, this index decreased to 0.66, 0.75 and 0.86 for the intradomestic, peridomestic and forest environments, respectively.

The predominance of *Lutzomyia* can be explained by the presence of a large number of species of the genus *Evandromyia*. This group of sandflies is very common in the savannah (*cerrado*) regions of Brazil. Saraiva et al. (2008) found that 26.3% of the sandflies in the state of Minas Gerais were from the genus *Evandromyia*. Another frequently identified genus was *Psathyromyia*, represented by six species (18.8%). This genus is found in preservation areas, particularly forest environments (Almeida et al. 2010, Margonari et al. 2010).

The species richness of the 32 sandfly species observed in Taquaruçu was consistent with that found by Andrade Filho et al. (2001). Vilela et al. (2011) observed greater species richness, with 48 identified species, whereas Lustosa et al. (1986) found only 14 species. The species richness observed in this study was greater during the rainy season than during the dry season. This pattern has also been observed in several studies performed in the Northeast region of Brazil (Barros et al. 2000, Martin & Rebêlo 2006). However, Rebêlo et al. (2001) and Marinho et al. (2008) observed more species during the dry season in the state of Maranhão (MA). During the dry season, the greatest species richness was found in the intradomestic environment, followed by the peridomestic and the forest environments. This may be because at this time of year, the humidity is very low and fires are frequent, making the home environment more favourable for some species of sandflies, such as *Bichromomyia flaviscutellata* (Mangabeira 1942). Martin and Rebêlo (2006) obtained different results in MA, with the highest species richness being observed in the peridomestic environment, followed by the forest and intradomestic environments.

During the rainy season, the greatest number of species was observed in the forest, followed by the peridomicile and the intradomicile areas. The highest similarity was found between the peridomicile and forest areas, most likely because the peridomicile areas were close to the forest, which may have facilitated the movement of many species between these environments.

A greater abundance of sandflies was observed in the home environment in both intra and peridomicile areas for both periods studied, suggesting an increased risk of leishmaniasis transmission to humans and their pets dur-

ing these periods. de Souza et al. (2004) found an increase in the number of human cases of ACL and AVL after an increase in sandfly numbers after rain. The higher density of sandflies in the home compared to natural environments, such as forests, suggests that the presence of domestic animals and humans can cause some species to significantly increase in number in human environments. The increases in population density or abundance around homes show the degree of adaptation that sandflies have undergone associated with increasing anthropogenic activity (Missawa & Lima 2006, Saraiva et al. 2011) and

TABLE
Species of sandflies collected with CDC light trap in the district of Taquaruçu, Palmas, Tocantins, in the dry (June-August 2007) and rainy season (November 2007 and January 2008)

Species	Dry season environment				Rainy season environment				Total
	n	Intradomicile	Peridomicile	Forest	n	Intradomicile	Peridomicile	Forest	
<i>Micropygomyia goiana</i>	32	28.3	14.5	7.8	166	43.8	24.5	13.2	198
<i>Siopemyia sordellii</i>	10	2.8	7.2	11.5	95	10.4	22.5	12.0	105
<i>Evandromyia carmelinoi</i>	17	2.8	14.5	19.2	71	7.3	12.9	15.8	88
<i>Evandromyia termitophila</i>	6	2.8	4.4	3.8	67	14.1	10.1	9.4	73
<i>Nyssomyia whitmani</i>	32	18.3	24.6	7.8	39	8.3	4.4	7.6	71
<i>Lutzomyia longipalpis</i>	24	24	7.2	7.8	12	3.7	2	0	36
<i>Micropygomyia micropyga</i>	0	0	0	0	24	1.6	8	0.6	24
<i>Evandromyia evandroi</i>	11	5.6	8.7	3.8	9	1	1.2	2.5	20
<i>Pressatia choti</i>	3	1.4	1.5	3.8	15	1	0.8	6.9	18
<i>Psathyromyia hermanlenti</i>	7	1.4	4.4	11.5	10	0	0.4	5.7	17
<i>Evandromyia lenti</i>	6	1.4	5.8	3.8	11	1.6	2.4	1.3	17
<i>Micropygomyia longipennis</i>	3	1.4	1.5	3.8	11	2.1	2.4	0.6	14
<i>Psathyromyia runoides</i>	2	0	0	7.8	10	0	2.4	2.5	12
<i>Psathyromyia aragaoi</i>	2	0	1.5	3.8	9	0	1.6	3.1	11
<i>Evandromyia walkeri</i>	0	0	0	0	9	0.5	1.2	3.1	9
<i>Psathyromyia brasiliensis</i>	0	0	0	0	8	0	0.4	5	9
<i>Bichromomyia flaviscutellata</i>	1	1.4	0	0	6	0	0	3.8	7
<i>Evandromyia sallesi</i>	1	1.4	0	0	5	0	1.2	1.3	6
<i>Micropygomyia echinatopharynx</i>	3	1.4	2.9	0	2	0.5	0.4	0	5
<i>Evandromyia teratodes</i>	1	0	0	3.8	2	0.5	0	0.6	3
<i>Evandromyia saulensis</i>	0	0	0	0	3	0	0	1.9	3
<i>Micropygomyia oswaldoi</i>	3	4.2	0	0	0	0	0	0	3
<i>Psathyromyia punctigeniculata</i>	0	0	0	0	3	1.6	0	0	3
<i>Micropygomyia rorotaensis</i>	0	0	0	0	3	0	0	1.9	3
<i>Pintomyia christenseni</i>	1	0	1.5	0	1	0	0	0.6	2
<i>Martinsmyia oliveirai</i>	0	0	0	0	2	0	0.8	0	2
<i>Psychodopygus davisii</i>	0	0	0	0	2	0.5	0	0.6	2
<i>Evandromyia bacula</i>	1	1.4	0	0	0	0	0	0	1
<i>Psychodopygus complexa/welcomei</i>	0	0	0	0	1	0	0.4	0	1
<i>Evandromyia corumbaensis</i>	0	0	0	0	1	0.5	0	0	1
<i>Psathyromyia lutziana</i>	0	0	0	0	1	0.5	0	0	1
<i>Brumptomyia brumpti</i>	0	0	0	0	1	0.5	0	0	1
%	-	100	100	100	100	100	100	100	100
Total	166	71	69	26	601	192	250	159	767
Species total	20	16	14	14	30	19	20	22	32

