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Major Article

Ecology of phlebotomine sand flies in an area of leishmaniasis occurrence in the Xakriabá Indigenous Reserve, Minas Gerais, Brazil

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Abstract

Introduction: Leishmaniasis is a complex vector-borne infectious diseases caused by protozoan parasites in the genus *Leishmania* and spread by hematophagous phlebotomine sand flies (Diptera: Psychodidae, Phlebotominae). The aim of this study was to investigate the phlebotomine fauna, endophily and exophily of the species found, and possible influence of climatic factors on their populations. **Methods:** The study was conducted in the Xakriabá Indigenous Reserve (XIR) in the municipality of São João das Missões in northern Minas Gerais state, Brazil. Insects were collected over three consecutive nights in the last week of each month for 12 months from July 2015 to May 2016 from four houses in four different villages. Two traps were set up in each house: one in the intra-domicile and another in the peri-domicile. **Results:** A total of 2,012 phlebotomine sand fly specimens representing 23 species and belonging to 10 different genera were captured and identified. Among the studied villages, Riacho do Brejo showed the highest density and diversity of phlebotomine sand flies. The species *Lutzomyia longipalpis* (80.3%) and *Nyssomyia intermedia* (7.3%), which are major vectors of visceral and cutaneous leishmaniasis, respectively, had the highest population densities, both in the intra- and peri-domicile. No correlation was observed between climatic factors and the density of phlebotomine sand flies. **Conclusions:** The results of the present study may contribute to a better understanding and targeting of the measures for preventing and controlling leishmaniasis by the authorities responsible for indigenous health.

Keywords: Phlebotomine. Sand fly. Leishmaniasis. Xakriabá indigenous reserve.

INTRODUCTION

Leishmaniasis is a complex infectious disease caused by protozoan parasites in the genus *Leishmania* Ross, 1903, and transmitted by hematophagous phlebotomine dipterans in the family Psychodidae, subfamily Phlebotominae[1], commonly known as sand flies. In the Americas, the transmission cycle of leishmaniasis is zoonotic; therefore, an animal reservoir is required to complete the life cycle and survive in the environment. Leishmaniasis has a broad clinic spectrum and epidemiologic diversity and can present as diverse clinical forms that depend on the *Leishmania* species and host immune response[2]. The two basic clinical forms of human leishmaniasis manifestations are visceral leishmaniasis (VL) and cutaneous leishmaniasis (CL)[3,4].

Leishmaniasis is considered as a significant public health problem, as it has a broad geographic distribution and is considered to be part of the group of neglected tropical diseases. It is strongly associated with poverty, prevalent in tropical areas where it coexists with other neglected tropical diseases, and has low visibility in the rest of the world; therefore, the pharmaceutical industry has not shown strong interest in developing and improving products for leishmaniasis treatment and prevention[5].

The transmission of leishmaniasis in indigenous areas of Brazil is not well-understood. In the Xakriabá Indigenous Reserve (XIR), located in the northern region of Minas Gerais (MG) state, autochthonous human cases of CL[6] and canine cases

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of VL (AM Rocha: unpublished data) have been reported since 2001. In the XIR, a neglected population exists in an endemic leishmaniasis area and there is little relevant information regarding the epidemiologic chain of these diseases; therefore, this study was conducted to assess various entomological aspects of leishmaniasis epidemiology in the XIR.

METHODS

Study Area

The XIR is in the municipality of São João das Missões (latitude, 14° 88' 51'' S; longitude, 44° 07' 73'' W) in the northern region of Minas Gerais state (**Figure 1**). It is in the upper middle zone of São Francisco, a microregion of the Peruaçu Valley, and borders the municipalities of Miravânia, Manga, and Itacarambi[6]. The XIR is organized into 32 villages which are distributed into five health base poles and has a population of 7,760, with more than 50% of people under the age of 24 years[7]. Because it is in the northern region of MG, transitional vegetation is observed between the savanna and Caatinga, with denser forest zones closer to the water sources and a semi-arid climate with long periods of drought.

