

## PHLEBOTOMINE FAUNA (DIPTERA: PSYCHODIDAE) IN AN AREA OF FISHING TOURISM IN CENTRAL-WESTERN BRAZIL

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### SUMMARY

The aim of this study was to identify behavioral aspects of the sandfly fauna of a fishing tourism area in the municipality of Bonito (MS). Monthly captures were undertaken from December 2009 to November 2010, using automatic CDC type light traps, from 18h00 to 06h00, in a forested area, a savannah area, peridomiciles and animal shelters near peridomiciliary areas. *Nyssomyia whitmani* was the most frequent out of a total of 6,699 specimens collected, belonging to 16 species, followed by *Psathyromyia bigeniculata* and *Lutzomyia longipalpis*, found in all the environments investigated, though in their greatest numbers in the animal shelters. *Ny. whitmani* exhibited its highest frequencies during the dry months, coincident with the fishing season, when the risk of transmission of cutaneous leishmaniasis for tourists and inhabitants increases. Noteworthy was the finding of two species naturally infected by flagellates: *Ny. whitmani* and *Pa. bigeniculata*. The local population and visiting tourists should be warned of the threat posed by leishmaniasis and the health authorities alerted to the need for adopting environmental sanitary measures, especially regarding such animal shelters as they seem to provide favorable conditions to the proliferation, maintenance and breeding opportunities of phlebotomines.

**KEYWORDS:** Sandflies; Leishmaniasis; Natural infection; Animal's shelters; Vectors; Ecotourism.

### INTRODUCTION

American visceral leishmaniasis (AVL) has been recorded in increasing numbers of human and canine cases in the state of Mato Grosso do Sul (MS), which is one of the states with the greatest incidences in the central-western region of Brazil<sup>4,6,26</sup>. Meanwhile, American cutaneous leishmaniasis (ACL) has been recorded in the majority of municipalities<sup>12,17</sup>. However, despite the wide distribution and growing number of human cases, epidemiological studies on leishmaniasis in MS are few and far between.

In Bonito (MS), which is a municipality classified at a moderate transmission level of leishmaniasis, studies have indicated the occurrence of both human and canine cases of AVL and ACL, and these diseases are spreading due to the implementation of ecotourism and livestock activities in the area<sup>4,16,24</sup>.

Three species of *Leishmania* (Ross) have already been reported in MS: *Leishmania (Leishmania) infantum chagasi* Cunha & Chagas, *Leishmania (Leishmania) amazonensis* Lainson & Shaw and *Leishmania (Viannia) braziliensis* Vianna and their respective vectors, *Lutzomyia longipalpis* (Lutz & Neiva), *Bichromomyia flaviscutellata* (Mangabeira) and *Nyssomyia whitmani* (Antunes & Coutinho), all of which are found

in both rural and urban areas<sup>3,11,12,17,24,25</sup>.

The Águas do Miranda district, has fishing tourism as its main economic source and presents socio-economic and environmental conditions favorable to the transmission of the endemic diseases under consideration. These facts together with the results of research into the canine population of the district, which have shown 40% out of the 92 animals as seropositive for *Leishmania* (VLB Nunes, unpublished data), have motivated the present study for the purpose to identify behavioral aspects of the sandfly fauna, including its species abundance, diversity, evenness, monthly distribution and natural infection by flagellates, to identify potential vectors of *Leishmania* spp.

### MATERIAL AND METHODS

**Study locality:** Águas do Miranda District (20° 45' 44.4"S, 56° 05' 42.8"W) is 75 km from the municipality of Bonito and 180 km from Campo Grande, the capital of the state of MS. The permanent human population consists of 450 inhabitants, which may rise to as many as 10,000 in the fishing season, from March to October. The local economy is based mainly on fishing and the tourist trade<sup>29</sup>.

The prevalent vegetation belongs to the great savannah ("cerrado")

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domain; however, it presents particularities associated with local environmental conditions, such as forest patches in areas with more fertile soil and a more plentiful supply of water. Noteworthy is the vegetation cover of the Bodoquena range, a typical forest associated with calcareous rocks known as Dry Forest or Submontane Semideciduous Seasonal Tropical Forest. The climate is tropical with an annual average temperature of 22 °C<sup>29,38</sup>.

