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Ecology of *Lutzomyia longipalpis* and *Lutzomyia migonei* in an endemic area for visceral leishmaniasis

Ecologia de *Lutzomyia longipalpis* e *Lutzomyia migonei* em uma área endêmica para Leishmaniose Visceral

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Abstract

The main vector for visceral leishmaniasis (VL) in Brazil is *Lutzomyia longipalpis*. However, the absence of *L. longipalpis* in a region of autochthonous VL demonstrates the participation of other species in the transmission of the parasite. Studies conducted in La Banda, Argentina, and São Vicente Férrer, Pernambuco State, Brazil, have correlated the absence of *L. longipalpis* and the presence of *L. migonei* with autochthonous cases of VL. In São Vicente Férrer, Pernambuco, there was evidence for the natural infection of *L. migonei* with *Leishmania infantum chagasi.* Thus, the objective of this work was to assess the ecology of the sand flies *L. longipalpis* and *L. migonei* in Fortaleza, an endemic area for VL. Insect capture was conducted at 22 sampling points distributed across four regions of Fortaleza. In total, 32,403 sand flies were captured; of these, 18,166 (56%) were identified as *L. longipalpis* and 14,237 (44%) as *L. migonei*. There were significant density differences found between the vectors at each sampling site (indoors and outdoors) (p <0.0001). These findings confirm that *L. migonei* and *L. longipalpis* are distributed throughout Fortaleza, where they have adapted to an indoor environment, and suggest that *L. migonei* may share the role as a vector with *L. longipalpis* in the transmission of VL in Fortaleza.

Keywords: *Lutzomyia longipalpis*, *Lutzomyia migonei*, visceral leishmaniasis, Fortaleza.

Resumo

O principal vetor de leishmaniose visceral (LV) no Brasil é *Lutzomyia longipalpis*. Entretanto, a ausência de *L. longipalpis* em área com casos autóctones de LV demonstra a existência de outras espécies na transmissão dessa doença. Estudo realizado na cidade de La Banda, Argentina, e São Vicente Férrer, Brasil, correlacionou a ausência de *L. longipalpis* e a presença de *Lutzomyia migonei* com casos autóctones de LV. Em São Vicente Férrer, foi comprovada a infecção natural de *L. migonei* por *Leishmania infantum chagasi.* Dessa forma, o objetivo deste trabalho foi avaliar a ecologia dos flebotomíneos *L. longipalpis* e *L. migonei* no município de Fortaleza, área endêmica para LV. A captura de flebotomíneos foi realizada em 22 pontos de coleta distribuídos nas quatro regiões do município de Fortaleza. No total, foram capturados 32.403 flebotomíneos. Destes, 18.166 (56%) eram da espécie *L. longipalpis* e 14.237 (44%) eram *L. migonei*. Houve diferença significativa de densidade entre os vetores em cada local de captura (intra e peri) (p<0,0001). Esses achados confirmam que, na cidade de Fortaleza, *L. migonei* e *L. longipalpis* estão bem distribuídos, bem como adaptados ao ambiente intradomiciliar e que *L. migonei*, possivelmente, compartilhe com *L. longipalpis* o papel de vetor da LV em Fortaleza.

Palavras-chave: *Lutzomyia longipalpis*, *Lutzomyia migonei*, leishmaniose visceral, Fortaleza.

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Introduction

Brazil accounts for approximately 90% of the reported cases of visceral leishmaniasis (VL) on the South American continent. The northeastern area of Brazil is considered most representative, with 48% of the cases (ZERPA et al., 2003; BERN et al., 2008; BRASIL, 2009). In the northeast, the state of Ceará is endemic for VL and is considered a priority for health surveillance and control by the Ministry of Health. The cities with the highest incidence of VL are Fortaleza, Sobral, Juazeiro, Barbalha and Caucaia. However, Fortaleza is the only city classified as an area of intense transmission, as 218 and 262 cases were reported in 2009 and 2010, respectively. Based on studies of vector competence and capacity, the main vector for VL in Brazil is *Lutzomyia longipalpis* (DEANE, 1956; LAINSON; RANGEL, 2005). However, studies conducted in Corumbá, State of Mato Grosso do Sul, in the Brazilian Midwest found *Lutzomyia cruzi* as the most prevalent species, both indoors and outdoors (GALATI et al., 1997). The natural infection of *L. cruzi* with *Leishmania infantum* (syn *Leishmania chagasi*) has also been documented in Corumbá (SANTOS et al., 1998). Although *L. cruzi* has become an accepted vector for VL, its range is restricted to Mato Grosso (RIBEIRO et al., 2007), Mato Grosso do Sul and Goiás (GALATI et al., 1997; GALATI, 2003).

