The phlebotomine fauna (Diptera: Psychodidae) of Guaraí, state of Tocantins, with an emphasis on the putative vectors of American cutaneous leishmaniasis in rural settlement and periurban areas

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Phlebotomine sandflies were captured in rural settlement and periurban areas of the municipality of Guaraí in the state of Tocantins (TO), an endemic area of American cutaneous leishmaniasis (ACL). Forty-three phlebotomine species were identified, nine of which have already been recognised as ACL vectors. Eleven species were recorded for the first time in TO. Nyssomyia whitmani was the most abundant species, followed by Evandromyia bourrouli, Nyssomyia antunesi and Psychodopagus complexus. The Shannon-Wiener diversity index and the evenness index were higher in the rural settlement area than in the periurban area. The evaluation of different ecotopes within the rural area showed the highest frequencies of Ev. bourrouli and Ny. antunesi in chicken coops, whereas Ny. whitmani predominated in this ecotope in the periurban area. In the rural settlement area, Ev. bourrouli was the most frequently captured species in automatic light traps and Ps. complexus was the most prevalent in Shannon trap captures. The rural settlement environment exhibited greater phlebotomine biodiversity than the periurban area. Ps. complexus and Psychodopagus ayrozai naturally infected with Leishmania (Vianna) braziliensis were identified. The data identified Ny. whitmani as a potential ACL vector in the periurban area, whereas Ps. complexus was more prevalent in the rural environment associated with settlements.

Key words: phlebotomine fauna - Nyssomyia whitmani - Psychodopagus complexus - Leishmania vectors

American cutaneous leishmaniasis (ACL) has undergone a clear geographical expansion in Brazil in recent decades, which is likely associated with environmental and climatic changes. In the context of this novel distribution, human cases have been recorded in environmentally impacted and deforested rural areas, including periurban regions of some Brazilian towns (MS/SVS 2007).

In most Brazilian endemic areas, ACL is associated with Leishmania (Vianna) braziliensis infection, which is transmitted by several sandfly species, including Psychodopagus wellcomei and Psychodopagus complexus, which are involved in the sylvatic cycle in the Amazon Basin, and Nyssomyia whitmani, Nyssomyia intermedia, Nyssomyia neivai and Migonemyia (Migonemyia) migonei, which are associated with the outskirts of cities and areas that have suffered from environmental impact in the Northeast, Southeast, South and Central Regions (Rangel & Lainson 2009).

In the state of Tocantins (TO), ACL presents an occupational epidemiological profile, affecting mostly males and young adults. In this context, the local transmission patterns are associated with deforestation for the construction of highways, railroads, hydroelectric dams and agricultural expansion, which has favoured the establishment of settlements and villages (Graser 2008, SESAU/TO 2010). A total of 6,497 cases of ACL were recorded in the period from 2001-2012 (Information System for Notifiable Diseases) (dtr2004.saude.gov.br/sinanweb/index.php).

The purpose of this paper is to contribute to the current knowledge of phlebotomine fauna in TO and to identify putative ACL vectors in a rural settlement area and in the periurban environment of Guaraí.

MATERIALS AND METHODS

Sandfly capture sites - Guaraí is located in the northwestern TO (Supplementary data) at coordinates S08°50’03” W48°30’37” and at an altitude of 259 m. The local population is estimated to be 23,445 inhabitants distributed over 2,268,155 km² with a demographic density of 10.23 inhabitants/km². The inhabitants’ main source of income is agricultural activity (Brazilian Institute of Geography and Statistics) (ibge.gov.br/cidadesat/link.php?uf=to). The city lies along the BR-153 highway, which connects the cities of Belém and Brasília, and is the major link between the Central and Northeast Regions of Brazil. The traffic along this highway is heavy, as it constitutes the main conduit of economic activity in the region (Government of the state of Tocantins) (atm-to.org.br/cidade.php?l=e6149e89399dba56fa890aaff1b0f138) (to.gov.br/tocantins/guarai/891).

Guaraí is within the Cerrado biome, which has a continuous canopy and tree cover ranging from 50-90%, with the most cover in the rainy season and least during the dry season (Brazilian Agricultural Research Corporation) (agencia.cnptia.embrapa.br/Agencia16/AG01/arvore/...
Sandflies were captured from January 2005-June 2008 using CDC light traps (HP model) (Pugedo et al. 2005) and Shannon traps (Sudia & Chamberlain 1962). The light traps were used in peri-domestic areas near animal shelters or in the forest every month for three consecutive nights from 06:00 pm-06:00 am. Light traps were placed in each ecotope 1 m above the ground. The captured specimens were fixed in 70% alcohol and identified in accordance with Galati (1995, 2003); the abbreviation of the species’ names conforms to Marcondes (2007). Shannon traps were used in MS 1 in a forested part of the rural settlement area over a period of 12 h (06:00 pm-06:00 am) monthly from March-June 2008. The anthropophily of the species was also evaluated in these captures. A single capture with a Shannon trap was made in November 2008 on four consecutive nights to search for natural Leishmania spp infection in phlebotomine females using multiplex polymerase chain reaction (PCR). The insects were kept alive in nylon cages (Barraud 1929) and were taken to the laboratory (Entomology Laboratory of the Center for Zoonosis Control of Guarai). For sandfly identification, males and the last abdominal segments of the females were cleared and mounted on slides. Males were identified by analysing the morphology of the genitalia (gonostyle, gonocoxite, structures of the parameres, pump and ejaculatory ducts) as well as the colour of the thighs and thoracic pleurae; the spermathecae, individual ducts and common duct were also observed in females. After identification, the insects were grouped into pools of 10 females and 10 males by species and locality. Male insects were used in diagnostic assays as negative controls. The pools of sandflies were kept at -20°C for DNA extraction.