**Capture sites:** A total of nine sites were sampled in different environments (Fig. 1): peridomiciliary areas near fruit trees, native grass and tuberous vegetables (A1), native species of trees, fruit trees and bamboos (A2) and bordered by a stream, within a bamboo grove, near fruit and ornamental trees (A3); area of savannah with selective extraction of timber and native species of trees (A4); gallery forests with some secondary vegetation which grew after the selective extraction of timber (A5, A6) and animal shelters such as a pigsty (A7), a henhouse (A8) and a perch (A9), near peridomiciliary areas.



Fig. 1 - Distribution of capture sites in the district of Águas do Miranda, in the municipality of Bonito, Mato Grosso do Sul, from December 2009 to November 2010, Brazil 04/30/2012. Source: Google Earth.

**Methodology:** The phlebotomines were captured on three consecutive nights, once a month, during the period from December 2009 to November 2010 using modified automatic light CDC traps<sup>23</sup> from 18h00 to 06h00. One trap was installed per night in each of the nine sites sampled.

The insects captured with the CDCs were transferred to nylon cages. The females were recaptured with polyethylene tubes, in which they were anaesthetized with sulfuric ether. Then, after dissection to expose the gut and spermathecae, under stereomicroscopy, they were examined under an optical microscope (400x) for identification of the phlebotomine species and investigation of flagellates. The male insects were kept in Petri dishes under refrigeration until their clarification in accordance with the technique given by FORATTINI (1973)<sup>13</sup>. Species identification was undertaken in accordance with GALATI (2003)<sup>18</sup> and the abbreviation of the species names follows MARCONDES (2007)<sup>22</sup>.

The flagellates found in the gut of the dissected females of two sand fly species were inoculated intradermally, in the hind legs of hamsters (*Mesocricetus auratus*). The animals were observed weekly during 12 months for checking the appearance of lesions. After euthanasia, the spleens of these animals were removed and inoculated into NNN culture medium (blood agar) for isolation of the parasites. Cultures were maintained at 25 °C and examined weekly for one month to observe if there was proliferation of flagellates.

The pluviometric data used in the analysis was obtained from the Aquidauna meteorological station, the nearest to Águas do Miranda district, at about 30 km away.

**Data analysis:** Species abundance was calculated for all the ecotopes investigated in accordance with ROBERTS & HSI (1979)<sup>31</sup>. Initially the Index of Species Abundance (ISA) was obtained by the application of the formula:  $ISA = a + R_j/k$ ; where: **a** = number of ecotopes investigated in which the given species was not present, multiplied by **c**; **c** being obtained as follows: a ranking of the species was established, ranging from 1.0 to N (attributing the value of 1.0 to the most abundant species), for each ecotope. The highest value obtained in the ranking of the species (taking all the ecotopes into consideration) + 1.0 = **c**; **R<sub>j</sub>** = the sum of the positions in the ranking of a particular species in all the ecotopes and **k** = the number of ecotopes sampled.

The Standard Index of Species Abundance (SISA) was used to convert ISA into a scale of 0 to 1.0. According to this, the most abundant species are those which are closer to 1.0. The formula used for the calculation is:  $SISA = c-ISA/c-1$ .

The diversity and evenness were obtained, respectively, by using Shannon's Diversity Index (H) and that of Pielou (J). In accordance with HAYEK & BUZAS (1997)<sup>19</sup>, the respective formulae are:

$$H = - \sum p (\ln p); p: \text{frequency of each species in a particular ecotope};$$
$$J = H/\ln s; s: \text{number of species in each ecotope}.$$

The project was submitted to the Ethics Committee on Animal Use in Research (CEUA) Anhanguera-UNIDERP University and approved according to opinion No. 63-006/09.

## RESULTS

A total of 6,699 phlebotomine specimens were captured, *Brumptomyia avellari* (Costa Lima), *Br. brumpti* (Larrousse), *Brumptomyia* sp., *Evandromyia* sp. (Cortezzi complex), *Ev. lenti* (Mangabeira), *Ev. termitophila* (Martins, Falcão & Silva), *Lutzomyia longipalpis* (Lutz & Neiva), *Sciopemyia sordellii* (Shannon & Del Ponte), *Nyssomyia neivai* (Pinto), *Ny. whitmani* (Antunes & Coutinho), *Psathyromyia aragaii* (Costa Lima), *Pa. campograndensis* Oliveira, Andrade Filho, Falcão & Brazil, *Pa. hermalenti* (Martins, Silva & Falcão), *Pa. bigeniculata* (Floch & Abonnenc), *Pa. punctigeniculata* (Floch & Abonnenc) and *Micropygomyia quinquefer* (Dyar) (Table 1).

