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Notes on the Sand fly fauna (Diptera: Psychodidae: Phlebotominae) in municipalities along the Peixe River, Midwest of Santa Catarina, Brazil

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ABSTRACT

We aimed to find out the sand fly species in five municipalities along the Peixe River in the west of Santa Catarina state, Brazil, and verify the presence of females naturally infected with *Leishmania* (*Leishmania*) *infantum* Nicolle. Phlebotomine were collected using a Falcão light trap and identified through morphology. A total of 149 specimens of ten phlebotomine species were identified, four of which were already reported with natural *Leishmania* spp. infection. However, using amplification of *L.* (*L.*) *infantum* kDNA through multiplex polymerase chain reaction (PCR) assay, no natural infection was observed in these specimens. The region is non-endemic for leishmaniasis and the low amount of collected specimens may have contributed to the negative results. However, the simultaneous presence of phlebotomine species reported in the literature as naturally infected with *Leishmania* spp. and a recent report of dogs naturally infected with *L.* (*L.*) *infantum* demonstrate the need to expand monitoring in this geographical region, particularly to detect sand flies potentially infected with *Leishmania* spp.

Keywords: Leishmaniasis, *Migonemyia migonei*, *Nyssomyia neivai*.

Notas sobre a fauna de flebotomíneos (Diptera: Psychodidae: Phlebotominae) em municípios ao longo do rio do Peixe, Centro-Oeste de Santa Catarina, Brasil

RESUMO

Nós objetivamos verificar a ocorrência de espécies de flebotomíneos em cinco municípios ao longo do rio do Peixe, no oeste de Santa Catarina, e verificar a presença de fêmeas naturalmente infectadas com *Leishmania* (*Leishmania*) *infantum* Nicolle. Flebotomíneos foram coletados através de armadilha luminosa de Falcão e identificados no nível de espécie através de chaves dicotômicas. Foi identificado um total de 149 espécimes de dez espécies de flebotomíneos, quatro dos quais de importância médica, pois já foram encontrados naturalmente infectados por *Leishmania* spp. Entretanto, usando a amplificação do kDNA de *L.* (*L.*) *infantum* através do ensaio de reação em cadeia da polimerase (PCR) multiplex, nenhuma infecção natural foi observada nestes espécimes. A região não é endêmica para a leishmaniose e a baixa quantidade de espécimes coletados pode ter contribuído para os resultados negativos. No entanto, a presença simultânea de espécies de flebotomíneos relatados na literatura como naturalmente infectados por *Leishmania* spp. e um relato recente de cães naturalmente infectados com *L.* (*L.*) *infantum* demonstram a necessidade de expandir o monitoramento nesta região geográfica, particularmente para detectar flebotomíneos potencialmente infectados com *Leishmania* spp.

Palavras-chave: Leishmaniose, *Migonemyia migonei*, *Nyssomyia neivai*.

Introduction

Phlebotomines (Diptera: Psychodidae: Phlebotominae) are vectors of the leishmaniasis etiological agent (ALVAR et al., 2012). Since the late 20th century, leishmaniasis has increasingly urbanized and has expanded into cities located in distinct geographical regions, including the northeast and southeast of Brazil (ALVES; BEVILACQUA, 2004), in addition to the increased number of cases in southern states (MARLOW et al., 2013).

In Santa Catarina, the first cases of American tegumentary leishmaniasis (ATL) were recorded in the western region of Quilombo and Coronel Freitas in 1987 (THIAGO; GUIDA, 1990). Since 2001, ATL has been considered as an emerging disease in this state because of the large number of autochthonous cases, particularly in the eastern region (MARLOW et al., 2013). In recent years, cases of canine visceral leishmaniasis (CVL) have also been documented in the eastern region of the state (Florianópolis) (STEINDEL et al., 2013), in the west (São Miguel do Oeste and Descanso) (MAZIERO et al., 2014), and recently in Erval Velho (OLIVEIRA, 2017).

