

ASPECTS OF THE ECOLOGY OF PHLEBOTOMINES (Diptera: Psychodidae: Phlebotominae) IN AN AREA OF CUTANEOUS LEISHMANIASIS OCCURRENCE, MUNICIPALITY OF ANGRA DOS REIS, COAST OF RIO DE JANEIRO STATE, BRAZIL

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SUMMARY

Over a complete two-year period, phlebotomine specimens were caught in an area of cutaneous leishmaniasis occurrence in the municipality of Angra dos Reis. A manual suction tube was used to catch phlebotomines on house walls, and also light traps in domestic and peridomestic settings and in the forest. This yielded 14,170 specimens of 13 species: two in the genus *Brumptomyia* and eleven in the genus *Lutzomyia*. *L. intermedia* predominantly in domestic and peridomestic settings, with little presence in the forest, with the same trend being found in relation to *L. migonei*, thus proving that these species have adapted to the human environment. *L. fischeri* appeared to be eclectic regarding location, but was seen to be proportionally more endophilic. *L. intermedia* and *L. migonei* were more numerous in peridomestic settings, throughout the year, while *L. fischeri* was more numerous in domestic settings except in March, April, May and September. From the prevalence of *L. intermedia*, its proven anthropophily and findings of this species naturally infected with *Leishmania (Viannia) braziliensis*, it can be incriminated as the main vector for this agent of cutaneous leishmaniasis in the study area, especially in the peridomestic environment. *L. fischeri* may be a coadjuvant in carrying the parasite.

KEYWORDS: Phlebotomines; Cutaneous Leishmaniasis; Angra dos Reis.

INTRODUCTION

The present study is the first of an intended series on the ecology of phlebotomines in areas of cutaneous leishmaniasis occurrence in the Serra do Mar, i.e. the coastal mountain range of the states of Rio de Janeiro and São Paulo.

The traditional communities present in the study area are characterized by their development of subsistence agriculture, non-mechanized fishing and a variety of differentiated economic activities that came to be introduced into their day-to-day lives after the opening of the Rio-Santos highway (BR 101), which facilitated growth of tourism and real-estate speculation. Consequently, these communities present low income and schooling levels, minimal access to the official healthcare network and a particular absence of adequate participation in the local economy⁴.

Land occupation has been driven by individuals coming in from more highly valued parts of the region and has generally taken place in a non-harmonious manner. Thus, over recent decades, this has favored proliferation of areas at risk of *Leishmania* infection. Consequently, phlebotomine species that have already adapted to domestic and peridomestic environments can transmit the parasite to domestic animals, which serve as reservoirs⁴.

Changes to the environment in many regions of Brazil have modified the epidemiological profile of leishmaniasis. Thus, wild mammals that are reservoirs for *Leishmania* have been able to invade peridomestic areas that are populated by phlebotomine species that have become adapted to environments modified by humans. Maintenance of cutaneous leishmaniasis in these ecologically altered areas clearly indicates that a cycle of secondary transmission in the peridomestic environment has evolved^{19,23}.

Currently, cutaneous leishmaniasis presents three characteristic epidemiological patterns: wild, occupational and leisure. In addition, it has rural and periurban characteristics relating to the migratory process, in which hill slopes have been occupied, with transformation of forested land to secondary and residual forests. In the states of Rio de Janeiro and São Paulo, *L. intermedia s. lat.* has been the vector, especially in settings of intense human action, affecting individuals of both sexes in the domestic environment³¹.

In view of the diversity of phlebotomine species and the peculiarities of the areas involved, with or without transmission of cutaneous leishmaniasis, studies on the bioecology of species that have been shown to be vectors or are suspected of being vectors can be expected to provide useful information for constructing indicators that contribute towards

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risk assessment, thereby giving rise to prevention and control measures that are more effective.

With the aim of learning more about the habits of phlebotomines, a series of captures was undertaken. The objectives were to determine the local phlebotomine fauna, the behavior of the main species (endophilic or exophilic), their monthly frequencies and occurrence in domestic and forested environments.

