



UNIVERSIDADE FEDERAL DOS VALES DO JEQUITINHONHA E MUCURI

Programa de Pós-Graduação em Biologia Animal

Gabriele Zaine Teixeira Debortoli

**ESTUDO DE FLEBOTOMÍNEOS NO MUNICÍPIO DE UNAÍ, ÁREA DE
TRANSMISSÃO DE LEISHMANIOSE VISCERAL NO ESTADO DE MINAS GERAIS**

**Diamantina
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Dissertação apresentada ao programa de Pós-Graduação em Biologia Animal da Universidade Federal dos Vales do Jequitinhonha e Mucuri, como requisito para obtenção do título de Mestre.

Orientadora: Profa. Dra. Thaís Rabelo dos Santos
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Dissertação apresentada ao
MESTRADO EM BIOLOGIA
ANIMAL, nível de MESTRADO,
como parte dos requisitos para
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RESUMO

As leishmanioses são zoonoses parasitárias negligenciadas, transmitidas por flebotomíneos, sendo uma das principais causas de morbidade e mortalidade em todo o mundo. A expansão geográfica da doença, aliada à urbanização do ciclo de transmissão, reforça a necessidade de investigações que integrem aspectos entomológicos, ambientais e climáticos. O município de Unaí, localizado no estado de Minas Gerais, é uma área de transmissão de leishmanioses, com registros de casos humanos e caninos da doença, mas sem dados entomológicos disponíveis sobre a fauna flebotomínea local. Esta dissertação tem como objetivo descrever a fauna do município, sua distribuição espacial e os fatores ambientais e climáticos associados à sua ocorrência. No Capítulo 1, apresenta-se o levantamento entomológico realizado ao longo de 2024, em dez residências distribuídas por sete bairros de Unaí/MG. As capturas mensais foram feitas com armadilhas luminosas do tipo HP, instaladas em ambientes intra e peridomiciliares. Os espécimes foram identificados morfolologicamente e os dados analisados por meio de modelos lineares de efeitos mistos, a fim de verificar associações com variáveis ambientais e climáticas. Foram capturados 504 flebotomíneos pertencentes a 11 espécies. *Lutzomyia longipalpis* foi a espécie mais abundante, seguida por *Lu. lenti* e *Lu. intermedia*. As áreas peridomiciliares apresentaram maior diversidade e abundância, associadas à presença de galinheiros, matéria orgânica e vegetação. Verificamos a associação positiva da temperatura com *Lu. longipalpis* e *Lu. lenti*, e efeito negativo da precipitação sobre *Lu. intermedia*. A predominância de machos (69,8%) sugere a existência de criadouros ativos nas proximidades das residências. Os resultados indicam que as condições ambientais favorecem a presença de vetores em locais com contato próximo entre humanos e animais, aumentando o risco de transmissão domiciliar. Esses achados ressaltam a importância da vigilância entomológica contínua e do manejo ambiental como estratégias complementares no controle das leishmanioses.

Palavras-chave: *Lutzomyia*; Biodiversidade; Leishmanioses; Vetor; Clima.

ABSTRACT

Leishmaniasis are neglected parasitic zoonoses transmitted by phlebotomine sand flies and are among the leading causes of morbidity and mortality worldwide. The geographic expansion of the disease, along with the urbanization of its transmission cycle, underscores the need for investigations that integrate entomological, environmental, and climatic aspects. The municipality of Unaí, located in the state of Minas Gerais, is an area of leishmaniasis transmission, with recorded cases in both humans and dogs, yet no entomological data on the local sand fly fauna have been available. This dissertation aims to describe the sand fly fauna in the municipality, its spatial distribution, and the environmental and climatic factors associated with its occurrence. Chapter 1 presents the entomological survey conducted throughout 2024 in ten households distributed across seven neighborhoods of Unaí, MG. Monthly collections were carried out using HP-type light traps installed in both indoor and peridomestic environments. Specimens were morphologically identified, and the data were analyzed using mixed-effects linear models to assess associations with environmental and climatic variables. A total of 504 sand flies belonging to 11 species were captured. *Lutzomyia longipalpis* was the most abundant species, followed by *Lu. lenti* and *Lu. intermedia*. Peridomestic areas showed greater diversity and abundance, associated with the presence of chicken coops, organic matter, and vegetation. A positive association with temperature was observed for *Lu. longipalpis* and *Lu. lenti*, while precipitation had a negative effect on *Lu. intermedia*. The predominance of males (69.8%) suggests the presence of active breeding sites near the residences. The results indicate that environmental conditions favor the presence of vectors in areas with close contact between humans and animals, increasing the risk of household transmission. These findings highlight the importance of continuous entomological surveillance and environmental management as complementary strategies for leishmaniasis control.

Keywords: *Lutzomyia*; Biodiversity; Leishmaniasis; Vector; Climate.

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1. INTRODUÇÃO

A leishmaniose compreende um grupo de doenças parasitárias negligenciadas causadas por protozoários do gênero *Leishmania* (Kinetoplastida: Trypanosomatidae), transmitidas principalmente pela picada de fêmeas infectadas de flebotomíneos (Diptera: Psychodidae: Phlebotominae) (Steverding, 2017; Ansari *et al.*, 2025). A Organização Mundial da Saúde (OMS) estima que mais de um bilhão de pessoas vivem em áreas endêmicas e estão sob risco de infecção. A leishmaniose apresenta-se em três formas clínicas principais: visceral (LV), cutânea (LC) e mucocutânea. A leishmaniose visceral, causada por *Leishmania infantum*, é considerada endêmica em várias regiões do Brasil, país responsável por aproximadamente 94% dos casos notificados nas Américas (WHO, 2023; PAHO, 2024).

Atualmente, são reconhecidas 1.067 espécies de flebotomíneos (Phlebotominae) em todo o mundo (Alencar, Scarpassa, 2018; Garcia *et al.*, 2025) das quais 557 ocorrem nas Américas. O Brasil abriga a maior diversidade de espécies de flebotomíneos registrada globalmente, com 300 espécies catalogadas no país, incluindo 98 no estado de Minas Gerais (Galati, 2018; Bánki *et al.*, 2021). *Lutzomyia longipalpis*, principal vetor da leishmaniose visceral no Brasil, possui ampla distribuição na América do Sul e apresenta elevada plasticidade ecológica, especialmente em ambientes urbanos e periurbanos (Sousa-Paula, Dantas-Torres, 2021). Refletindo essa ampla distribuição, o catálogo FIOCRUZ-COLFLEB, uma das maiores coleções de flebotomíneos do mundo, inclui 3.682 registros de *Lu. longipalpis* em todo o território brasileiro (Andrade-Filho *et al.*, 2017; Andrade-Filho *et al.*, 2022).

