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SPATIAL DISTRIBUTION AND TEMPORAL VARIABILITY OF PHLEBOTOMINAE AT THE CUIABÁ CAMPUS OF THE FEDERAL UNIVERSITY OF MATO GROSSO, BRAZIL

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ABSTRACT

Phlebotomine sand flies are vectors of *Leishmania* to humans and other animals. The aim of this study was to verify the spatial distribution and effect of some abiotic factors on the abundance and temporal variability of Phlebotominae at the Cuiabá campus of the Federal University of Mato Grosso (600,000 m^2). CDC traps were installed at distances of 1,000 to 300 m s from each other and at a height of 1.5 m from the ground, between 17:00 and 07:00, distributed at six sites on the campus. Monthly collections were made on three consecutive days from December 2008 to December 2011. The living sand flies captured were sacrificed in ethyl acetate and then screened under a stereoscopic microscope. The insects were then subjected to clarification and identified. The abiotic data of temperature and average relative humidity during the times when the traps remained at the insect collection sites were determined with a digital thermometer-hygrometer. A non-linear mathematical model was used to evaluate the association between the abiotic variables and the number of insects caught. Species of Phlebotominae with and without the capacity to transmit *Leishmania* were captured at the site during the study period. The abundance of phlebotominae presented spatial and temporal variability by virtue of abiotic and other variables. The most favourable conditions for the occurrence of Phlebotominae at the site were a temperature of 23.2 ºC and a relative humidity of 70%. Temperatures above 35 °C or below 18 °C and a relative humidity lower than 18% were unfavourable for the Phlebotominae.

KEY WORDS: Leishmaniasis; vector; population dynamics; abiotic factors.

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RESUMO

Distribuição espacial e variabilidade temporal de flebotomíneos no campus de Cuiabá da Universidade Federal de Mato Grosso

Flebotomíneos são insetos vetores de *Leishmanias* para humanos e outros animais. O objetivo do trabalho foi verificar a distribuição espacial e o efeito de alguns fatores abióticos sobre a abundância e a variabilidade temporal de flebotomíneos no *campus* de Cuiabá da Universidade Federal de Mato Grosso (600.000 m^2). Armadilhas CDC foram instaladas com distâncias entre si de 1.000 a 300 m, a 1,5 m de altura, das 17 h às 7 h, distribuídas em seis locais do *campus* para coletas mensais e em três dias consecutivos, de dezembro de 2008 a dezembro de 2011. Os flebótomos capturados vivos foram encaminhados ao Laboratório de Entomologia da UFMT, colocados em acetato de etila e, em seguida, foram triados sob observação em microscópio estereoscópico. Após esse procedimento, os insetos foram submetidos ao processo de clarificação e identificados. Os dados abióticos de temperatura e umidade relativa médias dos horários em que as armadilhas permaneceram nos locais de coleta de insetos foram determinados com um termohigrômetro digital. Para avaliar o efeito das variáveis abióticas sobre o número de insetos capturados, utilizou-se um modelo matemático não linear. Espécies de flebotomíneos com e sem a capacidade de transmissão das *Leishmanias* foram capturadas no local durante o período estudado. A abundância de flebotomíneos apresentou variabilidade espacial e temporal em razão de variáveis abióticas e de outras variáveis. As condições mais favoráveis à ocorrência de flebotomíneos no local foram: temperatura de 23,2ºC e umidade relativa de 70%. Temperaturas acima de 35°C ou abaixo de 18°C e umidade relativa inferior a 18% foram desfavoráveis para os flebotomíneos.

DESCRITORES: Leishmaniose; vetor; dinâmica populacional; fatores abióticos.

INTRODUCTION

The incidence of American tegumentary leishmaniasis and visceral leishmaniasis (VL), especially the latter in canine and human carriers, is growing in the State of Mato Grosso. VL is considered of extreme importance due to its high incidence and mortality, and is deemed a public health problem, occurring not only in the Americas, but also in Europe, Africa, Asia and the Middle East (23).

The first recordings of human cases of VL in Mato Grosso occurred in 1973, and by 1988 eight cases had been diagnosed in that period (5). Between 1992 and 1994 thirty-nine cases were investigated in the State, with thirty-five cases originating in the municipality of Várzea Grande and subsequently extending to other regions (7). An additional 138 new cases of human VL were recorded in the State in the period from January 1988 to December 2005 (20).