Statistical analysis - The index of species abundance (ISA) and the standardised index of species abundance (SISA) (Roberts & Hsi 1979) were calculated for the phlebotomine species collected in the rural and peri-urban MSs. Excel 2010 and Diversidade de Espécies v. 2.0 software (DivEs) were used to analyse the data. The data were entered into Tables organised by MS, species and period of capture. Each column was classified separately according to the number of specimens of each species. The values in each column were ranked, with the highest value classified as 1, the second as 2 and so on. ISA was calculated according to this formula:

\[ ISA = a + \frac{Rj}{k} \]

where \( k \) is the number of columns in the table (number of months of collection), \( a \) is the number of columns in which the species was absent multiplied by \( c \), \( c \) is the highest value of the ranking obtained in all columns + 1 and \( Rj \) is the sum of the values for each species.

The minimum and maximum limits of the index were determined according to the highest distribution classification, where the limit is different in each series of data. To avoid this variation and to standardise the index we used a scale of values between 0-1 to calculate SISA:

\[ SISA = \frac{c-ISA}{c-1} \]

Species abundance was considered high when the SISA value was close to the maximum value of 1. The results provide information on the relative abundance of species and on the temporal distribution of the collected individuals.

To analyse and compare the overall number of phlebotomine insects captured in the rural and urban areas, the DivEs software was used to analyse the data using the Shannon-Wiener diversity index (H) and the evenness index (J) (Rodrigues 2005).

PCR multiplex studies - To detect Leishmania spp infection, molecular analysis was performed on a total of 250 females and 40 males, which were grouped into pools of 10 specimens each. DNA was extracted as previously described (Pita-Pereira et al. 2005). Multiplex PCR was designed to simultaneously amplify the cacophony gene IVS6 region in Neotropical sandflies, which was used as an internal control for the polymerase enzyme activity and DNA extraction and the conserved kinetoplast DNA minicircle from Leishmania spp. The amplified products were further analysed by dot blot hybridisation with a biotinylated Leishmania (Viannia)-specific probe (Pita-Pereira et al. 2005). Rigorous procedures were used to prevent contamination: negative control groups (male sandflies) were included in the DNA extraction step, instrument and working areas were decontaminated with diluted chloride solution and ultraviolet light and artificially infected females were added as positive controls.

RESULTS

The Guarai exhibited diverse phlebotomine fauna with 43 identified species represented in 3,530 total specimens, 1,872 of which were male and of which were 1,658 female (male/female ratio = 1.12:1.0). Eleven phlebotomine species that had never been found in TO were recorded: Pintomyia (Pintomyia) damascenoi, Pressatia choti, Psathyromyia (Forattiniella) runoides, Psathyromyia (Xiphomyia) dreisbachi, Ps. complexus, Psychodopygus davisi, Psychodopygus ilanosmartinsi, Psychodopygus hirsutus hirsutus, Psychodopygus ayrozai, Psychodopygus paraensis and Trichophoromyia ubiquitatis.

Some species identified in both areas are considered to be putative vectors of leishmaniasis: Nyssomyia antunesi, Ny. whitmani, Ny. intermedia, Bichromomyia flaviscutellata, Ps. complexus, Ps. ayrozai, Ps. paraensis, Ps. hirsutus hirsutus, T. ubiquitatis and Mig. (Mig.) migoneti, which are vectors of ACL, and Lutzomyia (Lutzomyia) longipalpis and Mg. (Mig.) migoneti, which are vectors of American visceral leishmaniasis (AVL).
The SISA of the Phlebotominae showed that *Ny. whitmani* was the species with the highest index (0.9524) followed by *Evandromyia bourrouli* (0.9444), *Ny. antunesi* (0.9206), *Ps. complexus* (0.9206) and *Lu. longipalpis* (0.8571) (Table I). With regard to species abundance, *Ev. bourrouli* was the most represented species (1,000) in the rural settlement area, followed by *Ny. antunesi* (0.9682) and *Ps. complexus* (0.9206). In the periurban area, *Ny. whitmani* was the most abundant (1,000), followed by *Lu. longipalpis* (0.9512) and *Ps. complexus* (0.878) (Table II).

The H and the J analyses revealed that the indexes recorded in the rural settlement environment (H = 0.9641 and J = 0.6059) were higher than those of the periurban environment (H = 0.7758 and J = 0.5252) (Table III).