The greatest phlebotomine species richness (15) and frequency (95.35%) occurred in the henhouse (A8), representing almost the totality of the specimens captured during the period studied. On the other hand, the lowest species richness (3) occurred in the pigsty (A7) and the lowest

**Table 1**

Phlebotomines of both sexes captured with light traps in distinct environments, in the district of Águas do Miranda, Bonito municipality, Mato Grosso do Sul, Brazil, December 2009 to November 2010

Environments	Peridomiciles			Forest and savannah			Animal shelters			Total	%	SISA
	A1	A2	A3	A4	A5	A6	A7	A8	A9			
Species ecotopes	A1	A2	A3	A4	A5	A6	A7	A8	A9			
<i>Brumptomyia</i> sp.	1	-	1	-	-	-	-	-	-	2	0.03	0.164
<i>Br. avellari</i>	-	-	-	1	1	-	-	1	-	3	0.04	0.107
<i>Br. brumpti</i>	-	1	1	3	2	-	-	8	-	15	0.22	0.342
Cortelezzii complex	1	2	2	1	24	2	-	95	1	128	2.06	0.678
<i>Ev. lenti</i>	-	3	-	23	3	-	-	9	-	38	0.57	0.357
<i>Ev. termitophila</i>	-	-	-	-	-	-	-	1	-	1	0.01	0.014
<i>Lu. longipalpis</i>	4	5	1	18	5	9	8	885	1	936	13.80	0.853
<i>Mi. quinquefer</i>	-	-	-	-	-	-	-	4	-	4	0.07	0.043
<i>Ny. neivai</i>	-	1	1	-	-	25	-	323	23	373	6.44	0.457
<i>Ny. whitmani</i>	3	-	4	34	9	9	6	3270	7	3342	49.26	0.830
<i>Pa. aragaoi</i>	-	-	-	9	-	-	-	2	-	11	0.18	0.107
<i>Pa. campograndensis</i>	-	-	2	-	-	-	-	4	-	6	0.10	0.135
<i>Pa. hermanlenti</i>	-	-	-	3	1	-	-	4	-	8	0.12	0.164
<i>Pa. punctigeniculata</i>	-	-	-	-	1	-	-	8	-	9	0.13	0.114
<i>Pa. bigeniculata</i>	-	1	2	9	19	10	2	1773	4	1820	26.90	0.764
<i>Sc. sordellii</i>	-	-	-	1	1	-	-	1	-	3	0.06	0.107
Total	9	13	14	102	66	55	16	6388	36	6699	100	
%	0.13	0.19	0.21	1.52	1.00	0.82	0.24	95.35	0.54		100	
Shannon (H)	1.21	1.58	1.95	1.78	1.69	1.38	0.97	1.23	1.05	1.30		
Pielou (J)	0.87	0.88	0.94	0.77	0.73	0.85	0.89	0.45	0.65	0.47		

A1, A2, A3: peridomiciliary areas, A4: savannah, A5: gallery forest, A6: gallery forest, A7: pigsty, A8: henhouse, A9: perch. H: Shannon's diversity; J: Pielou's evenness; %: percentage, SISA: standardized index of species abundance.

frequencies in the peridomiciles (Table 1).

The highest diversity indices were recorded in peridomicile (A3) and in a savannah area (A4) and the lowest in the pigsty (A7). The highest values of the indices were low. The henhouse (A8) had the greatest species richness, but the evenness index was the lowest (Table 1).

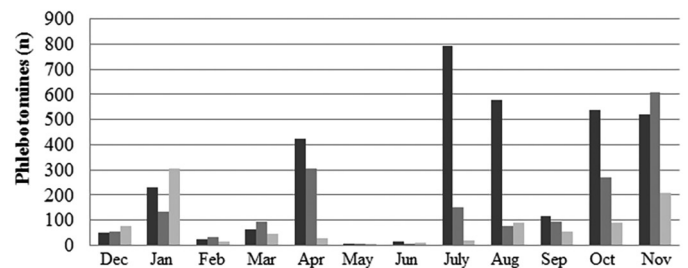
*Lu. longipalpis* was the most abundant species, followed by *Ny. whitmani*, *Pa. bigeniculata*, and the complex species *cortelezzii* and *Ny. neivai* (Table 1).