Despite frequent reports of cases of leishmaniasis, little is known about the sand fly fauna in the west of Santa Catarina. Several studies were conducted primarily in the eastern region in areas with the largest number of cases (MARCONDES et al., 2005; 2009; DIAS et al., 2013; GROTT et al., 2015). In contrast, studies in the western region described only a few species (MÜLLER et al., 2014) and even the agency responsible for epidemiological surveillance in Santa Catarina has little information about the phlebotomine species that occur in this region (DIVE, 2016). Studies conducted in the west of Santa Catarina did not aim to identify the phlebotomine species infected with *Leishmania* spp. despite descriptions of cases of human or dog leishmaniasis in the region. Therefore, this study aimed to identify the phlebotomine species that occur in some municipalities in the western region of Santa Catarina and verify the presence of females naturally infected with *Leishmania* (*L.*) *infantum*.

Materials and Methods

Samples were collected from seven locations in the municipalities of Joaçaba, Herval d'Oeste, Lacerdópolis, Herval Velho, and Ouro. These five municipalities are located on the margins of the "Peixe river", in the west of Santa Catarina (Figure 1). These locations were chosen because they were accessible and

they presented favorable characteristics to the phlebotomine species such as the presence of animal shelters (kennel, chicken coop), proximity to forest fragments (ALEXANDER, 2000) and the presence of dogs infected with *L.* (*L.*) *infantum* in these areas (OLIVEIRA, 2017).

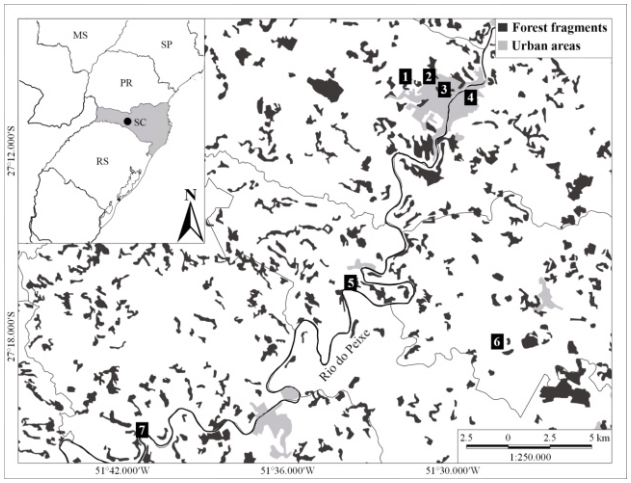

Figure 1. Sampled areas in the Middle West of Santa Catarina, Brazil. Municipalities: 1, 2, and 3 = Joaçaba; 4 = Herval d'Oeste; 5 = Lacerdópolis; 6 = Herval Velho; 7 = Ouro.

The vegetation comprises remnants of deciduous seasonal forests (VIBRANS et al., 2012). The region is predominantly rural, with an emphasis on family agriculture, but the urban area has increased in recent years. According to the climate classification system of Köppen, the climate of the municipalities is classified as wet mesothermic with hot summer (Cfa), with average annual temperature of 18°C, approximate annual precipitation of 2,000 mm, average annual relative humidity of 76%, and average altitude of approximately 520 m (ALVARES et al., 2013).

Samples were collected between September 2015 and March 2016, and each location contained samples collected over three nights during the period of new moon and when climatic conditions were favorable, that is, without rain and with low wind speed (ALEXANDER, 2000) totalizing 21 samples. For sample collection, Falcão light traps similar to CDC light traps were installed approximately 1.5 m from the ground at twilight, one near the animal shelters and/or dwelling and another inside each forest fragment near the animal shelters and/or dwelling, and were collected in the morning at twilight (adapted from SECRETARIA DE VIGILÂNCIA EM SAÚDE, 2003).

Characterization of sampled areas: **Sample site 1** (27° 9'43.77"S; 51°31'36.54"W): Trap 1, installed near the stable, distant approximately 30 m from the forest fragment. Trap 2, installed approximately 20 m inside the forest fragment and 50 m from the trap 1. **Sample site 2** (27° 9'31.59"S; 51°30'45.35"W: Trap 1, installed near the kennel and dwellings distant approximately 20 m from the forest fragment. Trap 2, installed approximately 10 m inside the forest fragment and 30 m from the trap 1. **Sample site 3** (27°10'6.41"S; 51°30'14.04"W): Trap 1, installed near the kennel and dwellings distant approximately 20 m from the forest fragment. Trap 2, installed approximately 20 m inside the forest fragment and 40 m from the trap 1. **Sample site 4** (27°10'14.21"S; 51°29'23.21"W): Trap 1, installed near the kennel and dwellings distant approximately 10 m from the forest fragment. Trap 2, installed approximately 10 m inside the forest fragment and 20 m from the trap 1. **Sample site 5** (27°16'24.23"S; 51°33'35.92"W): Trap 1, installed near the kennel, stable and dwellings, distant approximately 20 m from the forest fragment. Trap 2, installed approximately 10 m inside the forest fragment and 30 m from the trap 1. **Sample site 6** (27°18'14.31"S; 51°28'21.48"W): Trap 1, installed near the chicken coop and dwelling, distant approximately 10 me from the forest fragment. Trap 2, installed approximately 10 m inside the forest fragment and 20 m from the trap 1. **Sample site 7** (27°21'9.20"S; 51°41'24.11"W): Trap 1, installed near the kennel and dwelling, distant approximately 10 m from the forest fragment. Trap 2, installed approximately 20 m inside the forest fragment and 30 m from the trap 1.