More recently, studies from La Banda, Argentina, have correlated the absence of *L. longipalpis* and the presence of *L. migonei* with autochthonous VL cases (SALOMÓN et al., 2010). A similar pattern has been demonstrated in San Vicente Férrer, State of Pernambuco, in northeastern Brazil. In this study, the natural infection of *L. migonei* with *L. (L.) infantum chagasi* was documented, highlighting the potential role of this vector for parasite transmission in these areas (CARVALHO et al., 2010). The objective of this study was to gain insight into the transmission of *L. infantum* in Fortaleza by studying the ecology of *L. longipalpis* and *L. migonei* through the evaluation of entomological indicators of VL and discussing whether these sympatric species may share the role as vectors for *L. infantum* in urban areas.

Materials and Methods

Study area

The study was conducted in Fortaleza, the capital of Ceará State, which is located along the Atlantic coast at an average elevation of 21 m, covers an area of 313.8 km² and has 2,505,552 inhabitants. Fortaleza has the highest population density, at 8,001 inhabitants per km². The average annual temperature is 26°C. December and January are the warmest months, and July is the coolest, though the temperature differences between months are minor. The average rainfall is 1,600 mm and is concentrated between February and May. The wettest month is April (348mm), and the driest is November (13mm). The vegetation is typical of coastal areas and consists of mangroves and *restingas*. The remaining vegetation in the city is variable but includes many fruit trees (IBGE, 2008).

Capture of sand flies

Phlebotomine sand flies were captured from February of 2009 to January of 2010. The captures were performed in the following 22 districts within Fortaleza: the northern districts of Quitino Cunha (QC), Alvaro Weyne (AW), Farias Brito (FB), Aldeota (AD) and Jardim America (JA); the southern districts of Mondumbim (MD), Passaré (PA), Cajazeiras (CA), Planalto Airton Sena (PAS), Prefeito José Walter (PJW), Conjunto Palmeira (CP) and Paupina (PU); the eastern districts of Vicente Pizon (VP), Jardim das Oliveiras (JD), Edson Queiroz I (EDI), Edson Queiroz II (EDII), Cambeba (CB) and Lagoa Redonda (LR) and the western districts of Conjunto Ceara II (CCII), Democrito Rocha (DR), Siqueira (SQ) and Vila Manuel Sátiro (VMS) (Figure 1).

The districts were chosen according to the reported number of VL cases in the past 5 years and in accordance with features suggestive of vector presence, such as fruit trees, livestock and organic matter accumulation, as evaluated by environmental characterization forms. The captures were performed over 12 months using CDC light traps (SUDIA; CHAMBERLAIN, 1962) that were armed for four consecutive nights per month from 6 pm to 6 am. One collection point was chosen in each district. The traps were placed near animal shelters when possible, and two were placed at each residence, with one trap inside and another outside of the home (BRASIL, 2006). A total of 44 traps were used, with a minimum distance between sampling points (districts) of 1 km.

Identification of phlebotomine sand flies

The collected sandflies were sent to the Entomology Laboratory of the Health Secretariat of Ceará State for identification. The specimens were killed with ethyl acetate and placed in small Petri dishes in a solution of 10% potassium hydroxide for 2 hours. The sand flies were then submerged in acetic acid for 15 minutes and in lactophenol for 24 hours. The specimens were mounted onto slides with coverslips using Berlese fluid (VILELA et al., 2003). Males and females were classified using the key of Young and Duncan (1994).

