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Phlebotomine fauna in the urban area of Timóteo, State of Minas Gerais, Brazil

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ABSTRACT

This work is characterized by an entomological research and an investigation on whether seasonal behaviours can be associated to the phlebotomine fauna found in the urban area of Timóteo-MG – an endemic focus of tegumentary leishmaniasis (TL). The analysis of the seasonal behaviour of sand flies has taken into account the following climatic variables: rainfall, relative humidity and temperature. Automatic light traps were installed in households between 2009 and 2010. The sand fly species with the highest number captured was *Lutzomyia whitmani* (66.5%), a TL vector species, whose abundance has provided strong evidences that this species is the main vector of TL in the municipality of Timóteo, with its cycle of transmission developing in its urban area. Amongst the results observed in the analyses of seasonal behaviour, only temperature conveyed particular association between seasonal occurrence of sand flies and climate variables. The findings of this study may assist the local epidemiological surveillance agency in defining strategies and directing efforts for controlling these insects.

1. Introduction

Phlebotomine are insects of the order Diptera, family Psychodidae and contained within the subfamily Phlebotominae; some species of this group of insects transmit leishmaniasis which is associated with infectious forms of Leishmania spp. The first descriptions of phlebotomine sand flies in the Americas date back to 1907, and by 1940 around 33 species had been documented. With the indictment of some species of sand flies as vectors of Leishmania spp., researches focused on this insect have deepened, thus taking the extent of identified species and subspecies of sand flies in the Americas to approximately 500. Brazil records many species of phlebotomine sand flies, about 229 species and subspecies have already been identified nationwide (Aguiar and Medeiros, 2003).

Not surprisingly, such variety of phlebotomine sand flies is one of the main factors behind Brazil's high incidence of tegumentary leishmaniasis (TL). It is estimated that predominantly Brazil along with Iran, Peru, Saudi Arabia and Syria, account for 90% of all cases leishmaniasis reported worldwide (Alvar et al., 2012). Cases of TL in Brazil are on the rise; in the early 1980s autochthonous cases were reported in 19 of the Brazilian federal units, and by 2003 autochthonous cases of tegumentary leishmaniasis had been documented in all Brazilian states (Brasil, 2011).

The increasing incidence of TL over the years, directly coincides with migration and human occupation; often disorderly, they can be associated with the dilapidation of the native flora, construction of inadequate dwellings in inappropriate sites, poor sanitation, and allocation of animal shelters in peridomestic environments. Such actions have a direct impact upon the behaviour of, and in the habitat of both vectors and reservoir species for leishmaniasis, which conversely have allowed species selection and their adaptation to anthropic environments; thus partially explaining both occurrence and persistence of leishmaniasis in domestic and peridomestic spaces (Teodoro, 1996; Teodoro et al., 2001; Lima et al., 2002).

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Over the past decades, several areas of the Brazilian territory have been affected by the urbanization of leishmaniasis with growing new records of this disease being reported in humans. According to the Brazilian Ministry of Health (Brasil, 2011), in the state of Minas Gerais alone, outbreaks of TL between 2001 and 2010 have pushed the number of reported cases from 1116 to 1887. Greater understanding of the phlebotomine fauna in surroundings with recorded cases of human leishmaniasis is vital to identifying likely vectors, and to understand changes in the diversity of this fauna. Such knowledge, in turn, allows the identification of species unknown as to existing in areas affected by Leishmaniasis.

As a result of the adaptation likelihood of phlebotomine sand flies to urban environments, advanced studies on the potential variations of this fauna, could equally foretell changes in the behaviour patterns of the disease, as well as its associated risks to human habitations and peridomestic environments; thus allowing measures to curb population exposure. Moreover, analysis on the seasonal occurrence of sand flies is another key factor that has been receiving greater scholarly attention. The main climatic variables often associated with the occurrence of phlebotomine sand flies are temperature (Andrade Filho et al., 1998; Dias et al., 2007; Gomes et al., 1980; Mayo et al., 1998; Salomon et al., 2002; Saraiva et al., 2006), rainfall (Dias et al., 2007; Salomon et al., 2002), and relative humidity (Andrade Filho et al., 1998; Dias et al., 2007; Gomes et al., 1980; Saraiva et al., 2006). These variables have been applied to elucidating the behaviour of sand flies and, consequently, possible variants in cases of leishmaniasis affecting humans. Information of this nature contributes to the development of more effective controlling and deterrent measures to prevent TL from dispersing into areas at risk.

