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# Ecology of Lutzomyia (Nyssomyia) whitmani in an urban area in Brazil

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## **ABSTRACT**

## **Objectives**

To study the fauna, hours of activity and seasonality of phlebotomines in forest animal habitats in the Ingá Park, on the urban perimeter of the municipality of Maringá, Paraná State, Brazil.

## Methods

Phlebotomines were collected on the urban perimeter using Shannon traps from 6:00 p.m. to 6:00 a.m. and Falcão traps from 8:00 p.m. to 12:00 midnight, on two nights per month from October 1998 to September 2000. The Shannon traps were installed on the banks of the Moscados stream; the Falcão traps were placed in shelters for captive birds, forest mammals and reptiles.

#### Results

The Shannon traps yielded 13,656 phlebotomines, with predominance of *Lutzomyia whitmani* (98.7%). The Falcão traps yielded 4,040 phlebotomines, with *L. whitmani* again predominating (96.3%). *L. whitmani* was most frequently found between 12:00 midnight and 2:00 a.m. in the Shannon traps. This species was most frequently collected in the months of August (55.1%) in the Shannon traps, and in March 1999 (19.2%), November 1999 (15.9%) and August 2000 (20.6%) in the Falcão traps. More phlebotomines were collected in Falcão traps installed in shelters for captive mammals (84.0%).

## Conclusion

There was a clear predominance of L. whitmani in the Ingá Park. The greatest frequency of L. whitmani was in August from Shannon traps, and in March, August and November from Falcão traps. The peak activity of L. whitmani took place between 12:00 midnight and 2:00 a.m.

# Keywords

Psychodidae. Leishmaniasis, cutaneous. Ecology, vectors. Insect vectors. Seasonal variations. Urban zones. American tegumentary leishmaniasis. Phlebotominae sandflies. *Lutzomyia whitmani*.

## INTRODUCTION

Up to the middle of the 20<sup>th</sup> century, approximately 40,000 cases of leishmaniasis had occurred at various localities within Brazilian territory. Subsequently, there was a brief period of respite, but over the last two decades the number of cases has noticeably increased and cases are being notified from all Brazilian states. 7

In the State of Paraná, American tegumentary leishmaniasis had also been recorded since the beginning of the 20<sup>th</sup> century, with reports of cases up to 1958.<sup>5</sup> It started to be officially notified again from 1980, with the occurrence of cases in 276 of the state's 399 municipalities and concentration in its northern and western regions, despite the deforestation of more than 90% of the state's territory.<sup>5</sup>

The occurrence of tegumentary leishmaniasis in the urban zones of large and medium-sized cities has been noted in Brazil since the beginning of the 20<sup>th</sup> century. In the State of Paraná, there has been notification of cases of this disease in the urban areas of small cities (Cianorte) and medium-sized cities (Maringá).<sup>5</sup> Thus, there is an interest in studying the ecology of phlebotomines in the urban area of the municipality of Maringá, since these insects transmit the protozoa of the *Leishmania* genus that act as etiological agents for tegumentary leishmaniasis.<sup>1,2,6,8,10</sup> In the Ingá Park, the remaining native forest provides shelter for a variety of forest mammal species, which form a potential reservoir for

Leishmania, in addition to species kept in captivity, thereby making possible the existence of a complete enzootic cycle for Leishmania Ross, 1903.

In an earlier study in the Ingá Park and another two preserved areas within the urban perimeter of Maringá that are covered with native forest, phlebotomines were found to be present within the forest, especially within habitats for wild animals. In the present investigation, the aim was to expand the knowledge of phlebotomine fauna composition, the inclination of these insects to feed on blood from certain hosts, and the hours of the night and the months in which they are most frequent, in the Ingá Park.