Data from human and canine visceral leishmaniasis

Data relating to human and canine cases from February 2009 to January 2010 were obtained from the Secretary of Health and the Zoonosis Control Center of Fortaleza, respectively.

Statistical analysis

The index of home infestation (IID) and the relative abundance (RA) were calculated using the following formulas (BRASIL, 2006):

IID = Total households by positive sort/search site/technical × 100 ÷ Number of local searches;

RA = Number of *L. longipalpis* or *L. migonei* collected by household (outdoors or indoors) ÷ Total number of households surveyed (outdoors or indoors).

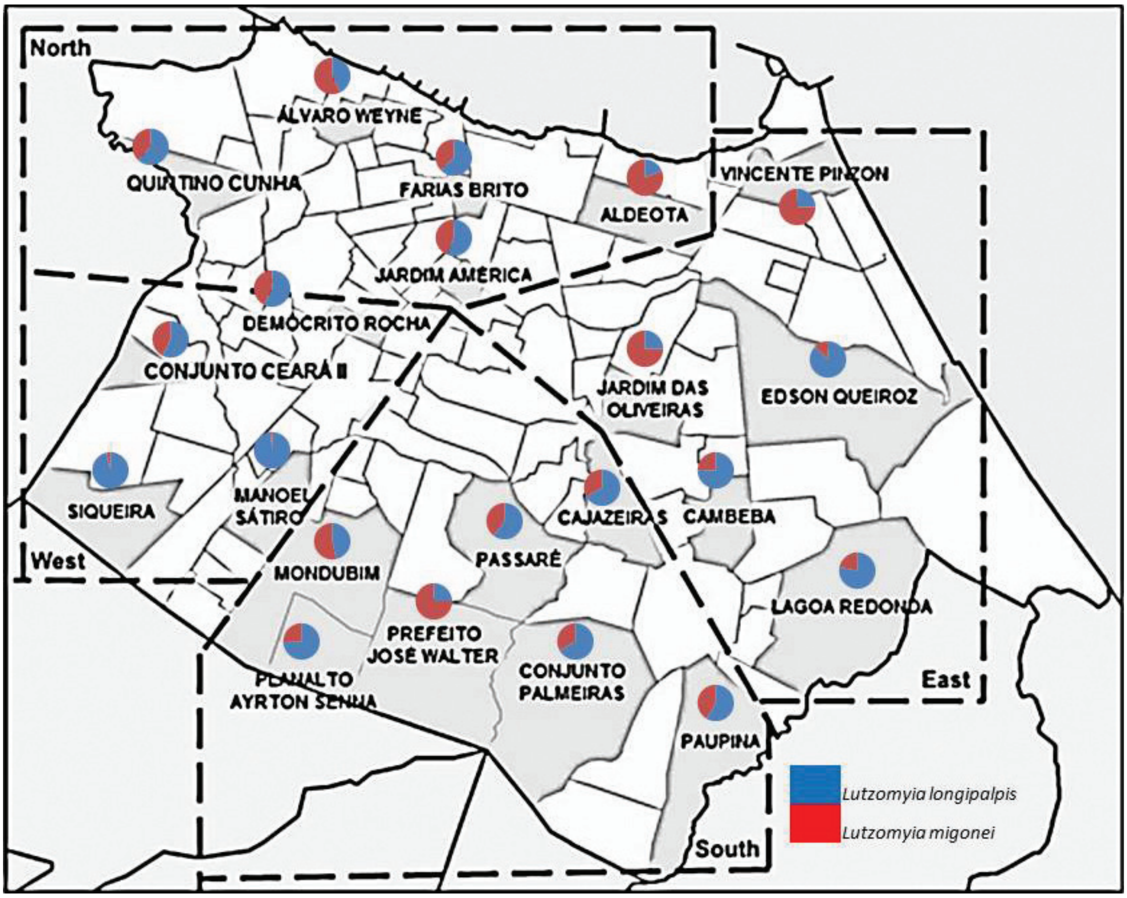

Figure 1. Distribution of *Lutzomyia longipalpis* and *Lutzomyia migonei* by collection point (neighborhood) in Fortaleza, Ceará, Brazil.