The distribution of the three most abundant species captured in all the ecotopes sampled is shown in Figure 2. *Lu. longipalpis* presented peaks in January and November, *Ny. whitmani* in July, August, October and November and *Pa. bigeniculatus* in November.

The monthly distribution of rainfall, average temperature and relative humidity is shown in Figure 3.

The rates of natural infection detected by optical microscopy were

■ *Ny. whitmani* ■ *Pa. bigeniculata* ■ *Lu. longipalpis*



**Fig. 2** - Number of specimens of both sexes collected per month of the species *Lu. Longipalpis*, *Ny. whitmani* and *Pa. bigeniculata*, in the district of Águas do Miranda, Bonito, Mato Grosso do Sul, December 2009 to November 2010.

0.07% (1/1418) for *Ny. whitmani* and 0.23% (1/408) for *Pa. bigeniculata*. The flagellates were observed in the hindgut and midgut.

The animals inoculated with the gut *Ny. whitmani* and *Pa. bigeniculata* containing flagellates did not develop a lesion during the

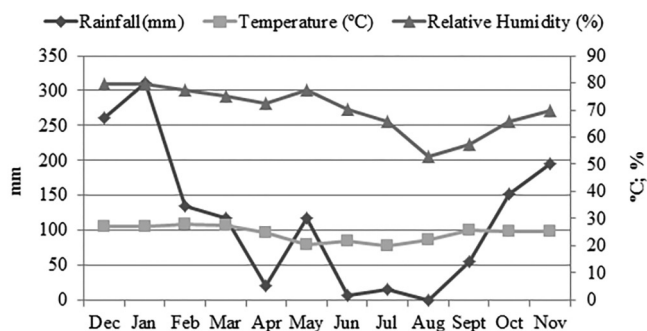


Fig. 3 - Monthly distribution of rainfall (mm), temperature (°C) and relative humidity (%) in the municipality of Bonito, from December 2009 to November 2010.

observation period and in the cultures in the hamsters' spleens showed no growth of flagellated form.

## DISCUSSION

The greatest frequency and species richness of the phlebotomines captured occurred in anthropic environments, probably attracted to the peridomicile due to blood meal sources represented by domestic and synanthropic animals. The predominance of *Ny. whitmani* in a henhouse (A8) near native forest suggests that this species has a close relationship with both wild and anthropic environments, in which the forest serves as a shelter and breeding place for adults, as do the shaded areas in the peridomicile<sup>7</sup>. Furthermore, the walls and roof of the henhouse, as well as the chickens can serve as substrates for males waiting for the opportunity to mate with the females seeking blood meal sources in this ecotope, since the males of hematophagous insects, dispersed throughout their habitats, may obtain a mating advantage by staying near the host and waiting for the females to arrive<sup>1</sup>. It is noteworthy that one specimen of this species, naturally infected by flagellates, was captured in this henhouse, suggesting that this ecotope is attractive to the synanthropic animals which constitute the *Leishmania* reservoir or that the infected sand fly had moved from the forest to the henhouse .

The highest diversity and evenness indices in Águas do Miranda, especially in the peridomiciliary environment, may demonstrate the presence of these insects in areas of preserved forests and anthropic action in these locations. The findings of this study differed from those of NUNES *et al.* (2008)<sup>24</sup> and ANDRADE *et al.* (2009)<sup>4</sup>, which found lower values in urban areas of the municipality of Bonito. The highest frequency of *Ny. whitmani* (49.26%) in the henhouse (A8) may explain the lowest evenness and diversity despite being the ecotope with the highest species richness.

The most abundant species calculated by SISA were *Lu. longipalpis* and *Ny. whitmani*, which showed a wide distribution of these species in the ecotopes sampled, this indicates that these species may be participating in the cycle of transmission of leishmaniasis agents in the area, also observed in others areas of the municipality of Bonito and the state of Mato Grosso do Sul<sup>4,17,24,25</sup>.