Os flebotomíneos estão presentes em todas as unidades federativas do Brasil, evidenciando sua notável adaptabilidade a diferentes condições ambientais (Almeida *et al.*, 2019). São dípteros de hábitos crepusculares e noturnos, cujas fêmeas hematófagas se alimentam de uma ampla variedade de hospedeiros vertebrados, incluindo mamíferos silvestres e domésticos (Azami-Conesa, Gómez-Muñoz, Martínez-Díaz, 2021). Diversos fatores ambientais, como a presença de matéria orgânica em decomposição, galinheiros e variáveis climáticas (temperatura, umidade, precipitação e velocidade do vento), influenciam diretamente a abundância e a distribuição espacial desses vetores (Aguiar-Martins *et al.*, 2021; Senanayake *et al.*, 2023).

No município de Unaí, estado de Minas Gerais, há registros de casos humanos e animais de LV e LC, com 16 casos confirmados de leishmaniose visceral e 228 de leishmaniose cutânea notificados no banco de dados nacional SINAN/DATASUS entre 2014 e 2024 (Ministério da Saúde, 2025). No entanto, até o momento, nenhum estudo anterior investigou sistematicamente a fauna flebotomínea local. Essa lacuna dificulta a compreensão da dinâmica

de transmissão local e compromete a elaboração de estratégias eficazes de vigilância e controle vetorial.

Frente a esse contexto, esta dissertação está estruturada em capítulo único, redigido no formato de artigo científico, conforme as normas da revista de submissão. O capítulo apresenta a investigação da fauna local de Unaí/MG, abordando sua distribuição espacial e os fatores ambientais e climáticos associados à sua ocorrência. Os resultados obtidos reforçam a importância da vigilância entomológica contínua e do manejo ambiental como estratégias complementares no controle das leishmanioses.

2. CHAPTER 1

Phlebotomine sand flies (Diptera: Psychodidae, Phlebotominae) fauna in Unaí, State of Minas Gerais, Brazil

Phlebotomine Sand Fly Fauna in Unaí, Minas Gerais, Brazil

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ABSTRACT

Background: Leishmaniasis are neglected parasitic diseases transmitted by phlebotomine sand flies, with significant impact on public health. The municipality of Unaí, in the state of Minas Gerais, reports human and canine cases of leishmaniasis but lacked entomological data. This study aimed to describe the local phlebotomine fauna, its spatial distribution, and the environmental and climatic factors associated with its occurrence.

Methods: Sampling was conducted monthly throughout 2024 in ten households across seven neighborhoods of Unaí. HP light traps were installed in both intradomestic and peridomestic areas. Specimens were morphologically identified and statistically analyzed to assess associations with environmental and climatic variables using mixed-effects linear models.

Results: A total of 504 phlebotomine specimens from 11 species were captured. *Lutzomyia longipalpis* was the most abundant, followed by *Lu. lenti* and *Lu. intermedia*. Peridomestic areas exhibited greater species diversity and abundance, associated with the presence of chicken coops, organic matter, and vegetation. Temperature was positively associated with *Lu. longipalpis* and *Lu. lenti*, while precipitation had a negative effect on *Lu. intermedia*. Males accounted for 69.8% of the specimens captured, suggesting active breeding sites near the households.

Conclusions: This is the first record of the phlebotomine fauna in Unaí. The results indicate that environmental conditions favor the presence of vectors in areas with close human and animal contact, increasing the risk of domiciliary transmission. The findings underscore the importance of continuous entomological surveillance and the implementation of environmental management measures as a complementary strategy for leishmaniasis control.

Keywords: *Lutzomyia*, biodiversity, leishmaniasis, insect vector, spatial distribution, climate

INTRODUCTION

Leishmaniasis comprises a group of neglected parasitic diseases caused by protozoa of the genus *Leishmania* (Kinetoplastida: Trypanosomatidae), primarily transmitted through the bite of infected female phlebotomine sand flies (Diptera: Psychodidae: Phlebotominae)^{1,2}. The World Health Organization (WHO) estimates that over one billion people live in endemic areas and are at risk of infection. Leishmaniasis presents in three main clinical forms: visceral (VL), cutaneous (CL), and mucocutaneous. Visceral leishmaniasis, caused by *Leishmania infantum*, is considered endemic in several regions of Brazil, which accounts for approximately 94% of reported cases in the Americas^{3,4}.

There are 1,067 recognized species of sand flies (Phlebotominae) worldwide^{5,6}, of which 557 occur in the Americas. Brazil is home to the greatest diversity of sand fly species recorded in the world, with 300 species recorded in the country, including 98 in the state of Minas Gerais^{7,8}. *Lutzomyia longipalpis*, the main vector of visceral leishmaniasis in Brazil, is widely distributed throughout South America and exhibits high ecological plasticity, particularly in urban and peri-urban environments⁹. Reflecting its broad distribution, the FIOCRUZ-COLFLEB catalog—one of the largest sand fly collections in the world—includes 3,682 records of *Lu. longipalpis* across the country^{10,11}.

Phlebotomine sand flies are present in all Brazilian federal units, reflecting their remarkable adaptability to diverse environmental conditions¹². These are crepuscular and nocturnal insects whose hematophagous females feed on a wide variety of vertebrate hosts, including wild and domestic mammals¹³. Several environmental factors—such as the presence of decomposing organic matter, chicken coops, and climatic variables including temperature, humidity, precipitation, and wind speed—directly influence the abundance and spatial distribution of these vectors^{14,15}.

Despite the occurrence of human and animal cases of both VL and CL in Unaí, Minas Gerais (Brazil), with 16 confirmed cases of visceral leishmaniasis and 228 cases of cutaneous leishmaniasis recorded in the national SINAN/DATASUS database between 2014 and 2024¹⁶, no prior studies have systematically surveyed the local sand fly fauna. This lack of baseline entomological data hampers a clear understanding of local transmission dynamics and limits the development of effective surveillance and vector-control strategies.

METHODS

Study area

The study was conducted in the municipality of Unaí (16°22'45"S, 46°53'45"W), located in the state of Minas Gerais, in southeastern Brazil. The municipality encompasses an area of 8,445.432 km², and its administrative center lies at an altitude of 640 meters above sea level¹⁷. The topography is predominantly flat. Unaí is part of the São Francisco River basin and features a humid tropical climate, with a mean annual relative humidity of 63.3%, temperatures ranging from 10 to 35°C, and an annual average temperature of 24°C. The mean annual rainfall is approximately 1,200 mm, with the rainy season extending from October to March.

Sand fly trapping or Sand fly collection

The selection of households was based on the occurrence of reported cases of visceral leishmaniasis in humans and/or dogs, as well as on ecological characteristics conducive to the development of phlebotomine sand fly populations. These characteristics included the presence of decomposing organic matter, shade-providing fruit trees, chicken coops, pigsties, and other domestic animals. Vector capture was conducted at ten households distributed across seven neighborhoods, totaling 20 HP light traps, described in detail by Pugedo et al.¹⁸ deployed over

two consecutive nights, from 6:00 p.m. to 6:00 a.m., during the last week of each month, from January to December 2024. In each household, two traps were installed — one in the intradomestic ecotope - inside the house and the other in the peridomestic ecotope - up to 100 meters from the house.

