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. American cutaneous leishmaniasis (ACL) has been recorded in the majority of municipalities. 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.

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.

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.

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.

**Capture sites:** A total of nine sites were sampled in different environments (Fig. 1): peridomestic 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 peridomestic 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.](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\(^1\) 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)\(^1\). Species identification was undertaken in accordance with GALATI (2003)\(^1^8\) and the abbreviation of the species names follows MARCONDES (2007)\(^2\).

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 Aquidauana 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)\(^1\). Initially the Index of Species Abundance (ISA) was obtained by the application of the formula: \( ISA = a + Rj/k; \) where: \( a \) = number of ecotopes investigated in which the given species was not present, multiplied by \( c; \) \( e \) 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 = \( c; \) \( Rj \) = 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, using Shannon’s Diversity Index (H) and that of Pielou (J). In accordance with HAYEK & BUZAS (1997)\(^1\), the respective formulae are:

\[
H = - \sum p_i \ln p_i; \quad J = H/\ln s
\]

\( s \) = 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 avellar (Costa Lima), Br. brumpti (Larrousse), Brumptomyia sp., Evandroplasia sp. (Cortelezzii complex), Ev. leni (Mangabeira), Ev. termedophila (Martins, Falcão & Silva), Lutzomyia longipalpis (Lutz & Neiva), Sciopemyia sordellii (Shannon & Del Ponte), Nyssomyia neivai (Pinto), Ny. whitmani (Antunes & Coutinho), Psathyromyia aragoai (Costa Lima), Pa. campograndensis Oliveira, Andrade Filho, Falcão & Brasil, Pa. hermalenti (Martins, Silva & Falcão), Pa. bigeniculata (Floh & Abonnenc), Pa. puncigeniculata (Floh & Abonnenc) and Microgyomyia 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...
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 cortelezii 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 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
The greatest frequency and species richness of the phlebotomines captured occurred in anthropic environments, probably attracted to the peridomestic 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. 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. 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 peridomestic 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) and ANDRADE et al. (2009), 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 other areas of the municipality of Bonito and the state of Mato Grosso do Sul.

The natural infection rate found for *Ny. whitmani* (0.07%) was lower than that recorded by GALATI et al. (1996) 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) and GALATI et al. (2003).

In this study, *Ny. whitmani* was more abundant in the cold, dry period, a result similar to that found by GALATI et al. (1996) in MS, and also in the state of Rio de Janeiro (RJ) by SOUZA et al. (2002). 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, 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) technic 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.

*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, *Pa. bigeniculata* presents pronotum, paratergite, prescutum, scutum, and scutellum brown, upper anepisternum straw and the other pleural off-white sclerites.

*Pa. shannoni* is considered in the United States an important arbovirus vector and has been captured naturally infected by *Leishmania mexicana* in peri-urban areas of Mexico, by *Leishmania* sp. in Guatemala and also developed experimental infection with *L. i. chagasi* when feeding on infected dog. *Ps. shannoni* s. lat has been associated with the transmission of *Endotrypanum schaudinni*, a trypanosomatid of sloths, and was found naturally infected by *Leishmania* sp. in Serra do Baturité, in the northeastern region of Brazil. 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.

*Ny. neivai* presents no widespread geographical distribution in the state of Mato Grosso do Sul, being found mainly in the southeastern and eastern mesoregions. 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\(^5,15,22,27,28\) and also in neighboring countries\(^45\).

The Cortelezzii complex includes the species *Ev. cortelezzii*, *Ev. sallesi* and *Ev. corumbaensis*, which are all found in MS\(^1,3,17\). 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*\(^1,3\), 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, perídomíciolitos e abrigos de animais próximos a áreas perídomícias. 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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