The analysis of the number of sand flies in relation to the local site characteristics (indoor and outdoor) and species (*L. longipalpis* and *L. migonei*) was performed using a generalized mixed model with Poisson distribution and a logarithmic link function, considering the residence as a random factor (DEMIDENKO, 2004). As the interactive effect between the two factors (location and species), a significant adjustment of the models with a single factor for each level of the other was performed. The significance level was 5%.

Relationships between the human or canine cases and sand fly density were determined using a Pearson correlation coefficient.

Results

From February 2009 to January 2010, 32,403 sand flies were captured. Of these, 18,166 (56%) were identified as *L. longipalpis* and 14,237 (44%) as *L. migonei* (Table 1). The presence of both species was confirmed in each neighborhood (Figure 1), though the sand fly density was heterogeneous (Table 1), ranging from 112.23 insects per trap in Farias Brito (FB) to 0.04 in Vicente Pizon (VP). The environmental characterization of each collection point is shown in Table 2. All sampling sites showed abundant vegetation, composed mainly of fruit trees. Regarding the presence of domestic animals, dogs and birds were commonly found in collection points. The index of home infestation in Fortaleza was 90.90% indoors and 95.45% outdoors for both species.

In Fortaleza, the relative abundance (RA) of *L. longipalpis* flies per household was 510 indoors and 315.72 outdoors. For *L. migonei*, the RA was 306.04 indoors and 342.90 outdoors.

Significant differences were observed between the two species, *L. migonei* and *L. longipalpis*, in each location (indoor and outdoor) ($p < 0.0001$) and between the two sites for each species, $p = 0.0012$ and < 0.0001, respectively (Figure 2).

There was no correlation between the number of human and canine VL cases and the sand fly density (*L. longipalpis* and *L. migonei*) (Tables 3 and 4).

Lutzomyia longipalpis

In this study, 18,166 specimens of *L. longipalpis* were captured; 11,220 (61.76%) were caught indoors, and 6,946 (38.24%) were caught outdoors (Table 1).

Significant differences in the density of *L. longipalpis* between sites and indoors and outdoors were observed, $p < 0.0001$ (Figure 2).

The district with the highest density of *L. longipalpis* was Farias Brito (FB), located in the northern region: the densities were 5.189 (46.25%) indoors and 1.575 (22.67%) outdoors. Lower densities were found in the district of Vicente Pizon, located in the eastern region, with values of 0 (0%) indoors and 1 (0.01%) outdoors (Table 1. The predominant sex was male, and the male/female ratio was 1.85.

Table 1. Number of *Lutzomyia longipalpis* and *Lutzomyia migonei* flies collected per site (indoor and outdoor), region (North, South, East and West) and sex (male or female) in Fortaleza, Ceará, Brazil.