In the rural settlement area, 2,515 specimens representing 39 species were captured, 13 of which were found exclusively in this environment: *Micropygomyia (Sauromyia) quinquefer*, *Lutzomyia (Tricholateralis) sherlocki*, *Pintomyia (Pintomyia) christenseni*, *Pi. (Pin.) damascenoi*, *Pr. choti*, *Evandromyia (Aldamyia) walkeri*, *Psathyromyia (Forattiniella) aragaoi*, *Pa. (Xiphomyia) dreisbachi*, *Psathyromyia (Psathyromyia) dendrophyla*, *Viannamyia furcata*, *Ps. hirsutus hirsutus*, *Ps. paraensis* and *T. ubiquitalis*. In this environment, *Ev. bourrouli*

| Species | ISA  | SISA | Position |
|---------|------|------|----------|
| Brumptomyia brumpti | 25.875 | 0.2103 | 25th |
| Micropygomyia (Sauromyia) peresi | 28.625 | 0.1230 | 29th |
| Micropygomyia (Sauromyia) longipennis | 29.250 | 0.1032 | 34th |
| Micropygomyia (Sauromyia) quinquefer | 31.875 | 0.0198 | 40th |
| Micropygomyia (Sauromyia) rorotensis | 29.500 | 0.0952 | 35th |
| Micropygomyia (Sauromyia) villelai | 20.375 | 0.3849 | 17th |
| Sciopemyia sordellii | 12.500 | 0.6349 | 10th |
| Lutzomyia (Lutzomyia) longipalpis | 5.500 | 0.8571 | 5th |
| Lutzomyia. (Tricholateralis) sherlocki | 30.250 | 0.0714 | 39th |
| Migonemyia (Migonemyia) migonei | 15.000 | 0.5556 | 12th |
| Pintomyia (Pintomyia) christenseni | 32.250 | 0.0079 | 41st |
| Pintomyia (Pintomyia) damascenoi | 30.250 | 0.0714 | 38th |
| Pressatia choti | 28.250 | 0.1349 | 27th |
| Trichopygomyia dasypogogeton | 14.750 | 0.5635 | 11th |
| Evandromyia (Aldamyia) carmelinai | 15.625 | 0.5357 | 13th |
| Evandromyia (Aldamyia) lenti | 17.875 | 0.4643 | 16th |
| Evandromyia (Aldamyia) evandroi | 17.000 | 0.4921 | 15th |
| Evandromyia (Aldamyia) termitophila | 22.000 | 0.3333 | 20th |
| Evandromyia (Aldamyia) walker | 23.625 | 0.2817 | 21st |
| Evandromyia (Evandromyia) bourrouli | 2.750 | 0.9444 | 2nd |
| Evandromyia (Evandromyia) pinotti | 20.875 | 0.3690 | 18th |
| Evandromyia (Evandromyia) begonae | 10.000 | 0.7143 | 8th |
| Evandromyia (Evandromyia) saulensis | 22.000 | 0.3333 | 19th |
| Psathyromyia (Forattiniella) ruionides | 25.000 | 0.2381 | 22nd |
| Psathyromyia (Forattiniella) aragaoi | 30.250 | 0.0714 | 37th |
| Psathyromyia (Xiphomyia) hermanlenti | 25.375 | 0.2262 | 24th |
| Psathyromyia (Xiphomyia) dreisbachi | 32.250 | 0.0079 | 43rd |
| Psathyromyia (Psathyromyia) shannoni | 28.625 | 0.1230 | 30th |
| Psathyromyia (Psathyromyia) dendrophyla | 32.250 | 0.0079 | 42nd |
| Viannamyia furcata | 28.750 | 0.1190 | 31st |
| Bichromomyia flaviscutellata | 7.875 | 0.7817 | 7th |
| Psychodopygus complexus | 3.500 | 0.9206 | 4th |
| Psychodopygus davisi | 11.750 | 0.6587 | 9th |
| Psychodopygus caustrei | 26.375 | 0.1944 | 26th |
| Psychodopygus llanosmartinsi | 6.750 | 0.8175 | 6th |
| Psychodopygus hirsutus hirsutus | 28.250 | 0.1349 | 28th |
| Psychodopygus ayrozai | 15.750 | 0.5317 | 14th |
| Nyssomyia antunesi | 29.625 | 0.0913 | 36th |
| Nyssomyia whitmani | 2.500 | 0.9524 | 1st |
| Nyssomyia intermedia | 28.875 | 0.1151 | 32nd |
| Trichophoromyia ubiquitalis | 28.750 | 0.2381 | 23rd |

*a*: vector species.
had the highest frequency among the collected species (29.82%), followed by *Ny. antunesi* (21.71%), *Ny. whitmani* (11.01%) and *Ps. complexus* (10.42%) (Table III). In the periurban area, 1,015 specimens were collected, belonging to 30 species, four of which were exclusive to this environment, including *Microgyomyia* (Sauromyia) peresi, *Microgyomyia* (Sauromyia) rorotaensis, *Evandromyia* (Aldamyia) termiotipha and *Psathyromyia* (Forattiniella) lutziana. *Ny. whitmani* was the most predominant species, representing more than half of the collected individuals (53.99%), followed by *Ev. bourrouli* (10.44%) and *Ps. complexus* (9.36%) (Table III).

When evaluating the overall number of specimens captured per ecotope, it was observed that the MS 2 forested area had the most individuals (n = 848), followed by other environments in which animals were present, such as chicken coops: MS 1 (n = 707), MS 2 (n = 695) and MS 4 (n = 581) (Supplementary data).