Since 2002, the municipality of Timóteo, in the state of Minas Gerais (BR), has been systematically recording human cases of TL, and up to 2010, 164 cases of tegumentary leishmaniasis have been accounted for. Aiming to better understanding the dynamics of the transmission of TL within the referred township, this research is aimed at studying the existing phlebotomine fauna in the urban area of Timóteo, and at analysing whether the occurrence of seasonality behaviours of sand flies can be associated to the municipality object of this study.

2. Materials and methods

2.1. Study area

This study was undertaken in the municipality of Timóteo, which is located in the metropolitan area of the *Vale do Aço*, east Minas Gerais; an area of approximately 144,381 km^2, and population of 81,119 inhabitants, of whom 99.9% live within the urban area (IBGE, 2010).

Timóteo enjoys a high-altitude tropical climate, with droughts in the winter and rains during summer; with the temperature reaching 15 °C in winter and 35 in summer. The terrain is rather uneven and characterized mainly by mountains; the native vegetation is regarded as tropical rainforest typical of the southeast of Brazil, with some riparian and gallery forests, which unfortunately are currently limited to a few scattered spots and confined within the neighbouring State Park of Rio Doce, one of Brazil's largest conservation areas of Atlantic Rainforest (PMT, 2011).

2.2. Choice of capture sites, sample collection and identification of phlebotomines

To investigate the phlebotomine fauna, automatic light traps were placed in some households within the urban area of Timóteo. The primarily target was habitations where human cases of TL had been reported between January 2002 and July 2009, followed by other residences that in the same period were free from TL. The allocation of sampling points and trap settings was oriented by the municipality's map, taking into account its census subdivisions, which total 95 census district areas (IBGE, 2009). Residences with reported cases of tegumentary leishmaniasis in the aforementioned period were pinpointed on the map, and then a circumference with a radius of 250 metres was drawn with the aid of CorelDraw® Graphics Suite X4. The maximal distance of 250 m was established considering that as one intention was to investigate the presence of sand flies nearby households with and without recorded cases of TL, and consequently the greater or lesser likelihood of transmission of *Leishmania*, so even a small distance would suffice to support the findings. Also, previous investigations carried out in the Americas describe movements within small radius as below 200 m (Alexander and Young, 1992), below 60 m (Casanova et al., 2005), and below 57 m (Chaniotis et al., 1974) and below 180 m (Galati et al., 2009). Moreover, as some households with recorded cases of TL were located very close to each other, defining a large radius would make it difficult to characterize the peridomicile in terms of the presence of sand flies.

After that, all households identified by isolated circumferences were singled out as sampling points, whilst areas identified by overlapping circumferences were considered as one distinct area out of which the residences that better represented the total area, in spatial terms, were visually selected.

In addition to the traps described above, additional trapping devices were also set up at residences located within the census district areas that up to July 2009 had no records of TL. 20% of a grand total of 75 census district areas free of reported cases of TL were randomly chosen, resulting in 15 districts. Then, in an arbitrarily manner, a respective street, avenue or square was elicited from each one of the 15 selected districts, where at the 15 corresponding selected residences, a trap was then set up (Fig. 1).

According to the above criteria, 30 traps, Falcão model (Falcão, 1981) (Fig. 2) were installed at households with recorded cases of TL, and 15 in residences that up to October 2009 were free of TL (Fig. 1). A 'Falcão' (Falcão, 1981) light trap was deployed in each household for two succeeding nights in every month between November 2009 and October 2010. The traps were installed at a maximum distance of 20 m from each residence, and at 150 cm from the ground; totalling 45 trapping devices. Priority was given to resting site of domestic animals and orchards. Traps were activated at 18:00 and collected at 06:00 of the following day. Specimen triage took place at Timóteo's Department of Health with support provided by the staff from the Centre for Zoonosis Control. All captured insects were sacrificed by refrigeration; male and females were separated, slide-mounted and identified following the classification proposed by Young and Duncan (1994).