The natural infection rate found for *Ny. whitmani* (0.07%) was lower than that recorded by GALATI *et al.* (1996)<sup>17</sup> in the Corguinho

municipality (MS) where a rate of 0.16% was recorded in 613 dissected females. Although both these rates are low, which is usually the case when only optical microscopy is used, the numbers of the infected sources of the parasite in the areas of the present study seem to be much lower than those at Corguinho, which may be explained as due to the higher level of anthropic activity in Águas do Miranda. Low frequencies of this species in the other animal shelters (pigsty and perch) corroborate the results found by NUNES (2008)<sup>24</sup> and GALATI *et al.* (2003)<sup>16</sup>.

In this study, *Ny. whitmani* was more abundant in the cold, dry period, a result similar to that found by GALATI *et al.* (1996)<sup>17</sup> in MS, and also in the state of Rio de Janeiro (RJ) by SOUZA *et al.* (2002)<sup>36</sup>. It is important to note that the greater part of this dry, cold period coincides with the fishing season (March to October), thus indicating the concomitant risk of the transmission of cutaneous leishmaniasis to both the local population and visiting tourists.

*Lu. longipalpis* was found in anthropic environments in which animals are reared, but is also habitual in other environments, both rural and urban, where AVL and canine visceral leishmaniasis (CVL) occur<sup>4,24,25</sup>, which suggests that this species may be the vector responsible for the transmission of *Le. i. chagasi* to the canine population of the district, which, in a research project undertaken in 2009, presented 40% seropositive dogs for *Le. i. chagasi* in a population of 92; the parasite isolated by the Polymerase Chain Reaction (PCR) technique being *Le. i. chagasi* (VLB Nunes, unpublished data). These observations have been found in other areas of the country and the public health authorities should be alerted, since that CLV cases precede human AVL and the dog has a fundamental role in the domestic transmission<sup>8,20</sup>.

*Pa. bigeniculata*, considered for long time as a junior synonym of *Pa. shannoni*, recently had its status of species resurrected. The difference between these two species is mainly the thoracic coloration, i.e. while *Pa. shannoni* presents pronotum and paratergite straw, prescutum, scutum, and scutellum brown, pleurae off-white, *Pa. bigeniculata* presents pronotum, paratergite, prescutum, scutum, and scutellum brown, upper anepisternum straw and the other pleural off-white sclerites<sup>33</sup>.

*Pa. shannoni* is considered in the United States an important arbovirus vector<sup>9</sup> and has been captured naturally infected by *Leishmania mexicana* in peri-urban areas of Mexico<sup>34</sup>, by *Leishmania* sp. in Guatemala<sup>32</sup> and also developed experimental infection with *L. i. chagasi* when feeding on infected dog<sup>37</sup>. *Ps. shannoni s. lat* has been associated with the transmission of *Endotrypanum schaudinni*, a trypanosomatid of sloths<sup>14</sup>, and was found naturally infected by *Leishmania* sp. in Serra do Baturité, in the northeastern region of Brazil<sup>30</sup>. The finding of *Pa. bigeniculata* naturally infected by flagellates, in a henhouse close to a forest, demonstrates the need to clarify its epidemiological significance in relation to anthropophily and the transmission of the leishmaniasis agent, especially because it presents close affinity with *Pa. shannoni* for which there are records of its infection, being either natural or experimental, by *Leishmania* spp.<sup>32,37</sup>.

*Ny. neivai* presents no widespread geographical distribution in the state of Mato Grosso do Sul, being found mainly in the southeastern and eastern mesoregions<sup>2,5</sup>. It is worth highlighting the considerable abundance of the species found in this study, especially in animal shelters, because it has been reported as naturally infected by *Leishmania* and is

suspected of involvement in the transmission of cutaneous leishmaniasis by *Le. braziliensis* in such Brazilian states<sup>15,22,27,28</sup> and also in neighboring countries<sup>10</sup>.

The Cortelezzii complex includes the species *Ev. cortelezzii*, *Ev. sallesi* and *Ev. corumbaensis*, which are all found in MS<sup>2,3,17</sup>. The only possible way to distinguish them with confidence is by using males. As only females were identified in this study (data not given), it was impossible to identify the species of this complex. Recent studies have reported the natural infection of *Ev. cortelezzii* and *Ev. sallesi* by *Leishmania*<sup>3,35</sup>, thus calling for studies on their vectorial capacity, since many of the localities where they have been captured are endemic for leishmaniasis, as in the area covered by the present study.