There is a diversified phlebotomine fauna in Mato Grosso State involved in the transmission of diseases due to the variety of ecotopes such as the Amazon Rainforest, Cerrado and Pantanal. The phlebotomine species capable of transmitting *Leishmania* to humans and animals in Mato Grosso were listed in 2007 (21, 22).

Many of these insects prefer modified environments, which offer them more shelter and food than natural environments (19). However the number of insects present in the environment at a given time, as is true with other living creatures, may vary due to either naturally or artificially occurring alterations in the environment over the course of time. In addition to the amount of food available, fluctuations of abiotic parameters such as the temperature and humidity of the air can lead to changes in the population of a given species present at a site (14, 19, 26).

The relationship between the occurrence of sand flies and some climatic elements, such as rainfall, temperature and humidity has been investigated in several studies (1, 4, 9, 10, 12, 25, 28, 29, 33, 34, 39). Significant correlations were found in some of these works, but not in others, indicating that other biotic or abiotic factors might modify the effect of the variables. Another hypothesis raised is that certain ranges of variation in climatic elements may be propitious or unpropitious for the abundance of sand flies at the site, which may also determine their seasonality (28).

When the growth of a biotic variable is at once influenced by several environmental parameters, such growth will be limited by whichever attribute is less favourable or available. Several other phenomena that simultaneously depend on various environmental factors display similar behaviour, obeying a principle that was originally propounded by Justus von Liebig in the 19^{th} century (17), known as Liebig's Law of the Minimum.

Some authors have implicitly or explicitly applied the Law of the Minimum using mathematical models to quantify the observed effects (6, 16, 18, 24, 32, 35 36, 38). The quantification may be made by using multiplicative models in which the effect of each environmental variable is represented by a factor that varies from 0 to 1. The maximum value of the biotic variable is multiplied by the factors that represent the environmental variables, penalising the maximum value as each factor moves away from the unit.

Bearing in mind the current importance of leishmaniasis, this study aimed to verify the spatial distribution and association between some abiotic factors and the abundance and temporal variability of the main vectors of leishmaniasis at the UFMT Cuiabá campus (600,000 m^2), by three years period.

MATERIAL AND METHODS

The study was conducted at the Federal University of Mato Grosso campus, in an area covering 600,000 m^2, situated in the Mesoregion of Cuiabá at a latitude of 15º36'31,37"S and longitude of 56º3'42"W.

Over the course of the last thirty years, the environment where the traps were installed, the Cuiabá campus of UFMT, has suffered major disturbances, originally consisting of natural cerrado, and now significant alterations due to anthropic actions, with streets, houses, street lighting at night and pedestrian and car traffic.

To select the collection points, consideration was given to peridomestic environments where a greater quantity of accumulated organic matter could be found on the ground, represented primarily by animal faeces, leaves and decomposing fruit, such as the mammal and bird zones of the zoo. As well as these sites, some areas where people circulate, including university employees, visitors and students, were also used as collection points. The traps were distributed at six locations on the campus, considering accessibility, total area cover, material availability and randomness: 1 – the bird zone of the zoo (high organic matter on the ground); 2 – the mammal zone of the zoo (high organic matter on the ground); 3 – the central security office (poor blood meal availability); 4 – the cultural centre (median blood meal availability); 5 – the School of Agronomy, Veterinary Medicine and Zootechnics (high blood meal availability); and 6 – the deans's offices building (median blood meal availability). The range of distances between traps was 1,000 to 300 m.

The phlebotominae were collected every month on three consecutive days, between December 2008 and December 2011. CDC traps (31) were used, installed at a height of 1.5 m, from 17:00 to 07:00 of the following morning, totalling 108 collection nights, 648 insect samples and 7,776 hours of the traps being in use.

The living sand flies captured were taken to the Entomology Laboratory of UFMT, sacrificed in ethyl acetate and then screened under a stereoscopic microscope. The insects were then subjected to clarification and identified (37).

The abiotic data of temperature and average relative humidity during the times when the traps remained at the insect collection sites were determined by the maximum and minimum readings obtained with a digital thermometer-hygrometer TFA 7429, installed between the mammal zone and the bird zone of the zoo.