The H and the J indexes of the evaluated ecotopes showed the highest values in the banana grove ecotope

| Species | Rural settlement area | Periurban area |
|---------|-----------------------|----------------|
| **Brumptomyia brumpti** | 25.75 0.2142 25th | 20.50 0.0487 21th |
| *Microgyomyia* (Sauromyia) peresi | - - - | 20.50 0.0487 23th |
| *Microgyomyia* (Sauromyia) longipennis | 32.00 0.0158 39th | 21.00 0.2439 26th |
| *Microgyomyia* (Sauromyia) quinquefer | 31.25 0.0396 34th | - - - |
| *Microgyomyia* (Sauromyia) rorotaensis | - - - | 21.00 0.2439 28th |
| *Microgyomyia* (Sauromyia) villetai | 24.00 0.2698 23th | 16.75 0.2317 17th |
| *Scopemyia* sordellii | 14.00 0.5873 9th | 11.00 0.5121 8th |
| *Lutzomyia* (Lutzolateralis) sherlocki | 9.00 0.746 9th | 2.00 0.9512 2th |
| *Migonomyia* (Migonomyia) migonei | 28.00 0.1428 30th - - - |
| *Pintomyia* (Pintomyia) christensi | 10.00 0.7142 10th | 20.00 0.0731 20th |
| *Pintomyia* (Pintomyia) damasceni | 32.00 0.0158 35th - - - |
| *Pressatia* choti | 28.00 0.1428 29th - - - |
| *Trichopygomyia* dasydopogeton | 24.00 0.2698 20th - - - |
| *Evandromyia* (Aldamyia) carmelinii | 23.75 0.2777 19th | 7.50 0.6829 7th |
| *Evandromyia* (Aldamyia) lenti | 24.00 0.2698 22th | 11.75 0.4756 11th |
| *Evandromyia* (Aldamyia) evandroi | 21.00 0.365 18th | 13.00 0.4146 13th |
| *Evandromyia* (Aldamyia) termitophila | - - - | 11.50 0.4878 10th |
| *Evandromyia* (Aldamyia) walker | 14.75 0.5634 14th - - - |
| *Evandromyia* (Evandromyia) bourrouli | 1.00 1.000 1th | 4.50 0.8292 4th |
| *Evandromyia* (Evandromyia) pinottii | 15.25 0.5476 15th | 21.00 0.2439 27th |
| *Evandromyia* (Evandromyia) begonae | 7.50 0.7936 7th | 12.50 0.439 12th |
| *Evandromyia* (Evandromyia) saulensis | 17.50 0.4761 16th | 21.00 0.2439 29th |
| *Psathyromyia* (Psathyromia) dendrophyla | 28.50 0.1269 31th | 16.00 0.2682 16th |
| *Psathyromyia* (Psathyromia) aragaoi | 28.00 0.1428 28th - - - |
| *Psathyromyia* (Psathyromia) lutziana | - - - | 20.50 0.0487 22th |
| *Psathyromyia* (Xiphomyia) hermanlenti | 32.00 0.0158 38th | 13.25 0.4024 14th |
| *Psathyromyia* (Xiphomyia) dreisbachi | 32.00 0.0158 37th - - - |
| *Psathyromyia* (Psathyromia) shannoni | 30.75 0.0555 32th | 21.00 0.2439 30th |
| *Psathyromyia* (Psathyromyia) dendrophyla | 32.00 0.0158 36th - - - |
| *Vianamyia* furcata | 25.00 0.238 24th - - - |
| *Bichromomyia* flaviscutellata | 4.50 0.8888 5th | 11.25 0.5 9th |
| *Psychodopygus* complexus | 3.50 0.9206 3th | 3.50 0.878 3th |
| *Psychodopygus* davisi | 9.50 0.7301 10th | 14.00 0.3658 15th |
| *Psychodopygus* clausrestri | 26.25 0.1984 26th | 21.00 0.2439 24th |
| *Psychodopygus* llanosmartinsi | 7.00 0.8095 6th | 6.50 0.7317 6th |
| *Psychodopygus* hirsutus hirsutus | 24.00 0.2698 21th - - - |
| *Psychodopygus* ayrozai | 11.50 0.6666 12th | 20.00 0.0731 18th |
| *Psychodopygus* paraisensis | 26.75 0.1825 27th - - - |
| *Nyssomyia* antunesi | 2.00 0.9682 2th | 5.00 0.8048 5th |
| *Nyssomyia* whitmani | 4.00 0.9047 4th | 1.00 1.000 1th |
| *Nyssomyia* intermedius | 31.25 0.0396 33th | 21.00 0.2439 25th |
| *Trichophoromyia* ubiquitalis | 17.50 0.4761 17th - - - |

*a*: vector species.
TABLE III
Total number, percentage and Shannon-Wiener diversity (H) and evenness (J) indexes of phlebotomines captured in light traps at rural settlement and periurban areas in the municipality of Guaraí, state of Tocantins, Brazil, January 2005-June 2008

