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Studies on Phlebotominae (Diptera: Psychodidae) in the Campus FIOCRUZ Mata Atlântica, Jacarepaguá, in the City of Rio de Janeiro, Brazil

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ABSTRACT

Introduction: The presence of American cutaneous leishmaniasis (ACL) in the communities of the Campus FIOCRUZ Mata Atlântica (CFMA) in the City of Rio de Janeiro initiated the investigation of the Phlebotominae fauna in the Atlantic Forest to determine the occurrence of putative ACL vectors associated with the enzootic cycle. **Methods:** For 24 consecutive months, sand flies were captured inside the forest and in the border area near the communities. **Results:** The following sand fly species were identified: *Brumptomyia brumpti*, *Brumptomyia cunhai*, *Brumptomyia nitzulescui*, *Lutzomyia edwardsi*, *Lutzomyia pelloni*, and *Lutzomyia quinquefer.* Other identified sand fly vectors, such as *Lutzomyia intermedia* (the predominant species), *Lutzomyia migonei*, *Lutzomyia whitmani*, *Lutzomyia fischeri*, and *Lutzomyia hirsuta hirsuta*, are associated with ACL transmission, and the vector for American visceral leishmaniases (AVL), *Lutzomyia longipalpis*, was also found. **Conclusions:** All sand fly vectors were found in both studied environments except for *Lutzomyia whitmani*, which was only identified in the forest. This study represents the first identification of *Lutzomyia longipalpis* in the CFMA, and the epidemiological implications are discussed.

Keywords: American Cutaneous Leishmaniasis. American Visceral Leishmaniasis. Rio de Janeiro City. *Lutzomyia longipalpis*. *Lutzomyia intermedia*.

INTRODUCTION

All the Brazilian states currently report human cases of American cutaneous leishmaniasis (ACL) in a significant number of municipalities. ACL is transmitted from a complex parasitosis that involves multiple parasites, vectors and vertebrate hosts in restricted ecologic niches[1].

In the State of Rio de Janeiro, the occurrence of ACL has been recorded since the beginning of the previous century. In the last few decades, ACL has been observed in epidemic outbreaks in different municipalities, including the economically developed City of Rio de Janeiro. In the capital of the state, the disease is particularly prevalent in the western areas[2] of the region around the Pedra Branca massif in the neighborhood of Jacarepaguá where the *Campus FIOCRUZ da Mata Atlântica* (*CFMA*) is located[3].

Approximately 55% of the CFMA consists of the Pedra Branca State Park, a preserved environmental area of the Atlantic Forest. Six communities comprise the remaining 45% of the CFMA: *Vianna do Castelo, Nossa Senhora dos Remédios, Faixa Azul, Fincão, Sampaio Correa*, and *Caminho da Cachoeira.* Cases of ACL from these communities historically represent approximately 8% of all human ACL cases recorded in Rio de Janeiro with evidence of intra- and peridomiciliary transmission[3].

The current study aims to improve the understanding of the eco-epidemiology of ACL at the CFMA by studying the Phlebotominae fauna, potential vectors in the forest environment associated with a zoonotic cycle, and the interface between ACL and the community of Caminho da Cachoeira.

METHODS

Study area

The CFMA is located in part of the former hospice Colonia Juliano Moreira in the neighborhood of Jacarepaguá in the City of Rio de Janeiro. The boundaries of the CFMA are defined

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by the following geographic points: Pedra Branca massif (to the east), Tijuca massif (to the west), the lagoons of Jacarepaguá, Camorim, and Tijuca (to the south), and the Serra do Valqueire range (to the north). The western region of the CFMA is an environmental conservation area with predominantly secondary Atlantic Forest vegetation, which corresponds to a dense shade-loving forest, and is located in the quadrant formed by the coordinates 22° 56' 26''S and 43° 28' 50'' W. The CFMA is home to wild animals such as toucans (Piciformes), opossums (Didelphimorphia), crocodiles *(Crocodylia*), and lizards (*Squamata*), especially in areas of preserved vegetation. Tamarins (Primates), sloths (Pilosa), and parrots (Psittaciformes) can also be found in this area.