Morphological identification of sand flies or identification of phlebotomine specimens

The samples were preserved in 70% ethanol and sent to the Animal Parasitology Laboratory at the Federal University of the Jequitinhonha and Mucuri Valleys. The preparation of captured phlebotomine sand flies followed the protocol described by Langeron¹⁹. Species identification was performed based on morphological characteristics, using the taxonomic key proposed by Young and Duncan²⁰. The main structures analyzed included the head (antennae, cibarium, pharynx, and palps), thorax (wings and legs), and abdomen (external and internal genitalia). For female specimens, the last two abdominal segments were dissected to allow visualization of the internal genitalia.

Sand fly mapping and distribution

For the spatial analysis of phlebotomine abundance, the Geographic Information System software QGIS[®] 3.26 Buenos Aires (QGIS Development Team, 2022) was used. The municipality of Unaí, Minas Gerais, was georeferenced (Figure 1), including the locations of streets layouts, river basins, and the locations of households where HP light traps were installed for sand fly collection. The selected households were distributed across seven neighborhoods with different environmental and urbanization characteristics: Cachoeira, Dom Bosco, Iuna, Mamoeiro, Mansões Sul, Novo Horizonte, and Santa Clara. Based on these data, a Kernel density map was generated to represent the spatial concentration of the vector across the

municipality's census sectors. Buffer zones with a 1.5 km radius were established around each household to indicate the potential influence areas for phlebotomine presence.

Environmental factors and their sources or Meteorological Data

The climatic data used in this study were obtained from the National Institute of Meteorology (INMET) online portal. Four predictive climatic variables were selected: temperature, relative humidity, wind speed, and precipitation. The values were retrieved from daily records of the Conventional Meteorological Station 83428, located in Unaí, Minas Gerais. For the analysis, the data were compiled as monthly averages based on the information available in the INMET database.

Calculation of sand fly diversity values

To assess species diversity across neighborhoods, the Shannon-Wiener diversity index was calculated using the following formula:

$$H = - \sum [(p_i) \cdot \log(p_i)]$$

Where, p_i is the proportion of individuals of species i in relation to the total number of individuals in the sample. This index considers the number of species and with the evenness of their relative abundances. Additionally, the Simpson diversity index was used to estimate the probability that two individuals randomly selected from a sample will belong to different species. The index was computed using the formula:

$$D = \sum_{i=1}^s \frac{n_i(n_i - 1)}{N(N - 1)}$$

Where, n_i is the number of organisms of a particular species and N the number of organisms of all species²¹.

Statistical analysis

Descriptive statistics were used to summarize the abundance of phlebotomine sand flies and climatic variables, including arithmetic mean, standard deviation, median, minimum, and maximum values. The normality of continuous data was assessed using visual inspection and statistical criteria. To compare differences in climatic conditions between months, a one-way analysis of variance (ANOVA) was applied to evaluate the probability of significance (p-value) for F-statistics.

To assess the association between the presence of *Lutzomyia* species (dependent variables) and environmental and biological predictors (independent variables), Generalized Linear Mixed Models (GLMMs) were fitted using a binomial distribution and logit link function. Separate models were developed for *Lu. longipalpis*, *Lu. lenti*, and *Lu. intermedia*. Fixed effects included area of capture (intradomestic or peridomestic), sex of the insect, average temperature (on the day of capture and for the previous 7, 15, and 30 days), average humidity, wind speed, and accumulated precipitation across the same periods. Neighborhoods and houses were included as nested random effects to account for clustering.

In the first stage, univariate analyses were conducted to screen for variables potentially associated with the outcome. Variables with p-values ≤ 0.25 were selected for inclusion in multivariate models. Collinearity among predictors was assessed using the Variance Inflation Factor (VIF), and highly correlated variables were excluded to avoid multicollinearity. Final multivariate models were obtained by sequentially excluding non-significant variables, while monitoring changes in model fit and effect estimates.

All models were fitted in Stata[®] version 16.1 (StataCorp LLC, College Station, TX, USA), and odds ratios (OR) and adjusted odds ratios (AOR) were presented with 95% confidence intervals. The level of statistical significance was set at 5%.

RESULTS

Table 1 highlights the main characteristics of the domestic environments of the ten households included in the study, which are frequently associated with the attraction and shelter of phlebotomine sand flies. The main differences involved the size of the outdoor area, presence of fruit trees, chicken coops, domestic animals, organic matter, and waste. The neighborhoods of Cachoeira (C6), Mamoeiro (C3), and Mansões Sul (C9) had more vegetated peridomestic areas, with greater shade and a higher number of animals, especially chickens (> 15). Household C3 stood out for harboring the highest diversity of domestic animal species, including dogs (n=3), cats (n=2), horses (n=3), one calf, and chickens (>15). In total, chicken coops were recorded in eight of the ten households, being more common in peripheral areas.

The influence of these environmental characteristics on the sand fly populations was reflected in the species composition and abundance observed across the sampled neighborhoods. Between January and December 2024, a total of 504 phlebotomine sand fly specimens were captured, representing 11 species, across 10 households distributed among seven neighborhoods in the municipality of Unaí, Minas Gerais (Table 2). A total of 352 males (69.8%) and 152 females (30.2%) were recorded, with an overall mean density of 0.98 specimens/trap-night.

Lutzomyia longipalpis was the most predominant species (39.7% of the total), followed by *Lu. lenti* (33.3%) and *Lu. intermedia* (15.3%). The three most frequent species exhibited statistically significant differences among neighborhoods (*Lu. longipalpis*, $p = 0.01$; *Lu. lenti*, $p < 0.0001$; *Lu. intermedia*, $p < 0.0001$).

Among the neighborhoods, Mamoeiro recorded the highest absolute number of phlebotomine sand flies (n = 268; 53.2% of the total), followed by Cachoeira (n = 137; 27.2%), which also exhibited the highest capture density (3.81 specimens/trap-night), likely favored by the

combination of a chicken coop, pigsty, and dense peridomestic vegetation. In contrast, Dom Bosco ($n = 1$) and Santa Clara ($n = 4$) showed the lowest captures.

Species diversity complemented the observed patterns of species abundance, varying substantially among neighborhoods. Assessed by the Shannon diversity index (H'), the highest indices were observed in Novo Horizonte ($H' = 1.6$), Mansões Sul ($H' = 1.4$), and Cachoeira ($H' = 1.3$), while the lowest were recorded in Dom Bosco ($H' = 0$) and Santa Clara ($H' = 0.6$). No statistical test was performed to compare diversity (H') across neighborhoods.