MATERIAL AND METHODS

Study area: The municipality of Angra dos Reis is situated in the Rio de Janeiro State (23° 59' 27" S and 44° 15' 52" W) and is 155 km from the state capital. It is one of the oldest settled areas on the coast of the state of Rio de Janeiro and on the entire Atlantic coastline of Brazil, occupying a narrow strip of land between the bay of Ilha Grande and the escarpment of the Serra do Mar (the coastal mountain range). Angra is a word in Portuguese meaning "wide-open cove or small bay that appears where the land rises high above the shoreline" (Fig. 1).

The climate is hot and humid, with a rainy season in the summer. The mean annual rainfall is 2,279 mm, but this is not evenly distributed over the year²⁴.

The peninsula of Angra dos Reis was discovered in 1502 and because of its natural beauty and strategic position, it became a privileged and sought-after space, both as the state capital and as the private enterprise capital. Within this context, three major projects were implemented: the nuclear power complex, the terminal of the bay of Ilha Grande (TEBIG) and the Rio-Santos coastal highway (BR 101). Of these, the construction of the highway, which crosses the entire length of the municipality, was the greatest cause of the transformations, both at a social and at an environmental level²⁶.

A house in the Camorim district of the municipality of Angra dos Reis was chosen to serve as a vector capture station. This house was

selected because two cases of cutaneous leishmaniasis had been found in the family living there, and because it was suitable for systematized phlebotomine capture, given that it had outhouses for domestic animals and a banana plantation, and was relatively close to the forest.

Every month from March 1996 to February 1998, the investigators spent two nights in the study area. Phlebotomines that landed on both the internal and the external walls of the house were caught using manual suction tubes between the following hours: 18:00 to 20:00, 21:00 to 23:00 and 00:00 to 02:00. A total of 288 hours were spent on this activity. The CDC light traps (a total of three), were also installed inside the home, in the peridomestic area (next to the chicken coop) and in the forest (around 300 meters from the house), always at the same sites and same times (18:00 to 06:00 the next morning) and one m above the ground. The traps were in position for a total of 576 hours at each collection site.

To analyze the monthly frequencies of the most numerous species at the three collection sites, the Williams mean (X_w) was calculated as described by HADDOW^{17,18}. The numbers of phlebotomines caught inside the house (internal walls and light traps) in the peridomestic area (external walls and light traps) and in the forest (light traps) were summed.

RESULTS

For two consecutive years, by summing up the results each month, 14,170 phlebotomines of 13 species were caught. There were two species of the genus *Brumptomyia* França & Parrot 1921 and eleven of the genus *Lutzomyia* França 1924 as listed in the following, in order of frequency, using the designations of YOUNG & DUNCAN³⁴:

Lutzomyia (Nyssomyia) intermedia (Lutz & Neiva, 1912)

Lutzomyia (Pintomyia) fischeri (Pinto, 1926)

Lutzomyia migonei (França, 1920)

Lutzomyia (Nyssomyia) whitmani (Antunes & Coutinho, 1939)

Lutzomyia (Pintomyia) pessoai (Coutinho & Barretto, 1940)

Lutzomyia monticola (Costa Lima, 1932)

Lutzomyia (Psychodopygus) ayrozai (Barretto & Coutinho, 1940)