Captured sand flies were taken to the Laboratory of Infectious and Parasitic Diseases of the University of the West of Santa Catarina (Unoesc). These sand flies were killed by freezing at −10°C. For identification, the whole body of male sand flies was prepared, whereas for female sand flies the last three segments of the abdomen and head were separated for identification, and the rest of the body was stored at −20°C for further molecular analysis. The preparation of sand flies followed Young & Duncan (1994), whereas the identification and nomenclature of sand flies followed the protocol used by Galati (2003).

Except for female specimens of *Brumptomyia* França and Parrot species, all other female specimens were subjected to molecular analysis to assess possible infection with *L.* (*L.*) *infantum*. For this purpose, specimens had their DNA extracted individually and six pools containing DNA of 6-10 sand flies in each tube, according to species, was used for the PCR. For DNA extraction, 10µL of buffer (10 mM Tris-HCl, 2 mM EDTA, and 0.2% Triton X-100) and 1µL of proteinase K (20 mg/mL) were added to each pool. The insects were macerated, 90µL of buffer was added to each sample, and the homogenates were incubated at 37ºC for 2 h. Next, proteinase K was inactivated at 95ºC for 30 min, and the samples were centrifuged at 5,000 g for 5 min.

For PCR, we used the goTaq DNA polymerase kit (5µL), 10 pmol of each primer to detect Phlebotominae DNA: 5Llcac [5'-GTGGCCGAACATAATGTTAG-3'] and 3Llcac [5'-CCACGAACAAGTTCA ACATC-3'] (LINS et al., 2002) and *L.* (*L.*) *infantum* kDNA: RV1, [5'-CTTTTCTGGTCCCGCGGGTAGG-3'] and RV2, [5'-CCACCTGGCTATTTTACACCA-3'] (LACHAUD et al., 2002), and 1 L of DNA (100 ng) at a final volume of 10 L. PCR conditions were as follows: 95°C for 5 min, 95°C for 1 min, 60°C for 30 s, 72°C for 30 s, and 72°C for 5 min. The reaction was previously standardized using different amounts of *L.* (*L.*) *infantum* DNA, equivalent to between 10^6 and 10^1 parasites per PCR reaction and equivalent amount of sand fly female DNA. Under all conditions, the PCR was able to detect the presence of DNA from the parasite. As positive control, a PCR was performed containing 10 ng of DNA from standard samples of *L.* (*L.*) *infantum*. As negative controls, two PCR reactions were made, one containing only water and the second using 100 ng of male sand fly DNA, thus free of potential infection with *Leishmania* sp. The PCR reactions were repeated only with the primer for the *L. (L.) infantum* kDNA to confirm the results. All samples were resolved in 2% electrophoresis gel and stained with ethidium bromide.

Results and Discussion

A total of 149 specimens of 10 species were collected (Table 1). The presence of *L.* (*L.*) *infantum* was investigated by analyzing 73 females of five species with epidemiological relevance: *Evandromyia edwardsi* (Mangabeira), *Martinsmyia alphabetica* (Fonseca), *Migonemyia migonei* (França), *Nyssomyia neivai* (Pinto) and *Pintomyia fischeri* (Pinto). The DNA of *L.* (*L.*) *infantum* was not detected (Figure 2). However, the occurrence of phlebotomine species described in the literature with vectorial capacity is evidence for broader and continuous monitoring. *Pintomyia fischeri* is a vector of ATL and has been found to be naturally infected with *Leishmania* (*Viannia*) sp. (PITA-PEREIRA et al., 2005) and is described as potential vector of *L.* (*L.*) *infantum* (GALVIS-OVALLOS et al., 2017).