| Species                        | Rural settlement area | Periurban area |
|--------------------------------|-----------------------|----------------|
|                                | M (n)  | F (n)  | Total n (%) | M (n)  | F (n)  | Total n (%) |
| Brumptomyia brumpti            | 2      | 2      | 4 (0.16)    | 1      | 0      | 1 (0.10)    |
| Micropygomyia (Sauromyia) peresi | -      | -      | -           | 1      | 0      | 1 (0.10)    |
| Micropygomyia (Sauromyia) longipennis | 1      | 0      | 1 (0.04)    | 1      | 0      | 1 (0.10)    |
| Micropygomyia (Sauromyia) quinquefer | 0      | 1      | 1 (0.04)    | -      | -      | -           |
| Micropygomyia (Sauromyia) rorotaensis | -      | -      | -           | 1      | 0      | 1 (0.10)    |
| Micropygomyia (Sauromyia) villelai | 2      | 2      | 4 (0.16)    | 4      | 0      | 4 (0.39)    |
| Sciopemyia sordellii           | 53     | 21     | 74 (2.94)   | 15     | 42     | 57 (5.62)   |
| Lutzomyia (Lutzomyia) longipalpis | 0      | 1      | 1 (0.04)    | -      | -      | -           |
| Mignonemyia (Mignonemyia) migonei | 8      | 10     | 18 (0.72)   | 1      | 1      | 2 (0.20)    |
| Pintomyia (Pintomyia) christenseni | 0      | 1      | 1 (0.04)    | -      | -      | -           |
| Pintomyia (Pintomyia) damascenoi | 0      | 2      | 2 (0.08)    | -      | -      | -           |
| Presatia choti                 | 20     | 32     | 52 (2.07)   | 0      | 2      | 2 (0.20)    |
| Trichophoromyia dasydopogon    | 8      | 0      | 8 (0.32)    | 1      | 0      | 1 (0.10)    |
| Evandromyia (Alamyia) carmelinoi | 1      | 5      | 6 (0.24)    | 8      | 14     | 22 (2.17)   |
| Evandromyia (Alamyia) lenti     | 2      | 2      | 4 (0.16)    | 7      | 6      | 13 (1.28)   |
| Evandromyia (Alamyia) evandroi  | 1      | 4      | 5 (0.20)    | 4      | 5      | 9 (0.99)    |
| Evandromyia (Alamyia) termitophila | -    | -      | -           | 2      | 10     | 12 (1.18)   |
| Evandromyia (Alamyia) walkeri   | 4      | 10     | 14 (0.56)   | -      | -      | -           |
| Evandromyia (Evandromyia) bourrouli | 538    | 212    | 750 (29.82) | 83     | 23     | 106 (40.44) |
| Evandromyia (Evandromyia) pinottii | 8      | 0      | 8 (0.32)    | 1      | 0      | 1 (0.10)    |
| Evandromyia (Evandromyia) begonae | 0      | 94     | 94 (3.74)   | 0      | 8      | 8 (0.79)    |
| Evandromyia (Evandromyia) saulensis | 2      | 5      | 7 (0.28)    | 0      | 1      | 1 (0.10)    |
| Psathyromyia (Forattiniella) runoides | 1      | 1      | 2 (0.08)    | 3      | 6      | 9 (0.89)    |
| Psathyromyia (Forattiniella) aragaoi | 1      | 0      | 1 (0.04)    | -      | -      | -           |
| Psathyromyia (Forattiniella) lutiana | -    | -      | -           | 1      | 0      | 1 (0.10)    |
| Psathyromyia (Xiphomyia) hermanlenti | 0      | 1      | 1 (0.04)    | 7      | 8      | 15 (1.48)   |
| Psathyromyia (Xiphomyia) dreisbachi | 0      | 1      | 1 (0.04)    | 1      | 0      | 1 (0.10)    |
| Psathyromyia (Psathyromyia) shannoni | 0      | 1      | 1 (0.04)    | -      | -      | -           |
| Psathyromyia (Psathyromyia) dendrophyla | 0      | 1      | 1 (0.04)    | -      | -      | -           |
| Vianamyia farcata              | 0      | 4      | 4 (0.16)    | -      | -      | -           |
| Bichromomyia flaviscutellatae   | 109    | 60     | 169 (6.72)  | 7      | 4      | 11 (1.08)   |
| Psychodyopus complexus         | 47     | 215    | 262 (10.42) | 17     | 78     | 95 (9.36)   |
| Psychodyopus davisi            | 13     | 50     | 63 (2.50)   | 4      | 5      | 9 (0.89)    |
| Psychodyopus clausrei          | 0      | 9      | 9 (0.36)    | 0      | 1      | 1 (0.10)    |
| Psychodyopus llanosmartinsi    | 11     | 73     | 84 (3.34)   | 6      | 23     | 29 (2.86)   |
| Psychodyopus hirsutus hirsutus | 0      | 4      | 4 (0.16)    | -      | -      | -           |
| Psychodyopus ayozae            | 5      | 16     | 21 (0.83)   | 0      | 2      | 2 (0.20)    |
| Psychodyopus paraenisi         | 0      | 3      | 3 (0.12)    | -      | -      | -           |
| Nyssomyia antunesi             | 124    | 422    | 546 (21.71) | 11     | 28     | 39 (3.84)   |
| Nyssomyia whitmani             | 244    | 33     | 277 (11.01) | 478    | 70     | 548 (53.99) |
| Nyssomyia intermedia           | 1      | 0      | 1 (0.04)    | 1      | 0      | 1 (0.10)    |
| Trichophoromyia ubiquitalis    | 0      | 1      | 1 (0.04)    | -      | -      | -           |

Total 1,202 1,313 2,515 (100) 670 345 1,015 (100)

H 0.9641 0.7758
J 0.6059 0.5252

a: vector species; F: female; M: male.
ment area chicken coop at MS 2 (42.01%). *Ny. whitmani* was the most prevalent in the other ecotopes near domestic animal shelters in the periurban area, such as the chicken coop at MS 1 (44.62%), the pig sty at MS 2 (49.52%) and the chicken coop at MS 3 (56.63%), followed by *Lu. longipalpis* in all ecotopes (Supplementary data).

