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* Psychodopygus 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* Psychodopygus ayrozai *naturally infected with* Leishmania (Viannia) 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* - *Psychodopygus 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 (Viannia) braziliensis* infection, which is transmitted by several sandfly species, including *Psychodopygus wellcomei* and *Psychodopygus 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 S08º50'03'' W48º30'37'' and at an altitude of 259 m. The local population is estimated to be 23,445 inhabitants distributed over 2,268,155 km^2 with a demographic density of 10.23 inhabitants/km^2. 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) (atmto.org.br/cidade.php?l=e6149e89399dba56fa890afff1b0f138) (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/

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AG01_58_911200585234.html). The sandfly capture sites are referred to as monitoring stations (MSs). In the rural settlement area, the MSs bordered the forests of the Agricultural Project Pedra Branca, MS 1 (S08º40'08'' W48º24'76'') and MS 2 (S08º39'99'' W48º24'73''), approximately 40 km from the town centre (Supplementary data). In the periurban area, two residences were selected, MS 3 (S08º48'55.9" W48º30'25") and MS 4 (S08º49'09" W48º30'21.6"). These MSs have rural characteristics, such as the presence of animal shelters and a small area with banana plants (Supplementary data).

Sandfly capture and taxonomy - 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 peridomestic 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 Guaraí). 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 -20ºC 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 periurban 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:

$$ISA = a + Rj/k$$

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:

$$SISA = c\text{-}ISA/c\text{-}1$$

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 Guaraí 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 llanosmartinsi*, *Psychodopygus hirsutus hirsutus*, *Psychodopygus ayrozai*, *Psychodopygus paraensis* and *Trichophoromyia ubiquitalis*.

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. ubiquitalis* and *Mig. (Mig.) migonei*, which are vectors of ACL, and *Lutzomyia (Lutzomyia) longipalpis* and *Mg. (Mig.) migonei*, 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*

TABLE I

Index of species abundance (ISA) and standardised index of species abundance (SISA) obtained from the captures with automatic light traps in municipality of Guaraí, state of Tocantins, Brazil, January 2005-June 2008

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) rorotaensis	29.500	0.0952	35th
Micropygomyia (Sauromyia) villelai	20.375	0.3849	17th
Sciopemyia sordellii	12.500	0.6349	10th
Lutzomyia (Lutzomyia) longipalpis[a]	5.500	0.8571	5th
Lutzomyia. (Tricholateralis) sherlocki	30.250	0.0714	39th
Migonemyia (Migonemyia) migonei[a]	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 dasydopogeton	14.750	0.5635	11th
Evandromyia (Aldamyia) carmelinoi	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) pinottii	20.875	0.3690	18th
Evandromyia (Evandromyia) begonae	10.000	0.7143	8th
Evandromyia (Evandromyia) saulensis	22.000	0.3333	19th
Psathyromyia (Forattiniella) runoides	25.000	0.2381	22nd
Psathyromyia (Forattiniella) aragaoi	30.250	0.0714	37th
Psathyromyia (Forattiniella) lutziana	29.250	0.1032	33rd
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 furcate	28.750	0.1190	31st
Bichromomyia flaviscutellata[a]	7.875	0.7817	7th
Psychodopygus complexus[a]	3.500	0.9206	4th
Psychodopygus davisi	11.750	0.6587	9th
Psychodopygus claustrei	26.375	0.1944	26th
Psychodopygus llanosmartinsi	6.750	0.8175	6th
Psychodopygus hirsutus hirsutus[a]	28.250	0.1349	28th
Psychodopygus ayrozai[a]	15.750	0.5317	14th
Psychodopygus paraensis[a]	29.625	0.0913	36th
Nyssomyia antunesi[a]	3.500	0.9206	3rd
Nyssomyia whitmani[a]	2.500	0.9524	1st
Nyssomyia intermedia[a]	28.875	0.1151	32nd
Trichophoromyia ubiquitalis[a]	25.000	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 *Micropygomyia (Sauromyia) peresi*, *Micropygomyia (Sauromyia) rorotaensis, Evandromyia (Aldamyia) termitophila* 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