To evaluate the association between the considered abiotic variables X_i (temperature, T, in ºC and relative humidity, RH, in %) and the number of insects captured (N), it was assumed that this number is affected by several variables or factors, each of which may be in a condition of influence regardless of the number of insects. Therefore, there is a maximum number of insects captured (N_{max}) for each condition or value of those variables. The actual number observed may be equal or less than N_{max} conditioned by the studied variable, because the other intervening factors may impose more limitations. The association with an isolated variable should be determined by taking into account only the maximum value that it conditions. As the maximum number conditioned by a variable such as temperature varies in function of the temperature, there is a record number of insects (N_{rec}) that can be captured when the temperature is most favourable (optimum temperature). Thus, the following adaptation of Wang & Engel's mathematical model (1998) followed:

$$N_{max} = N_{rec} \, . \, f(X_1) \, . \, f(X_2)$$

where N_{rec} is the absolute record of the number of insects recorded, or the quantity of insects that can occur when the abiotic variables X_1 and X_2 are simultaneously most favourable. $f(X_i)$ is a factor that varies from one, when the abiotic variable X_1 is most favourable, to zero, when it is entirely unfavourable. $f(X_i)$ is given by:

If $X_i > X_{Bi}$, $f(X_i)=0$;

If $Xi<Xbi$, $f(Xi)=0$; Se $X_{bi} < X_i, < XB_i$, $f(X_i)=\dfrac{2 \,.\, (X_i - X_{bi})^{\alpha} \,.\, (X_{oi} - X_{bi})^{\alpha} - (X_i - X_{bi})^{2.\alpha}}{(X_{oi} - X_{bi})^{2.\alpha}}$

If$X_{bi} < X_i < Xi_B$, $f(Xi) = \dfrac{2 \,.\, (Xi - Xbi)\alpha \,.\, (Xoi - Xbi)\alpha - (Xi - Xbi)2.\alpha}{(Xoi - Xbi)2.\alpha}$

where X_{Bi} is the value of the variable $X_{i,}$ above which there is no occurrence of insects, X_{oi} is the value of the variable $X_{i,}$ most favourable for the occurrence of insects, X_{bi} is the value of the variable X_i, below which there is no occurrence of insects, and

$$\alpha = \frac{\mathrm{Ln}(2)}{\mathrm{Ln}\ \dfrac{(X_{Bi} - X_{Bi})}{(X_{oi} - X_{oi})}}$$

The parameters N_{max} were adjusted to the observed data (*N*) considering initial values of X_{bi} and X_{Bi} which were found as the lowest and highest value of *X*, respectively, in which *N>0,* after the data pairs (X_i, *N*) have been ordered in growing order of $X_{i.}$ The initial value of N_{rec} was the greatest value of *N* observed in the whole study. The initial value of X_o was the observed value of X_i corresponding to the initial N_{rec} value. Then an iterative process was applied to find the values of X_{bi}, X_{Bi}, X_{oi} and N_{rec}, minimizing the differences between the values of the calculated and observed numbers of insects, considering that these differences could not be less than zero.

RESULTS

Throughout the study period, 449 specimens of phlebotominae were captured, belonging to two genera: *Brumptomyia,* with two species and *Lutzomyia* distributed among 14 species, of which 283 (63%) were male and 166 (37%) female (Table 1).

In the first year, 349 phlebotomine sand flies were collected, distributed in two genera: *Brumptomyia* with two species and *Lutzomyia* with 11 species, of which 63% were male and 37% female.

In the second year, 79 sand flies were captured: 54 males (68%) and 25 females (32%). There were two species of the genus *Brumptomyia* and nine of the genus *Lutzomyia.*

In the third year, the phlebotomine fauna totalled 21 specimens: 10 males (48%) and 11 females (52%). They were distributed in two genera: *Brumptomyia* with two species and *Lutzomyia* with eight species.

The species *L. cruzi, L. whitmani, L. carmelinoi* and *L. lenti* were reported in every collection period.

Of the known vector species of *Leishmania, L. longipalpis* occurred only in the first year, whereas *L. cruzi* and *L. whitmani* were found every year.

The most abundant species were *L. cruzi* with 269 (61%) and *L. whitmani* with 84 (19%), especially in the bird and mammal areas of the zoo (Table 1).

Table l. Number of Phlebotominae captured in Cuiabá campus of the Federal University of Mato Grosso, at six locations, from December 2008 to December 2011.