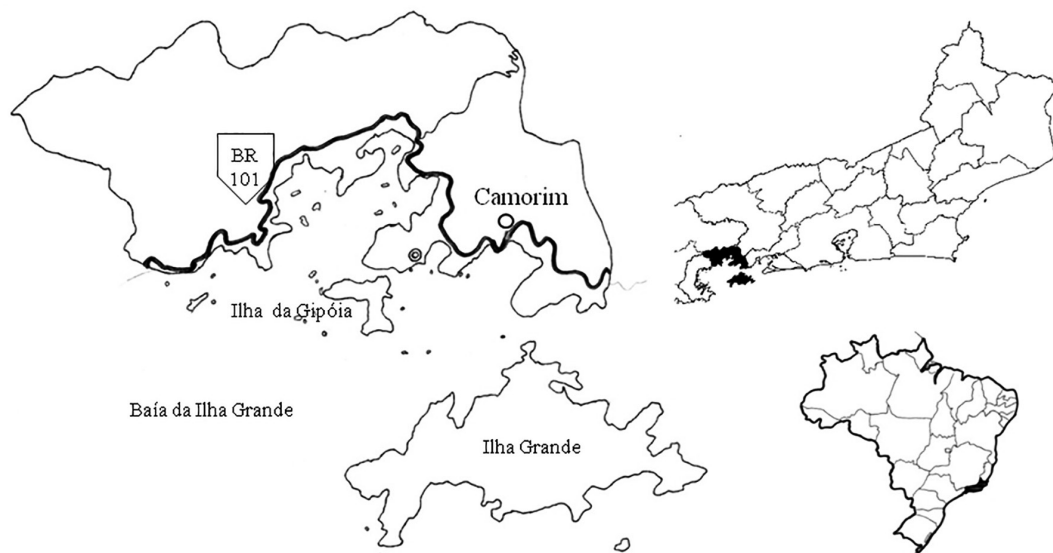


Fig. 1 - Map showing the location of the study area, in the Camorim district of the municipality of Angra dos Reis, state of Rio de Janeiro, Brazil.

Lutzomyia (Psathyromyia) shannoni (Dyar, 1929)
Lutzomyia edwardsi (Mangabeira, 1941)
Lutzomyia barrettoi (Mangabeira, 1942)
Brumptomyia avellari (Costa Lima, 1932)
Lutzomyia aragaoi (Costa Lima, 1932)
Brumptomyia guimaraesi (Coutinho & Barretto, 1941)

Table 1 presents the monthly frequencies of the species *L. intermedia*, *L. fischeri*, *L. migonei* and *L. whitmani*, which together accounted for 99.6% of the total number of specimens caught. The number of females was overall greater than the number of males: 57% versus 43%. *L. intermedia* showed balanced division, with only slightly more females; *L. migonei* showed a significantly greater number of males, while *L. fischeri* showed a predominance of females. Regarding the specimens caught when they landed on the internal and external walls of the house, *L. intermedia* was seen to be proportionally more exophilic, such that 70% of the examples were collected from the external walls, while *L. fischeri* was more endophilic, such that 72% of its specimens were caught on the internal walls of the house. *L. migonei* was seen to be clearly exophilic, such that 97% were on the external walls.

Among the phlebotomines caught using light traps, the predominance of *L. intermedia* was again greatest in the peridomestic area, with smaller numbers inside the house and a few specimens in the forest. *L. fischeri* presented similar numbers inside and outside the house. In the forest, it had much smaller number, but nonetheless it was the predominant species. At this collection site, the presence of *L. whitmani* was also noteworthy.

Also in relation to Table I and equally in Fig. 2, it was noted that the four most important species had higher mean numbers during the hot and humid period of the year, i.e. between October and January, with maximum peaks in December for *L. intermedia* and January for *L. fischeri* and *L. migonei*. The predominance of *L. intermedia* was clear in all months, both inside the house and in the peridomestic area. In the cooler and drier part of the year, from May to August, it presented a certain balance with *L. fischeri*, but from August onwards inside the house and from September onwards in the peridomestic area, the means gradually increased until reaching their maximum peak in December. Even though the means for *L. fischeri* were much lower than those of *L. intermedia*, *L. fischeri* showed significant presence inside the house, with means that gradually increased up to the maximum peak, in January. In the peridomestic area, the means were more balanced over the year, such that the presence of *L. fischeri* was usually slightly greater than that of *L. migonei*, except in the hot and humid period, when the latter predominated. In the forest, *L. fischeri* predominated except between April and July, when there was a balance with *L. whitmani*, which registered more significant means between December and February.

DISCUSSION

In the Americas, American cutaneous leishmaniasis is widespread, affecting all countries except for Uruguay and Chile¹⁶. In Brazil, this disease extends across all states of the federation, with a tendency towards urban areas. Its extent is especially related to environmental changes introduced through human action, in areas of continuous population flow, especially in areas with high levels of tourism such as the region of the Green Coast, i.e. the coastline of the states of Rio de Janeiro and São Paulo³.

In the southeastern region of Brazil, a process of geographical expansion of the endemic area of cutaneous leishmaniasis has been witnessed. This has probably taken place as a result of the introduction of the parasite into new areas by means of migration of infected people and domestic animals^{4,12,22,27,28}.

In most areas with *Leishmania (Viannia) braziliensis* transmission in the southeastern and northeastern regions of Brazil, there is absolute predominance of *L. intermedia* or *L. whitmani*, followed by *L. fischeri* and *L. migonei*, in domestic environments^{10,13,29,30,33}.