TABLE II

Index of species abundance (ISA) and standardised index of species abundance (SISA) obtained from the captures with automatic light traps for environment in municipality of Guaraí, state of Tocantins, Brazil, January 2005-June 2008

	Rural settlement area			Periurban area		
Species	ISA	SISA	Position	ISA	SISA	Position
Brumptomyia brumpti	25.75	0.2142	25th	20.50	0.0487	21th
Micropygomyia (Sauromyia) peresi	-	-	-	20.50	0.0487	23th
Micropygomyia (Sauromyia) longipennis	32.00	0.0158	39th	21.00	0.2439	26th
Micropygomyia (Sauromyia) quinquefer	31.25	0.0396	34th	-	-	-
Micropygomyia (Sauromyia) rorotaensis	-	-	-	21.00	0.2439	28th
Micropygomyia (Sauromyia) villelai	24.00	0.2698	23th	16.75	0.2317	17th
Sciopemyia sordellii	14.00	0.5873	13th	11.00	0.5121	8th
Lutzomyia (Lutzomyia) longipalpis[a]	9.00	0.746	9th	2.00	0.9512	2th
Lutzomyia (Tricholateralis) sherlocki	28.00	0.1428	30th	-	-	-
Migonemyia (Migonemyia) migonei[a]	10.00	0.7142	10th	20.00	0.0731	20th
Pintomyia (Pintomyia) christenseni	32.00	0.0158	35th	-	-	-
Pintomyia (Pintomyia) damascenoi	28.00	0.1428	29th	-	-	-
Pressatia choti	24.00	0.2698	20th	-	-	-
Trichopygomyia dasydopogeton	9.50	0.7301	9th	20.00	0.0731	19th
Evandromyia (Aldamyia) carmelinoi	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 (Forattiniella) runoides	28.50	0.1269	31th	16.00	0.2682	16th
Psathyromyia (Forattiniella) aragaoi	28.00	0.1428	28th	-	-	-
Psathyromyia (Forattiniella) 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 (Psathyromyia) shannoni	30.75	0.0555	32th	21.00	0.2439	30th
Psathyromyia (Psathyromyia) dendrophyla	32.00	0.0158	36th	-	-	-
Viannamyia furcate	25.00	0.238	24th	-	-	-
Bichromomyia flaviscutellata[a]	4.50	0.8888	5th	11.25	0.5	9th
Psychodopygus complexus[a]	3.50	0.9206	3th	3.50	0.878	3th
Psychodopygus davisi	9.50	0.7301	10th	14.00	0.3658	15th
Psychodopygus claustrei	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[a]	24.00	0.2698	21th	-	-	-
Psychodopygus ayrozai[a]	11.50	0.6666	12th	20.00	0.0731	18th
Psychodopygus paraensis[a]	26.75	0.1825	27th	-	-	-
Nyssomyia antunesi[a]	2.00	0.9682	2th	5.00	0.8048	5th
Nyssomyia whitmani[a]	4.00	0.9047	4th	1.00	1.000	1th
Nyssomyia intermedia[a]	31.25	0.0396	33th	21.00	0.2439	25th
Trichophoromyia ubiquitalis[a]	17.50	0.4761	17th	-	-	-

a: vector species.

at MS 4 (H = 1.1238 and J = 0.7063), followed by the forest ecotope at MS 1 (H = 1.0850 and J = 0.6819), the forest ecotope at MS 2 (H = 0.9755 and J = 0.6131) and the chicken coop ecotope at MS 1 (H = 0.9563 and J = 0.6011) (Supplementary data).