The spatial distribution of phlebotomine species across the sampled neighborhoods is shown in the Kernel density maps (Figure 2). *Lutzomyia longipalpis*, *Lu. lenti*, and *Lu. intermedia* exhibited the highest spatial densities, with hotspots primarily concentrated in the Mamoeiro neighborhood.

In addition to environmental characteristics, climatic variables were analyzed for their potential influence on sand fly abundance. Descriptive statistics of the monthly climatic variables recorded between January and December 2024 in the municipality of Unaí, Minas Gerais, are presented in Table 3. For each sampling event, climatic averages were calculated based on the days when traps were installed and for the preceding 7, 15, and 30 days.

Mean values for temperature (26.9°C to 27.3°C), relative humidity (60.4% to 67.5%), mean wind speed (1.0 to 1.1 m/s), and accumulated precipitation (0.0 to 3.5 mm) showed limited variation across the periods analyzed, with no statistically significant differences ($p > 0.05$).

The climatic variables remained stable throughout the study, indicating no direct influence on the abundance of phlebotomine sand flies in the sampled areas.

The influence of these climatic and environmental factors on the abundance of the main species was further explored through mixed-effects linear regression analysis (Table 4). The analysis revealed that the abundance of *Lu. longipalpis* was significantly higher in peridomestic areas (AOR = 2.01; $p = 0.01$) and among males (AOR = 3.12; $p < 0.0001$). Among the climatic

variables, the mean temperature over the previous 15 days was positively associated with the abundance of *Lu. longipalpis* (AOR = 2.03; $p < 0.0001$), whereas the mean temperature over the previous 30 days was negatively associated (AOR = 0.42; $p < 0.0001$). No significant associations were observed with humidity, wind speed, or precipitation.

The abundance of *Lu. lenti* was significantly higher in intradomestic areas (AOR = 3.82; $p < 0.0001$) and among females (AOR = 1.76; $p = 0.040$). Among the climatic variables, the mean temperature over the previous 30 days showed a positive association (AOR = 1.71; $p = 0.001$), whereas the mean temperature over the previous 15 days was negatively associated (AOR = 0.64; $p = 0.010$). No significant associations were observed with humidity, wind speed, or precipitation for the abundance of *Lu. lenti*.

For *Lu. intermedia*, abundance was higher in peridomestic areas (AOR = 3.04; $p = 0.010$). No statistically significant association was observed with sex, although the odds ratio suggested a trend toward higher abundance among males (AOR = 1.45; $p = 0.235$). Among the climatic variables, precipitation on the day of collection (AOR = 0.16; $p = 0.026$) was negatively associated.

Temperature was the only climatic variable significantly associated with the abundance of *Lu. longipalpis* and *Lu. lenti*, while precipitation was associated with *Lu. intermedia* abundance, with distinct effects across species and at different temporal scales. No significant associations were observed with humidity or wind speed for the species analyzed.

DISCUSSION

To our knowledge, this is the first entomological survey conducted in Unaí, Minas Gerais, Brazil, offering novel insights into the species composition, spatial distribution, and climatic variables influencing sand fly populations in the region. These findings are particularly relevant

given the high incidence of visceral leishmaniasis in Minas Gerais, highlighting the public health importance of understanding vector ecology in endemic areas²²⁻²⁴.

The dynamics of leishmaniasis transmission have been significantly influenced by the ecological plasticity of phlebotomine vectors, which enables their adaptation to anthropized and urban environments. Environmental degradation (deforestation) caused by agricultural expansion and other changes (migration, irregular land occupation, poor sanitation, and the increase of domestic animals in the peridomestic environment) has been associated with changes in the transmission patterns of leishmaniasis observed²⁵, favoring their establishment in peridomestic areas where they find shelter and food sources^{26,27}.

In the study, the neighborhoods of Mamoeiro and Cachoeira recorded the highest sand fly abundance. These sites share typical peridomestic characteristics (such as the presence of poultry coops, domestic animals, organic matter, and proximity to vegetation), which create favorable conditions for the presence and maintenance of phlebotomine populations²⁸⁻³⁰. Chicken coops and pigsties are recognized as resting places for adults of both sexes, where females also take their blood meals^{31,32}. Moreover, these ecotopes provide shade, moisture, and soil rich in organic matter, offering suitable conditions for breeding sites of immature sand flies^{33,34}.

Several aspects observed in the current study appear to favor the domiciliary transmission of VL: (a) the high number of male sand flies captured indicates the presence of active breeding sites in the areas surrounding the sampled households; (b) peridomestic areas showed significantly higher species diversity and sand fly abundance compared to intradomestic environments; (c) synanthropic species such as *Lu. intermedia* and *Lu. whitmani* were more abundant in animal shelters located close to human dwellings.

The highest species richness (11 species) and total abundance (324 individuals) were recorded in peridomestic areas, supporting findings from other Brazilian municipalities that have demonstrated similar ecological preferences^{35,36}.

Lutzomyia longipalpis, the primary vector of *Le. infantum* and the most abundant species in this study (39.7% of captures), was detected in all sampled neighborhoods. Its predominance over other species in areas where VL is endemic has been reported in different Brazilian regions³⁷⁻⁴⁰.

The second most abundant vector species, *Lu. intermedia*, a known vector of *Le. braziliensis*, also showed a broad distribution across both intradomestic and peridomestic ecotopes in Unaí. Environmental changes resulting from anthropogenic activity may have contributed to the domiciliation of *Lu. intermedia*. The generalist behavior of *Lu. intermedia* explains its higher occurrence observed in peridomestic areas. Its greater reproductive capacity around households could explain its high density and predominance over other species in this environment⁴¹.

The probability of capturing *Lu. longipalpis* was 2.20 times higher in peridomestic areas compared to intradomestic environments, and for *Lu. intermedia*, this likelihood was 3.35 times higher. These findings highlight the preference of both species for transitional ecotopes, where vegetation, animal shelters, organic matter, and host availability coexist.

Indeed, *Lu. longipalpis* appears to be fully adapted to anthropogenic environments, as observed in other areas²⁷.

Despite the presence of confirmed vectors of CL and VL, other species found in this study also warrant attention. *Lu. lenti*, *Lu. sallesi*, and *Lu. sordellii* have been described as infected by *Le. braziliensis* and *Le. infantum*^{40,42-44}.

Lutzomyia lenti was found approximately three times more frequently in intradomestic environments compared to peridomestic ones, echoing urban patterns reported in other endemic regions with environmental and socioeconomic characteristics similar to those of the studied

areas, suggesting that the species exhibits endophilic behavior, residing in habitats close to its preferred hosts. In Campo Grande, this species was closely associated with peridomestic areas⁴⁵ and domestic shelters in rural areas of Mato Grosso do Sul⁴⁶. Although Brazil et al.⁴⁷ stated that *Lu. lenti* is refractory to *Leishmania* infection, this species has been found naturally infected with *Le. infantum*, thus providing further evidence of its participation in the epidemiological cycle of VL^{44,48,49}.