FORATTINI & OLIVEIRA¹⁴ raised the hypothesis that *L. intermedia s. lat.* (*intermedia* complex formed by *L. intermedia s. str.* and *L. neivai*) was the main transmitter of *Leishmania braziliensis*, while GOMES *et al.*¹⁵ corroborated the suspicions of FORATTINI *et al.*¹³ that this species carried the parasite in periurban areas.

Lutzomyia intermedia (Lutz & Neiva 1912) and *L. neivai* (Pinto 1926) are captured in various parts of Brazil and show a remarkable intraspecific and intrapopulation variation gradient⁷. *L. neivai* was considered synonymous junior of *L. intermedia*²¹, both with allopatric distribution in regions west of the Serra do Mar, in the states of São Paulo and Paraná and sympatric speciation in the Serra do Mar, in the state of São Paulo^{6,20}.

American cutaneous leishmaniasis acquired epidemic characteristics on the northern coastline of the State of São Paulo beginning in the 1990s. From secondary data, a descriptive study of the disease in the four municipalities making up this region over the period from 1993 to 2005 was conducted. The frequency of phlebotomine capture in the probable transmission locations was analyzed. Among the 2,758 phlebotomines captured, *Lutzomyia intermedia s. lat.* predominated (80.4%) inside homes and in areas surrounding them¹¹.

In 1978, ARAÚJO FILHO⁸ studied an outbreak of the disease in Ilha Grande, state of Rio de Janeiro and showed that *L. intermedia* predominated over the other species. On that occasion, this author reported that *L. flaviscutellata*, the vector for *Leishmania amazonensis*, was present.

AGUIAR *et al.*⁴ studied the phlebotomine fauna of Paraty, a municipality on the coast of the state of Rio de Janeiro and took the view that *L. intermedia* could be considered to be a potential vector for *Leishmania braziliensis*, given its high prevalence, anthrophilic nature and high degree of eclectic behavior, as well as because this species had already been incriminated in other areas of the southeastern region of Brazil. The insignificant presence of this species in wild environments and its adaptation to environments modified by humans led these authors to conclude that *L. intermedia* was transmitting the parasite in domestic and peridomestic settings.

AZEREDO-COUTINHO *et al.*⁹ reported on an unusual case of human infection by *Leishmania amazonensis* that occurred in the historical city of Paraty. This species, which typically occurs in Amazônia, had already been identified in the states of Goiás, Mato Grosso, Bahia, Minas Gerais, São Paulo, Paraná and Santa Catarina, and it was also recently held responsible for the development of an autochthonous case of diffuse cutaneous leishmaniasis, which is a rare and severe form of the disease^{9,31}.

Table 1

Monthly averages of phlebotomines caught when they landed on internal (Int) or external (Ext) walls of the house, or caught in CDC traps installed inside the house (Dom), in the peridomestic area (Per) or in the forest (For), added the monthly results from March 1996 to February 1998; Angra dos Reis, state of Rio de Janeiro