## **METHODS**

The Ingá Park, where the research was carried out, is a preserved area covered with native forest, located on the urban perimeter of the municipality of Maringá, in the State of Paraná, with an area of 47.43 hectares. The forest is of dense tropical to subtropical transition type, with partial leaf fall from some tree species in the less favorable winter season, when a slight drought takes place. The municipality of Maringá is located in the northwest of the state at the latitude of 23°25′ south and longitude of 51°25′ west of Greenwich. It has average annual precipitation of between 1500 and 1600 mm and an average annual temperature of 20 to 21°C, with average maximums of 27 to 28°C and minimums of 16 to 17°C. In the least rainy three months (June, July and August), the precipitation measured is 200 to 250 mm and in the rainiest three months (December, January and February) it is 500 to 550 mm.

The phlebotomine collections were carried out using Shannon and Falcão traps. During the period when the research was carried out, infrastructure work was being performed inside the Ingá Park, which made it difficult to access the Shannon trap installation localities. Thus, it was decided to move the Shannon trap locations, such that the Shannon trap collections were divided into two stages. In the first stage, collections were done from October 1998 to September 1999. On two nights per month, from 6:00 p.m. to 6:00 a.m., collections were done using Shannon traps installed on the banks of the Moscados stream (Figure 1). On another two nights, from 8:00 p.m. to 12:00 midnight, collections were done using Falcão traps installed in shelters for captive birds, mammals and reptiles.

In the second stage, from October 1999 to September 2000, the Shannon traps were installed on a kiosk used for recreation, beside the lake formed by the Moscados stream (Figure 1). At this new locality, there was no interference between the collections via the two trap types. Thus, on two nights of each month, collection was done simultaneously for the two collection methods, at the same times as for the first stage.

The Falcão traps (FT) were distributed in the following manner (Figure 1):

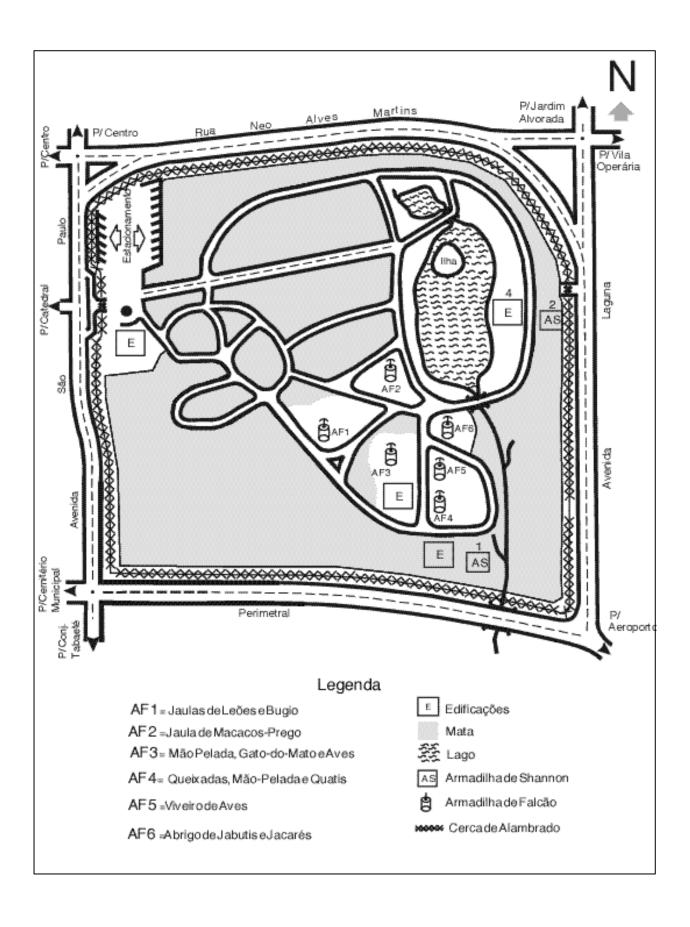


Figure 1 - Localities for phlebotomine collection in the Ingá Park, on the urban perimeter of Maringá, State of Paraná, southern Brazil.