Studies indicate that the occurrence and seasonal fluctuation of sand fly populations can be modulated by climatic factors such as temperature, relative humidity, precipitation, and wind speed, which influence their development, flight activity, and survival⁵⁰⁻⁵².

Lutzomyia longipalpis was found throughout the entire sampling period, with high abundance during summer (n = 65) and autumn (n = 96), likely due to rainfall preceding these seasons (October to January), which may lead to vegetation growth, reduced solar radiation, increased soil moisture, and consequently, better insect development. Prolonged dry periods are likely to result in arid conditions that hinder the development of both immature and adult stages.

Similar results were found by Oliveira et al.⁵³, who observed high abundance of *Lu. longipalpis* after a one- to two-month lag in rainfall. Indirectly supporting this, there is evidence that the period of highest visceral leishmaniasis transmission occurs during and shortly after the rainy season, when the insect's population density increases⁵⁴.

This study also provides evidence of a positive association not only between *Lu. longipalpis* and precipitation, but also with temperature (previous 15 – AOR: 2.03), and a negative association (AOR: 0.42) with lagged temperature (previous 30), indicating that modest short-term warming increases the likelihood of capturing the species. The negative association with temperature may suggest an upper tolerance limit for this vector^{32,55}, suggesting that prolonged heat may suppress population abundance, possibly due to increased mortality or reduced activity at suboptimal thresholds.

Previous studies have demonstrated that temperature conditions influence the dynamics of *Lu. longipalpis*⁵⁰. Martins et al.⁵⁶ reported that female sand flies modulate their behavior in response to ambient temperature, exhibiting reduced activity at lower temperatures and increased host-seeking behavior in warmer conditions as blood digestion progresses.

Lutzomyia intermedia was present in almost every month of the study period, with peaks during the warmer and rainier months (summer and autumn); similar results were found by Virgens et al.⁵⁷, Condino et al.⁵⁸. Unlike *Lu. longipalpis*, which responded to temperature variation, *Lu. intermedia* was primarily influenced by precipitation, reflecting distinct ecological preferences between the species. Rainfall is one of the key environmental factors influencing the population dynamics of phlebotomine sand flies⁵⁹. Precipitation on the day of collection was associated with a marked decrease in the occurrence of this species, possibly due to reduced flight activity during heavy rainfall⁶⁰.

Studies on the diversity and distribution of phlebotomine species provide key elements to clarify the epidemiology of leishmaniasis⁶¹, offering essential information to effectively guide and implement preventive measures and improve community health. Environmental management is an important preventive control strategy, as this method was effective in reducing the population density of *Lu. longipalpis* in a study conducted in Brazil⁶².

A limitation of this study was the absence of molecular screening for *Leishmania* DNA in female specimens of *Lu. longipalpis*, *Lu. intermedia*, and *Lu. whitmani*, species recognized as vectors of leishmaniasis in various regions of Brazil. This analysis was not performed due to budget constraints. Therefore, future studies in this region are strongly recommended to assess natural infection rates in sand fly populations of epidemiological concern, which would significantly enhance the understanding of local transmission dynamics and support targeted control strategies.

CONCLUSIONS

This study provides the first record of sand fly fauna in Unaí, Minas Gerais, revealing the predominance of *Lu. longipalpis*, *Lu. lenti*, and *Lu. intermedia*. Peridomestic environmental characteristics significantly influence the abundance and spatial distribution of these vectors, while climatic variables such as temperature and precipitation modulate their populations in a species-specific and time-dependent manner. The abundance of vector species in areas with conditions favorable for breeding and in close contact with humans and animals reinforces the potential risk of domiciliary transmission of leishmaniasis. The findings highlight the importance of entomological surveillance integrated with environmental management measures as a vector control strategy.

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TITLE AND LEGEND

Figure 1. Sand Fly Collection Sites in the Municipality of Unaí, Minas Gerais, Brazil

Figure 2. Spatial distribution and abundance intensity (Kernel density) of phlebotomine sand fly species captured in Unaí, Minas Gerais, Brazil.

Table 1. Description of the peridomestic environment and presence of domesticated animals in sampled households across neighborhoods of Unaí, Minas Gerais, Brazil.

Table 2. Sand fly species composition and abundance in seven neighborhoods in Unaí, Minas Gerais, Brazil.

Table 3. Descriptive statistics of monthly climatic variables recorded between January and December 2024 in Unaí, Minas Gerais, Brazil

Table 4. Variation in phlebotomine abundance analyzed using Generalized Linear Mixed Models (binomial family; logit link), with neighborhoods and houses included as a random effect. Unaí, Minas Gerais, Brazil

Figure 1. Sand Fly Collection Sites in the Municipality of Unaí, Minas Gerais, Brazil