2.3. Analysis

The data were compiled in tables, based on a calculation of the proportion and prevalence of sand flies according to species, sex, and place of capture. The seasonal behaviour analysis was based upon the proportional distribution of sand flies (by totals and by sex) given their capture within periods aggregated in trimesters; the proportions were compared using the χ^2 test (chi-squared). Both descriptive and Pearson correlation analyses were also carried out on both: proportions of captured sand flies (by totals and by gender), and climatic variables: temperature (°C), rainfall (%) and humidity (mm). The data pursuant to climatic variables contained within the collection period (November 2009 to October 2010) was derived from the National Institute of Meteorology (2009) website; these climatic variables were then examined by descriptive statistics (mean, median, and standard deviation) according to the

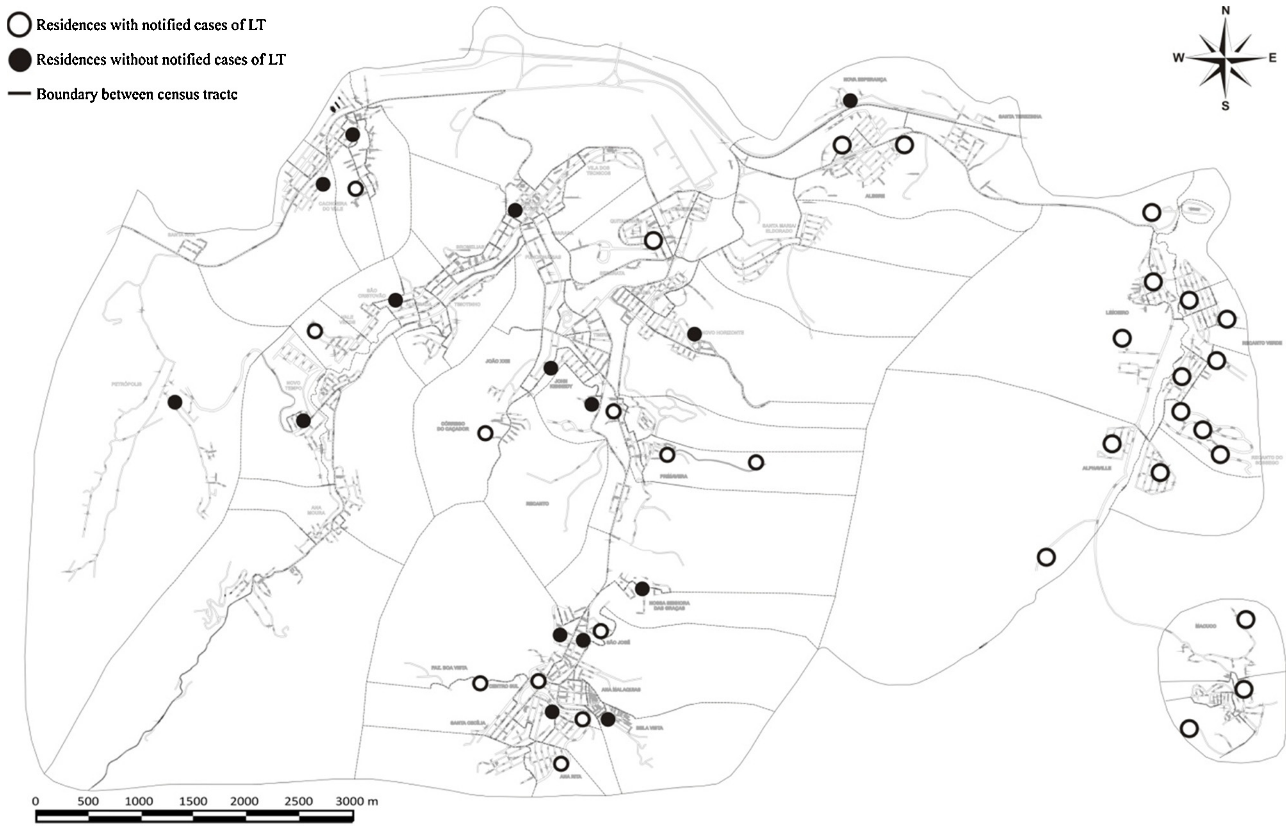

Fig. 1. Localization of the selected residences for the instalation of the traps used in the capture of phlebotomines, Timóteo City, Minas Gerais State, November/2009 until October/2010.

trimester in which the sand flies were captured. Correlation analyses were carried out using linear, exponential, logarithmic and geometric models, whilst the data arrangement of the climatic variables took into account different 'timings': during collection, 24 h, 1 week, 2 weeks and 30 days prior to collection. Both temperature and humidity variables were examined in terms of mean and median deviations, whereas for rainfall only the mean values were taken into account; primarily because rainfall data systematically returned null. Data analyses were performed on Microsoft® Office Excel 2007 and XLSTAT (2009) version 1.02. The interpretations were made by adopting a significance level of 5% ($\alpha = 0.05$). For the purposes of this study, the collection of specimen in the urban area of the municipality of Timóteo/MG was authorized by the Ministry of the Environment – approval recorded under number 20481-1 dated June 8th, 2009.