Spe	Local	Sex	Years/Months												Total	
			1996 - 97										1997 - 98			
			Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb		
<i>L. intermedia</i>	Wall	Int	F	76	24	16	19	34	27	62	65	79	134	191	125	838
		M	11	8	-	-	-	1	3	7	15	23	13	18	113	
	Ext	F	267	75	62	56	44	59	66	76	128	203	239	182	1,457	
		M	127	33	19	17	24	28	30	32	84	126	168	172	860	
	Dom	F	115	45	35	23	44	46	97	93	144	194	135	146	1,117	
		M	12	4	3	1	1	7	21	47	63	94	78	53	384	
	CDC	Per	F	122	39	21	27	32	37	43	201	297	338	401	299	1,857
		M	289	114	81	89	62	68	93	321	524	708	416	326	3,091	
	For	F	-	-	-	-	1	1	-	-	-	-	-	-	2	
		M	-	-	1	-	1	-	-	-	-	2	-	-	4	
<i>L. fischeri</i>	Wall	Int	F	11	24	14	22	35	46	35	59	48	84	65	54	497
		M	-	-	-	-	1	1	-	-	-	1	2	-	5	
	Ext	F	7	10	9	4	15	10	26	10	19	32	29	20	191	
		M	1	-	-	-	2	2	3	5	4	4	3	5	29	
	Dom	F	34	16	5	12	4	9	38	81	114	147	283	181	924	
		M	2	-	-	1	-	-	6	3	8	7	3	8	38	
	CDC	Per	F	84	40	36	18	16	31	56	71	79	83	88	53	655
		M	13	3	2	3	-	5	29	26	35	44	51	38	249	
	For	F	23	3	-	2	1	2	9	17	20	34	21	18	150	
		M	29	4	1	3	1	4	13	19	21	42	31	26	194	
<i>L. migonei</i>	Wall	Int	F	-	-	-	1	-	1	-	-	3	1	1	7	
		M	-	-	-	-	-	-	-	-	-	-	1	1	2	
	Ext	F	6	2	2	1	-	2	7	4	16	32	14	24	110	
		M	21	10	7	4	3	6	15	12	22	44	20	29	193	
	Dom	F	6	2	-	1	-	2	4	4	7	9	5	6	46	
		M	3	1	-	-	-	-	2	1	4	5	3	7	26	
	CDC	Per	F	11	2	-	3	2	6	8	17	28	43	40	189	
		M	38	7	5	16	8	11	20	47	79	155	185	124	695	
	For	F	-	-	-	-	-	-	1	-	-	-	-	-	1	
		M	2	-	-	-	-	-	-	-	1	1	-	-	4	
<i>L. whitmani</i>	Wall	Int	F	-	-	-	-	-	-	-	-	-	-	-	-	
		M	-	-	-	-	-	-	-	-	-	-	-	-	-	
	Ext	F	-	-	-	-	-	-	-	-	-	-	-	-	-	
		M	-	-	-	-	-	-	-	-	-	-	-	-	-	
	Dom	F	-	-	-	-	-	-	-	-	-	-	-	-	-	
		M	-	-	-	-	-	-	-	-	-	-	-	-	-	
	CDC	Per	F	1	-	-	-	-	-	1	2	-	3	1	9	
		M	-	-	-	-	-	-	-	2	-	1	-	1	6	
	For	F	7	5	3	-	-	-	1	4	5	16	12	10	63	
		M	4	2	1	1	3	3	4	9	12	19	24	31	113	