Monitoring stations

Two monitoring stations (MS) were established: one station in the forest (MSf) 500 meters from the second station and other transition area (MSt) at the border with the Caminho da Cachoeira community[3]. The MSt was adjacent to the peridomiciliary area of the last house in the community, which contained four kennels and a chicken coop at a distance of approximately 9 meters (**Figures 1A** and **1B**).

Sandfly captures

Four HP luminous traps were used at each MS[4]. Four captures were made at each MS every week on consecutive nights for 24 consecutive months (from January 2009 to December 2010) between 5p.m. and 9 a.m. In the forest, the specimens were captured in an area with rocks, grottos, and some fruit trees. After the phlebotomines were caught, they were mounted individually and identified[5]. The meteorological data for the dates of collection were obtained from the National Institute of Meteorology[6]. The study was authorized by the State Institute of Forests/State Park of Pedra Branca (IEP/RJ/PR n°10/06).

Statistical analysis of the collected data

The index species abundance (ISA) and the standardized mean species abundance (SISA)[7] were calculated. The trend of occurrence of Phlebotominae vectors during the period of the study was determined by calculating the Williams' averages[8].

RESULTS

During the study, 3,249 specimens of Phlebotominae (male and female) representing thirteen species from the genera *Brumptomyia* and *Lutzomyia* were captured: *Brumptomyia brumpti*, *Brumptomyia nitzulescui*, *Brumptomyia cunhai*, *Brumptomyia* sp., *Lutzomyia intermedia*, *Lutzomyia migonei*, *Lutzomyia hirsuta hirsuta*, *Lutzomyia quinquefer*, *Lutzomyia fischeri*, *Lutzomyia edwardsi*, *Lutzomyia longipalpis*, *Lutzomyia pelloni*, and *Lutzomyia whitmani.* The specific identification of *Brumptomyia* sp. was not possible because only female specimens were captured.

The *Lutzomyia* genus represented 98% of the specimens. *L. intermedia*, *L. migonei*, *L. h. hirsuta*, *L. fischeri*, and *L. whitmani* were identified to be vectors of ACL, and *L. longipalpis* was identified to be a vector of AVL.

Combining data from both stations (MSf and MSt), *L. intermedia* was the most abundant species (SISA = 0.55) followed by *L. migonei* (SISA = 0.21) and *L. h. hirsuta* (SISA = 0.13).

Captures at MSt were more common and accounted for 93% of all the specimens captured. At this station, *L. intermedia* was the most abundant species (SISA = 0.86) followed by *L. migonei* (SISA = 0.27). Three other vectors were found less frequently: *L. h. hirsuta* (SISA = 0.05), *L. fischeri* (SISA = 0.04), and *L. longipalpis* (SISA = 0.01). Captures at MSf accounted for only 7% of all the captured specimens. Similar to MSt,