3. Results and discussions

A total of 1958 specimens from 17 phlebotomine species (Table 1) were collected during the catching stage. Out of this total, 1905 (97.3%) were captured in households with reported human cases of TL, and only 53 (2.7%) in households with no recorded case of human TL; this difference therefore being statistically significant ($p < 0.001$) (Table 1). *Lutzomyia whitmani*, *Lutzomyia quinquefer* and *Lutzomyia intermedia* were predominant in those two groups of households. Proportionally, in homes with human cases of TL, *L. whitmani* was the most abundant species ($p < 0.001$), whereas in homes with no recorded human cases, the number of captured sand flies was more homogeneous amongst the three most abundant species (Table 2).

A comparison between those two types of environments revealed that *L. whitmani* prevailed (67.1%) in households with recorded cases of TL rather than in households with no records of it (45.2%); this difference being statistically significant ($p = 0.001$). Yet, it differed from that observed for the species *L. quinquefer* and *L. intermedia*, which mostly prevailed in homes with no reported human cases of TL (30.2% and 22.6%, respectively), the difference being statistically significant only for *L. quinquefer* ($p = 0.003$) (Table 1). Amongst the leading captured species, only *L. quinquefer* is neither suspected of, nor does it play a vector proven role in the transmission of TL.

According to entomological studies carried out in the municipality of São Jorge Ivaí-PR (Teodoro and Kühl, 1997) and in the city of Belo Horizonte-MG (Saraiva et al., 2011), the species *L. whitmani* and, *L. intermedia* have been reported as dominant in anthropic environments where autochthonous cases of TL have been recorded. The predominance of *L. whitmani* was also reported in a study conducted in the city of Puertom Iguazú, Argentina (Fernández et al., 2012). These species represent the vectors of leishmaniasis species best adapted to new environmental conditions, having been linked to the transmission of *Leishmania* spp. in various parts of Brazil, especially in the south-eastern region (Andrade Filho et al., 2007). Studies in others states of Brazil have found *L. whitmani* to be the vector of *L. braziliensis* (Luz et al., 2000; Queiroz et al., 1994), similarly research carried out in the Vale do Rio Doce region have concluded that *L. whitmani* was the predominant species in that area between (Mayrink et al., 1979).

Lutzomyia whitmani was found to be predominant in the urban area of Timóteo, which corroborates the findings of previous studies carried out in this municipality (Andrade Filho et al., 1997; Souza et al., 2009). This conclusion may be feasibly explained by this species great capability to adapt to domestic environments. The capture of high densities of *L. whitmani* has also been reported in similar studies carried out in the states of Minas Gerais

Table 1
Frequency of captured phlebotomines, according to species and type of residence, Timóteo city, Minas Gerais State, Brazil. From November/2009 until October/2010.

Species	Residences with notified cases of LT	(%)	Residences without notified cases of LT	(%)	Total	(%)
Brumptomyia spp.	2	0.1	–	–	2	0.1
Brumptomyia avellari	6	0.3	–	–	6	0.3
Brumptomyia cunhai	2	0.1	–	–	2	0.1
Brumptomyia nitzulescu	1	0.1	–	–	1	0.1
Lutzomyia edwardsi	2	0.1	–		2	0.1
Lutzomyia lenti	2	0.1	–	–	2	0.1
Lutzomyia sallesi	6	0.3	1	1.9	7	0.4
Lutzomyia capixaba	2	0.1	–	–	2	0.1
Lutzomyia oswaldoi	1	0.1	–	–	1	0.1
Lutzomyia quinquefer[a]	286	15.0	16	30.2	302	15.4
Lutzomyia migonei	18	0.9	–	–	18	0.9
Lutzomyia whitmani[a]	1279	67.1	24	45.3	1303	66.5
Lutzomyia intermedia[a]	251	13.2	12	22.6	263	13.4
Lutzomyia fischeri	21	1.1	–	–	21	1.1
Lutzomyia pessoai	18	0.9	–	–	18	0.9
Lutzomyia choti	5	0.3	–	–	5	0.3
Lutzomyia aragaoi	2	0.1	–	–	2	0.1
Lutzomyia longispina	1	0.1	–	–	1	0.1
TOTAL (%)[b]	1905 (97.3)	100.0	53 (2.7)	100.0	1958	100.0

[a] Comparison between the proportion of captured phlebotomines species according to the type of residence: *L. quinquefer* ($\chi^2 = 9.106$; GL = 1; $p = 0.003$), *L. whitmani* ($\chi^2 = 11.065$; GL = 1; $p = 0.001$), *L. intermedia* ($\chi^2 = 3.973$; GL = 1; $p = 0.046$).
[b] Comparison between the proportion of total captured phlebotomines according to the type of residence: $\chi^2 = 3503.48$; GL = 1; $p < 0.0001$.