L. = *Lutzomyia* Spe = species.

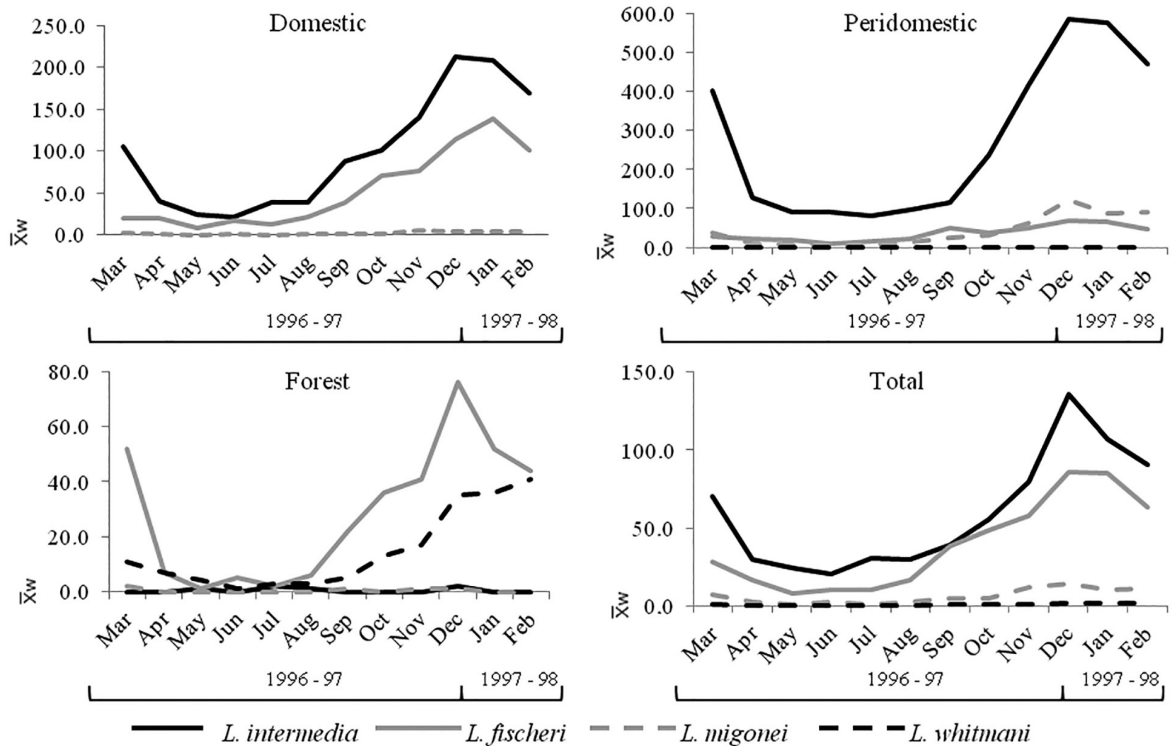


Fig. 2 - Monthly averages (\bar{X}_w) of phlebotomines caught inside the house, in the peridomestic area or in the forest added to the monthly results from March 1996 to February 1998; Angra dos Reis, Rio de Janeiro State.

It is important to remember that in Brazil, human migrations to new agricultural areas and to urban centers have contributed towards propagating various diseases. Leishmaniasis is no different in this respect, given that large outbreaks of this disease have occurred especially in recently colonized areas. Thus, the changes to the natural environment caused by humans have had a huge impact on the behavior of the phlebotomine fauna, such that some species have disappeared while others have adapted to the human environment. Situations like this can be demonstrated in many foci of cutaneous leishmaniasis in the states of Rio de Janeiro, São Paulo and Paraná. In these regions that have been occupied for longer times, the environment changes were accompanied by adaptation of the components of the transmission cycle. In this manner, the changes induced selection of the phlebotomine species and reservoirs, which became better-fitted for survival under the new conditions represented by residual forests close to human homes in rural zones or on the periphery of urban zones with or without rural characteristics^{3, 27, 32}.

As a consequence of the drastic changes to the environment caused by human interference, some wild mammals that are reservoirs for *Leishmania* invaded domestic areas in which some phlebotomine species with eclectic feeding habits could be found. Through this, a transmission cycle with the potential to affect humans was established^{28, 32}.

From research carried out so far in the southeastern and southern regions of Brazil, it can be said that *L. neivai* and *L. migonei* are the phlebotomine species with the greatest capacity for adaptation to human environments and therefore have the greatest possibility of adaptation to the domestic environment. The proof of this is the ever smaller numbers

of specimens found in forested areas. Another important factor is the male/female ratio in the peridomestic environment, which suggests that the natural shelters and breeding sites of these species are nearby, given that, as is well known, the males do not have much capacity for flight and appear in large numbers in the peridomestic area motivated by mating⁵. However, in the district of Posse, in a rural zone of the municipality of Petrópolis, at a distance of 112 km from the city of Rio de Janeiro, and in the municipality of Mesquita, a periurban region on the Gericinó massif, state of Rio de Janeiro, investigations demonstrated that *L. intermedia* also occurred in significant numbers in the residual forests. In Mesquita, a hypothesis of three transmission cycles was also formulated (domestic, extradomestic and wild). The authors highlighted the sloth as a possible reservoir for *Leishmania braziliensis*, which would act as a link between the wild and peridomestic environments, while dogs and horses would participate in the domestic cycle, with *L. intermedia* as the main vector^{23, 32}. However, in all the locations where this species predominates, the numbers reveal that there is greater activity in the domestic and peridomestic environments, thus proving the trend towards domestic adaptation of this species².

Over the years, in Angra dos Reis, increasing devastation of the forest and growth of banana plantations (where the inhabitants frequently construct their houses, following a habit that started with the irregular division of the land into plots in the 1960s) have led to decreases in the phlebotomine and forest animal fauna. However, the density of *L. intermedia* in the domestic and peridomestic environments has increased: in these settings, survival of the species has been ensured through the close feeding relationship with humans and domestic and synanthropic animals.

In the study area, out of the 13 species obtained, six species (*L. intermedia*, *L. migonei*, *L. fischeri*, *L. whitmani*, *L. pessoai* and *L. ayrozai*) had already been found naturally infected with *Leishmania braziliensis* and *Leishmania naiffi*^{25,31}. It has also been suggested that all of these species are transmitters in the epidemiological chain of cutaneous leishmaniasis and that they frequent both the domestic environment and remnant forest^{1,27,28}.