The District of Águas do Miranda revealed a diverse sandfly fauna, with 16 species, some of them proven vectors of leishmaniasis agents in Brazil and others that have been described as naturally infected and which may, therefore, act as potential vectors. In light of the above, the local population and visiting tourists should be warned of the threat posed by leishmaniasis and the health authorities alerted of the need for adopting environmental sanitary measures, especially regarding such animal shelters, as they seem to provide favorable conditions to the proliferation, maintenance and breeding opportunities of phlebotomines.

## RESUMO

### Fauna flebotomínea (Diptera: Psychodidae) em área de turismo pesqueiro no Centro-Oeste do Brasil

O objetivo deste estudo foi identificar aspectos do comportamento da fauna flebotomínea de área de turismo pesqueiro localizada no município de Bonito (MS). Foram realizadas capturas mensais no período de dezembro de 2009 a novembro de 2010, utilizando armadilhas luminosas automáticas do tipo CDC das 18:00h às 6:00h, em matas, área de cerrado, peridomicílios e abrigos de animais próximos a áreas peridomiciliares. De um total de 6.699 espécimes coletados, pertencentes a 16 espécies, *Nyssomyia whitmani* foi a mais frequente, seguida de *Psathyromyia bigeniculata* e *Lutzomyia longipalpis*, encontradas em todos os tipos de ambientes, porém com maior expressão em abrigos de animais. *Ny. whitmani* apresentou frequências mais elevadas nos meses mais secos, coincidentes com a estação da pesca, o que eleva o risco de transmissão da leishmaniose tegumentar a turistas e moradores da área. Importante ressaltar o encontro de duas espécies naturalmente infectadas por flagelados: *Ny. whitmani* e *Pa. bigeniculata*. A população local e turistas devem ser advertidos da ameaça que representam as leishmanioses e as autoridades de saúde alertadas para adoção de medidas de saneamento ambiental, principalmente com relação aos abrigos de animais, que parecem fornecer condições favoráveis para a proliferação, manutenção e reprodução de flebotomíneos.

## CONFLICT OF INTEREST

The authors declare there is no conflict of interests.

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## REFERENCES

- Alexander B, de Carvalho RL, McCallum H, Pereira MH. Role of the domestic chicken (*Gallus gallus*) in the epidemiology of urban visceral leishmaniasis in Brazil. *Emerg Infect Dis*. 2002;8:1480-5.
- Almeida PS, Leite JA, Araújo AD, Batista PM, Touro RB, Araújo VS, et al. Fauna of phlebotomine sand flies (Diptera, Psychodidae) in areas with endemic American cutaneous leishmaniasis in the State of Mato Grosso do Sul, Brazil. *Rev Bras Entomol*. 2013;57:105-12.
- Andrade AR, Dorval ME, Andrade SM, Marques A, Lima-Junior MS, Silva BAK, et al. First report of natural infection of phlebotomines for *Leishmania (Leishmania) chagasi* captured in Ponta Porã, on the border between Brazil and Paraguay. *Asian Pac J Trop Dis*. 2011;1:253-8.
- Andrade AR, Nunes VL, Galati EA, de Arruda CC, Santos MF, Rocca ME, et al. Epidemiological study on leishmaniasis in an area of environmental tourism and ecotourism, state of Mato Grosso do Sul, 2006-2007. *Rev Soc Bras Med Trop*. 2009;42:488-93.
- Andrade-Filho JD, Galati EA, Falcão AL. *Nyssomyia intermedia* (Lutz & Neiva, 1912) and *Nyssomyia neivai* (Pinto, 1926) (Diptera: Psychodidae: Phlebotominae) geographical distribution and epidemiological importance. *Mem Inst Oswaldo Cruz*. 2007;102:481-7.
- Brasil. Ministério da Saúde. Sistema de Informação de Notificação de Agravos de Notificação (SINAN). Brasília; Ministério da Saúde, 2010. Available from: [http://portal.saude.gov.br/portal/arquivos/pdf/2\\_lv\\_casos\\_14\\_10\\_10.pdf](http://portal.saude.gov.br/portal/arquivos/pdf/2_lv_casos_14_10_10.pdf)
- Casanova C. A soil emergence trap for collection of phlebotomine sand flies. *Mem Inst Oswaldo Cruz*. 2001;96:273-5.
- Colla-Jacques FE, Casanova C, Prado AP. Study of sand fly fauna in an endemic area of American cutaneous leishmaniasis and canine visceral leishmaniasis in the municipality of Espírito Santo do Pinhal, São Paulo, Brazil. *Mem Inst Oswaldo Cruz*. 2010;105:208-15.
- Comer JA, Tesh RB, Modi GB, Corn JL, Nettles VF. Vesicular stomatitis virus, New Jersey serotype: replication in and transmission by *Lutzomyia shannoni* (Diptera: Psychodidae). *Am J Trop Med Hyg*. 1990;42:483-90.
- Córdoba-Lanús E, De Grosso ML, Piñero JE, Valladares B, Salomón OD. Natural infection of *Lutzomyia neivai* with *Leishmania* spp. in northwestern Argentina. *Acta Trop*. 2006;98:1-5.
- Dorval ME, Alves TP, Cristaldo G, Rocha HC, Alves MA, Oshiro ET, et al. Sand fly captures with Disney traps in area of occurrence of *Leishmania (Leishmania) amazonensis* in the State of Mato Grosso do Sul, mid-western Brazil. *Rev Soc Bras Med Trop*. 2010;43:491-5.
- Dorval ME, Oshiro ET, Cupollilo E, Castro AC, Alves TP. Ocorrência de leishmaniose tegumentar americana no Estado do Mato Grosso do Sul associada à infecção por *Leishmania (Leishmania) amazonensis*. *Rev Soc Bras Med Trop*. 2006;39:43-6.
- Forattini OP. Entomologia médica. São Paulo; Edgard Blücher; 1973. p. 658.
- Franco AM, Grimaldi G Jr. Characterization of *Endotrypanum* (Kinetoplastida: Trypanosomatidae), a unique parasite infecting the neotropical tree sloths (Edentata). *Mem Inst Oswaldo Cruz*. 1999;94:261-8.
- Galati EA, Fonseca MB, Marassá AM, Bueno EF. Dispersal and survival of *Nyssomyia intermedia* and *Nyssomyia neivai* (Diptera: Psychodidae: Phlebotominae) in a cutaneous leishmaniasis endemic area of the speleological province of the Ribeira Valley, state of São Paulo, Brazil. *Mem Inst Oswaldo Cruz*. 2009;104:1148-58.