Region	Collection site	*Lutzomyia longipalpis*								*Lutzomyia migonei*							
		Indoor				Outdoor				Indoor				Outdoor			
		M	F	Total	%	M	F	Total	%	M	F	Total	%	M	F	Total	%
North	QC	114	44	158	1.41	145	73	218	3.14	42	33	75	1.11	79	78	157	2.09
	AW	58	27	85	0.76	46	24	70	1.01	62	50	112	1.66	47	50	97	1.29
	FB	3293	1896	5189	46.25	967	608	1575	22.67	1525	1275	2800	41.62	663	548	1211	16.13
	AD	123	95	218	1.94	64	36	100	1.44	560	479	1039	15.45	178	138	316	4.21
	JA	159	93	252	2.25	396	164	560	8.06	57	68	125	1.86	300	218	518	6.90
South	MD	2057	1242	3299	29.40	340	181	521	7.50	808	710	1518	22.57	1391	1505	2896	38.56
	PA	97	91	188	1.68	87	54	141	2.03	48	31	79	1.17	68	68	136	1.81
	CA	324	116	440	3.92	422	140	562	8.09	172	105	277	4.12	148	74	222	2.96
	PAS	68	38	106	0.94	32	19	51	0.73	14	16	30	0.45	15	9	24	0.32
	PJW	36	33	69	0.61	221	114	335	4.82	100	96	196	2.91	725	335	1060	14.11
	CP	90	34	124	1.11	242	79	321	4.62	46	28	74	1.10	104	43	147	1.96
	PU	66	31	97	0.86	76	31	107	1.54	36	22	58	0.86	49	39	88	1.17
East	VP	0	0	0	0.0	1	0	1	0.01	0	0	0	0.0	2	1	3	0.04
	JO	83	58	141	1.26	67	41	108	1.55	3	1	4	0.06	1	0	1	0.01
	EQI	4	0	4	0.04	3	4	7	0.10	2	8	10	0.15	2	5	7	0.09
	EQII	41	38	79	0.70	54	58	112	1.61	11	4	15	0.22	2	11	13	0.17
	CB	44	21	65	0.58	46	9	55	0.79	14	3	17	0.25	16	6	22	0.29
	LR	82	49	131	1.17	990	441	1431	20.60	39	26	65	0.97	271	104	375	4.99
West	CCII	77	23	100	0.89	171	83	254	3.66	53	65	118	1.75	86	61	147	1.96
	DR	145	60	205	1.83	134	51	185	2.66	67	44	111	1.65	36	25	61	0.81
	SQ	67	32	99	0.88	73	44	117	1.68	2	1	3	0.04	2	3	5	0.07
	VMS	106	65	171	1.52	79	36	115	1.66	1	0	1	0.01	1	3	4	0.05
Total		7134	4086	11220	100	4656	2290	6946	100	3662	3065	6727	100	4186	3324	7510	100
					18166								14237				

Table 2. Environmental characteristics of sites, in Fortaleza, Ceará, Brazil.

Collection site	DOMESTIC ANIMALS							VEGETATION		
	Canine	Feline	Porcine	Veal	Equine	Bird	Others	Vegetable garden	Garden	Fruit trees
Aldeota		X					Tamarin		X	X
Álv. Weyne	X					X				X
Cajazeiras	X					X				X
Cambeba	X			X		X				X
Conj. Ceará II						X				X
Conj. Palmeira	X			X		X				X
Demócrito Rocha	X									X
Ed Queiroz I	X							X		X
Ed Queiroz II	X				X	X				X
Farias Brito	X							X	X	X
Jardim América						X				X
Jardim das Oliveiras	X		X		X	X			X	X
Lagoa Redonda	X					X			X	X
Modumbim	X					X		X		X
Palpina	X		X			X				X
Passaré	X					X	Fox		X	X
Planalto Airton Senna							Rabbit	X		X
Quitino Cunha	X		X			X				X
Siqueira	X		X			X				X
Vicente Pizon						X				X
Vila Man Sátiro	X									X
José Walter	X					X				X

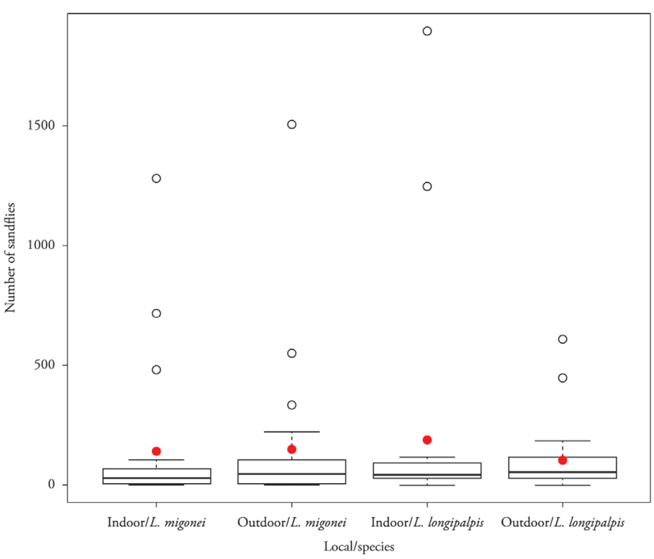

Figure 2. Box-plot of the number of female sandflies by local (indoor and outdoor) and species (*Lutzomyia longipalpis* and *Lutzomyia migonei*). Fortaleza, Ceará, Brazil.

