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# Phlebotomine fauna in the Ponta Porã city: epidemiological importance in border line between Brazil and Paraguay

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## ARTICLE INFO

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# ABSTRACT

Objective: To identify the urban phlebotomine sandfly fauna in Ponta Porã, Mato Grosso do Sul State, Brazil. Methods: The captures were undertaken from April 2009 to March 2010 with CDC light traps in 14 ecotopes (intra and peridomicile) in different areas of the city, Shannon traps being used in areas with abundant vegetation. Results: A total of 707 specimens were captured with CDC light traps (565 males and 142 females) and 155 specimens (112 males and 43 females) with Shannon traps, a total of 862 phlebotomines. The specimens captured belonged to eight species: Lutzomyia longipalpis (Lutz & Neiva, 1912), Evandromyia cortelezzii (Brethes, 1923), Sciopemyia sordelli (Shannon & Del Ponte, 1927), Pintomyia pessoai (Coutinho & Barretto, 1940); Pintomyia monticola (Costa Lima, 1932); Brumptomyia brumpti (Larousse, 1920); Nyssomyia whitmani (Antunes & Coutinho, 1939) and Psathyromyia shannoni (Dyar, 1929). Lutzomyia longipalpis, the main vector of Leishmania (Leishmania) chagasi, was the species most frequently captured (97.03%) and also the most abundant according to the standardized abundance index (SAI)=0.86. Conclusions: The highest species richness was captured, with CDC light traps, inside the domiciles and the species diversity and evenness in the peridomicile, clearly indicating a preference for anthropic environments.

# 1. Introduction

Visceral and cutaneous leishmaniases are parasitic diseases caused by several protozoal species of the genus Leishmania that determine different clinical forms[1]. All the species of the genus are transmitted by the bite of infected females of the Phlebotominae subfamily, belonging to the genus *Lutzomyia* in the New World and Phlebotomus in the Old World[2].

The State of Mato Grosso do Sul (MS) has been presenting high rates of infection of Leishmania (Leishmania) chagasi, the agent of visceral leishmaniasis, in both humans and dogs, other human infections by *Leishmania* spp, agents of cutaneous leishmaniasis, and also a great number of

*Leishmania* sp. vectors[3–5].

The environmental changes caused by the intense migratory process, the increasing urbanization and socioeconomic pressures have triggered the expansion of endemic areas and the occurrence of new outbreaks. Sandfly species become resistant to adverse conditions and approach the peridomicile increasingly, facilitating the transmission of these diseases<sup>[6]</sup>.

Ponta Porã was considered a silent area or a region without transmission of visceral leishmaniasis (VL) until the appearance of the first human case in 2007. Thereafter, it was included among the vulnerable cities that, according to the Ministry of Health, do not present autochthonous cases of human and/or canine visceral leishmaniasis, but meet one or more of the following criteria: cities neighboring those reporting VL cases; those having intense migratory flux; or those belonging to the same road axis as cities with disease cases[7].

The aim of this study was to identify the species of phlebotomine sandflies found in the urban area of Ponta Porã, on the Brazil-Paraguay border, so as to provide information for the leishmaniasis control program.

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# 2. Material and methods

# 2.1 Area of study

Ponta Porã is located in southern Mato Grosso do Sul State, in the micro-region of Dourados (22°53′10″ S, 55°42′32″ W, 655 m above sea level), with a population of 75 941 inhabitants in 2009.

The municipality's total area is 5328.62 km² and climate tropical with a dry winter and heavy concentration of rainfall from November through March. The average monthly temperature throughout the year is 23 °C and the typical vegetation is savannah and Atlantic Forest. Ponta Porã, which presents a growing tourist industry, has a common land border with Pedro Juan Caballero city, Paraguay, with no intervening geographical barriers[8].