Selection of sampled villages and residences

Three villages selected for the study (Riacho do Brejo: 14° 81' 64'' S, 44° 19' 95'' W, Brejo do Mata Fome: 14° 84' 16'' S, 44° 20' 67'' W, and Prata: 14° 83' 42'' S, 44° 16' 38'' W), which have an average altitude of 633.25 m, have reported cases of autochthonous canine visceral leishmaniasis, while the other village (Riachinho: 14° 88' 44'' S, 44° 20' 46'' W) had no case records from the combination of serological results obtained by enzyme-linked immunosorbent assay and indirect immunofluorescence (AM Rocha: unpublished data). For insect capture, residences recognized as favorable to the vector and with the following environmental characteristics were selected: a) presence of organic matter such as decomposed dry leaves and animal feces, b) occurrence of areas with high humidity because of the presence of trees, c) presence of animal enclosures (e.g., chicken coop or pigsty) close to human habitation, and d) considerable shade from fruit trees[8,9].

Phlebotomine sand fly capture

Centers for Disease Control (CDC) light traps were used to capture the insects with the goal of obtaining the greatest possible diversity of sand flies. Capture was conducted over three consecutive nights during the last week of each month for 12 months (June 2015 to May 2016). Pairs of traps were set in each house, with one inside and one outside (close to the animal enclosures, fruit trees, and accumulation of organic matter), and were operated from 18:00 to 6:00. Following the capture period, the support with a screen carrying the entomological material