these increases may be associated with cases of leishmaniasis (Rebêlo et al. 2001, de Souza et al. 2004).

Slightly more *Nyssomyia whitmani* specimens were captured in the home during the rainy season. This species is a vector of *Leishmania braziliensis* in some regions of Brazil (Luz et al. 2000, Saraiva et al. 2011). The preference of *Ny. whitmani* for domestic environments suggests that this species is well adapted to these areas in Taquaruçu and may be involved in the transmission of leishmaniasis, as previously observed by Martin and Rebêlo (2006) and Mayo et al. (1998) in other areas.

Lutzomyia longipalpis is the main vector of *Leishmania infantum chagasi* (Gontijo & Melo 2004) and has been reported in all regions of Brazil and in many countries of South America (Souza et al. 2009, Salomón et al. 2011). It is the most frequent and perhaps the best adapted species with respect to living with humans and domestic animals (Rebêlo et al. 1999). This species was found during both the dry and wet seasons and showed a preference for the dry period and household areas, particularly intradomicile areas. In Taquaruçu, the domestic environment and dry period appear to be more favourable for the transmission of AVL. This is important for epidemiological surveillance because of the risk of introduction of AVL to the district of Taquaruçu.

Other species were observed in addition to *Ny. whitmani* and *Lu. longipalpis*, though at lower frequencies; however, they included a number of important vectors for leishmaniasis, such as *Psychodopygus davisii*, *Psychodopygus complexus/Ps. wellcomei* and *Bi. flaviscutellata*. *Bi. flaviscutellata* is a zoophilic species and is considered to represent the vector of *Leishmania amazonensis* (Shaw 1972). The occurrence of this species in intradomicile areas is worrisome because according to Rebêlo et al. (1999), it appears that this species is adapting not only to secondary forests, but also to the home environment.

Evandromyia corumbaensis and *Evandromyia sallesi* belong to the *cortelezzii* complex, along with *Evandromyia spelunca* Carvalho, Sanguinette, Brazil & Andrade Filho 2011 and *Evandromyia cortelezzii* (Brêthes 1923) (Carvalho et al. 2011). This complex has a wide geographical distribution in Brazil (Carvalho et al. 2009) and two species, *Evandromyia sallesi* and *Ev. cortelezzii*, were recently found to be naturally infected with *Leishmania* (Carvalho et al. 2008, Saraiva et al. 2009).

Ps. wellcomei is present in the Amazon in Pará and in the forest zone of Pernambuco (Ward et al. 1973, Dantas-Torres et al. 2010). This sandfly is responsible for the transmission of *L. braziliensis* (Ryan et al. 1987). The distribution of *Ps. complexus* is restricted to the Amazon Basin and MA (Young & Duncan 1994, Martin & Rebêlo 2006). In this study, the only specimen of *Ps. wellcomei/Ps. complexus* collected could not be further identified because no males were found and females of these species are indistinguishable from each other (Young & Duncan 1994). Both species may be involved in the transmission of cutaneous leishmaniasis in northern Brazil (Souza et al. 1996). Despite this taxonomic uncertainty, detection of *Ps. wellcomei* or *Ps. complexus* can now be reported in TO.

The highest value of H' was observed in the forest, followed by the peridomicile and intradomicile areas

during the rainy season. This finding differs from those of Martin and Rebêlo (2006), who found greater diversity in intradomicile areas, followed by forest and peridomicile areas. The increase in the number of species in the forest during the rainy season was most likely due to greater structural variety with regard to the available habitats and ecological niches (Ricklefs 1996).

The dry season was characterised by a more even distribution of sandflies among the environments than the rainy season, consistent with the findings of Barros et al. (2000). These findings indicate that individuals are more evenly distributed in the dry forest environment than in the other environments studied.

Among studies previously conducted in TO, 58 species of sandflies had been reported (Lustosa et al. 1986, Andrade Filho et al. 2001, Carvalho et al. 2010, Vilela et al. 2011). Of the species collected, four (*Martinsmyia oliveirai*, *Psathyromyia runoides*, *Pressatia choti* and *Ps. wellcomei/Ps. complexa*) were first observed in TO. Thus, with the information presented here, the number of species of sandflies reported in TO is increased to a total of 62.

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