The Shannon trap captures at the MS1 rural settlement area yielded 1,996 specimens, including 190 males (18%) and 906 (82%) females, resulting in a female/male ratio of 4.55:1.0. Among the 14 species identified, those from the *Psychodopygus* genus were the most prevalent, including *Ps. complexus*, *Ps. llanosmartinsi* and *Ps. ayrozai*. All of these species were observed biting humans during the captures. Among these species, *Ps. complexus* (42.61%) was predominant (Table IV).

A total of 290 specimens were collected and used for PCR and dot blot hybridisation. Sandflies of the same species were analysed in pools of 10; there were 23 *Ps. complexus* pools (3 male and 20 female pools) and six *Ps. ayrozai* pools (1 male and 5 female pools) (Table V). The results showed that four of 25 (16%) female pools were positive for *Leishmania* (*Viannia*) sp. infection. In the *Ps. complexus* pools, only three of 20 were found to be positive (15%). In the *Ps. ayrozai* pools (n = 5), one pool (20%) was positive for infection (Supplementary data).

**DISCUSSION**

Initial studies of phlebotomine fauna in TO focused on the description of species (Barreno 1946, Martins et al. 1962, 1964, 1975); further entomological studies identified 32 sandfly species in the state (Lustosa et al. 1968, Andrade Filho et al. 2001), including the new species *Micropygomyia (Silvamyia) echinatopharynx* and *Martinsmyia reginae* (Andrade Filho et al. 2004, Carvalho et al. 2010). These findings indicate a great diversity of sandfly species in TO. Recent investigations of ACL and AVL vectors in the municipality of Porto Nacional recorded 48 sandfly species, 22 of which were the first records of these species in the state (Vilela et al. 2011).

The present study identified 43 phlebotomine species in Guarai, 11 of which were newly recorded species, underscoring the rich biodiversity of the Brazilian *Cerrado*. Among the identified sandfly species, *Ev. bourrouli* was predominant in the rural settlement area and presented the second highest percentage in the periurban area. This species was found in various environments and ecotopes, such as preserved forest and animal shelters (chicken coops and pig sties), which underscores its eclectic behaviour. In previous studies carried out in TO, this species was the most frequent at Porto Nacional along with

| Table V |
|---|
| **Phlebotomine molecular diagnostic evaluation for infection with *Leishmania (Viannia)* braziliensis and positive polymerase chain reaction hybridisation results with female pools of *Psychodopygus complexus* and *Psychodopygus ayrozai* by traps in the municipality of Guarai, state of Tocantins, Brazil** |
| Traps | *Ps. complexus* (p/n) | *Ps. ayrozai* (p/n) | Females (p/n) |
|---|---|---|---|
| CDC light - HP model | 1/5² | 0/0 | 2/5 |
| Shannon | 2/15³ | 1/5⁴ | 2/20 |
| Total | 3/20⁴ | 1/5⁴ | 4/25 |

the numbers in superscript represent the groups of females positive related to each species of phlebotomine. n: total number of females evaluated; p: number of positive females.
Evandromyia sallesi (Andrade Filho et al. 2001). However, it is important to emphasise that this species has not been reported to transmit pathogens to humans.

Of the species identified in this study, 11 were recorded in TO for the first time, including Ps. complexus, Ps. davisi, Ps. hirsutus hirsutus, Ps. ayrozai, Ps. paraensis and T. ubiquitalis, which are putative ACL vectors in Brazil; other sandfly vectors of leishmaniasis that had already been recorded in TO were also found in Guaraí, including Ny. antunesi, Nyssomyia flaviscutellata, Ny. whitmani, Ny. intermedia, Mig. (Mig.) migonei and Lu. longipalpis.

Previous studies developed in TO identified Ny. whitmani as the main vector of L. (V) braziliensis, especially in areas affected by hydroelectric construction and agricultural activities, where this sandfly has been found inside and outside residences, near animal shelters and even within forest boundaries (Carvalho 2008, Vilela et al. 2008, 2011). Ny. whitmani is associated with the transmission of L. (V) braziliensis in most Brazilian endemic areas, occupying different types of plant cover and modified environments (da Costa et al. 2007, Rangel 2010).

Ny. whitmani was the most abundant species among the species collected in the present study. Furthermore, it was the most abundant ACL vector in periurban areas and one of the most represented species in the rural settlement areas. This species was found in all ecotopes, with a particularly high density in animal shelters (chicken coops and pig styts) from residences in the periurban environment. Studies performed in Porto Nacional discussed the capacity of Ny. whitmani to adapt to environmental changes by expanding its transmission cycle (Vilela et al. 2011).

Of the ACL vectors observed in Guaraí, it is important to highlight the identification of Ps. complexus, an important L. (V) braziliensis vector in the low-altitude forests of the state of Pará (PA) in the Amazon Region of northern Brazil (Souza et al. 1996, Lainson & Shaw 2005, Garecz et al. 2009, Rangel & Lainson 2009). This sandfly species has been recorded outside the Amazon Region, but there has been no evidence to suggest its involvement in L. (V) braziliensis transmission in the states of Pernambuco (Andrade et al. 2005) and Mato Grosso (Azevedo et al. 2002, Ribeiro et al. 2007).