Species	Location*												Subtotal		Total
	1		2		3		4		5		6				
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	
B. brumpti	-	-	-	-	1	-	-	-	3	1	2	2	6	3	9
Brumptomyia sp.	-	-	-	1	-	-	1	1	8	1	3	2	12	5	17
L. amazonensis	-	-	-	-	-	-	-	-		-	1	-	1	-	1
L. carmelinoi	-	-	2	4	-	-	-	-	2	3	1	1	5	8	13
L. cruzi	1	1	2	-	-	-	-	-	111	35	76	48	191	84	275
L. dreisbachi	-	-	-	-	-	-	-	-	-	1	-	1	-	2	2
L. hermanlenti	-	-	-	-	-	-	-	-	2	6	1	-	3	6	9
L. lenti	1	-	-	1	-	-	-	-	2	2	1	-	4	3	7
L. longipalpis	-	-	-	-	-	-	-	-	1	-	6	1	7	1	8
L. saulensis	-	-	-	-	-	-	-	-	-	1	-	-	-	1	1
L. shannoni	-	-	-	1	-	-	-	-	-	-	-	-	-	1	1
L. sordellii	-	-	-	-	-	-	-	-	1	-	-	1	1	1	2
L. termitophila	-	1	1	2	-	-	-	-	-	-	-	-	1	3	4
L. walkeri	-	-	-	-	-	-	-	-	-	1	-	-	-	1	1
L. whitmani	2	1	2	1	3	2	1	1	19	20	17	15	44	40	84
Lutzomyia sp.	-	-	2	-	-	1	-	-	2	3	4	3	8	7	15
Total	4	3	9	10	5	3	2	2	151	74	112	74	283	166	449

*1 – the School of Agronomy, Veterinary Medicine and Zootechnics; 2 – the dean's office building; 3 – the central security office; 4 – the cultural centre; 5 – the bird zone of the zoo; and 6 – the mammal zone of the zoo.

There was a significant difference ($p<0.001$) by the chi-squared test in the number of insects of the species *L. cruzi* captured in 2008/2009 in relation to 2009/2010, and in 2009/2010 compared to 2010/2011. In the same species, there was a significant difference between the number of males and the number of females caught in the bird zone of the zoo in 2008/2009 and in 2009/2010 ($p<0.001$), as well as in the mammal zone of the zoo in 2008/2009.

There was a significant difference by the chi-squared test in the number of insects of the species *L. whitmani* captured in 2008/2009 in relation to 2009/2010 (p<0.001), and in 2009/2010 compared to 2010/2011 (p<0.005). There was no significant difference between the number of male and number of female *L. whitmani* insects caught in any year or site.

There were nights without insect captures in all the months of all the years. Eight of ten values of trapped insect numbers above ten were observed in the first year. In the first year, the maximum number of trapped insects was observed in April. In the second year, the maximum number of trapped insects was observed in June. In the third year, the maximum number of trapped insects was observed in May. So, there was no regular monthly pattern of insect abundance.

In Figure 1 and Figure 2, the diamonds represent the observed number of insects captured and the average temperature and relative humidity values at the time of collection. The curves (max) correspond to the maximum number predicted from application and fit to the model (38), considering each abiotic variable independently, as described in the methodology.

According to the results obtained, the most propitious conditions for the occurrence of Phlebotominae are a temperature of 23.2 ºC and relative humidity of 70%, when the number of insects captured can reach 40. Temperatures above 35 °C or below 18 °C and a relative humidity lower than 18% are unfavourable for capturing the insects. These limits correspond to the intersections of the line (max) with the horizontal axis (See Figures 1 and 2).

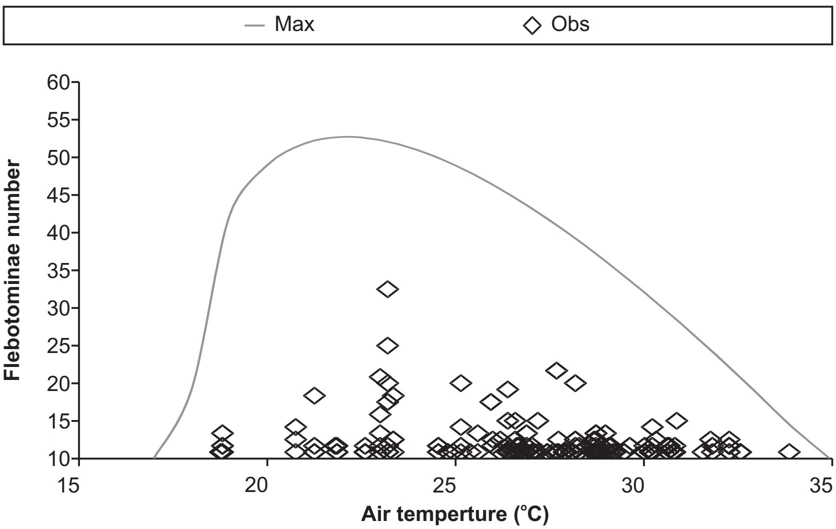

Figure 1. Number of Phlebotominae captured (obs), air temperature measured at time of capture in the UFMT campus, Cuiabá, from 2008 to 2011, and predicted maximum number (max) determined from the mathematical model of mean temperature association.