(Mayrink et al., 1979; Passos et al., 1993), Bahia (Azevedo et al., 1996), and Ceará (Queiroz et al., 1994; Azevedo et al., 1990), which likewise have emphasized *L. whitmani* susceptibility to domestication. Unlike the observations recorded in Timóteo, researchers have identified *L. intermedia* as dominant in the states of Rio de Janeiro (Rangel et al., 1986; Aguiar et al., 1996) and Sao Paulo (Condino et al., 1998), and as being the main vector in these states due to its high degree of anthropophily and capability to adapt to domestic environments. Studies carried out in Araçuaí (Gontijo et al., 2002) and Alto Caparaó (Saraiva et al., 2006), municipalities in the state of Minas Gerais, have drawn attention to the abundance of *L. intermedia* in those areas, having also linked this specie's adaptation capabilities to high levels of anthropogenic modification.

Researchers have indicated both *L. intermedia* and *L. whitmani* as the main vectors of TL in the Brazilian south-eastern region. Overall, the main explanation for a greater abundance of these two species has been linked to *L. intermedia* and *L. whitmani*'s adaptability to anthropic environments. Nonetheless, studies carried out in the state of Rio Grande do Sul (Silva and Grunewald, 1999) and in the northern of the state of Paraná (Teodoro et al., 1993) have found the specie *L. migonei* as being predominant in these two regions, and therefore confirmed as a likely vector of *Leishmania*.

The steep amount of *L. whitmani* found in the urban area of the municipality of Timóteo suggests that this species is the main vector of *Leishmania*, a condition favoured by its adaptation to anthropic environment. However, other factors (environmental, behavioural and ecological) should be elucidated so that a clearer account for this variation on species' density from region to region can be attained.

In addition to the species already implicated as vectors of *Leishmania* spp., one cannot underestimate the incidence of other species in the urban area of Timóteo as plausible vectors, such as *L. fischeri*, *L. pessoai*, *L. lenti* and *L. sallesi*. It has been suggested that the species *L. pessoai* and *L. fischeri* participate in the transmission cycle of *Leishmania* in the south-eastern Brazil, mainly due to their high anthropophily, high density and the invasion of households by these species in regions with endemic levels of tegumentary leishmaniasis (Rangel and Lainson, 2003). Recent studies have reported

Table 2
Distribution of predominant species of captured phlebotomines according to the type of residence and sex, Timóteo City, Minas Gerais State, Brazil. From November/2009 until October/2010.

Species/residences	Sex				Total
	Male	%	Female	%	
Residences with human cases of LT[1]					
L. whitmani[a3]	635	49.6	644	50.4	1279
L. intermedia[b3]	115	45.8	136	54.2	251
L. quinquefer[b3]	47	16.4	239	83.6	286
Residences without human cases of LT[2]					
L. whitmani[#4]	15	62.5	9	37.5	24
L. intermedia[*4]	9	75.0	3	25.0	12
L. quinquefer[#*4]	4	25.0	12	75.0	16
Total of captured phlebotomines					
Residences with human cases of LT	846	44.4	1.059	55.6	1905
Residences without human cases of LT	28	52.8	25	47.2	53
Total of captured phlebotomines[5]	874	44.6	1084	55.4	1958