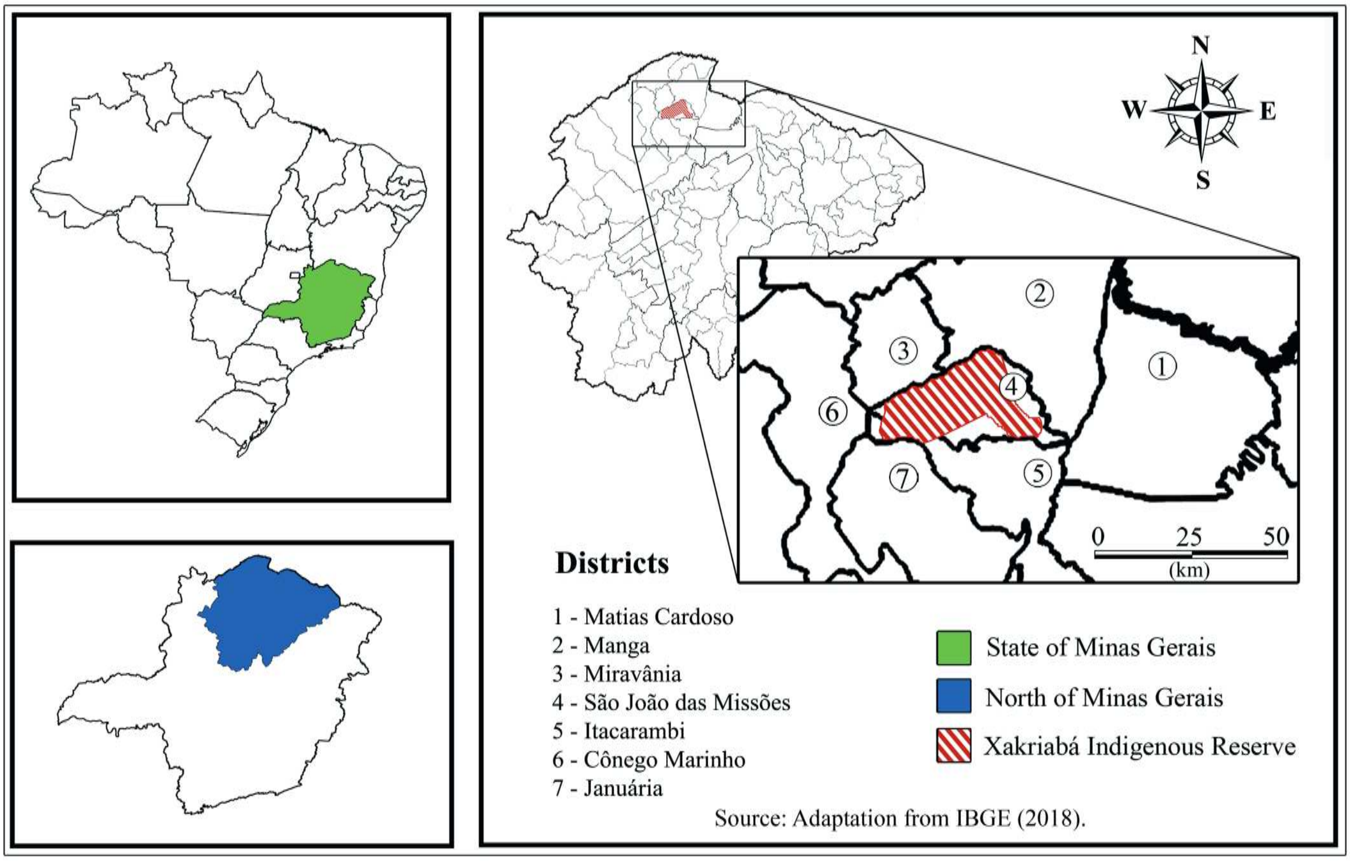

FIGURE 1: Geographic location of the Xakriabá Indigenous Reserve.

was uncoupled from the traps and placed inside plastic bags containing chloroform for 20 min to kill the insects. They were then sorted under magnifying glasses to remove any insects not relevant to the study. Both males and females insects were stored in hemolysis tubes containing 70% ethanol and transported to the Leishmaniasis Laboratory of the René Rachou Institute, where they were prepared and assembled between glass slides and coverslips for taxonomic identification.

Sand fly preparation for internal and external examination

Preparation of the captured sand flies was carried out according to Langeron's protocol[10] with some modifications. Initially, the sand flies were submerged in 10% potassium hydroxide solution for 2–3 h in a Petri dish and identified. Next, the sand flies were placed in 10% acetic acid for 20 min and washed by soaking in distilled water three times for 15 min each time. The sand flies were removed from the distilled water and immersed in lactophenol for 24 h and then assembled on a microscope slide in Berlese Liquid. This procedure enabled examination of the internal and external taxonomic structures of the flies with an optical microscope (Axe scope, Zeiss, Oberkochen, Germany).

Sand flies assembled on the microscope slides were identified according to the classification proposed by Galati[11] and compared with reference materials deposited in the Collection of Phlebotomine Sand flies of the René Rachou Institute. The microscope slides were properly labeled with the species name, capture date, gender, capture locality, and trap number. Various genera and subgenera of sand flies have the same initial letter; therefore, to avoid confusion, the abbreviations proposed by Marcondes[12] were used.

Climatic data

Climatic data for the study area, including temperature (ºC), relative air humidity (%), and rainfall (mm), were obtained monthly during the study period from the National Institute of Meteorology website. The data were collected from the automatic weather station of Mocambinho, located in the municipality of Itacarambi, MG (latitude 15º 08' 61'' S and longitude 44º 01' 61'' W), approximately 30 km from the XIR.

Statistical analysis

Data were organized in Microsoft Excel® (Office 2003, Microsoft, Seattle, WA, USA) spreadsheets and statistical analysis was performed with SPSS software version 22 (SPSS, Inc., Chicago, IL, USA). When necessary, the results were log-transformed to achieve a normal distribution. Statistical analysis was performed for species present in samples with diversities higher than 5% of the total sand flies captured. An unpaired Student's *t*-test was used to compare the mean values of the sand fly population densities according to gender and intra- or peri-domicile behavior. The means in different villages were compared by analysis of variance (ANOVA), followed by post-hoc analysis using a Student's *t*-test to compare adjacent means. Relationships between climatic variables (mean temperature, mean rainfall, and mean relative air humidity) and mean monthly population density of the sand flies were determined using the Pearson correlation test. Differences were considered significant when the p value was < 0.05.

RESULTS

During the 12-month study period, the phlebotomine fauna comprised 2,012 specimens, representing 23 species belonging to 10 genera, and are presented in **Table 1**. *Lutzomyia longipalpis* appeared most frequently (1,615 specimens), corresponding to 80.3% of the sand flies collected, as shown in **Table 1**, followed by *Nyssomyia intermedia* accounting for 7.3% of flies. Two other species of medical importance were found, *Migonemyia migonei* and *Pintomyia pessoai*, but at low abundances of 0.55% (11 specimens) and 0.05% (one specimen), respectively, among the total phlebotomine population density. In general, the number of females (779) was lower than the number of males (1,234), with a male/female ratio 1.58. In Student's *t*-test, no significant difference ($p = 0.09$) was observed between the population density of collected males (0.32 ± 0.03) and females (0.26 ± 0.02).