Migonemyia migonei and *N. neivai* have been described as vectors of *Leishmania* (*Viannia*) *braziliensis* Vianna (PITA-PEREIRA et al., 2005; 2009) and *L.* (*L.*) *infantum* (DE CARVALHO et al., 2010; SALOMÓN et al., 2010; DIAS et al., 2013). *Evandromyia edwardsi* was found to be infected with *L.* (*V.*) *braziliensis* in ATL transmission (SUCEN, 2005).

Table 1. Phlebotomine species collected in the seven locations between September 2015 and March 2016, in the Middle West of Santa Catarina, Brazil.

	1		2		3		4		5		6		7		Total	Total		
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	Total	%
Brumptomyia cunhai	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	2	2	1,34
Br. guimaraesi	-	-	4	-	-	-	-	-	1	-	-	-	-	-	1	4	5	3,36
Br. mangabeirai	1	-	5	-	25	-	-	-	2	-	-	-	-	-	33	-	33	22,15
Br. nitzulescui	-	-	-	-	-	-	-	-	-	-	-	-	1	-	1	-	1	0,67
Brumptomyia sp.	-	-	-	7	-	10	-	-	-	2	-	-	-	2	-	21	21	14,09
Evandromyia edwardsi	-	-	-	9	-	-	1	2	-	-	-	-	-	-	1	11	12	8,05
Martinsmyia alphabetica	-	1	1	2	-	-	-	-	-	2	1	-	-	1	2	6	8	5,37
Migonemyia migonei	-	-	2	-	3	-	1	-	1	-	-	-	-	-	7	-	7	4,70
Nyssomyia neivai	-	-	-	-	-	-	-	-	-	-	-	-	1	1	1	1	2	1,34
Pintomyia fischeri	-	1	-	35	-	14	-	1	-	-	-	1	-	-	-	52	52	34,90
Psathyromyia lanei	3	2	-	-	-	-	-	-	-	1	-	-	-	-	3	3	6	4,03
Total	4	4	12	53	28	26	2	3	4	5	1	1	2	4	49	100	149	100

1, **2**, and **3** = Joaçaba; **4** = Herval d'Oestes; **5** = Lacerdópolis; **6** = Erval Velho; **7** = Ouro. ♂ = Male; ♀ = Female. % = percentage.

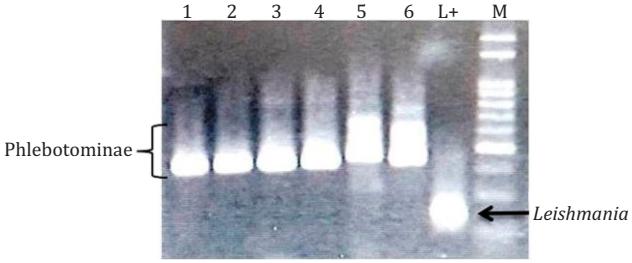

Figure 2. Detection of *Leishmania (Leishmania) infantum* in phlebotomines' females collected in the Midwest of Santa Catarina, Brazil. Lanes 1-6, pools with phlebotomines' females; lane L+, positive control of *L. (L.) infantum*; lane M, molecular weight marker (50 bp DNA ladder).

Sandflies were found only in traps installed inside the forest fragments. The failure of peridomiciliary collection may have been due to the light coming from public lighting and/or nearby residences, which was much more intense than that coming from the light trap because even the light reflected by the moon on full moon nights can undermine the success of sampling (ALEXANDER, 2000).

Although some of the phlebotomine species have already been reported in the west (THIAGO; GUIDA, 1990; MÜLLER et al., 2014; DIVE, 2016) and east of Santa Catarina (MARCONDES et al., 2005; 2009; GROTT et al., 2015), this is the first study in the Midwest of the state that has contributed to the record and knowledge of the distribution of sand fly species, a poorly studied fauna in the state. Despite the negative result for *L.* (*L.*) *infantum* infection, the occurrence of some vector species of *Leishmania*, especially *L.* (*V.*) *braziliensis* and *L.* (*L.*) *infantum*, shows that these species may act in the maintenance of a wild cycle of leishmaniasis, reinforcing the need to broaden the study of these vectors in the region.

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