# 2.2 Collections

Phlebotomine sand flies were captured with CDC automatic light traps fortnightly installed at seven sites in Ponta Porã, both intradmicile and in the peridomiciles, totaling 14 ecotopes. Collection was carried out in the period between April 2009 and March 2010, from 6 pm to 6 am, disregarding the summer time change. The sites were chosen so that they could comprise the whole urban area, considering the healthcare regions already defined for the municipality actions. The traps were so installed: Site 1: inside and outside a property measuring 2500 m<sup>2</sup>, a place used for breeding horses located at Jardim Ivone; Site 2: in the intra- and peridomicile of a property (123 m<sup>2</sup>) located at Jardim das Paineiras; Site 3: a house located in São João neighborhood; collections occurred both inside the house and in the peridomicile (in the hen-house); Site 4: Pousada do Bosque Hotel, located in the central area, where there is a remaining forest with preserved headspring and three residences - the traps were installed inside and outside the hen-house of one of the residences; Site 5: a house located at Bairro Granja, near a military area, 50 meters far from a stream; Site 6: an area of 230 m<sup>2</sup> where the traps were installed inside the house and in the peridomicile of a hen-house; Site 7: an area of 500 m<sup>2</sup> at Bairro São Francisco, with extensive swine and poultry breeding.

At Pousada do Bosque, captures using white Shannon traps, with a stand containing fluorescent lamps connected to a 12-volt battery were also carried out. Phlebotomine sand flies were captured manually with the aid of collector tubes labeled

with the collecting time and inserted in a damp plaster pot until exam.

The phlebotomine sand flies captured were separated from other insects; males were placed on Petri dishes and taken to the Parasitology Laboratory of the Federal University of Mato Grosso do Sul, where they were clarified and then arranged on plates for identification.

Dissection was used for studying the flagellates in the females' digestive tract. This method is the most commonly used in natural infection research and requires skill to be performed. The females were immobilized in refrigerator and placed on a dry blade for removal of wings and paws. Dissection was performed under a stereoscopic microscope with the aid of a scalpel. A drop of sterile saline (0.9%) was placed on the plates, where dissection was carried out. By making two sections in the final portion of the abdomen, the digestive tract was removed with zigzag movements, covered with glass slide and examined under an optical microscope (400 × magnification) for flagellates research and phlebotomine identification using head, thorax and abdomen structures in accordance with Galati's keys<sup>[9]</sup>.

The standard abundance index (SAI) of Roberts & His[10] was used to calculate species abundance; Williams' geometric mean<sup>[11]</sup> to check the seasonality of the most frequent species; the Shannon Index (*H*), to analyze the species diversity in a given environment and Pielou's Index (*J*) 12 to measure the contribution of each species to the community of a particular ecotope.

The analyze of correlation between the collected variables was performed by pearson linear correlation test. The significant level was 5% (*P*<0.05).

Data from the meteorological station in Ponta Porã, available at the National Institute for Space Research (NISR) database 13 were used to interpret the climatic results.

# 3. Results

A total of 707 phlebotomine sandfly specimens were captured with CDC automatic light traps and 155 using Shannon traps, total of 862 phlebotomines. Of that total, 565 were males and 142 females captured whit CDC, in a 3.97:1 ratio, belonging to three sub–tribes, five genera and five species: Lutzomyiina – Lutzomyia longipalpis (Lutz & Neiva, 1912) (Lu. longipalpis) and Evandromyia cortelezzii (Brethes, 1923) (Ev. cortelezzii); Bumptomyiina – Brumptomyia brumpti (Larousse, 1920) (Br.

**Table 1.**Phlebotomines captured fortnightly with CDC automatic light traps, by species, sex, ecotope, species diversity (*H*) and evenness (*J*) indices, in 14 environments of Ponta Porã city, Mato Grosso do Sul State, Brazil, from April 2009 to March 2010 (continues).