16. Galati EA, Nunes VL, Cristaldo G, Rocha HC. Aspectos do comportamento da fauna flebotomínea (Diptera:Psychodidae) em foco de leishmaniose visceral e área adjacente, estado de Mato Grosso do Sul, Brasil. *Rev Patol Trop*. 2003;32:235-61.
17. Galati EA, Nunes VL, Dorval ME, Oshiro ET, Cristaldo G, Espíndola MA, *et al*. Estudo dos flebotomíneos (Diptera: Psychodidae) em área de leishmaniose tegumentar no estado de Mato Grosso do Sul, Brasil. *Rev Saúde Pública*. 1996;30:115-28.
18. Galati EA. Classificação de Phlebotominae e morfologia e taxonomia Phlebotomine. In: Rangel EF, Lainson R, organizadores. *Flebotomíneos do Brasil*. Rio de Janeiro; Fiocruz, 2003. p. 23-51.
19. Hayek LA, Buzas MA. *Surveying natural populations*. New York; Columbia University; 1997. p. 347-89.
20. Krauspenhar C, Beck C, Sperotto V, Silva AA, Bastos R, Rodrigues L. Leishmaniose visceral em um canino de Cruz Alta, Rio Grande do Sul, Brasil. *Cienc Rural*. 2007;37:907-10.
21. Marcondes CB, Bittencourt IA, Stoco PH, Eger I, Grisard EC, Steindel M. Natural infection of *Nyssomyia neivai* (Pinto, 1926) (Diptera: Psychodidae, Phlebotominae) by *Leishmania (Viannia) spp.* in Brazil. *Trans R Soc Trop Med Hyg*. 2009;103:1093-7.
22. Marcondes CB. A proposal of generic and subgeneric abbreviations of phlebotomines sandflies (Diptera: Psychodidae: Phlebotominae) of the world. *Entomol News*. 2007;118:351-6.
23. Natal D, Marucci D, Reis IM, Galati EA. Modificação da armadilha CDC com testes para coletas de flebotomíneos (Diptera). *Rev Bras Entomol*. 1991;35:697-700.
24. Nunes VL, Galati EA, Cardozo C, Rocca ME, Andrade AR, Santos MF, *et al*. Estudo de flebotomíneos (Diptera: Psychodidae) em área urbana do município de Bonito, Mato Grosso do Sul, Brasil. *Rev Bras Entomol*. 2008;52:446-51.
25. Oliveira AG, Galati EA, de Oliveira O, de Oliveira GR, Espindola IA, Dorval ME, *et al*. Abundance of *Lutzomyia longipalpis* (Diptera: Psychodidae: Phlebotominae) and urban transmission of visceral leishmaniasis in Campo Grande, state of Mato Grosso do Sul, Brazil. *Mem Inst Oswaldo Cruz*. 2006;101:869-74.
26. Oliveira AL, Paniago AM, Dorval ME, Oshiro ET, Leal CR, Sanches M, *et al*. Foco emergente de leishmaniose visceral em Mato Grosso do Sul. *Rev Soc Bras Med Trop*. 2006;39:446-50.
27. Oliveira DM, Reinhold-Castro KR, Bernal MV, Legriffon CM, Lonardon MV, Teodoro U, *et al*. Natural infection of *Nyssomyia neivai* by *Leishmania (Viannia) spp.* in the State of Paraná, Southern Brazil, detected by multiplex polymerase chain reaction. *Vector Borne Zoonotic Dis*. 2011;11:137-43.