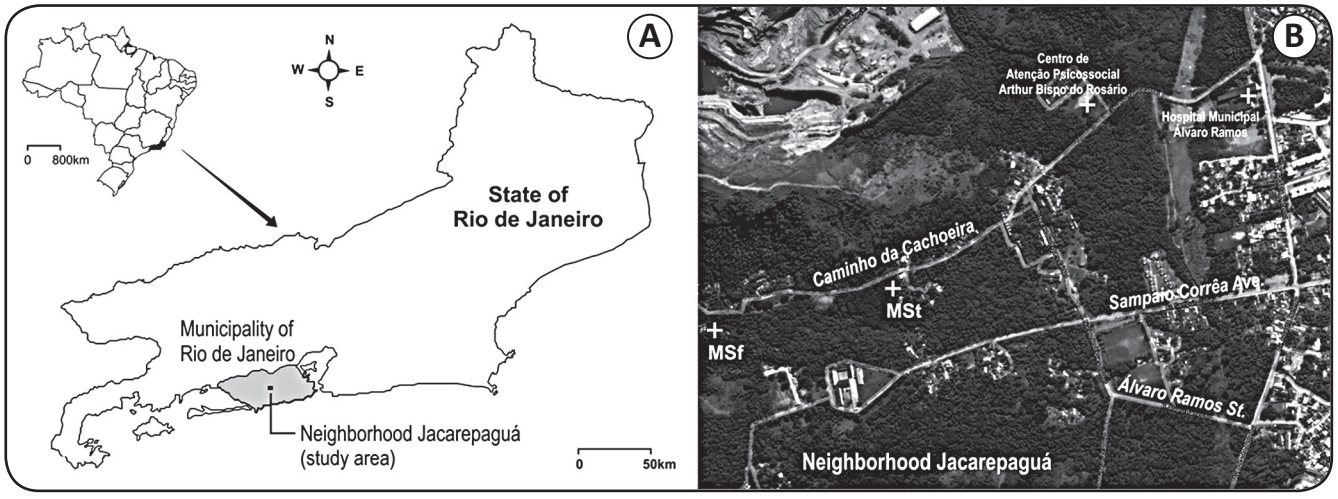

FIGURE 1 - A: Map of the Jacarepaguá neighborhood of Rio de Janeiro City, State of Rio de Janeiro. B: FIOCRUZ Atlantic Forest Campus and collection sites (MSt and MSf) in Caminho da Cachoeira. FIOCRUZ: Fundação Oswaldo Cruz; **MSt:** transition area monitoring station; **MSf:** forest monitoring station**.**

L. intermedia was the most abundant species (SISA = 0.23). The following vectors were also collected in this environment at low abundance: *L. migonei* (SISA = 0.10), *L. longipalpis* (SISA = 0.03), *L. fischeri* (SISA = 0.01), and *L. whitmani* (SISA = 0.01). **Table 1** shows detailed SISA data.

Seasonal fluctuations existed in vector species, and only *L. intermedia* was captured throughout the year. The Williams averages ($X_{W)}$ of *L. intermedia* was 168.9 with peak frequencies in February and September. *L. migonei* was not found in January and May, but it was the most frequent species in September even though its X_W (7.3) was lower than that of *L. intermedia* (**Figures 2A** and **2B**). *L. fischeri*, *L. h. hirsuta*, and *L. whitmani* were occasionally found in different months. *L. fischeri* was the most frequently captured species in September with an X_W of 0.6 (**Figure 2C)**, but *L. h. hirsuta* was the most frequently captured species in October (X_W = 1.8) (**Figure 2D**), and *L. whitmani* was the most frequently captured species in March, May and October (X_W = 0.2) (**Figure 2E**).

Lutzomyia longipalpis was found only in March, August, October, and November, its X_W was 0.4, and its frequency increased in November (**Figure 2F**).

The temperature and humidity remained constant throughout the two-year capture period. On the capture days, no significant variations were detected, and the average temperature was approximately 25°C. Air humidity remained above 80% on average. In December 2009 and April 2010, no captures were made due to intense rain (**Figure 3**).

DISCUSSION

In the epidemiological discussion of ACL transmission in southeastern Brazil, *L. intermedia* is one of the main vectors for human infection followed by *L. migonei*[9] [10] [11]. In the State of Rio de Janeiro, both species are currently associated with ACL transmission. *L. intermedia* is more frequently found in all areas with reported human cases[1] [3] [11] [12] [13] [14] [15] [16].

The first studies on ACL transmission at the CFMA showed the occurrence of *L. intermedia* and *L. migonei*, which are both proven vectors of *Leishmania* (*Vianna*) *braziliensis* in the community of Caminho da Cachoeira and are present in the domicile, intradomicile, and peridomicile[3] [17].

Previous studies have suggested that both the Phlebotominae *L. intermedia* and *L. migonei* are experiencing a process of domiciliation[1] [12] [18]. *L. intermedia* shows strong evidence of adaptation to domiciliary environments with intense anthropic action[1] [13] [19] [20]. In areas in which they occur in sympatry, *L. migonei* may also participate in the transmission cycle of ACL and may act more intensely in the maintenance of canine cutaneous leishmaniasis[11] [14] and maintain the enzootic cycle[21].