FT1 – in a cage divided in two, housing a pair of lions (*Panthera leo*) in one half and a howler monkey (*Alouatta fusca*) in the other;

FT2 – in a shelter containing a large number of capuchin monkeys (Cebus apella);

FT3 – in a pair of coupled shelters containing a raccoon (*Procyon cancrivorus*), two tiger cats (*Leopardus tigrinus*) and one bird (*Penelope obscura*);

FT4 – in a shelter containing a pair of peccaries (*Tayassu pecari*), which were substituted by a raccoon (*Procyon cancrivorus*) in April 1999 that, in its turn, was substituted by a large number of coatis (*Nasua nasua*) (close to 100), in April 2000;

FT5 – in a shelter containing a large number of forest bird species;

FT6 – in an area closed off by wire netting, containing several tortoises (*Geochelone chilensis*), which were substituted by caimans (*Caiman* spp.) in April 1999.

The light source for the Shannon traps was a 100W incandescent light bulb. The phlebotomines were collected from the internal and external walls of the trap by two persons using glass tubes containing cotton steeped in chloroform. Hour by hour, the insects collected in these traps were packed in small cardboard boxes and subsequently prepared and identified in the laboratory.

The Falcão traps were provided with 3W light bulbs 3W. After collection, the insects were killed using chloroform and also packed into small cardboard boxes, for subsequent preparation and identification in the laboratory.

The changes in animal distribution made during the period that the work was performed were made by the administration of the Ingá Park, with the aim of the animals' wellbeing.

To draw up the graph of monthly phlebotomine distribution, the Williams geometrical average  $(MG_W)^4$  for the collections done was utilized. The numbers of phlebotomines captured in the different traps were compared via variance analysis. The chi-squared test was utilized for comparing the numbers of phlebotomines of each sex. The significance level considered was p=0.05, with the utilization of the Statistica software, version 5.5.

#### RESULTS

The species collected are reported in the Table.

**Table** – Phlebotomines collected in the Ingá Park, on the urban perimeter of Maringá, from October 1998 to September 2000.

	Shanno	n Trap	Falcão Trap				
Species / Sex	Female	Male	Subtota	l Female	Male	Subtotal Total	
Lutzomyia whitmani	10,063	3,410	13,473	2,161	1,727	3,888	17,361
Lutzomyia neivai	8	16	24	27	21	48	72
Lutzomyia migonei	54	46	100	29	46	75	175

Lutzomyia fischeri	5	0	5	1	0	1	6	
Lutzomyia monticola	17	0	17	8	2	10	27	
Lutzomyia firmatoi	1	1	2	4	1	5	7	
Lutzomyia cortelezzi	7	1	8	2	0	2	10	
Lutzomyia sp.	9	2	11	3	0	3	14	
Lutzomyia pessoai	0	0	0	1	1	2	2	
Lutzomyia shannoni	0	0	0	0	1	1	1	
Brumptomyia								
brumpti	13	1	14	1	3	4	18	
Total	10,177	3,477	13,654	2,237	1,802	4,039	17,693	
No. of collection								
hours	576	576	576	192	192	192	-	
Hourly average	17.7	6.0	23.7	11.7	9.4	21.0	-	

It was noted that the quantities of phlebotomines collected in the Shannon and Falcão traps were 13,654 (hourly average of 23.7) and 4,039 (hourly average of = 21.0), respectively (Table). It was also observed that L. whitmani accounted for 98.7% of the phlebotomines (13,473 specimens) collected from the Shannon traps and 96.3% (3,888 specimens) from the Falcão traps. The numbers of species collected in the Falcão and Shannon traps were 10 and 8, respectively. More female phlebotomines were collected in the Shannon traps than males (p<0.01), but this was not seen in the Falcão traps.

Figure 2 shows that the annual distribution of L. whitmani (MG<sub>W</sub>) was similar for the two trap types. In the Shannon traps, L. whitmani was most frequent in November 1998, August 1999 and April and August 2000. In the Falcão traps, L. whitmani was most frequent in March, July and November 1999 and March/April and August 2000.