For *Lu. longipalpis*, the ratio male/female was 2.31, with a significant difference between males (0.30 ± 0.03) and females (0.20 ± 0.02) ($p = 0.0056$). As in *Lu. longipalpis*, *Ny. intermedia* showed a significant difference ($p = 0.01$) in gender (males: 0.02 ± 0.01 and females: 0.05 ± 0.01), but females predominated the gender ratio (male/female) by 0.16.

Regarding sand fly behavior, 975 (48.5%) individuals were captured outside of the houses and 1036 (51.5%) were captured inside the houses, with no significant difference ($p = 0.33$) between the means of the population density of the intra-domicile (0.31 ± 0.03) and peri-domicile behaviors (0.27 ± 0.03) (**Figure 2A**). A similar condition was observed for *Lu. longipalpis* ($p = 0.16$) for intra-domicile (0.27 ± 0.03) and peri-domicile behaviors (0.22 ± 0.02) (**Figure 2B**). For *Ny. intermedia*, a significant difference was observed ($p = 0.03$) in intra-domicile (0.02 ± 0.01) and peri-domicile behaviors (0.05 ± 0.01) (**Figure 2D**).

Examination of the population density of sand flies among villages revealed significant differences ($p < 0.0001$) by variance analysis. Student's *t*-test for multiple comparisons showed a significantly higher sand fly density in Riacho do Brejo village, followed by Prata village, and the villages, Brejo do Mata Fome and Riachinho, which showed similar results (**Figure 3A**). For *Lu. longipalpis*, Riacho do Brejo village showed the highest population density, while Brejo do Mata Fome village showed the lowest density (**Figure 3B**). No differences were observed between the Riachinho and Prata villages. The population density of *Ny. intermedia* showed a heterogeneous distribution between villages ($p < 0.0001$). The villages Brejo do Mata Fome and Riacho do Brejo showed similar population densities which were higher than those in other areas (Prata and Riachinho) (**Figure 3C**).

Climatic data obtained from the National Institute of Meteorology indicated that during the 12-month study period (June 2015–May 2016), the annual averages of temperature, relative humidity, and rainfall were 26.14 ± 2.12°C, 57.11 ± 9.35%,

TABLE 1: Phlebotomine sand fly fauna collected with light traps (CDC) by gender in Brejo do Mata Fome, Prata, Riachinho, and Riacho do Brejo villages in the Indigenous Land Xakriabá from June 2015 to May 2016.

Species	Males	%	Females	%	Total	%
Brumptomyia avellari	2	100.00	0	0.00	2	0.10
Brumptomyia brumpti	1	100.00	0	0.00	1	0.05
Cortelezzii complex	0	0.00	41	100.00	41	2.04
Evandromyia cortelezzii	2	100.00	0	0.00	2	0.10
Evandromyia evandroi	2	33.30	4	66.70	6	0.30
Evandromyia lenti	30	54.50	25	45.50	55	2.73
Evandromyia sallesi	9	100.00	0	0.00	9	0.45
Evandromyia termitophila	0	0.00	1	100.00	1	0.05
Lutzomyia ischnacantha	9	52.90	8	47.10	17	0.84
*Lutzomyia longipalpis**	1.127	69.80	488	30.20	1.615	80.27
Lutzomyia renei	6	22.20	21	77.80	27	1.34
Martinsmyia minasensis	0	0.00	1	100.00	1	0.05
Micropygomyia capixaba	0	0.00	5	100.00	5	0.25
Micropygomyia goiana	8	30.80	18	69.20	26	1.29
Micropygomyia oswaldoi	1	50.00	1	50.00	2	0.10
Micropygomyia peresi	6	54.50	5	45.50	11	0.55
Micropygomyia quinquefer	3	11.50	23	88.50	26	1.29
*Migonemyia migonei**	6	54.50	5	45.50	11	0.55
*Nyssomyia intermedia**	20	13.60	127	86.40	147	7.31
Pintomyia misionensis.	1	50.00	1	50.00	2	0.10
Pintomyia pessoai	1	100.00	0	0.00	1	0.05
Psathyromyia bigeniculata	1	100.00	0	0.00	1	0.05
Psathyromyia shannoni	0	0.00	1	100.00	1	0.05
Sciopemyia sordellii	0	0.00	2	100.00	2	0.10
Total	**1.234**	**61.30**	**779**	**38.70**	**2.012**	**100.00**

*species of medical importance.

and 43.6 ± 110.2 mm, respectively. Analysis of the total population density captured monthly in relation to the monthly climatic data revealed no variation in the study period for temperature and relative air humidity. However, increased sand fly density was observed 1–2 months after an increase in precipitation (**Figure 4**). Notably, the average precipitation during the studied period (43.6 mm) was similar to the annual average of the three previous years (41.3, 47.5, and 39.2 mm in 2012, 2013, and 2014, respectively).