Regarding seasonality, previous studies in the Caminho da Cachoeira community (CFMA) reported a higher frequency of *L. intermedia* in warmer months (January and February)[3]. This result differs partly from the present study, in which the species was captured throughout the year but more frequently in the

TABLE 1 - Total species percentages and index of abundance of the species collected in the transition area and forest in the FIOCRUZ Atlantic Forest Campus, Jacarepaguá neighborhood, City of Rio de Janeiro, State of Rio de Janeiro, from January 2009 to December 2010.

	MSt					MSf			
Species	N	%	SISA	ranking	Species	N	%	SISA	ranking
Lutzomyia intermedia	2,701	89.76	0.86	1	*Lutzomyia intermedia*	124	51.67	0.23	1
Lutzomyia migonei	259	8.61	0.27	2	*Lutzomyia hirsuta hirsuta*	29	12.08	0.20	2
Lutzomyia quinquefer	9	0.30	0.10	3	*Brumptomyia brumpti*	26	10.83	0.13	3
Brumptomyia sp.	2	0.07	0.09	4	*Lutzomyia migonei*	10	4.17	0.10	4
Brumptomyia brumpti	8	0.27	0.07	5	*Brumptomyia* sp.	5	2.08	0.07	5
Lutzomyia hirsuta hirsuta	5	0.17	0.05	6	*Lutzomyia quinquefer*	4	1.67	0.04	6
Lutzomyia fischeri	16	0.53	0.04	7	*Brumptomyia nitzulescui*	22	9.17	0.03	7
Brumptomyia cunhai	3	0.10	0.03	8	*Lutzomyia edwardsi*	2	0.83	0.03	8
Lutzomyia pelloni	2	0.07	0.02	9	*Lutzomyia longipalpis*	14	5.83	0.03	9
Brumptomyia nitzulescui	1	0.03	0.01	10	*Lutzomyia pelloni*	1	0.42	0.01	10
Lutzomyia edwardsi	2	0.07	0.01	11	*Lutzomyia fischeri*	2	0.83	0.01	11
Lutzomyia longipalpis	1	0.03	0.01	12	*Lutzomyia whitmani*	1	0.42	0.01	12
Total	3,009	100.0			Total	240	100.0		

FIOCRUZ: *Fundação Oswaldo Cruz*; **MSt:** transition area monitoring station**; MSf:** forest monitoring station**; N:** absolute number**. SISA:** standardized mean species abundance**.**