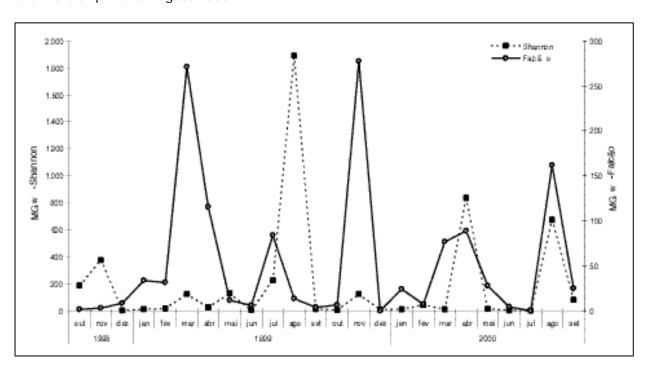
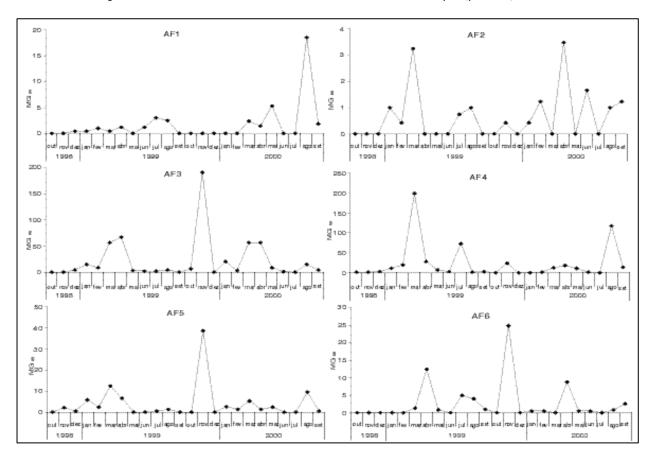


Figure 2 - Seasonal distribution of the collections of *Lutzomyia whitmani* in Shannon traps and the set of six Falcão traps, in the Ingá Park, on the urban perimeter of Maringá, from October 1998 to September 2000.

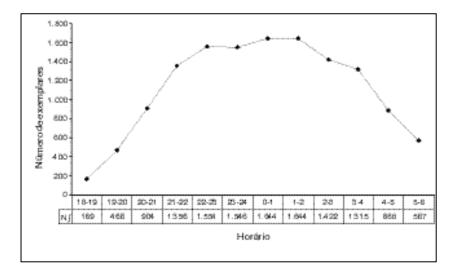
Figure 3 shows the distribution of *L. whitmani* (MG<sub>w</sub>) in each of the Falcão traps. The quantity of phlebotomines collected in FT4 was significantly different from the quantities collected in FT1, FT2, FT5 and FT6 ( $p \le 0.05$ ). Although the quantities of this phlebotomine in the traps FT3 and FT4 were not statistically different from each other (p = 0.95), the quantity of phlebotomines collected in FT3 was not statistically different from what was collected in the other traps ( $p \le 0.18$ ).



**Figure 3** - Seasonal distribution of *Lutzomyia whitmani* specimens collected in Falcão traps (FT) in shelters for captive forest animals in the Ingá Park, on the urban perimeter of Maringá, from October 1998 to September 2000.

In FT3, located in the coupled shelters containing forest animals (raccoon, tiger cats and a bird), an average of 7.8 phlebotomines were collected per hour. During the period when collections were done, there were changes in the animals kept in the shelter where FT4 was installed, thus giving rise to three sub-periods. From October 1998 to March 1999, when the shelter was housing a pair of peccaries, an hourly average of 10.9 phlebotomines was collected. From April 1999 to March 2000, when a raccoon was substituted for the pair of peccaries, the hourly average was 4.6 phlebotomines. From April to September 2000, a large number of coatis was substituted for the raccoon and the hourly average was 19.1 phlebotomines. Analysis of the distribution of *L. whitmani* during these three sub-periods showed that there was no significant difference between them. Over these same sub-periods, there was also no observed difference in the distribution of phlebotomines among the other Falcão traps.

In Figure 4, it can be seen that *L. whitmani* was present in the Shannon traps from 6:00 p.m. to 6:00 a.m.