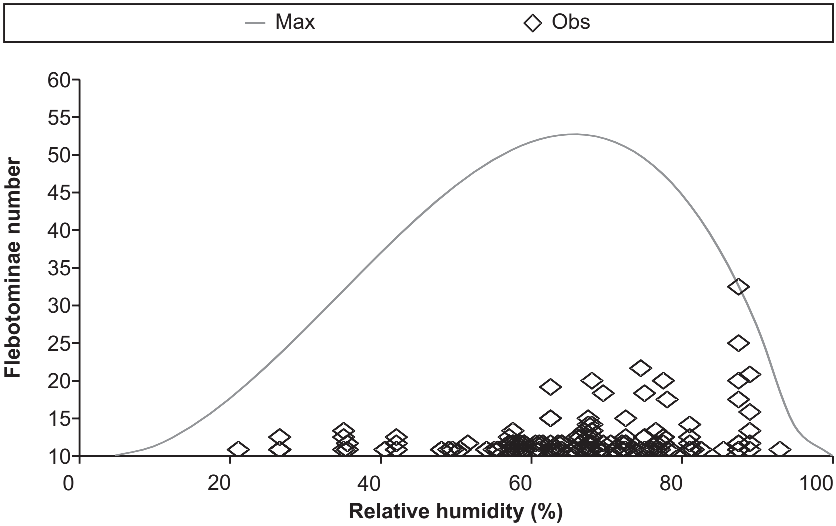

Figure 2. Number of Phlebotominae captured, relative humidity measured at time of capture in the UFMT campus, Cuiabá, from 2008 to 2011, and predicted maximum number (max) determined from the mathematical model of mean relative humidity association.

DISCUSSION

The species of phlebotomine sand flies capable of transmitting *Leishmania*, namely *L. cruzi*, *L. longipalpis* and *L. whitmani*, like those found in this study were also captured in Mato Grosso State and reported in every type of vegetation and in different modified areas (22).

Molecular biology was used (30) to determine the presence of *Leishmania infantum* (*syn, Leishmania chagasi*) in six foxes, *Cerdocyon thous* Linnaeus, 1766, and one bush dog, *Speothos venaticus* (Lund, 1842), kept at the same locality of this work, UFMT Zoo, and underlined the significance of these animals as hosts of disease agents of veterinary and public health importance, especially as they dwell in environments of public recreation. Another case of infection by *Leishmania infantum* (*syn. Leishmania chagasi*) in a bush dog (*S. venaticus*) was reported from the same site (3).

The diversity of species captured may be related to the process of urbanisation and disease transmission. This can be influenced by modifications of the habitat caused by anthropic action and the species' ability to adapt to intradomestic or peridomestic conditions, their capture being common in houses, chicken coops, kennels, rubbish bins, barns, grottos and other sites (11, 26, 27).

The progressive reduction in the number of sand flies caught over the course of the study period may be attributed to enhanced environmental management, with improved cleaning around the buildings, the removal and prevention of any build-up of waste, wood and organic matter on the ground (leaves and fruit) and the drainage of some areas, provided at least every three months. It has been shown that areas with moist ground and build-up of organic matter of vegetable and animal origin provide fertile conditions for the formation of breeding grounds and the concentration of Phlebotominae in the peridomestic environment (8).

The higher percentage of males caught of the *L. cruzi* species might be explained by their being attracted to the light source when they accompany females for mating (2), but the results obtained for *L. whitmani* do not corroborate this premise.

The male predominance can be understood because they emerge before the females, liberating pheromones attracting them to fecundation. This behaviour suggests that male proximity to hosts is not only higher but longer lasting. Because only females have haematophagous habits, they are the transmission agents (11, 15).