Notes: [1] Different letters in the same column indicates significant statistical difference ($\alpha = 0.05$) between the proportion of captured species of phlebotomines in residences with human cases of LT ($\chi^2 = 1629.3$; GL = 2; $p < 0.0001$). [2] Different symbols in the same column indicates significant statistical difference ($\alpha = 0.05$) between the proportion of captured species of phlebotomines in residences without human cases of LT ($\chi^2 = 6.401$; GL = 2; $p = 0.041$). [3] Comparison of the proportions of species of phlebotomines captured in residences with human cases of LT according to sex: *L. whitmani* ($\chi^2 = 0.13$; GL = 1; $p = 0.72$), *L. intermedia* ($\chi^2 = 3.51$; GL = 1; $p = 0.06$), *L. quinquefer* ($\chi^2 = 257.79$; GL = 1; $p = 0.046$). [4] Comparison of the proportions of species of phlebotomines captured in residences without human cases of LT according to sex: *L. whitmani* ($\chi^2 = 3.00$; GL = 1; $p = 0.08$), *L. intermedia* ($\chi^2 = 6.00$; GL = 1; $p = 0.01$), *L. quinquefer* ($\chi^2 = 8.00$; GL = 1; $p = 0.005$). [5] Comparison of the total proportion of phlebotomines captured according to sex: $\chi^2 = 10.98$; GL = 1; $p < 0.001$.

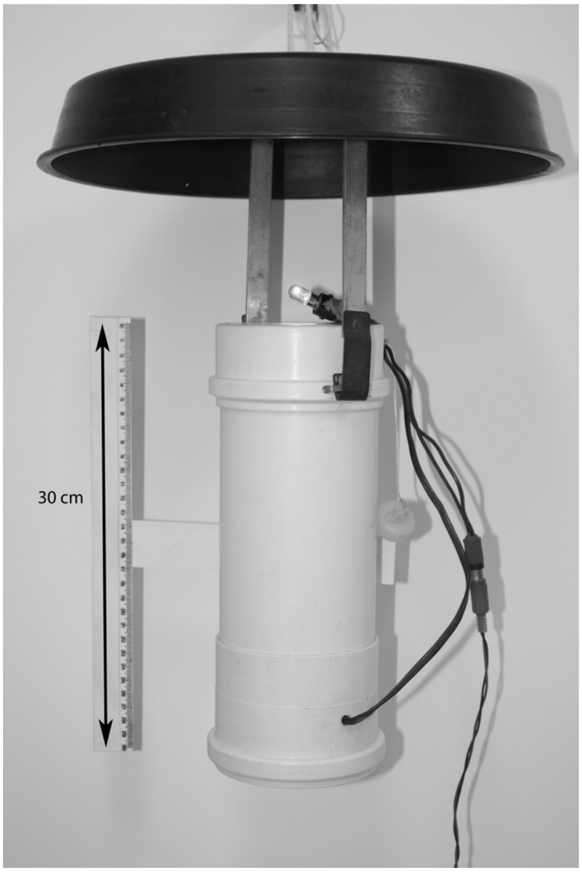

Fig. 2. Image of a Falcão trap installed at households.

the presence of *L. pessoai* and *L. fischeri* with natural infection of *Leishmania* in the locality of Divinópolis in Minas Gerais (Margonari et al., 2010). The species *L. lenti* also was found naturally infected with *L. braziliensis* in this last municipality (Margonari et al., 2010). With respect to *L. sallesi* in a study taken place in Corinto and Lassance, both in the state of Minas Gerais, Saraiva et al. (2009) observed the presence of natural infection of *Leishmania chagasi* in *L. sallesi.*

These accounts have drawn attention to the possibility of new vectors in the transmission cycle of *Leishmania* species. Accordingly, should this hypothesis be confirmed, these species may also be acting as vectors in the urban area of Timóteo.

Within the phlebotomine fauna found in his study, a higher proportion of females (55.4%) to males (44.6%) was identified; this difference being statistically significant ($p < 0.001$). Increased occurrence of females was also observed in households where human cases had been reported ($p < 0.001$); in households with no recorded cases, the number of trapped males was higher than that of females, nevertheless the difference was not statistically significant ($p = 0.56$). Interesting to note that in residences where Leishmaniasis had been reported, captured female *L. quinquefer* was fivefold higher than those of males, but only threefold higher in households with no reported cases (Table 2). The literature used dedicated to describe sand flies' population by sex, does not satisfactorily elucidate the difference between male and females' densities (Dias et al., 2007; Domingos et al., 1998; Nascimento et al., 2013; Rocha et al., 2013). In some instances, inclusive, there have been records of considerably higher prevalence of one sex over the other (Dias et al., 2007; Nascimento et al., 2013). Either way, the findings associated to females suggest the possibility of blood meal, and the consequential transmission of *Leishmania* spp. in the event of infection vector.