The analysis of species frequency per ecotope showed that *Ev. bourrouli* was present in all ecotopes and predominated in the rural settlement area, as well as in the banana grove ecotope at MS 4 in the periurban area. *Ny. antunesi* was the predominant species in the rural settle-

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

	Rural settlement area			Periurban area		
Species	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	-	-	-	0	1	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	4	11	15 (0.60)	6	7	13 (1.28)
Lutzomyia (Lutzomyia) longipalpis[a]	53	21	74 (2.94)	15	42	57 (5.62)
Lutzomyia (Tricholateralis) sherlocki	0	1	1 (0.04)	-	-	-
Migonemyia (Migonemyia) migonei[a]	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)	-	-	-
Pressatia choti	0	3	3 (0.12)	-	-	-
Trichopygomyia dasydopogeton	20	32	52 (2.07)	0	2	2 (0.20)
Evandromyia (Aldamyia) carmelinoi	1	5	6 (0.24)	8	14	22 (2.17)
Evandromyia (Aldamyia) lenti	2	2	4 (0.16)	7	6	13 (1.28)
Evandromyia (Aldamyia) evandroi	1	4	5 (0.20)	4	5	9 (0.89)
Evandromyia (Aldamyia) termitophila	-	-	-	2	10	12 (1.18)
Evandromyia (Aldamyia) walkeri	4	10	14 (0.56)	-	-	-
Evandromyia (Evandromyia) bourrouli	538	212	750 (29.82)	83	23	106 (10.44)
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) lutziana	-	-	-	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)	-	-	-
Viannamyia furcata	0	4	4 (0.16)	-	-	-
Bichromomyia flaviscutellata[a]	109	60	169 (6.72)	7	4	11 (1.08)
Psychodopygus complexus[a]	47	215	262 (10.42)	17	78	95 (9.36)
Psychodopygus davisi	13	50	63 (2.50)	4	5	9 (0.89)
Psychodopygus claustrei	0	9	9 (0.36)	0	1	1 (0.10)
Psychodopygus llanosmartinsi	11	73	84 (3.34)	6	23	29 (2.86)
Psychodopygus hirsutus hirsutus[a]	0	4	4 (0.16)	-	-	-
Psychodopygus ayrozai[a]	5	16	21 (0.83)	0	2	2 (0.20)
Psychodopygus paraensis[a]	0	3	3 (0.12)	-	-	-
Nyssomyia antunesi[a]	124	422	546 (21.71)	11	28	39 (3.84)
Nyssomyia whitmani[a]	244	33	277 (11.01)	478	70	548 (53.99)
Nyssomyia intermedia[a]	1	0	1 (0.04)	1	0	1 (0.10)
Trichophoromyia ubiquitalis[a]	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 1,096 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 (Barreto 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 rhe 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 Guaraí, 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 Guaraí, state of Tocantins, Brazil

Traps	*Ps. complexus* (p/n)	*Ps. ayrozai* (p/n)	Females (p/n)
CDC light - HP model	1/5[2]	0/0	2/5
Shannon	2/15[1]	1/5[1]	2/20
Total	3/20[3]	1/5[1]	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.

TABLE IV

Number of phlebotomine according to sex, captured monthly in Shannon traps at rural settlement area - monitoring station 1, municipality Guaraí, state of Tocantins, Brazil, March-June 2008

	March		April		May		June		
Species	M (n)	F (n)	M (n)	F (n)	M (n)	F (n)	M (n)	F (n)	Total n (%)
Sciopemyia sordellii	-	-	-	-	-	1	-	-	1 (0.09)
Lutzomyia (Lutzomyia) sherlocki	-	1	-	-	-	-	-	-	1 (0.09)
Evandromyia (Aldamyia) walkeri	-	-	-	-	-	-	-	1	1 (0.09)
Evandromyia (Evandromyia) bourrouli	-	-	-	-	-	-	-	1	1 (0.09)
Psathyromyia (Psathyromyia) dendrophyla	-	-	-	-	6	2	-	-	8 (0.72)
Bichromomyia flaviscutellata[a]	-	-	-	-	-	-	-	3	3 (0.27)
Psychodopygus complexus[a]	42	280	-	41	-	1	7	96	467 (42.60)
Psychodopygus davisi	21	17	1	3	-	2	5	18	67 (6.11)
Psychodopygus claustrei	-	7	-	3	-	-	-	1	11 (1)
Psychodopygus llanosmartinsi	78	165	-	60	-	-	-	7	310 (28.28)
Psychodopygus ayrozai[a]	19	130	-	17	-	-	1	6	173 (15.78)
Psychodopygus paraensis[a]	-	-	-	2	-	-	-	-	2 (0.18)
Nyssomyia antunesi[a]	1	8	-	1	8	30	-	1	49 (4.47)
Nyssomyia whitmani[a]	-	-	-	-	1	1	-	-	2 (0.18)
Total	161	608	1	127	15	37	13	134	1,096 (100)

a: vector species; F: female; M: male.