Lutzomyia migonei

A total of 14,237 specimens of *L. migonei* were captured; 6,727 (47.25%) of these were caught indoors and 7,510 (52.75%) outdoors (Table 1). These values were significantly different (p = 0.0012). Similar to *L. longipalpis*, the density of *L. migonei* was the highest in FB, as 2,800 (41.62%) samples were collected indoors and 1,211 (16.13%) were collected outdoors. The district of VP had the lowest neighborhood density of 0 (0%) indoors and 3 outdoors (0.04%). Male flies were predominant, but the male/female ratio was slightly lower, at 1.23, than it was for *L. longipalpis*. There was no correlation between the number of cases and vector density in this region (Tables 3 and 4).

Discussion

The urbanization of visceral leishmaniasis has been extensively studied and is related to environmental changes caused by human action, the intense process of rural-urban migration and the interaction between susceptible individuals (LAINSON, 1989; SILVA et al. 1997; DIAS et al. 2003; TAUIL, 2006; MAIA-ELKHOURY et al., 2008). However, an important factor determining the expansion and urbanization of VL is the enormous adaptability of the main vector, *L. longipalpis*, to a changing environment (RANGEL; VILELA, 2008; RANGEL; LAINSON, 2009).

This present study, conducted in Fortaleza, documented the presence of these species at each of the 22 sites examined, which supports the wide distribution of these vectors. The species appear to be fully adapted to the urban areas of Fortaleza due to the negligible differences in density between the studied area. This adaptation is of concern due to the increased interactions between the vector and susceptible individuals, who could make it difficult to prevent and control disease. This study also demonstrated a

Table 3. Correlation among canine and human cases, sex of *Lutzomyia longipalpis* and *Lutzomyia longipalpis* specimens collected indoors and outdoors.

		Human cases	male	female	Indoor	Outdoor	Total
Canine cases	Pearsons´ Correlation	0.162	0.078	0.099	0.139	–0.095	0.086
	p	0.472	0.73	0.662	0.537	0.673	0.704
	N	22	22	22	22	22	22
Human cases	Pearsons´ Correlation		–0.056	–0.072	–0.144	0.199	–0.062
	p		0.804	0.751	0.522	0.374	0.784
	N		22	22	22	22	22

Values of $p \leq 0.05$ indicate significant correlation.

Table 4. Correlation among canine and human cases, sex of *Lutzomyia migonei* and *Lutzomyia migonei* specimens collected indoors and outdoors.

		Human cases	Male	Female	Indoor	Outdoor	Total
Canine cases	Pearsons´ Correlation	0.162	0.104	0.18	0.047	0.212	0.141
	P	0.472	0.644	0.424	0.834	0.344	0.532
	N	22	22	22	22	22	22
Human cases	Pearsons´ Correlation		–0.036	–0.029	–0.147	0.088	–0.033
	P		0.872	0.898	0.515	0.697	0.884
	N		22	22	22	22	22

Values of $p \leq 0.05$ indicate significant correlation.

high vector density (56%), as there were significant differences between *L. longipalpis* and *L. migonei* densities (p<0.0001). These results corroborate those of Oliveira et al. (2006, 2008), who found *L. longipalpis* to be the most prevalent species (92.2%) in Campo Grande city, Mato Grosso do Sul State, and to have a high density and degree of adaptation in this city.

With regard to the socio-environmental factors that may contribute to the emergence of sand flies, the work by Fernández et al. (2010) in the city of Posadas in the Misiones province of Argentina found that factors related to the routine capture of sand flies, such as the presence or absence of electric light, may influence vector sample density. We observed large differences in the density of each vector species among collection points with similar environmental characteristics, such as vegetation and the presence of domesticated animals. The largest number of specimens was collected in the northern area of FB and the southern area of MD. These large densities may be due to the size of the property and/or the diversity of plant and animal species because these collection points had the largest areas and the greatest plant and animal diversity. Thus, further analyses of the local environmental characteristics must be included in future studies that use local capture. The smallest number of collected specimens of both species occurred in the VP district, which is justified by its location near the beach, in the presence of strong winds.