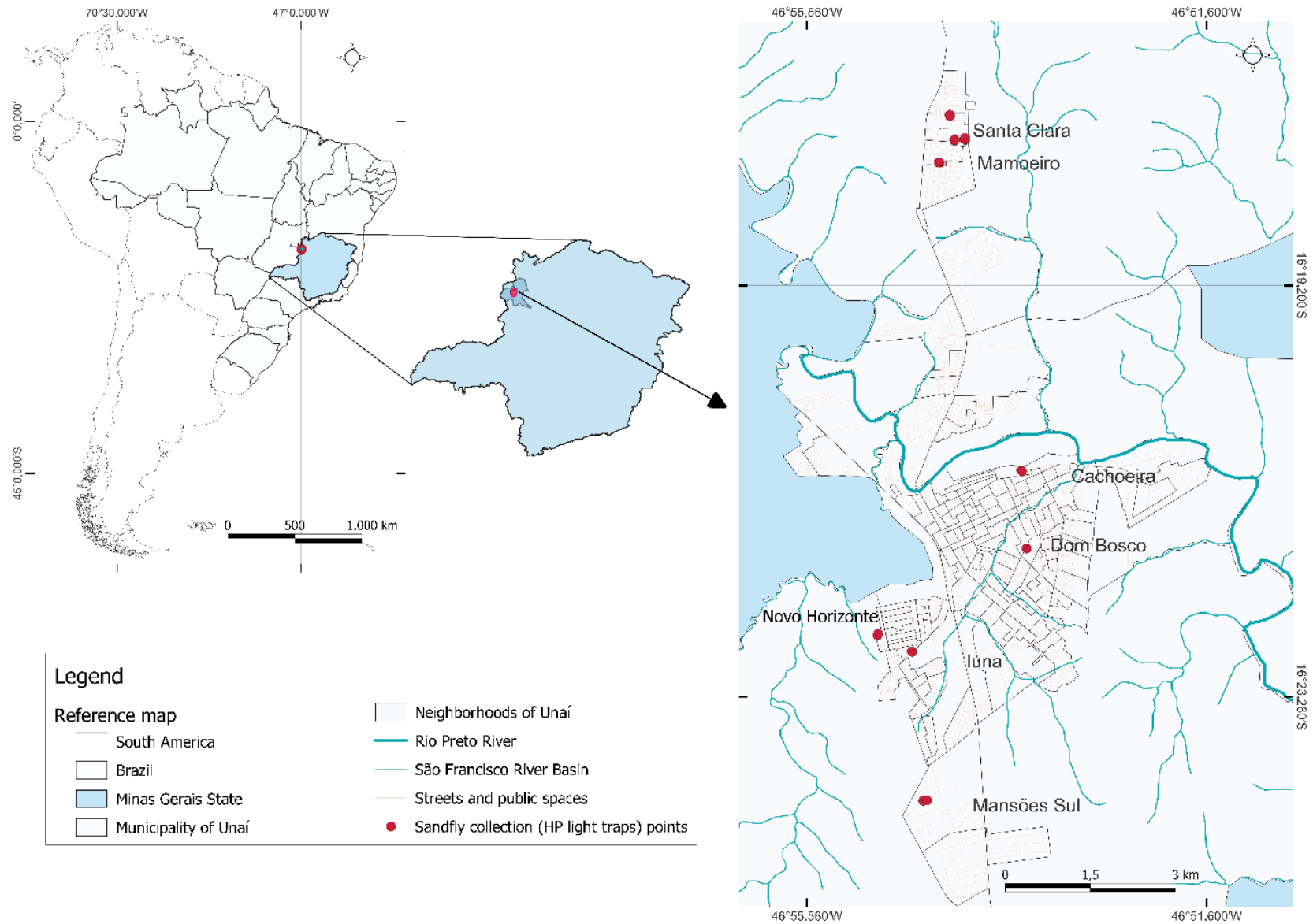


Figure 2. Spatial distribution and abundance intensity (Kernel density) of phlebotomine sand fly species captured in Unaí, Minas Gerais, Brazil.

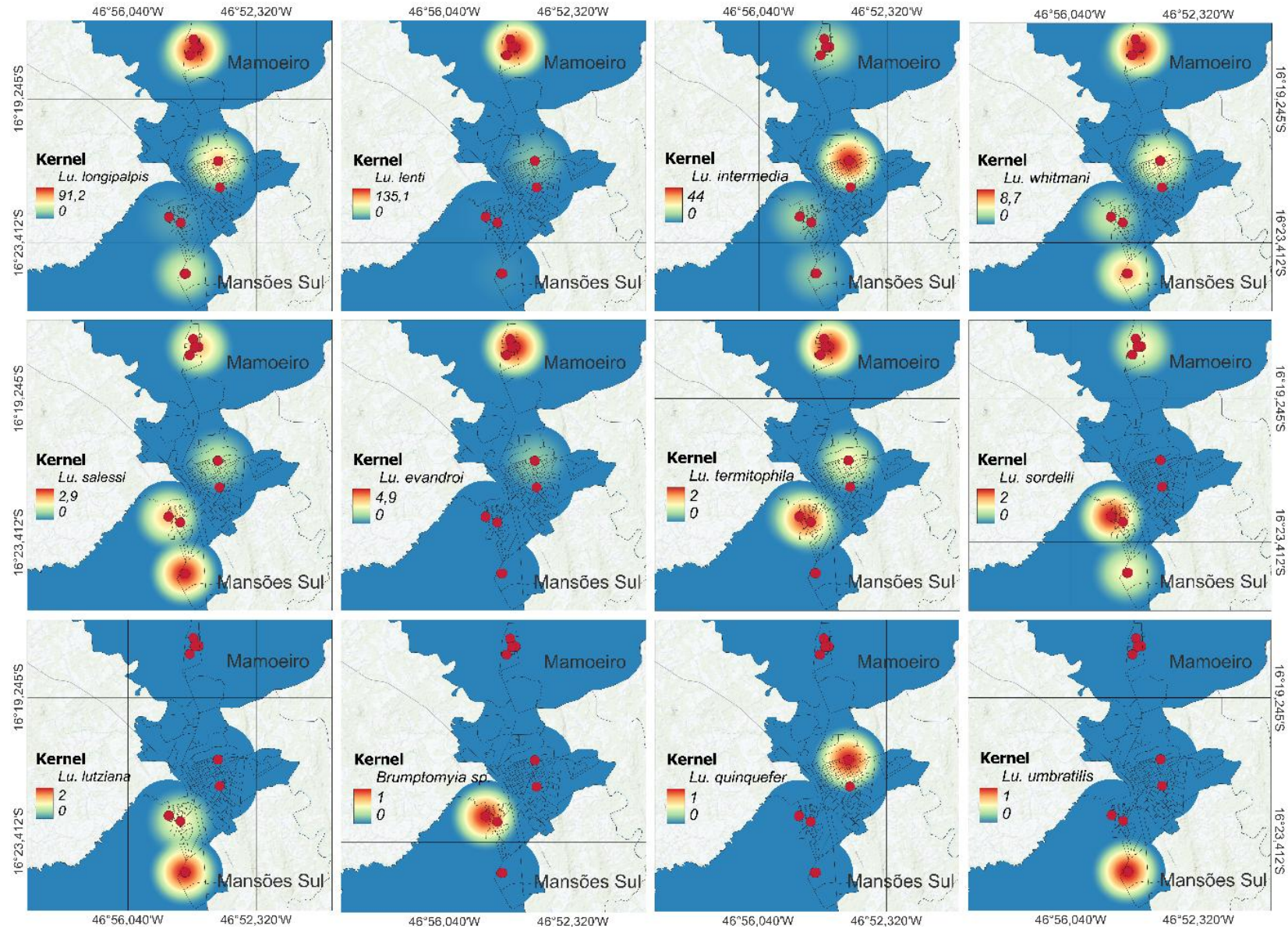


Table 1. Description of the peridomestic environment and presence of domesticated animals in sampled households across neighborhoods of Unaí, Minas Gerais, Brazil.