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 stys) 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, Garcez et al. 2009, Rangel & Lainson 2009). This sandfly species has been recorded outside of 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 peridomiciliary 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 Guaraí, 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) forattinii* (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) lindenbergi* 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.) lindenbergi* 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) echinatopharynx* 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 trainning 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 flebótomo do estado de Goiás, Brasil, e chave para determinação das espécies afins (Diptera: Psychodidae). *Rev Bras Biol 6*: 427-434.

Barretto 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, 68 pp.

Carvalho GML, Brazil RP, Sanguinette CC, Andrade Filho JD 2010. Description of a new phlebotomine species, *Martinsmyia 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 Dir Malariol Saneam 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.

Garcez 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 Jurití, Pará state, Brazil. *Cad Saude Publica 25*: 2291-2295.

Gomes AC, Galati EAB 1989. Aspectos ecológicos da leishmaniose tegumentar americana. 7. Capacidade vetorial flebotomínea em ambiente florestal primário do Sistema da Serra do Mar, 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 FEG Cox, D Wakelin, SH Gillespie, DD Despommier, *Topley and Wilson's microbiology and microbial infections*, 2nd ed., Hodder Arnold, London, p. 313-349.

Lainson R, Shaw JJ, Silveira FT, de Souza AAA, Braga RR, Ishikawa EAY 1994. The dermal leishmaniases of Brazil, with special reference to the eco-epidemiology of the disease in Amazonia. *Mem Inst Oswaldo Cruz 89*: 435-443.

Lustosa ED, Naves HP, Carvalho MESD, Barbosa W 1968. Contribuição ao conhecimento da fauna flebotomínica do estado de Goiás - 1984-1985. Nota Prévia I. *Rev Patol Trop 15*: 7-11.

Marcondes CB 2007. A proposal of generic and subgeneric abbreviations for Phlebotomine sandflies (Diptera: Psychodidae: Phlebotominae) of the world. *Entomol News 118*: 351-356.

Martins AV, Falcão AL, Silva JE 1962. Nota sobre os flebotomíneos do estado de Goiás, com a descrição de duas espécies novas e da fêmea de *Lutzomyia longipennis* (Barreto, 1946) e a redescrição do macho de *L. evandroi* (Costa Lima & Antunes, 1936) (Diptera: Psychodidae). *Rev Bras Malariol Doencas Trop 14*: 379-394.

Martins AV, Falcão AL, Silva JE 1964. Um novo flebótomo do estado de Goiás, *Lutzomyia teratodes* sp.n. (Diptera: Psychodidae). *Rev Bras Biol 24*: 321-324.

Martins AV, Falcão AL, Silva JE 1975. Descrição de fêmea de *Lutzomyia teratodes* Martins, Falcão & Silva 1964 (Diptera: Psychodidae: Phlebotominae). *Rev Bras Biol 35*: 515-517.

MS/SVS - Ministério da Saúde/Secretaria de Vigilância em Saúde 2007. *Manual de Vigilância da Leishmaniose Tegumentar Americana*, 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) braziliensis* in Rio de Janeiro (Brazil) revealed by a PCR multiplex non-isotopic hybridization assay. *Acta Trop 99*: 905-913.

Pugedo HR, 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 de 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.

SESAU/TO - 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. *Psychodopygus 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, Grajauskas AM, Carvalho BM, Brahim LRN, Kozlowsky D, Rangel EF 2008. Sand fly survey in the influence area of Peixe Angical hydroeletric 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 leishmaniases in impacted area by hydroelectric plant, state of Tocantins, Brazil. *PLoS ONE 6*: e27721.