	Hou	House 1 Hou		use 2 House 3		Pousada do		House 4		House 5		House 6		Total		Total	
Phlebotomines							Bos	sque									
	I	P	I	P	I	P	I	P	I	P	I	P	I	P	_ M F	MF	%
	M F	M F	M F	M F	M F	M F	M F	M F	M F	M F	M F	M F	M F	M F			
Br. brumpti								- 01							- 01	01	0.14
Ev. cortelezzii			- 02				- 03								- 05	05	0.71
Lu. longipalpis	02 -	01 -	62 14	79 10	157 50	59 06	106 30	89 16	- 02	- 01	02 -	01 -			557 129	686	97.03
Ny. whitmani			- 01	- 02			- 01								04 04	08	1.13
Ps. shannoni							- 02	04 01							04 03	07	0.99
Total	02 -	01	62 17	79 12	157 50	59 06	106 36	93 18	- 02	- 01	02 -	01 -			565 142	707	100
Shannon (H)	0	0	0.19	0.11	0	0	0.22	0.23	0	0	0	0	0	0	0.17	% ma	ales 79.92
Pielou (J)	0	0	0.17	0.15	0	0	0.16	0.21	0	0	0	0	0	0	0.11	% fer	males 20.08

I: Intradomicile; P: Peridomicile; M: male; F: female.

brumpti); Psychodopygina—Nyssomyia whitmani (Antunes & Coutinho, 1939) (Ny. whitmani) and Psathyromyia shannoni (Dyar, 1929) (Ps. shannoni). Lu. longipalpis was the most frequently found species, accounting for 686 (97.03%) of the sandflies captured.

The highest species (4 species) occurred in the intradomicile, and the highest diversity (H=0.23) and evenness (J=0.21) indices in the peridomicile of the Pousada do Bosque (Table 1). At site 6 no specimen was captured. *Lu. longipalpis* was the only species captured at all the other sites, occurring exclusively in eight of the 14 ecotopes sampled throughout the urban area of Ponta Porã (Table 1).

Lu. longipalpis was the most abundant species, presenting a standardized abundance index (SAI)=0.86, followed by Ny. whitmani (0.11) (Table 2).

**Table 2.**SAI according to species of phlebotomines captured in Ponta Porã city, Mato Grosso do Sul Sate, Brazil, from April 2009 to March 2010.

Species	SAI	Position
Lu. longipalpis	0.86	1st
Ny. whitmani	0.11	2nd
Ps. Shannoni	0.07	3rd
Ev. cortelezzii	0.07	3rd
Br. Brumpti	0.02	5th

Table 3 shows the number of phlebotomine sandflies collected monthly from April 2009 to March 2010. The Williams' geometric mean was used to quantify the frequency and regularity of the most abundant species presented in Figure 1

## and Table 3.

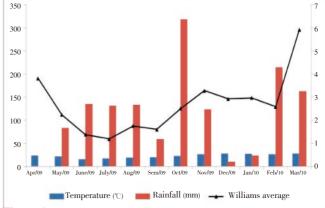


Figure 1.

Mean monthly temperature (°C), monthly rainfall (mm) and monthly frequency (Williams' ceometric mean) of *Lu. longipalpis* captured in 14 ecotopes of Ponta Porã, MS, Brazil, from April 2009 to March 2010.

There was positive and significant correlation (r=0.7755; P=0.003) between temperature and Williams' geometric mean. When analyzed monthly rainfall and Williams' geometric mean, there was no correlation (r=-0.04494; P=0.8897). No correlation was also observed between the variables temperature and monthly rainfall (r=-0.1304; P=0.6862).

With the Shannon trap, 155 specimens were captured (112 males and 43 females); 2.6:1 ratio. These samples belong to two sub–tribes, six genera and seven species: *Lutzomyiina – Lu. longipalpis, Ev. cortelezzii, Sciopemyia sordellii* (Shannon & Del Ponte, 1927), *Pintomyia pessoai* (Coutinho & Barretto, 1940) and *Pintomyia monticola* (Costa Lima, 1932) and *Psychodopygina*:

Table 3
Monthly distribution of phlebotomines captured fortnightly with CDC light traps in 14 environments of Ponta Porã city, Mato Grosso do Sul State, Brazil, from April 2009 to March 2010.