The present study provides evidence for the presence of Ps. complexus in all evaluated ecotopes, including animal shelters in the periurban area. Although Ny. whitmani is thought of as the most important ACL vector in TO (Rangel & Lainson 2009, Vilela et al. 2011), the discovery of naturally L. (V) braziliensis-infected Ps. complexus, the spatial distribution of this sandfly in forest and peridomiciary ecotopes, its predominance in Shannon traps and its relatively high abundance in settlements and periurban areas, together with epidemiological evidence reported in the literature, suggest that Ps. complexus may also play a role in the transmission cycle of ACL in the rural settlement areas of Guaraí.

Ps. ayrozai, another sandfly species that is naturally infected by L. (V) braziliensis in Guarái, has been suggested as a putative vector of Leishmania (Viannia) naiffi in PA (Lainson et al. 1994, Lainson & Shaw 2005). Ps. ayrozai is an anthropophilic species (de Aguiar et al. 1985, Gomes & Galati 1989) and experimental infection studies have revealed its susceptibility to Leishmania (Leishmania) forattini (Barretto et al. 1986, Lainson & Shaw 2005). Despite reports showing the vector competence of Ps. ayrozai, this species was not found frequently in this study and no further evidence was found of its participation in local transmission cycles.

Ny. antunesi, which was found in the rural settlement area, has been proven to be a Leishmania (Leishmania) lindenberghi vector in the northern PA and has been identified as anthropophilic in previous studies conducted in TO by Andrade Filho et al. (2001); however, cases of human infection by L. (V) lindenberghi have not been reported in this study area thus far.

The ecoepidemiology of ACL in Brazil is strongly related to the transmission cycle of L. (V) braziliensis and the vector Ny. whitmani, particularly in environmentally impacted areas, rural environments and the periphery of cities from all geographical regions. This new epidemiological profile has resulted from drastic environmental changes and the capacity of this sandfly to adapt to new ecological niches.

The Cerrado biome has experienced significant environmental impacts caused by deforestation. In this context, the establishment of highly populated, poor rural settlement groups and periurban areas without adequate infrastructure typically results in close contact between people and pathogen vectors.

This scenario has been frequently observed in TO and in this context, Ny. whitmani and Ps. complexus, as putative L. (V) braziliensis vectors, likely maintain two transmission cycles in Guaraí: one related to the periurban area and the other in settlements in rural environments.

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REFERENCES

Andrade Filho JD, Galati EAB, de Andrade WA, Falcão AL 2004. Description of Micropygomyia (Silvamyia) echinopharynx sp. nov. (Diptera: Psychodidae) a new species of Phlebotomine sand fly from the state of Tocantins, Brazil. Mem Inst Oswaldo Cruz 99: 609–615.

Andrade Filho JD, Valente MB, de Andrade WA, Brazil RP, Falcão AL 2001. Flebotomíneos do estado de Tocantins, Brasil (Diptera: Psychodidae). Rev Soc Bras Med Trop 34: 323–329.

Andrade MS, Valença HF, Silva AL, Almeida FA, Almeida EL, Brito MEF, Brandão-Filho SP 2005. Sandfly fauna in a military training area endemic for American tegumentary leishmaniasis in the Atlantic rain forest region of Pernambuco, Brazil. Cad Saude Publica 21: 1761–1767.

Azevedo ACR, Souza NA, Meneses CRV, Costa WA, Costa SM, Lima JB, Rangel EF 2002. Ecology of sand flies (Diptera: Psychodidae:
Phlebotominae) in the north of the state of Mato Grosso, Brazil. *Mem Inst Oswaldo Cruz* 97: 459-464.

Barraud PJ 1929. A simple method for carriage of living mosquitoes over long distances in the tropics. *Indian J Med Res* 17: 281-285.

Barreto MP 1946. Uma nova espécie de flebótomos do estado de Goiás, Brasil, e chave para determinação das espécies afins (Diptera: Psychodidae). *Rev Bras Biol* 6: 427-434.

Barreto AC, Vexenat JA, Peterson NE 1986. The susceptibility of wild caught sand flies to infection by a subspecies of *Leishmania mexicana* isolated from *Proechimys iheringi* denigratus (Rodentia, Echimyidae). *Mem Inst Oswaldo Cruz* 81: 235-236.

Carvalho BM 2008. Estudos sobre os flebotomíneos (Diptera: Psychodidae: Phlebotominae) do município de Porto Nacional, estado de Tocantins, Monografia de Graduação em Ciências Biológicas, Universidade Estácio de Sá, Rio de Janeiro, 88 pp.

Carvalho GML, Brazil RP, Sanguinette CC, Andrade Filho JD 2010. Description of a new phlebotomine species, *Martinsomyia reginae* sp. nov. (Diptera: Psychodidae: Phlebotominae) from a cave in the state of Tocantins, Brazil. *Mem Inst Oswaldo Cruz* 105: 336-340.

da Costa SM, Cechinel M, Bandeira V, Zannuncio JC, Lainson R, Rangel EF 2007. *Lutzomyia* (*Nyssomyia*) *whitmani* s.l. (Antunes & Coutinho, 1939) (Diptera: Psychodidae: Phlebotominae): geographical distribution and the epidemiology of American cutaneous leishmaniasis in Brazil - Mini-review. *Mem Inst Oswaldo Cruz* 102: 149-153.

de Aguiar GM, Vilela ML, Schuback PD, Soucasaux T, de Azevedo ACR 1985. Aspectos da ecologia dos flebótomos do Parque Nacional da Serra dos Órgãos, Rio de Janeiro. IV. Frequência mensal em armadilhas luminosas (Diptera, Psychodidae, Phlebotominae). *Mem Inst Oswaldo Cruz* 80: 465-482.