Neighborhoods	House	General characteristics	Domesticated animals (number)
Cachoeira	C6	Located in the northeastern periphery of the city. Extensive peridomestic area with medium to large trees, some of them fruit-bearing, abundant organic matter, and high humidity. Collection point closest to the Rio Preto River (approximately 665 meters away).	Dogs (1); Chickens (>15); Pigs (1)
Dom Bosco	C1	Located in a residential neighborhood near the city center. Two medium-sized fruit trees. Predominantly urbanized environment, with a smaller peridomestic area compared to the other sites.	Dogs (4); Cats (3)
Iuna	C10	Located in the southwestern periphery of the city. Chicken coop and presence of domestic animals.	Dogs (1); Chickens (<15); Cats (2)
Mamoeiro	C3	Located in the northern periphery of the city Peridomestic area adjacent to a well-preserved expanse of residual Cerrado, rich in organic matter, humidity, and with the presence of domestic animals and wild mammals. Presence of some fruit trees and household waste in the yard.	Dogs (3); Cats (2); Horses (3); Calves (1); Chickens (>15)
	C4	Located in the northern periphery of the city Extensive peridomestic area with medium to large trees, some of them fruit-bearing.	
	C7	Located in the northern periphery of the city Presence of a chicken coop and dogs, with a dirt yard rich in organic matter.	Dogs (2); Chickens (>15)
	C8	Located in the northern periphery of the city Chicken coop and presence of organic matter.	Dogs (2); Chickens (<15)
Mansões Sul	C9	Located in the southern periphery of the city. Largest peridomestic area compared to the other sites; medium and large trees, some of them fruit-bearing. Presence of a chicken coop.	Dogs (2); Chickens (>15)
Novo Horizonte	C2	Located in the southwestern periphery of the city. Peridomestic area bordered by native vegetation and small to medium-sized trees (some fruit-bearing), with abundant organic matter and humidity. Open sewage and stormwater drainage are located 30 meters away.	
Santa Clara	C5	Located in the northern periphery of the city Extensive peridomestic area with medium to large trees, some of them fruit-bearing. Abundant organic matter in the yard and small chicken coop.	Dogs (1); Chickens (<15)

Table 2. Sand fly species composition and abundance in seven neighborhoods in Unaí, Minas Gerais, Brazil.

Species	Neighborhoods and Sex																					Total	RA*	p-value**		
	Cachoeira			Dom Bosco			luna			Mamoeiro			Mansões Sul			Novo Horizonte			Santa Clara							
	M	F	Total	M	F	Total	M	F	Total	M	F	Total	M	F	Total	M	F	Total	M	F	Total	M	F	Total		
<i>Lutzomyia longipalpis</i>	48	9	57	0	1	1	3	0	3	77	18	95	33	4	37	4	2	6	0	1	1	165	35	200	39.7	0.01
<i>Lutzomyia lenti</i>	16	10	26	0	0	0	0	0	0	83	50	133	3	2	5	0	1	1	1	2	3	103	65	168	33.3	< 0.0001
<i>Lutzomyia intermedia</i>	36	8	44	0	0	0	0	0	0	8	7	15	7	2	9	1	8	9	0	0	0	52	25	77	15.3	< 0.0001
<i>Lutzomyia whitmani</i>	5	0	5	0	0	0	0	0	0	8	1	9	3	3	6	0	3	3	0	0	0	16	7	23	4.6	0.245
<i>Lutzomyia sallesi</i>	1	0	1	0	0	0	0	0	0	2	0	2	2	1	3	0	2	2	0	0	0	5	3	8	1.6	0.059
<i>Lutzomyia evandroi</i>	0	1	1	0	0	0	0	0	0	3	2	5	0	0	0	0	0	0	0	0	0	3	3	6	1.2	0.868
<i>Lutzomyia sp.</i>	0	1	1	0	0	0	0	0	0	0	4	4	0	0	0	0	0	0	0	0	0	0	5	5	1.0	0.938
<i>Lutzomyia termitophila</i>	1	0	1	0	0	0	0	1	1	0	2	2	0	0	0	1	0	1	0	0	0	2	3	5	1.0	< 0.01
<i>Lutzomyia sordellii</i>	0	0	0	0	0	0	0	0	0	0	1	1	1	0	1	1	1	2	0	0	0	2	2	4	0.8	< 0.01
<i>Lutzomyia lutziana</i>	0	0	0	0	0	0	0	1	1	0	0	0	0	2	2	0	0	0	0	0	0	0	3	3	0.6	< 0.001
Not identified	0	0	0	0	0	0	0	0	0	2	0	2	0	0	0	0	0	0	0	0	0	2	0	2	0.4	-
<i>Brumptomyia sp.</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	1	1	0.2	< 0.01
<i>Lutzomyia quinquefer</i>	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0.2	0.848
<i>Lutzomyia umbratilis</i>	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	1	0	1	0.2	0.333
Total	137			1			5			268			64			25			4			352	152	504		
Trap-nights	36			36			36			144			36			36			36			360				
Density (average no.) of sand flies	3.81			0.03			0.14			1.86			1.78			0.69			0.11			0.98				
Minimum	0			0			0			0			0			0			0			1				
Maximum	57			1			3			133			37			9			3			200				
Species richness	7			1			3			8			8			7			2			11				
Species diversity																										
Shannon-Wiener Index	1.31			0			0.95			1.16			1.40			1.63			0.56			1.43				
Simpson Index	0.69			0			0.70			0.61			0.64			0.80			0.50			0.70				

M: male; F: female

* Relative abundance

** Kruskal-Wallis test (Neighborhoods)

Table 3. Descriptive statistics of monthly climatic variables recorded between January and December 2024 in Unaí, Minas Gerais, Brazil

Climatic variables	Arithmetic mean	Standard deviation	Median	Minimum	Maximum	p-value*
Temperature average (°C)						
Collections days	27.0	3.4	26.2	22.6	31.8	0.851
Previous 7	27.2	3.6	26.4	24.4	35.0	
Previous 15	26.9	3.7	26.4	21.7	29.8	
Previous 30	27.3	3.8	26.3	21.7	35.7	
Humidity average (%)						
Collections days	60.4	12.2	63.5	42.0	76.0	0.979
Previous 7	63.1	13.7	66.0	56.5	86.0	
Previous 15	67.5	13.6	70.0	36.0	83.5	
Previous 30	64.7	14.2	67.3	35.0	95.0	
Wind speed average (m/s)						
Collections days	1.0	0.0	1.0	1.0	1.0	0.859
Previous 7	1.1	0.2	1.0	1.0	2.1	
Previous 15	1.0	0.3	1.0	1.0	1.4	
Previous 30	1.1	0.2	1.0	1.0	2.1	
Accumulated precipitation (mm³)						
Collections days	0.0	0.0	0.0	0.0	0.0	0.876
Previous 7	1.7	1.9	0.0	0.0	5.1	
Previous 15	2.2	4.5	0.0	0.0	14.8	
Previous 30	3.5	6.2	0.0	0.0	21.7	

*Anova (probability of significance for F value)

Table 4. Variation in phlebotomine abundance analyzed using Generalized Linear Mixed Models (binomial family; logit link), with neighborhoods and houses included as a random effect. Unaí, Minas Gerais, Brazil.