The high prevalence of *L. intermedia* has also been proven. Even with negative data from investigation of natural infection, the adaptive capacity of *L. intermedia* to environments modified by humans and its proven anthropophily allows it to be suggested that this species can be incriminated as the main vector for *Leishmania (Viannia) braziliensis* and that the transmission takes place in the domestic/peridomestic environment.

In relation to monthly frequency, *L. intermedia* presented great predominance in all the months of the year, both in the domestic and in the peridomestic environment. There was a gradual increase in frequency over the hotter and more humid period (between October and January, with mean temperatures ranging from 26 to 29 °C and relative air humidity from 84 to 87%). The maximum peak of activity was in December, whereas for *L. fischeri* and *L. migonei*, the highest peaks were in January.

The presence of *L. fischeri* in domiciliary environments, represented by much higher numbers of females, leads to the supposition that this species is not yet undergoing a process of domestic adaptation. Thus, it still maintains its natural shelters and breeding sites in the residual forest, as proven by the balance between the sexes at this study location, with slightly higher numbers of males. It can therefore be said that this species is eclectic regarding its blood meal location. Considering also that this species presented significant numbers at all three collection sites and that the distance from the house to the forest was around 300 meters, the data suggests that this is a species with greater dispersion.

Recently, in the municipality of Porto Alegre, in the state of Rio Grande do Sul, PITA-PEREIRA *et al.*²⁵ found naturally infected *L. fischeri* specimens in a periurban area by means of the PCR technique. In the present study area, even though there were no observations of natural infection due to *Leishmania sp.*, important epidemiological factors relating to *L. fischeri* were observed, especially its high anthropophily, degree of eclecticism and endophilia, along with levels of occurrence in foci of cutaneous leishmaniasis in the Brazilian Southeastern region that have always been notable. Hence, the results suggest that this species may act as a secondary vector for *Leishmania braziliensis* in the domestic/peridomestic environment. Since its population remains predominantly in the forest, it may participate in transmission within its natural enzootic cycle.

In relation to *L. migonei*, it was found that this species had greater adaptation to the peridomestic area, particularly in outhouses where domestic animals are kept. This was corroborated by the small number of specimens found in the forest, along with the greater presence of males in the peridomestic area. These factors demonstrate that this species is well adapted to environments that have been subjected to human influence.

Even with a small number of specimens, the presence of *L. whitmani* stood out. This species was only caught in light traps, and in more

significant numbers in the forest. It has been recorded in the state of Rio de Janeiro at low rates, but in studies conducted in the municipality of Posse, state of Rio de Janeiro, SOUZA *et al.*³² the hypothesis that *L. whitmani* may be exerting pressure on the ecological niche of *L. intermedia* was raised.

RESUMO

Aspectos da ecologia dos flebotomíneos (Diptera: Psychodidae: Phlebotominae) em área de ocorrência de leishmaniose tegumentar, Município de Angra dos Reis, orla marítima do estado do Rio de Janeiro, Brasil

Durante dois anos completos foram feitas capturas de flebotomíneos em área de leishmaniose tegumentar no município de Angra dos Reis. Utilizou-se tubo de sucção manual, para as capturas dos flebotomíneos pousados nas paredes da casa, além de armadilhas luminosas, no domicílio, peridomicílio e na mata. Foram obtidos 14.170 exemplares, de treze espécies, duas do gênero *Brumptomyia* França & Parrot 1921 e onze do gênero *Lutzomyia* França 1924. *L. intermedia* teve supremacia no peridomicílio e no domicílio, com pouca presença na mata, o mesmo ocorreu com *L. migonei*, comprovando a adaptação dessas espécies ao ambiente humano. *L. fischeri* aparece com característica eclética quanto ao local, mostrando-se proporcionalmente mais endófila. *L. intermedia* e *L. migonei* foram mais numerosas no peridomicílio, durante todos os meses do ano, enquanto *L. fischeri*, excetuando os meses de março, abril, maio e setembro, foi mais numerosa no domicílio. Pela prevalência, comprovada antropofilia e por ter sido encontrada infectada naturalmente por *Leishmania (Viannia) braziliensis*, *L. intermedia* pode ser incriminada como o principal vetor desse agente da leishmaniose tegumentar na área de estudo, sobretudo no ambiente peridomiciliar. *L. fischeri*, pelas características apresentadas, pode ser um coadjuvante na veiculação do parasita.

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