**Figure 4** - Hourly frequency of *Lutzomyia whitmani* in Shannon traps in the Ingá Park, on the urban perimeter of Maringá, from October 1998 to September 2000.

### DISCUSSION

The species *L. shannoni* can now be added to the nine species of phlebotomine previously noted in the Ingá Park. <sup>16</sup> The almost absolute domination of *L. whitmani* over the other species corroborates what was previously observed in this park. <sup>16</sup> The high prevalence of this species has been observed in endemic areas of tegumentary leishmaniasis in northern Paraná. <sup>6,11-16</sup> The infection of *L. whitmani* by *Leishmania* (*Viannia*) *braziliensis* that has been proven in the States of Paraná and Ceará highlights the importance of this species in the epidemiology of that disease.

The hourly average number of phlebotomines collected in the Shannon traps (23.7 specimens) was similar to what was collected among the Falcão traps (hourly average of 21.1). However, the distribution of the Falcão traps among a greater number of ecotypes favored the collection of a greater number of species.

The concentration of phlebotomines in the shelters for captive forest animals, especially mammals (FT3 and FT4) suggests that these insects are strongly attracted to such animals, except in the cases of primates and felines (lion). This fact has already been noted in the Ingá Park. <sup>16</sup> The results suggest that the larger quantities of phlebotomines collected in FT3 and FT4 may have been due to the presence of mammals in the shelters where these traps were placed. However, according to Campbell-Lendrum et al<sup>3</sup> (1999) and Quinnel et al<sup>9</sup> (1991), the order of preference that these insects show in relation to hosts is a function of the size or density of such hosts.

The small number of phlebotomines collected in the shelter housing the lions and howler monkey (FT1) was different from what was previously collected, <sup>16</sup> when this same shelter was housing a jaguar and yielded the greatest number of phlebotomines of all the shelters for captive forest animals. The low attractive power of the pair of lions for phlebotomines may be explained by the fact that this mammal does not form part of the native Brazilian fauna. The presence of a howler monkey in the cage coupled to the cage of the pair of lions suggests that primates attract few phlebotomines. This was also seen in the trap installed in the shelter for the capuchin monkeys (FT2). This was observed

previously, 16 in the same shelter, thereby giving evidence that these insects are little attracted towards primates.

The time of greatest frequency of *L. whitmani* was from 12:00 midnight to 2:00 a.m. However, in the Ingá Park there is a risk of infection by *Leishmania* throughout the night, especially between 8:00 p.m. and 5:00 a.m. In the endemic areas in northern Paraná, times of greatest frequency of phlebotomines similar to those observed in the present investigation have been observed.<sup>11,13</sup>

In the collections done using Falcão traps, the majority of L. whitmani specimens (52.2%) were collected during months that are normally hotter and rainier (March, April and November), although a considerable proportion (31.1%) were collected in colder and drier months (June, August and September). Thus, seasonality similar to what has previously been observed in rural areas of northern Paraná for L. whitmani using the same trap type can be confirmed. The results from the present work also show that, in the collections performed in the Shannon traps, the greatest number of specimens of L. whitmani (55.1%) was collected in one of the driest and coldest months of the year (August). This therefore differs from the seasonality observed previously, which was also in the rural area of this region, and using the same trap type.

The results from the present investigation show that there is clear predominance of *L. whitmani* in the Ingá Park, since this species had almost absolute prevalence using both collection methods. The results suggest that raccoons, coatis, peccaries and tiger cats attract more phlebotomines that the other mammals, forest birds and reptiles present in the Ingá Park. The frequency of *L. whitmani* increases up to 12:00 midnight, remains constant until 2:00 a.m. and thereafter gradually decreases. In the collections using Falcão traps, *L. whitmani* is most frequent in March, April and November, when the temperatures and rainfall in northern Paraná are higher. In the collections using Shannon traps, *L. whitmani* is more frequent in August, when the temperatures and rainfall are lower. It is recommended that people be prohibited from entering and remaining in the Ingá Park during the night, so as to avoid infection by *Leishmania*. Further evaluation through studies on the attraction of phlebotomines towards forest animals needs to be undertaken.

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