Variables	OR*	95% Confidence Interval	SE**	z	p-value	AOR***	95% Confidence Interval	p-value
<i>Lutzomyia longipalpis</i>								
Area (Intra reference)	2.03	(1.21 - 3.41)	0.53	2.68	0.01	2.01	(1.16 - 3.48)	0.01
Sex (Female reference)	3.00	(1.84 - 4.90)	0.08	4.38	< 0.0001	3.12	(1.87 - 5.21)	< 0.0001
Temperature average (°C)								
Collections days	1.07	(0.97 - 1.19)	0.05	1.43	0.15			
Previous 7	0.99	(0.84 - 1.16)	0.08	-0.17	0.87			
Previous 15	1.14	(0.90 - 1.44)	0.14	1.15	0.25	2.03	(1.44 - 2.88)	< 0.0001
Previous 30	0.74	(0.59 - 0.93)	0.09	-2.59	0.01	0.42	(0.28 - 0.61)	< 0.0001
Humidity average (%)								
Collections days	0.99	(0.97 - 1.01)	0.01	-1.40	0.16			
Previous 7	0.99	(0.97 - 1.01)	0.01	-0.82	0.42			
Previous 15	0.99	(0.96 - 1.01)	0.01	-0.95	0.34			
Previous 30	1.02	(0.99 - 1.05)	0.02	1.42	0.16	1.02	(0.97 - 1.06)	0.32
Wind speed average (km/h)								
Collections days	0.64	(0.41 - 0.98)	0.14	-2.04	0.04			
Previous 7	1.60	(1.00 - 2.57)	0.38	1.97	0.05	1.10	(0.64 - 1.89)	0.74
Previous 15	0.33	(0.16 - 0.70)	0.13	-2.90	0.01			
Previous 30	0.28	(0.11 - 0.69)	0.13	-2.78	0.01			
Accumulated precipitation (mm³)								
Collections days	1.00	(0.96 - 1.04)	0.02	-0.17	0.86			
Previous 7	1.04	(1.00 - 1.07)	0.02	2.00	0.05	0.60	(0.28 - 1.27)	0.18
Previous 15	1.04	(1.00 - 1.09)	0.03	1.64	0.10			
Previous 30	1.01	(0.96 - 1.07)	0.04	0.49	0.63			
<i>Lutzomyia lentii</i>								
Area (Peri reference)	3.71	(2.21 - 6.20)	0.97	5.00	< 0.0001	3.82	(2.19 - 6.67)	< 0.0001
Sex (Male reference)	1.87	(1.14 - 3.06)	0.47	2.47	0.01	1.76	(1.03 - 3.01)	0.04
Temperature average (°C)								
Collections days	0.86	(0.77 - 0.96)	0.05	-2.82	0.01			
Previous 7	0.99	(0.82 - 1.19)	0.09	-0.10	0.92			
Previous 15	0.78	(0.60 - 1.02)	0.11	-1.81	0.07	0.64	(0.46 - 0.90)	0.01
Previous 30	1.47	(1.16 - 1.87)	0.18	3.15	< 0.001	1.71	(1.24 - 2.36)	0.001
Humidity average (%)								
Collections days	1.02	(1.00 - 1.04)	0.01	1.73	0.08			
Previous 7	1.00	(0.97 - 1.03)	0.01	-0.04	0.97			
Previous 15	1.00	(0.97 - 1.03)	0.02	-0.27	0.79			
Previous 30	0.94	(0.91 - 0.97)	0.02	-3.69	< 0.0001	0.97	(0.93 - 1.02)	0.22
Wind speed average (m/s)								
Collections days	1.45	(0.91 - 2.31)	0.34	1.58	0.12	1.15	(0.60 - 2.23)	0.68
Previous 7	0.45	(0.27 - 0.74)	0.12	-3.09	< 0.001	0.85	(0.38 - 1.89)	0.69
Previous 15	4.08	(1.36 - 12.27)	2.29	2.50	0.01			
Previous 30	5.56	(1.46 - 21.19)	3.79	2.51	0.01			
Accumulated precipitation (mm³)								
Collections days	1.03	(0.98 - 1.08)	0.03	1.25	0.21			
Previous 7	0.93	(0.89 - 0.97)	0.02	-3.48	< 0.0001	0.98	(0.92 - 1.05)	0.51
Previous 15	0.93	(0.89 - 0.97)	0.03	-3.08	< 0.001			
Previous 30	0.93	(0.88 - 0.99)	0.03	-3.08	< 0.001			
<i>Lutzomyia intermedia</i>								
Area (Intra reference)	3.35	(1.45 - 7.78)	1.44	2.82	0.01	3.04	(1.30 - 7.13)	0.01
Sex (Female reference)	1.25	(0.70 - 2.23)	0.37	0.75	0.45	1.45	(0.79 - 2.65)	0.24
Temperature average (°C)								
Collections days	1.14	(1.00 - 1.30)	0.08	1.97	0.05	0.80	(0.57 - 1.12)	0.19
Previous 7	1.19	(0.97 - 1.46)	0.12	1.65	0.10			
Previous 15	1.26	(0.93 - 1.70)	0.20	1.47	0.14			
Previous 30	0.98	(0.73 - 1.33)	0.15	-0.10	0.92			
Humidity average (%)								
Collections days	0.99	(0.97 - 1.02)	0.01	-0.80	0.43			
Previous 7	1.00	(0.97 - 1.03)	0.02	0.05	0.96			
Previous 15	1.01	(0.97 - 1.05)	0.02	0.57	0.57			
Previous 30	1.05	(1.00 - 1.11)	0.03	1.94	0.05	1.08	(0.99 - 1.19)	0.09
Wind speed average (km/h)								
Collections days	1.53	(0.89 - 2.64)	0.43	1.54	0.13	0.84	(0.33 - 2.15)	0.72
Previous 7	1.39	(0.72 - 2.71)	0.47	0.97	0.33			
Previous 15	1.98	(0.43 - 3.33)	0.63	0.35	0.73			
Previous 30	1.02	(0.28 - 3.73)	0.67	0.04	0.97			
Accumulated precipitation (mm³)								
Collections days	0.95	(0.90 - 1.00)	0.03	-1.90	0.06	0.16	(0.03 - 0.80)	0.03
Previous 7	1.03	(0.99 - 1.08)	0.02	1.29	0.20	2.21	(0.49 - 9.92)	0.30
Previous 15	1.04	(0.98 - 1.11)	0.03	1.28	0.20			
Previous 30	1.08	(1.01 - 1.15)	0.04	2.32	0.02			

* OR: odds ratio; ** SE: Standard error; *** AOR: adjusted odds ratio

3. CONCLUSÕES

Os resultados apresentados no capítulo, elaborado no formato de artigo científico, revelam o primeiro levantamento da fauna de flebotomíneos em Unaí, Minas Gerais, evidenciando a predominância das espécies *Lutzomyia longipalpis*, *Lu. lenti* e *Lu. intermedia*. A análise das capturas indica que características ambientais peridomiciliares influenciam significativamente a abundância e a distribuição espacial dessas espécies, enquanto variáveis climáticas como temperatura e precipitação modulam suas populações de forma espécie-específica e dependente do tempo. A abundância de espécies vetoras em áreas com condições favoráveis à reprodução e em contato próximo com humanos e animais reforça o potencial risco de transmissão domiciliar das leishmanioses na região. Dessa forma, os achados contribuem com novos dados para o cenário epidemiológico local e reforçam a importância da vigilância entomológica integrada a medidas de manejo ambiental como estratégia de controle vetorial.

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