The criterion used for the distribution of traps has emphasized, even though the number of traps used was different, that in households where human cases of TL had been reported there was a higher prevalence of phlebotomine sand flies, a greater diversity of species, and presence of confirmed or suspected vectors. Overall, studies focused on surveying the phlebotomine fauna tend to concentrate on areas with incidence of human cases of tegumentary leishmaniasis (Dias et al., 2007; Mayo et al., 1998; Salomon et al., 2002; Saraiva et al., 2006; Condino et al., 1998), however, to ascertain which areas are at greatest risk of transmission, comparisons of different environments should be drawn (e.g., areas with and without incidence of human TL), as was the case of this study. These results, coupled with the presence of vector species, are strongly indicative of autochthonous human cases of TL in the urban area of the municipality of Timóteo.

In this study, the ratio of captured sand flies within each trimester revealed a statistical difference ($p < 0.0001$), notably May to July recorded the lowest ratio (14.9%) indicating a significant reduction in the presence of vectors compared to the other periods. In fact, May to July includes the winter season at Timóteo city and when the lowest temperatures may occur. The periods November to January and February to April recorded the highest proportions (31.0 and 28.9%, respectively) (Table 3). These periods correspond to the hottest months of the year (Table 3), which could have favoured the capture of a greater number of sand flies. According to Aguiar and Soucasaux (1984), sand fly concentrations tend to increase during hot and humid months, and equally decrease during the coldest and dry months of the year.

Dias et al. (2007) reported that, under natural conditions, variations in temperature and humidity can affect the presence of sand flies since they are very sensitive to desiccation. A small variation of these factors in microhabitats is enough to alter the dynamics of sand flies' population (Dias et al., 2007).

According to Brazil and Brazil (2003), the duration of the different stages of the life cycle of the phlebotomine depends upon temperature, humidity and food availability. Under laboratory conditions, the optimum temperature for the development of most neotropic species ranges between 25° and 27°, and any thermal conditions slightly above this range imply in a faster cycle.

A study by Rutledge and Ellenwood (1975) carried out under natural conditions during the rainy season, has shown that moderate rainfall benefits sand flies, whilst intensive rainfall can deter its life cycle as flooding saturates the soil, compromising breeding sites, and some stages of its life cycle (egg, larvae, and pupae).

With regard to the impact of rainfall and humidity upon the populations of sand flies, results were less clear. Values of mean and median rainfall were extremely low in all periods analyzed; however, during the periods of greater rainfall, November to January (0.30 mm) and February to April (0.22 mm) we had the highest occurrences of sand flies (Table 3). As for humidity, the values for the periods studied showed no clear differences; however, it is noteworthy that the mean and median humidity was always above 50%.

The correlation analyses concerning absolute frequencies of captured sand flies and climatic data revealed no significant differences between the models tested; thus, for the purposes of this study only the correlation results will be presented using the linear regression model. As a variable, temperature had the best-fit correlation for both mean and median values, having the occurrence of sand flies increased with higher temperatures (mean or median) (Table 4). Overall, these authors have observed that as the period used for calculating the mean and median temperatures draw near to the time of collection, the correlation adjustment have concurrently improved. However, only the data referring to the exact time of collection has shown some statistical significance for the linear regression coefficient (average temperature: $b = 31.5$,

Table 3
Frequency, percentage of captured phlebotomines and descriptive statistic of climate data, according to period of collection, Timóteo City, Minas Gerais State. From November/2009 until October/2010.

Variable	Period				Total
	November–January	February–April	May–July	August–October	
Captured phlebotomines					
Total	607	566	291	494	1958
%[a,b]	31.0^{a}	28.9^{ac}	14.9^{b}	25.2^{c}	100.0
Females					
Total	441	294	154	195	1084
%[b,c,e]	$40.7^{a\#}$	$27.1^{b\#}$	$14.2^{c\#}$	$18.0^{c\#}$	100.0
Males					
Total	166	272	137	299	874
%[b,d,e]	19.0^{a*}	$31.1^{b\#}$	$15.7^{a\#}$	34.2^{b*}	100.0
Temperature (°C)					
Mean	24.4	25.3	20.5	21.4	–
Standard deviation	3.2	3.5	3.2	3.7	–
Median	23.7	24.8	20.3	21.1	–
Coefficient of variation (%)	0.13	0.14	0.16	0.17	–
Rainfall (mm)					
Mean	0.30	0.22	0.08	0.01	–
Standard deviation	1.94	1.74	1.20	0.24	–
Median	0.00	0.00	0.00	0.00	–
Coefficient of variation (%)	6.49	7.77	15.77	17.26	–
Humidity (%)					
Mean	72.1	68.8	74.7	60.5	–
Standard deviation	14.0	14.6	11.2	13.8	–
Median	74.0	70.0	77.0	61.0	–
Coefficient of variation (%)	0.19	0.21	0.15	0.23	–

[a] $\chi^2 = 160.9$; GL = 3; $p < 0.0001$.
[b] Different letters in the same line indicates significant statistical difference ($\alpha < 0.05$).
[c] $\chi^2 = 240.6$; GL = 3; $p < 0.0001$.
[d] $\chi^2 = 114.4$; GL = 3; $p < 0.0001$.
[e] Different symbols in the same column indicates significant statistical difference ($\alpha < 0.05$).