DISCUSSION

The phlebotomine sand fly fauna found in Riacho do Brejo, Riachinho, Prata and Brejo do Mata Fome villages in the XIR demonstrated high levels of diversity, with 23 species collected, which belong to 10 genera. The species *Ev. sallesi* and *Ev. cortelezzii* were identified through the male sand flies. The females were identified as part of the *cortelezzii* complex, due to the morphological similarity between the species[13]. The number of species collected corresponded to 23.7% in a total of 97 species of sand flies already recorded in Minas Gerais[14-17]. Among the species collected, four were medically important, and vectors of leishmaniasis: *Lu. longipalpis, Ny. intermedia, Mg. migonei*. The species *Lu. longipalpis* transmits VL and the other species transmit CL.

The species showing the highest population density during the studied period was *Lu. longipalpis*, totaling 80.2% of the samples, which has also been found in many endemic areas of visceral leishmaniasis in Brazil[18-26]. This species was followed

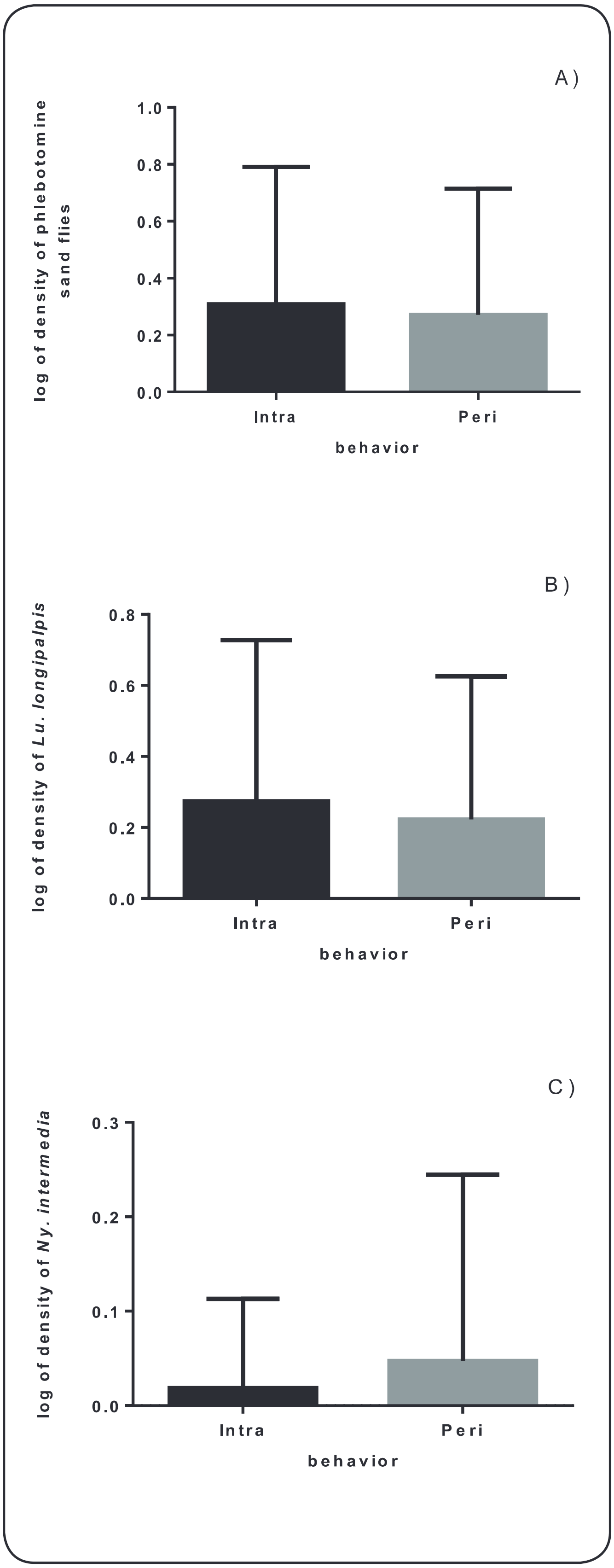

FIGURE 2: Relationship between population density and behavior (intra- and peri-domicile) **(A)**, *Lu. longipalpis* **(B)**, *Ny. intermedia* **(C)** captured with light traps (CDC) in Brejo do Mata Fome, Prata, Riachinho, and Riacho do Brejo villages located in the Xakriabá Indigenous Reserve from June 2015 to May 2016 (p = 0.33; p = 0.16; p = 0.03 – Unpaired Student's *t*-test).

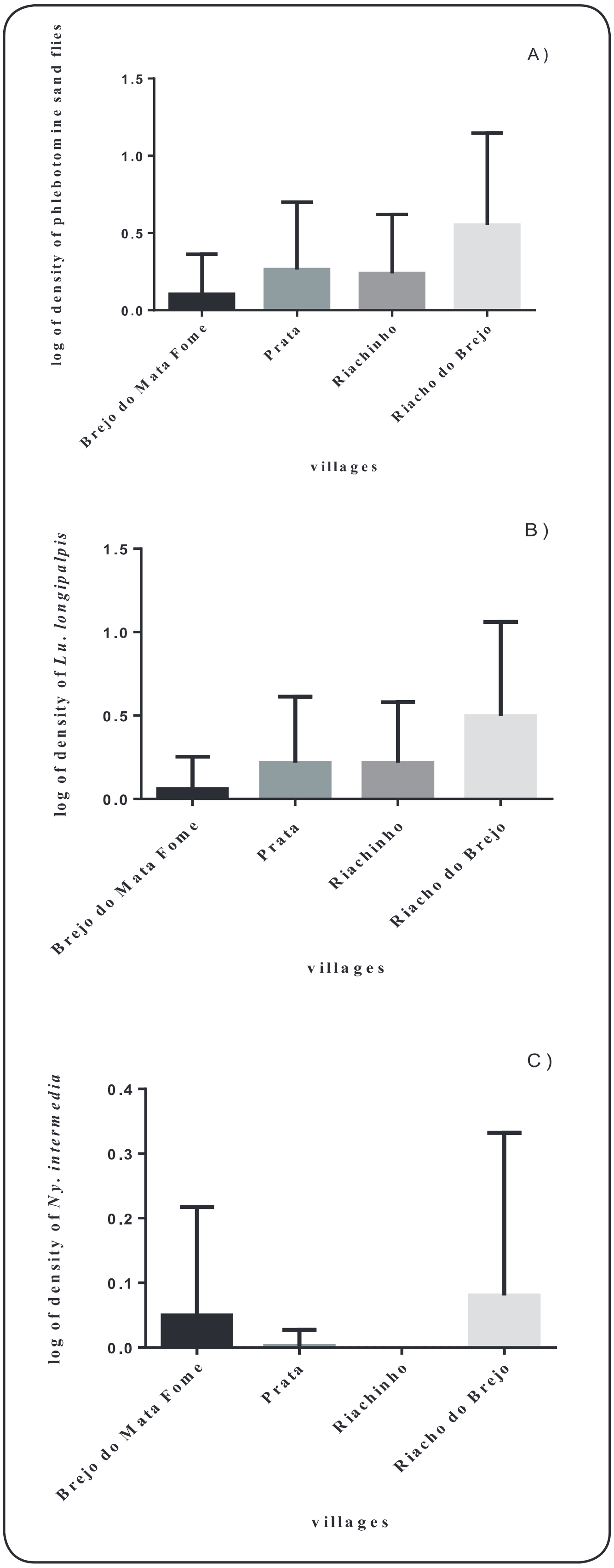

FIGURE 3: Relationship between phlebotomine sand fly population densities **(A)**, *Lu. longipalpis* **(B)**, *Ny. intermedia* **(C)** captured with light traps (CDC) in Brejo do Mata Fome, Prata, Riachinho, and Riacho do Brejo villages located in the Xakriabá Indigenous Reserve from June 2015 to May 2016 ($p < 0.0001$; $p < 0.0001$; $p < 0.0001$; one-way ANOVA).