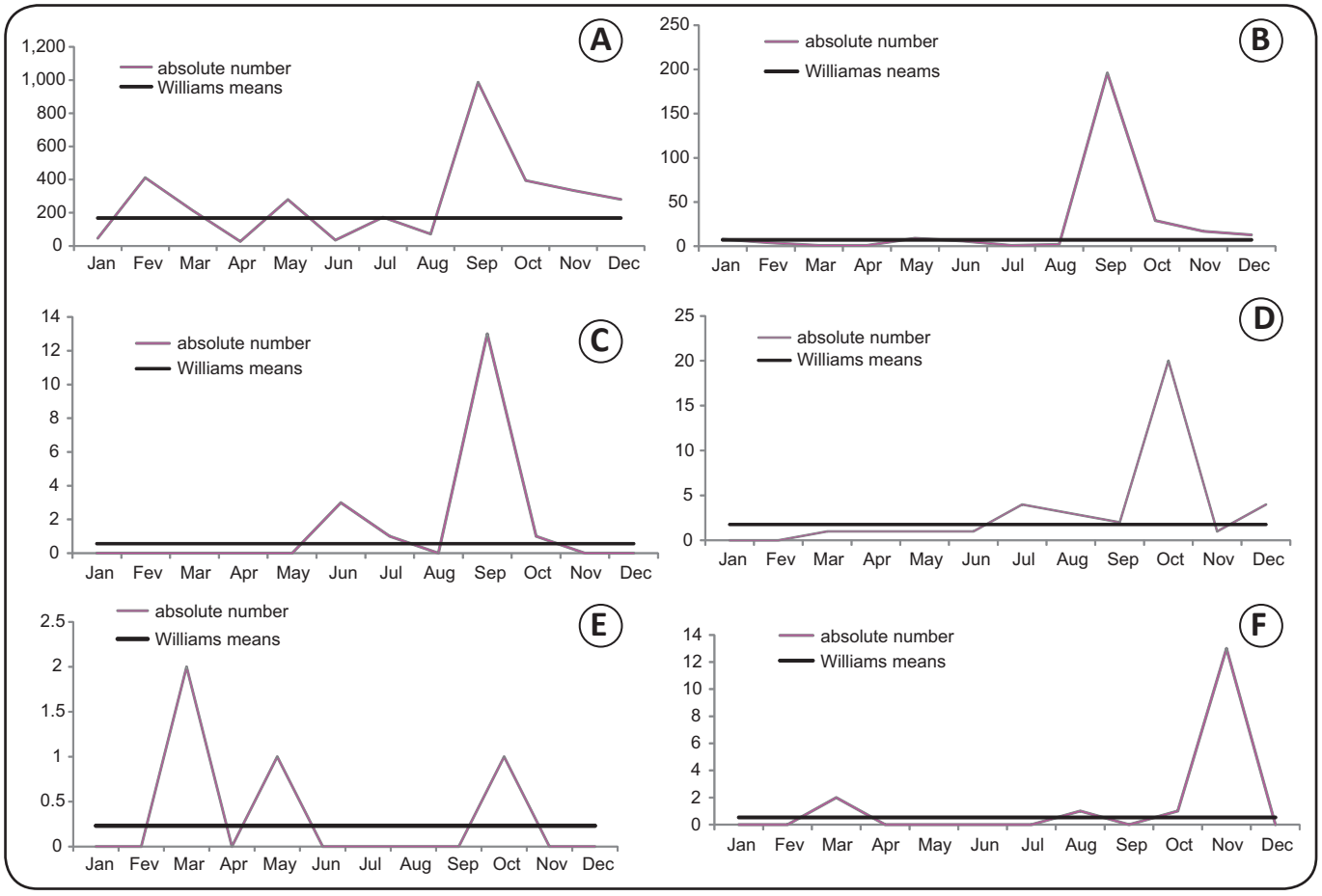

FIGURE 2 - Seasonal fluctuations and Williams averages of the main leishmaniasis vectors in the FIOCRUZ Atlantic Forest Campus, Jacarepaguá neighborhood, Rio de Janeiro City, State of Rio de Janeiro, from January 2009 to December 2010. A: *Lutzomyia intermedia*; **B:** *Lutzomyia migonei*; **C:** *Lutzomyia fischeri*; **D:** *Lutzomyia hirsuta hirsuta*; **E:** *Lutzomyia whitmani*; **F:** *Lutzomyia longipalpis.* **FIOCRUZ:** *Fundação Oswaldo Cruz*.

spring and summer months. *L. migonei*, *L. fischeri*, *L. h. hirsuta*, and *L. whitmani* were also found occasionally during this period. Previous studies at the CFMA showed that *L. migonei* was captured in the domiciliary environment in all months but less frequently in months with higher temperatures[3]. Previous studies in the area have reported divergent data that suggest that the monthly frequency of these Phlebotominae vectors might not be seasonal. Instead, the seasonal distribution of Phlebotominae vectors could be influenced by the characteristics of their environment. Studies in which the environment was managed to diminish contact with Phlebotominae vectors indicate that the environment can influence seasonal distributions of vectors[3].