Lutzomyia migonei is a species found mainly in the wild or in outdoor environments and is mainly associated with the transmission of cutaneous leishmaniasis, as this species is endowed with a remarkable degree of anthropophilicity (QUEIROZ et al. 1994; PITA-PEREIRA et al., 2005; RANGEL; LAINSON, 2009). However, recent studies have found that *L. migonei* participates in the transmission of VL. Indeed, *L. migonei* possesses the three essential characteristics of vector competence, including anthropophily, distribution coincident with human cases and evidence of natural infection with *L. (L.) infantum chagasi* (KILLICK-KENDRICK, 1990). Studies in La Banda, Argentina, have shown *L. migonei* to be the predominant species (93%) in areas with indigenous cases of VL and an absence of *L. longipalpis* and *L. cruzi*, the last of which is another potential vector of VL in Central Brazil (SALOMÓN et al., 2010). An enzootic cycle of VL and accidental transmission to humans was suggested for this sand fly vector (SALOMÓN et al., 2010). There are similar reports from San Vicente Ferrer in the State of Pernambuco, Brazil, and the natural infection of this species by *L. (L.) infantum chagasi* has been documented (CARVALHO et al., 2007, 2010).

In the epidemiological scenario of VL in Brazil, the city of Fortaleza has been highlighted by urban transmission, with high numbers of human and canine cases of the disease. The simultaneous occurrence of *L. migonei* and *L. longipalpis* in the local transmission of VL in this city, a fact already detected by the actions of entomological State Department of Health, is also worth mentioning.

The results of this study suggest changes in the urban behavior of *L. migonei*; this vector was found at a high density (44%) in all collection points but specifically with a high level of infestation both indoors (90.90%) and outdoors (95.45%), suggestive of an adaptation to the environment in and around residences. In previous studies, it was observed that *L. migonei* had a wild behavior, as it was found in abundance in forests, usually in areas with abundant vegetation (RANGEL; LAINSON, 2003). Based on the analysis of entomological indicators (index of relative abundance and level of home infestation), there were no differences in density between the species, as both were found at high densities indoors and outdoors. This suggests that these two species share the role of vector in the transmission of VL. Due to the scant difference in the densities of these species between the regions studied, they are assumed to be fully adapted to the urban area of Fortaleza. Considering the high prevalence of canines in the city of Fortaleza, this adaptation process is of concern with regard to the increased interaction among the vector, the domestic reservoir and susceptible individuals, making it challenging to prevent and control this disease.

The male-to-female ratio in this study was 1.23 for *L. migonei* and 1.85 for *L. longipalpis*, indicating the predominance of males described previously (CASTELLÓN et al. 1989; CABANILLAS; CASTELLÓN, 1999; XIMENES et al., 2000; CORTEZ et al., 2007). This could be because males are born earlier than females (CHANIOTIS, 1967) or due to the courtship behavior between males and females (KELLY; DYE, 1997).

Although we found no correlation between *L. migonei* and *L. longipalpis* density and the occurrence of human or canine VL, *L. migonei* and *L. longipalpis* cannot be considered to be excluded from participating in the transmission of VL in Fortaleza because additional factors, such as parasite load in the vector population, determine disease transmission (MEDLEY, 1992). Additionally, the delayed reporting of cases or the incubation period of the disease, which can vary up to one year, may explain this lack of correlation (ZERPA et al., 2003; BERN et al. 2008).

The results of this study in combination with information in the literature may suggest *L. migonei* as a potential vector of VL in Fortaleza. However, research into the natural infection of *L. migonei* by *Leishmania* sp. could in fact evaluate the real role of each species, i.e., *L. longipalpis* and *L. migonei*, in the local epidemiology of VL.

It is essential to further study the behavior and distribution of this species for the correct implementation of vector control and prevention of VL.

Within this context and considering the possibility of another sand fly vector transmitting VL, the analysis of entomological indicators should be performed periodically, especially to assess progress in epidemiology and environmental assistance in expanding areas of transmission.

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