Species	2009											2010			%
	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec		Jan	Feb	Mar	Total	
Br. brumpti	-	-	-	-	-	-	-	1	-		-	-	-	1	0.14
Ev. cortelezzii	-	-	-	-	1	2	-	-	2		-	-	-	5	0.71
Lu. longipalpis	135	34	13	2	7	12	44	56	55		60	64	204	686	97.03
Ny. whitmani	-	-	-	-	-	-	-	2	2		4	-	-	8	1.13
Ps. shannoni	2	3	-	-	-	2	-	-	-		-	-	-	7	0.99
Total	137	37	13	2	8	16	44	59	59		64	64	204	707	100

Table 4
Phlebotomines captured monthly with Shannon trap between 6 pm and 10 pm in an urban area of Ponta Porã city, MS, Brazil, from April 2009 to March 2010.

Species	6 pm to 7 pm		7 pm t	7 pm to 8 pm		8 pm to 9 pm		9 pm to 10 pm		Total				
	M	F	M	F	M	F	M	F	M	F	MF	% MF		
Ev. cortelezzii	_	2	_	_	-	-	-	_	-	2	2	1.3		
Lu. longipalpis	2	-	1	-	13	3	-	-	16	3	19	12.3		
Ny. whitmani	-	3	-	4	5	3	1	-	6	10	16	10.3		
Pi. monticola	-	2	-	-	-	-	-	-	-	2	2	1.3		
Pi. pessoai	8	4	9	4	1	2	-	2	18	12	30	19.3		
Ps. shannoni	35	3	15	5	18	4	4	-	72	12	84	54.2		
Sc. sordellii	_	_	_	_	-	_	-	2	-	2	2	1.3		
Total	45	14	25	13	37	12	5	4	112	43	155	100		

M=male; F = female.

Ny. whitmani and Ps. shannoni. The most frequently captured species were Ps. shannoni (54.2%) and Pi. pessoai (19.2%). The highest numbers of phlebotomine sandflies (38.1%) occurred between 6pm and 7 pm, when 45.0 % of Ps. shannoni specimens and 40.0% of Pi. pessoai were captured; however, Lu. longipalpis (84.2%) and Ny. whitmani (50.0%) presented their highest frequencies between 8 pm and 9 pm. Only females of Ev. cortelezzii, Pi. monticola and Sc. sordellii were captured; among the species both sexes of which were captured, females predominated over males only for Ny. whitmani (62.5%), the former being the less frequent for Lu. longipalpis (6.25%), Pi. pessoai (40.0%) and Ps. shannoni (14.3%).

A female of *Ev. cortelezzii* captured on Shannon traps, installed in the central area of the city was found to be infected by flagellates in the midgut and hindgut of the digestive tract, accounting for 14.3% of all samples of this species.

# 4. Discussion

Lu. longipalpis is among the species most frequently caught in Ponta Porã. In studies carried out by Oliveira et al[3] this species was not the most abundant; however, its gradual adaptation to urban areas in different regions of Mato Grosso do Sul is noteworthy[4,14].

It is important to emphasize that this species is the main vector of the etiological agent of visceral leishmaniasis in the State and participates actively in the epidemiological chain of this disease in Brazil[2]. Our findings are consistent with those of the literature regarding the expansion of the geographical distribution of *Lu. longipalpis*, this expansion probably being a direct consequence of changes in the ecosystems caused by human predatory actions and deforestation[15,16].

The second most abundant species, *Ny. whitmani*, is involved in the transmission of cutaneous leishmaniasis in several regions of Brazil, It was present both indoors and in areas surrounding homes, which shows that it has become adapted to human–modified environments<sup>[17]</sup>. In Mato Grosso do Sul, it has been found in several towns, including Antonio Joao, which borders on the municipality of Ponta Porã<sup>[18]</sup>. This species is widespread, representing an ample adaptation to different ecological niches and triggering behavioral changes in different populations<sup>[19–24]</sup>.