The data from the current study reflect other scenarios previously discussed for *L. whitmani*, which has been suggested to be the most important ACL vector in Brazil but has been infrequently observed in Rio de Janeiro[9][10][21][22][23]. However, in Posse (a rural area in Petropolis, RJ), this species was found in the peridomicile and the forest in higher densities than it has been found in other areas of Rio de Janeiro. Therefore, we hypothesized that in the rural areas of the State of Rio de Janeiro, *L. whitmani* and *L. intermedia* may both transmit ACL[14][15]. However, *L. whitmani* is clearly not epidemiologically relevant in the CFMA.

For two other species of Phlebotominae recorded in this study, *L. h. hirsuta* and *L. fischeri*, although they are associated with ACL transmission cycles in other Brazilian states (the latter recorded with natural infection by *L.* (*V.*) *braziliensis*)[24], the current data do not show epidemiological significance for either in the CFMA[1].

The occurrence of *B. brumpti*, *B. nitzulescui*, and *B. cunhai* species, which have been previously found in other studies in Rio de Janeiro, corroborates the proximity of the Atlantic Forest[13] because these species occur mainly in forested areas.

Studies performed in Rio de Janeiro observed *L. intermedia* in different habitats and with an eclectic taste for different mammals, including wild animals. Therefore, the transmission cycles of ACL could be occurring in different habitats (domestic and sylvatic) and may be influencing the epidemiological characteristics of the disease[13][20].

The presence of *L. intermedia* and *L. migonei* in the forest and their association with the enzootic transmission cycle of