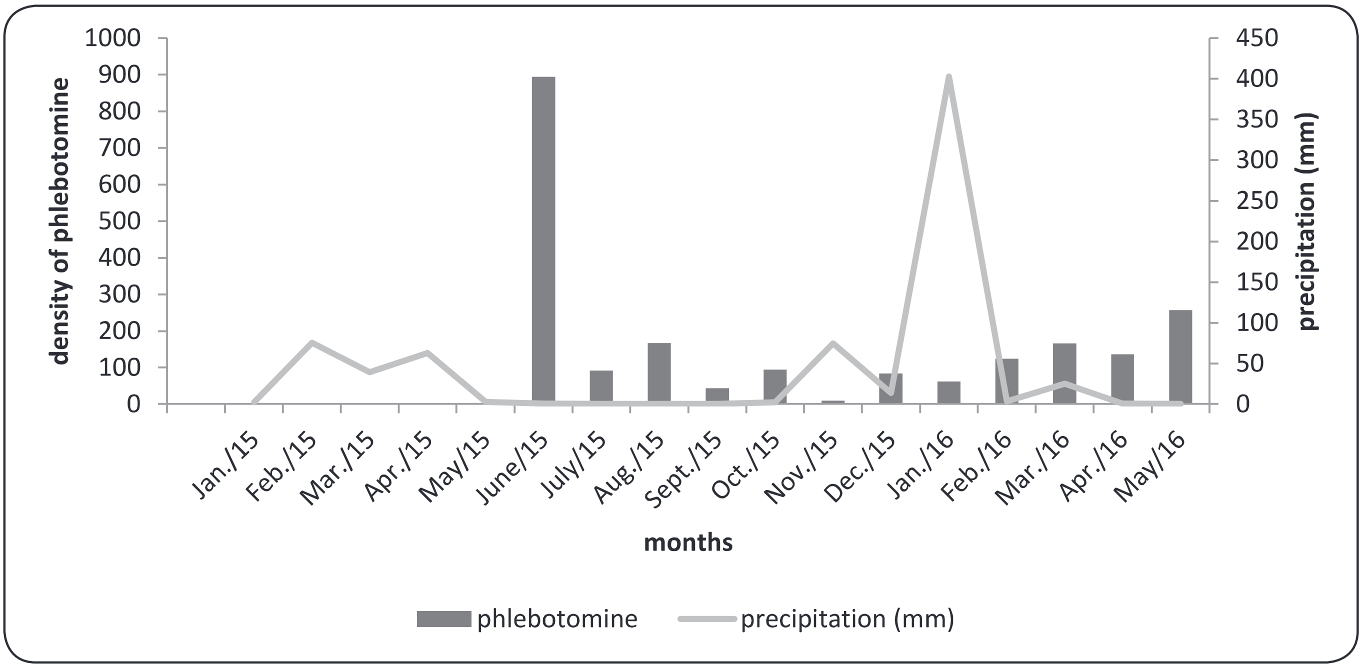

FIGURE 4: Population density of phlebotomine sand flies captured monthly with light traps (CDC) and mean of the monthly climatic variable of rainfall in Brejo do Mata Fome, Prata, Riachinho, and Riacho do Brejo villages located in the Xakriabá Indigenous Reserve from June 2015 to May 2016.

by *Ny. intermedia* with a density of 7.3%. The other species of medical importance, *Mg. migonei* and *Pi. pessoai*, presented very low population densities with values of only 0.55% (11 specimens) and 0.05% (one specimen), respectively.

Rego et al.[27] studied the same XIR but different villages and ecotopes (trails in the woods) and captured 8,046 specimens of sand flies from 11 different genera and 28 species. Comparison of the fauna collected by Rego et al.[27] with those identified in the present study showed that species such as *Lu. cavernicola* and *Ev. spelunca*, among others, observed in this previous study were not collected in the present study; other species such as *Mi. oswaldoi*, *Pa. bigeniculata*, and *Pa. shannoni* were found in the present study but not in the previous study. Other differences in the faunal diversity in this study compared to the one by Rego et al.[27] include the presence of *Pi. pessoai* and the absence of *Nyssomyia whitmani*, which are both vectors of CL. Notably, only a few specimens of *Pi. pessoai* and *Ny. whitmani* were identified. These results were expected, possibly because of the different location of the villages and ecotopes, as the present study was carried out in the peri-domicile and intra-domicile, while Rego et al.[27] focused on peri-domiciles and trails where rocky outcrops areas with craters in the soil exist, propitious to shelter some phlebotomine sand fly species.