Galati EAB 1995. Phylogenetic systematics of Phlebotominae (Diptera: Psychodidae) with emphasis on American groups. *Bol Din Malarial Sanaem Amb* 35: 133-142.

Galati EAB 2003. Morfologia e taxonomia. In EF Rangel, R Lainson (orgs.), *Flebotomíneos do Brasil*, Fiocruz, Rio de Janeiro, p. 23-206.

Garavez LM, Soares DC, Chagas AP, Souza GCR, Miranda JFC, Fraiha H, Flöeter-Winter LM, Nunes HM, Zampiere RA, Shaw JJ 2009. Etiology of cutaneous leishmaniasis and anthropophilic vectors in Juriti, Pará state, Brazil. *Caf Saude Publica* 25: 2291-2295.

Gomes AC, Galati EAB 1989. Aspectos ecológicos da leishmaniose tegumentar americana. 7. Capacidade vetorial flebotomínea em ambientes florestais do Parque do Seringal em região do Vale do Ribeira, estado de São Paulo, Brasil. *Rev Saude Publica* 23: 136-142.

Graser C 2008. *Leishmaniose tegumentar americana* no estado do Tocantins: situação epidemiológica e ocupacional, Monografia de Especialização em Saúde do Trabalhador e Ecologia Humana, Escola Nacional de Saúde Pública Sergio Arouca, Rio de Janeiro, 21 pp.

Lainson R, Shaw JJ 2005. New World leishmaniasis. In FG Cox, D Wakelin, SH Gillespie, DD Despommier, D Wakelin, SH Gillespie, DD Despommier, Topley and Wilson’s, 2nd ed., MS/SVS, Brasília, 180 pp.

Pita-Pereira D, Alves CR, Souza MB, Brazil RP, Bertho AL, Barbosa AF, Britto CC 2005. Identification of naturally infected *Lutzomyia intermedia* and *Lutzomyia migonei* with *Leishmania* (*Viannia* jiroveci) in Rio de Janeiro (Brazil) revealed by a PCR multiplex non-isotopic hybridization assay. *Acta Trop* 99: 905-913.

Puged PO, Barata A, França-Silva AJ, Silva JC, Dias ES 2005. HP: an improved model of suction light trap for the capture of small insects. *Rev Soc Bras Med trop* 38: 70-72.

Rangel EF 2010. *Lutzomyia* (*Nyssomyia*) *whitmani* and the eco-epidemiology of American cutaneous leishmaniasis in Brazil. Workshop Genética e Biologia Molecular de Insetos Vetores de Doenças Tropicais, 13-17 September 2010, Recife, p. 25-28.

Rangel EF, Lainson R 2009. Proven and putative vectors of American cutaneous leishmaniasis in Brazil: aspects of their biology and vectorial competence. *Mem Inst Oswaldo Cruz* 104: 937-954.

Ribeiro ALM, Missawa N, Zeilhofer P 2007. Distribution of phlebotomine sandflies (Diptera: Psychodidae) of medical importance in Mato Grosso state, Brazil. *Rev Inst Med Trop Sao Paulo* 49: 317-321.

Roberts DR, Hsi BP 1979. An index of species abundance for use with mosquito surveillance data. *Environ Entomol* 8: 1007-1013.

Rodrigues WC 2005. DivEs - Diversidade de espécies. Versão 2.0. Available from: ebras.bio.br.

SESAUTO/ - Secretaria da Saúde do Estado do Tocantins, Núcleo de Leishmanioses, Coordenadoria de Doenças Vetoriais e Zoonoses 2010. Informe entomoepidemiológico das leishmanioses, 26ª a 29ª Semana Epidemiológica, 27 June 2010-24 July 2010, SESAU, Palmas, p. 1-7.

Souza AAA, Ishikawa E, Braga R, Silveira F, Lainson R, Shaw JJ 1996. *Psychodopyges complexus*, a new vector of *Leishmania braziliensis* to humans in Pará state, Brazil. *Trans R Soc Trop Med Hyg* 90: 112-113.

Sudia WD, Chamberlain RW 1962. Battery operated light trap, an improved model. *Mosq News* 22: 126-129.

Vilela ML, Azevedo ACR, Costa SM, Costa WA, Motta-Silva D, Graujskas AM, Carvalho BM, Brahim LRN, Koizovsky D, Rangel EF 2008. Sand fly survey in the influence area of Peixe Angical hydroelectric plant, state of Tocantins, Brazil, 6th International Symposium on Phlebotomine Sandflies, 27-31 October 2008, Lima, 55 pp.

Vilela ML, Graser-Azevedo C, Carvalho BM, Rangel EF 2011. Phlebotomine Fauna (Diptera: Psychodidae) and putative vectors of leishmaniasis in impacted area by hydroelectric plant, state of Tocantins, *Brazil. PLoS ONE* 6: e27721.