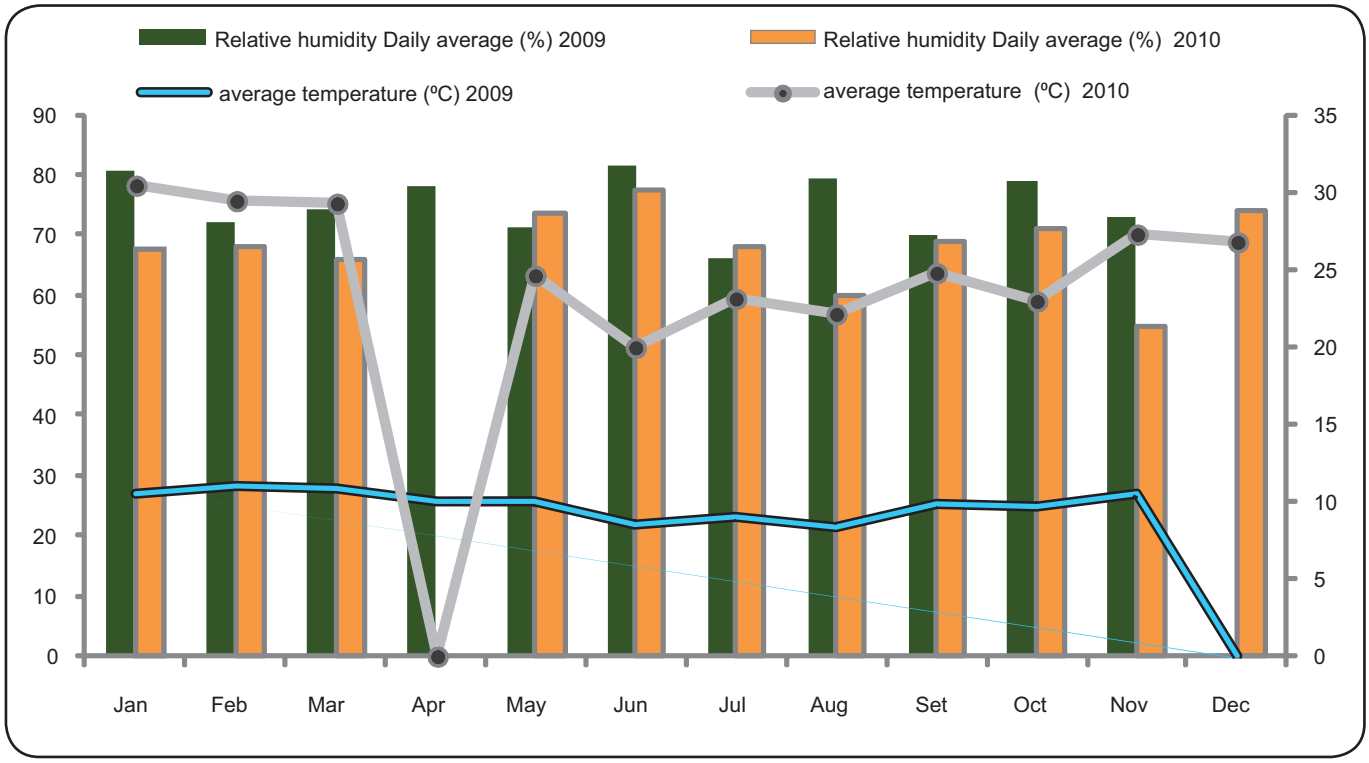

FIGURE 3 - Fluctuations in temperature and relative humidity on the collection days in the FIOCRUZ Atlantic Forest Campus, Jacarepaguá neighborhood, Rio de Janeiro City, State of Rio de Janeiro, from January 2009 to December 2010. In April 2009 and December 2010, no collections were performed due to heavy rain. **FIOCRUZ:** *Fundação Oswaldo Cruz***.**

L. (*V.*) *braziliensis* can be confirmed, similarly to the domiciliary environment. The presence of animal shelters (mainly chicken coops) in the peridomicile may attract Phlebotominae from the forest. Considering the presence of both species in the monitoring stations (forest and transition area), it can be assumed that they are gradually adapting to the human-modified environment. Previous studies in the same locality illustrated that *L. intermedia* and *L. migonei* showed great ability to adapt to anthropic environments because they were caught in association with humans and domestic animals. Thus, the presence of these two important ACL vectors and their high abundance within domestic and peridomestic environments may be related to animal shelters. This evidence, along with our observations, strongly suggests that transmission of ACL may be occurring in domiciliary areas[3].

As previously discussed, educating the general population on public health and environmental management are important components of the surveillance and prevention of ACL in the area under study[3].

Lutzomyia longipalpis was the main vector of AVL in Brazil when the first autochthonous human cases were recorded in Rio de Janeiro (1977). *L. longipalpis* was commonly found on the continental coast of the Pedra Branca massif in the Bangu and Realengo neighborhoods where it comprised approximately 97% of all captured Phlebotominae[25]. During the same time, the density of this species was low, and in some places it was not found at all[26].

Lutzomyia longipalpis has now been found in the CFMA (coastal slopes of Pedra Branca massif) for the first time; it was captured in the forest and at the border of the peridomicile of the residences. Previous studies performed since 2004 at the CFMA in the Caminho da Cachoeira community[3] have not reported this species in the areas under study (intradomicile, peridomicile, and in the border area with the forest).

The observation of *L. longipalpis* in the forest indicates that a wild population may have gradually adapted to the domiciliary environment. Other studies have mentioned finding this species in wild environments in the State of Rio de Janeiro[27] [28]. This sand fly species has adapted to different habitats and is widely distributed in the Americas in different ecotypes from Argentina to Mexico[5]. Based on the current evidence, *L. longipalpis* is considered to be a cryptic species, which could explain its ability to survive in different environments suffering from reproductive isolation[16] [28] [29] [30] [31] [32] [33] [34] [35] [36] [37] [38] [39] [40].

The altitude of the Pedra Branca massif may act as a geographical barrier between the continental and coastal sites and may interfere with the population dynamics of the vector. There are, however, two records of human cases of AVL at the Colônia Juliano Moreira in the 1980s[27].

According to the classification of the Manual of Vigilance and Control of AVL, by the Ministry of Health, the CFMA may be considered a receptive vulnerable area for the transmission of AVL. The main risk factor is the occurrence of the vector

following environmental patterns observed in other AVL transmission areas.

In the face of this evidence, it is important that the CFMA is monitored with the particular purpose of following the expansion of *L. longipalpis* in the direction of residential areas; we would also like to note the importance of keeping dogs under surveillance to evaluate canine enzootic transmission.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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