Female sand flies act as agents that transmit leishmaniasis through their hematophagous feeding habits; although we captured a lower number of females than males in this study, a significant difference between the population density was not observed. A significant predominance of males, however, was observed for *Lu. longipalpis*, with a ratio of 2.3 (M/F). In fact, the predominance of males of *Lu. longipalpis* in the field was consistent with those reported previously[17,25,27,20,28,29,30] in contrast, Rego et al.[27] found a larger number of female sand flies than males in Imbaúbas village with a ratio of 2.13 females/males. The highest proportion of males captured with CDC light traps, particularly for *Lu. longipalpis*, can be explained by the natural behavior of males that accompany females during their movements to ensure fertilization. However, this result was not observed for *Ny. intermedia* and a higher density of females was found compared to males, which has also been observed previously[27,31,32].

As observed in the present study, the species most commonly sampled in the peri-domicile were *Lu. longipalpis* (51.08%) and *Ny. intermedia* (31.79%), in contrast to the results found by Rego et al.[27]. Interestingly, the density and diversity of the total sand fly faunas between peri- and intra-domicile areas were equivalent, supporting previous observations by Resende et al.[29]. However, previous studies reported a higher density in the peri-domicile[22,24,33]. The population density was also equivalent between the intra- and peri-domicile, specifically for *Lu. longipalpis*. Additionally, the population density of *Ny. intermedia* was higher on the peri-domicile, which agrees with some previous studies[31,32].

These results suggest the endophilic behavior of the vectors and emphasize the probability of leishmaniasis transmission in the intra-domicile environment[19, 22-24,29]. Thus, sand fly species with the greatest epidemiological importance for leishmaniasis persistence in the XIR are *Lu. longipalpis* and *Ny. intermedia*, as they have a high vectorial capacity and anthropophilia[34-36]. Additionally, they showed high densities in both intra- and peri-domicile areas.

The variability in sand fly diversity and population density observed in the different villages may be associated with environmental differences. Compared to other villages evaluated in the study, Riacho do Brejo showed a greater variety of animal enclosures, such as pens for chickens, pigs, and dogs, as well as fruit trees such as banana and guava trees, providing excellent conditions for phlebotomine reproduction and population growth[37,38]. Brejo do Mata Fome was much more artificially modified and showed the lowest population density and lowest sand fly faunal diversity, corroborating the previous results of Feitosa et al.[39].

The interference of climatic factors with the sand fly population has been studied previously. Variables such as temperature, humidity, and rainfall were found to influence sand fly populations in different manners depending on the region. Rutledge & Ellenwood[40] suggest that sand fly seasonality is related to rainfall distribution patterns, which modify breeding site conditions in the soil. These insects are typically found at high densities during warm and humid months[41-44] or in drier months, as observed by Zelédon, Murrillo, and Gutierrez[45] in an area of Costa Rica and Galati et al.[46] in Mato Grosso do Sul state, Brazil. Barata et al.[21] showed that in Porteirinha, MG, a municipality relatively close to that in the current study, climatic factors interfered with the population density of sand flies, particularly the factors of rainfall and humidity, but not temperature. Similar results were observed previously[20,26,47]. However, in the present study the sand fly density tended to increase after rainy periods, particularly after March and April 2015 and January 2016, when higher peaks of rainfall occurred and there was a consequent increase in sand fly abundance in subsequent months; this tendency was also observed in previous studies[20, 26,47].

Although the correlation between abundance and climatic variables is crucial for understanding the risk dynamics in each locality, the biological plasticity of a species and different biotic and abiotic factors also explain the different results obtained in different geographic areas[48]. As the eco-epidemiological profile of VL and CL is complex and shows specific characteristics in each transmission area, the entomological results presented in this study improve the understanding and targeting of prevention and control measures that can be applied in the peri- and intra-domicile of the XIR villages by the responsible authorities.

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Conflict of interests: The authors declare that there is no conflict of interest.

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