28. Pita-Pereira D, Souza GD, Zwestsch A, Alves CR, Britto C, Rangel EF. First report of *Lutzomyia (Nyssomyia) neivai* (Diptera: Psychodidae: Phlebotominae) naturally infected by *Leishmania (Viannia) braziliensis* in a periurban area of south Brazil using a multiplex polymerase chain reaction assay. *Am J Trop Med Hyg*. 2009;80:593-5.
29. Prefeitura Municipal de Bonito. Relatório de planejamento da Prefeitura Municipal de Bonito. Bonito; Prefeitura Municipal, 2009.
30. Queiroz RG, Vasconcelos IA, Vasconcelos AW, Pessoa FA, de Sousa RN, David JR. Cutaneous leishmaniasis in Ceara State in Northeastern Brazil: incrimination of *Lutzomyia whitmani* (Diptera: Psychodidae) as a vector of *Leishmania braziliensis* in Baturité municipality. *Am J Trop Med Hyg*. 1994;50:693-8.
31. Roberts DR, Hsi BP. An index of species abundance for use with mosquito surveillance data. *Environ Entomol*. 1979;7:1007-13.
32. Rowton E, de Mata M, Rizzo N, Navin T, Porter C. Vectors of *Leishmania braziliensis* in the Petén, Guatemala. *Parassitologia*. 1991;33:501-4.
33. Sábio PB, Andrade AJ, Galati EA. Assessment of the taxonomic status of some species included in the Shannoni Complex, with the description of a new species of *Psathyromyia* (Diptera: Psychodidae: Phlebotominae). *J Med Entomol*. 2014;51:331-41
34. Sánchez-García L, Berzunza-Cruz M, Becker-Fausser I, Rebollar-Téllez EA. Sand flies naturally infected by *Leishmania (L.) mexicana* in the peri-urban area of Chetumal city, Quintana Roo, México. *Trans R Soc Trop Med Hyg*. 2010;104:406-11.
35. Saraiva L, Carvalho GM, Gontijo CM, Quaresma PF, Lima AC, Falcão AL, *et al*. Natural infection of *Lutzomyia neivai* and *Lutzomyia sallesi* (Diptera: Psychodidae) by *Leishmania infantum chagasi* in Brazil. *J Med Entomol*. 2009;46:1159-63.
36. Souza NA, Andrade-Coelho CA, Vilela ML, Peixoto AA, Rangel EF. Seasonality of *Lutzomyia intermedia* and *Lutzomyia whitmani* (Diptera: Psychodidae: Phlebotominae), occurring sympatrically in area of cutaneous leishmaniasis in the State of Rio de Janeiro, Brazil. *Mem Inst Oswaldo Cruz*. 2002;97:759-65.
37. Travi BL, Ferro C, Cadena H, Montoya-Lerma J, Adler GH. Canine visceral leishmaniasis: dog infectivity to sand flies from non-endemic areas. *Res Vet Sci*. 2002;72:83-6.
38. Veloso HP, Rangel-Filho LR, Lima JC. Classificação da vegetação brasileira, adaptada a um sistema universal. Rio de Janeiro; Instituto Brasileiro de Geografia e Estatística; 1991.

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