Considering that the temperature and relative humidity were measured at night, without solar radiation incidence, the main microclimatic factor, and that the digital thermometer-hygrometer was installed at conventional screen height, the weather conditions were assumed to be macroclimatic, i.e., one site's measurements were assumed valid for the whole UFMT campus.

The greatest number of phlebotominae observed in the traps was 27 males of *L. cruzi* in the bird zone of the zoo, on 28/04/2009, when the average air temperature was 23.2 ºC and the relative humidity was 68%. Therefore, the greatest number of insects caught on that day was less than the predicted number for the most propitious conditions. Although the temperature conditions were optimal for collection, the humidity conditions were not. Moreover, capturing the insects may have been hindered by other variables such as the availability of food at the site at that time.

Throughout the whole study the numbers of insects of both sexes, of all species captured and in all the sites, remained less than the maximum predicted value and are identified in Figure 1 and Figure 2 as the points located below the max curves. These points below the max curves represent the captures made when other biotic or abiotic factors, such as availability of food, were more limiting than the air temperature or humidity for the occurrence of phlebotominae, in accordance with the Law of the Minimum (13, 24). This interpretation extends to all the points in Figure 1 and Figure 2 that are below the line (max), at any temperature between 18 ºC and 35 ºC, and at any relative humidity between 18% and 100%.

The notion that there could be minimum and maximum limiting factors and an optimal level for each factor that influences the occurrence of phlebotomine sand flies was propounded when it was considered that moderate levels of rainfall were propitious for insects, but excessive levels would be detrimental as breeding grounds are destroyed and pupae in the soil are killed (28).

Literature review shows contradictory results, as high concentrations of phlebotomine sand flies have been found in warm and humid conditions (1, 12, 29), yet also in drier conditions (39). The correlation between the number of Phlebotominae captured and rainfall and humidity was significant, whereas temperature did not have a significant effect on the density of the insects (4). The temperature, humidity and rainfall do not constitute determining factors of the density of adult phlebotominae (9, 10). The number of phlebotomine sand flies was greater when the rainfall and temperature were higher (33, 34). The difficulties in interpreting these apparently contradictory results can be overcome when they are analysed with a model focussed on the levels at which the various factors favour or hinder the occurrence of the insects at a site, as made here with Law of Minimum.

It is important to highlight that the association with temperature was analysed from a macroclimatic perspective, that is to say, considering the temperature records used in the study as being representative of the situation in all the collection sites where traps were installed. Use of these results is, therefore, restricted to similar situations, in which the occurrence of these insects is to be inferred based on the general or regional ambient temperature. Such inferences may not be valid when one considers the temperature measured in the actual trap site, which is subject to the influences of the vegetation, artificial sources of heat and nearby buildings, configuring the microclimate. The natural habitats of phlebotomine sand flies are characterised by having a small variation in temperature and humidity, which favours the presence of these insects, as they are very sensitive to desiccation (10). Thus, a small variation in these factors in the microhabitats would be sufficient to alter the dynamics of the phlebotomine population.

The development of different insects was influenced by the temperature according to a similar mathematical model to that used in this study (16, 18, 6). Satisfactory results were obtained with that same mathematical model, analysing the behaviour of fungi (35).

An analogous procedure employed in this study was used to determine the minimum and maximum limits and the optimum value of an environmental factor that influenced a biotic variable (24). The authors used two lines of regression, one to the left and another to the right of the data set, as per the method proposed in (32). The maximum point was determined as the crossover point of the ascending and descending lines. The factor value, from those studied (24) required to reach the maximum point was considered as the optimum factor value.

Species of phlebotominae with and without the capacity to transmit *Leishmania* were captured at the Cuiabá campus of UFMT during the period from 2008 to 2011.

The abundance of phlebotominae at the campus presents spatial and temporal variability by virtue of abiotic and other variables.

The most propitious conditions for the occurrence of phlebotominae are a temperature of 23.2 ºC and a relative humidity of 70%.

Temperatures above 35 °C or below 18 °C and a relative humidity lower than 18% are unfavourable for the phlebotominae.

This is a first approach of parameters *T* and *RH* for Phlebotominae, based on all the species captured from June 2011, to April 2012 in the study area. According to the ecological niche theory, each species has its own minimum, optimum and maximum for the studied variables. However, phylogenetic characteristics could be both the cause and the consequence of specific *T* and *RH* limits, giving some similarities within genera and subfamilies. More data, unavailable at present, are required to determine eventual specific *T* and *RH* parameters.

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