Table 4
Linear correlation between the number of captured phlebotomines and climate data (mean and median) in different time-points, Timóteo city, Minas Gerais state, Brazil. From November/2009 until October/2010.

Variable	Time-points in relation to data collection[a]/parameters of the model														
	During			24 hours before			One week before			Two weeks before			30 days before		
	b	p	R^2	b	p	R^2	b	p	R^2	b	p	R^2	b	p	R^2
Mean temperatures	31.5	0.032	4793	21.8	0.052	32.7	24.3	0.053	3253	28.6	0.063	3039	22.4	0.203	1565
Median temperatures	33.2	0.009	5025	21.1	0.057	3157	25.2	0.049	3331	30.9	0.056	3175	27.0	0.151	1946
Mean humidity	−2.3	0.667	1.93	−0.4	0.935	0.07	−7.5	0.175	1756	−5.8	0.265	1225	−6.8	0.255	1275
Median humidity	−1.0	0.825	0.51	−0.5	0.887	0.21	4.7	0.354	8.63	−5.2	0.266	1219	−6.0	0.252	1290
Mean rainfall	2718	0.300	1067	−50.1	0.568	3.37	−1836	0.346	8.91	−1671	0.422	6.56	−1769	0.44	6.00

[a] b = coefficient of regression; p = p-values; R^2 = coefficient of determination.

$p = 0.032$; median temperature: $b = 33.3$, $p = 0.009$). Positive correlation between the mean temperature in the month of capture and the number of trapped sand flies had been previously identified by Saraiva et al. (2006); though no clarification was given about the correlation used in their analyses.

Such results show that temperature was the variable better conversant for eventual seasonal occurrences of sand flies, being helpful to defining strategies for controlling these insects in urban areas.

The frequency distribution of male and females according to the periods of capture revealed similar proportions to males' insects than to females (Table 3). Taking into account the scale of variation between periods of high and low capture, it was observed higher variance for females (65.1%) than males (54.1%). For females, the period from November to January recorded the highest proportion (40.7%) ($p < 0.05$); whilst February to April (31.1%) and August to October (34.2%) recorded the highest proportions to males' insect, however no statistical difference was observed ($p > 0.05$) (Table 3).

By analysing the prevalence of males and females in the same period, it was observed difference statistically significant between the ratios in the periods November to January ($p < 0.0001$) and August to October ($p < 0.0001$), but in the first period females were more prevalent (40.7%), and in the last, males were identified as most prevalent (34.2%) (Table 3). The correlation analysis considering individual populations of males and females did not present good fits for any tested model.

No other comparison studies investigating males and females caught in different months of a year have come to our attention. Therefore, further studies are required to better assess whether the prevalence of males and females phlebotomine follows a distinct pattern of seasonal occurrence.

4. Conclusions

The outcomes of this study suggest that the transmission cycle of *Leishmania* spp. is occurring in the urban area of the municipality of Timóteo. Such an assumption is grounded on the sheer diversity of phlebotomine fauna found in the urban area of the municipality object of this study, whereby *L. whitmani*, *L. intermedia* and *L. quinquefer* were found to be predominant, a situation that has been observed for over 10 years. Another important aspect is the large number of females vector species *(L. whitmani* and *L. intermedia)* found in households with reported cases of TL, suggesting the existence of transmission in peridomestic environments and the urbanization of the disease.

The analysis of climatic variables presented no clear pattern of seasonal behaviour, suggesting the no-seasonality in the behaviour of phlebotomine sand flies, which is mainly explicated by its great capability to adapt to anthropic and peridomestic environments, where food is easily accessible and there are suitable conditions for its reproduction.

The results of this study have so far allowed us to assist the epidemiological surveillance service of Timóteo in implementing prevention and controlling measures against TL, such as prioritizing areas where cases of TL have been reported.

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