The species diversity index of phlebotomine sandflies ranged from H=0 to H=0.23. Its highest value was found in the peridomicile of Pousada do Bosque, a place with an accumulation of garbage and profuse surrounding vegetation, an environment favorable to species richness, as observed by Oliveira *et al*[3].

The most numerous capture of specimens occurred at Site 3, followed by those at Pousada do Bosque and Site 2. All three domiciles are located in the central area of the city and have in common nearby hen–houses and decomposing organic matter, features favorable to vector development, especially of *Lu. longipalpis* that is usually found in anthropic environments. Further study should be undertaken in the area so that the relationship between capture sites, quantity of cases reported and number of specimens captured may be established.

In relation to the proportion that each species contributes to the community of a particular ecotope, it can be observed that the highest value was seen around the peridomicile of Pousada do Bosque. It is worth noting that this site is located in the central area of Ponta Porã, which may favor the spread of leishmaniasis in the urban core. The presence of only

one species can be observed at sites where J=0; it being important to highlight that the higher the value, the greater the occurrence of equal proportion between the species.

The correlation between the mean temperature and rainfall in the region over the period analyzed and the frequency and regularity of the most abundant species collected (*Lu. longipalpis*) was investigated. On the scale, had a close—to—1 value, indicating maximum abundance. These data have already been observed by some authors in studies conducted in other regions of Mato Grosso do Sul State[4,6].

This species was captured throughout the year. Control measures against the vector in these periods are highly to be recommended[25].

Although acknowledging that the study of seasonality shows reliable and consistent data only after at least two consecutive years of investigation to compare the results, thus reducing the margin of error of atypical years, this study indicated a density reduction of phlebotomine sandflies in the cold months, probably due to unfavorable environmental conditions for immature forms.

Although the requirements of vector incrimination of *Ev. cortelezzii* have not been met, the increase of its frequency in the peridomicile, and the environmental conditions favorable to the persistence of the enzootic cycle of Leishmania are factors indicating its vector potential. Even though, more studies about this species should be conducted as suggested by Carvalho *et al*[26].

Among the species captured in Shannon trap, useful capture method for analyzing the anthropophilic species, there is the presence of *Lu. longipalpis* and *Ny. whitmani*, both involved in cutaneous and visceral leishmaniasis transmission. *Ps. shannoni* predominated among the species. This species has been found to be attracted by humans in several regions,in Mato Grosso do Sul, its epidemiologic importance has been observed in several studies, because eventually can feed on man[6,27,28].

Even if this species is not associated with leishmaniasis transmission, it should be studied as it is an important arbovirus transmitter<sup>[29]</sup>.

This study was undertaken in an urban area, which accounts for the low number of specimens of some of the species captured, the natural habitat of which is the forest environment.

In brief, the eight species captured in this study are part of the already known phlebotomine sandfly fauna of Mato Grosso do Sul, three of them, known as vectors of Leishmania sp agents: Ny. whitmani and Pi. pessoai, are vectors of cutaneous leishmaniasis agents, and Lu. longipalpis, the most abundant species, is the main vector of the LV agent. The high frequencies of this last species in the anthropic environment associated with the presence of canine cases of visceral leishmaniasis 25 represent favorable conditions for the expansion and urbanization of human cases.

Due to the geographical location of the area studied and considering its epidemiologic importance as a city of LV transmission, it is essential to be aware of the vector's dispersal and the need to conduct entomological studies and sample surveys for canine information so that the data may serve as a basis for the design of strategies for prevention and control before the disease spreads further, as has already been occurring in many cities of Mato Grosso do Sul.

# **Conflict of interest statement**

We declare that we have no conflict of interest.

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