Tropical Ecology **58**(1): 87–93, 2017 ISSN 0564-3295

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Diversity of phlebotomine sand flies (Diptera, Psychodidae) in Brazilian 'Campo rupestre' vegetation of the Espinhaço Range, Minas Gerais, Brazil

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Abstract: The present study details for the first time the diversity of phlebotomine sand flies in the *Campo rupestre* vegetation in the Espinhaço Range, considered by the United Nations Educational, Scientific and Cultural Organization as the seventh Brazilian biosphere reserve. Flies were captured using HP light traps for twelve months from October 2012 to September 2013. The traps were exposed continuously for 40 hours each month, in four fixed sites. A total of 295 specimens were captured, being 184 female and 111 male, distributed in 9 species: *Lutzomyia cipoensis* (10.17%), *Lu. diamantinensis* (0.34%), *Lu. evandroi* (1.02%), *Lu. goiana* (1.35%), *Lu. ischyracantha* (69.49%), *Lu. lenti* (14.24%), *Lu. pessoai* (0.34%), *Lu. quinquefer* (1.69%), *Lu. whitmani* (0.34%) and unidentified *Lutzomyia* spp. (1.02%). Of these, only *Lu. ischyracantha* and *Lu. lenti* were classified as constant species in this environment. The presence of tegumentary leishmaniasis vector species reinforces the necessity of conducting more studies in order to understand the distribution and participation of these insects regarding the epidemiology of leishmaniasis in this Brazilian phytophysiognomy.

Key words: Biodiversity, *Campo rupestre* vegetation, espinhaço range, lutzomyia, phlebotomine, vector ecology.

Handling Editor: David Jenkins

Introduction

The *cerrado* (tropical savannah) is a vast tropical and subtropical biome covering more than 20 percent of Brazil. It includes a number of ecosystems from tall closed forests through marshlands to open grassland (Klink & Machado 2005). *Campo rupestre* vegetation is a characteristic formation of *cerrado* that occurs at altitudes above 900 m and consists primarily of a more or less continuous herbaceous level growing on rock outcrops, with many small shrubs and subshrubs with perennial leaves growing in crevices or in other sites that have shallow soil (Vasconcelos 2011).

In Brazil, *Campo rupestre* vegetation is distributed in regions of the Espinhaço Mountain Range, an extensive and continuous zone in the states of Minas Gerais and Bahia (Rapini *et al.* 2008). This group of mountains is considered by the United Nations Educational, Scientific and Cultural Organization as the seventh Brazilian biosphere reserve for its enormous diversity and biological importance (UNESCO 2005).

Campo rupestre vegetation is not a homogeneous vegetation type, but rather a complex

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mosaic of environments with distinct physical characteristics which are determined by the local topography, slope, microclimate, and the nature of the substrate (Giulietti *et al*. 2000; Oliveira-Filho *et al*. 1989; Rizzini 1976; Scarano 2007). These singular characteristics enable the formation of a great variety of microenvironments in small areas, making these sites highly biodiverse (Rapini *et al*. 2008).

Since there are few or no studies about sand flies in Brazilian *Campo rupestre* vegetation, the present work aimed to know the fauna of these insects and their seasonal distribution in order to contribute to the understanding of the epidemiology of leishmaniasis in this region as well as of the Brazilian biodiversity. Although the study area did not present reported cases of leishmaniasis, the municipality where it is located is considered endemic for the cutaneous and visceral forms.

Materials and methods

Study area

The present study was conducted in an area of *Campo rupestre* vegetation inserted in Espinhaço Range, located in the municipality of Diamantina (18°14'17"S–43°36"W, at 1288 m altitude), Minas Gerais state, Brazil (Fig. 1). This vegetation is characterized principally by a herbaceous-shrub physiognomy with a high degree of endemism. The region has extensive areas of exposed rock and a wide variety of substrates (Vasconcelos 2011). The local climate is semi-humid, with irregular annual rainfall between 600 and 1500 mm (Franco & Gimenes 2011). Average temperatures vary between 13 ºC in the dry season (April to September) and 30 ºC in the rainy season (October to March), although large variation may occur between years (Machado *et al*. 2013).

Characterization of collection sites

Four collection sites were selected for this study (Fig. 2). The sites were inserted on quartzitic rock outcrops, typical of the phytophysiognomy of *Campo rupestre*, with the presence of woody vegetation or herbaceous and shrub near them.

Capture and identification of specimens

Captures were performed with HP light traps from October, 2012 to September, 2013, except March and May, 2013. The traps were exposed continuously, at 4 fixed sites [18.19535S–43.56869W, 1302 m, 18.19536S–43.56868W, 1306m, 18.19926S–43.56705W, 1337 m, 18.19929S–43.56565W, 1323 m] (Fig. 2), during 40 h/month, between 04:00 pm (day 1) and 8:00 am (day 3). Captured specimens were transported to the Laboratório de Parasitologia, Universidade Federal dos Vales do Jequitinhonha e Mucuri, and then killed by freezing. Males and females were placed in hemolytic tubes containing 70% ethanol, prepared and assembled on slides according to the

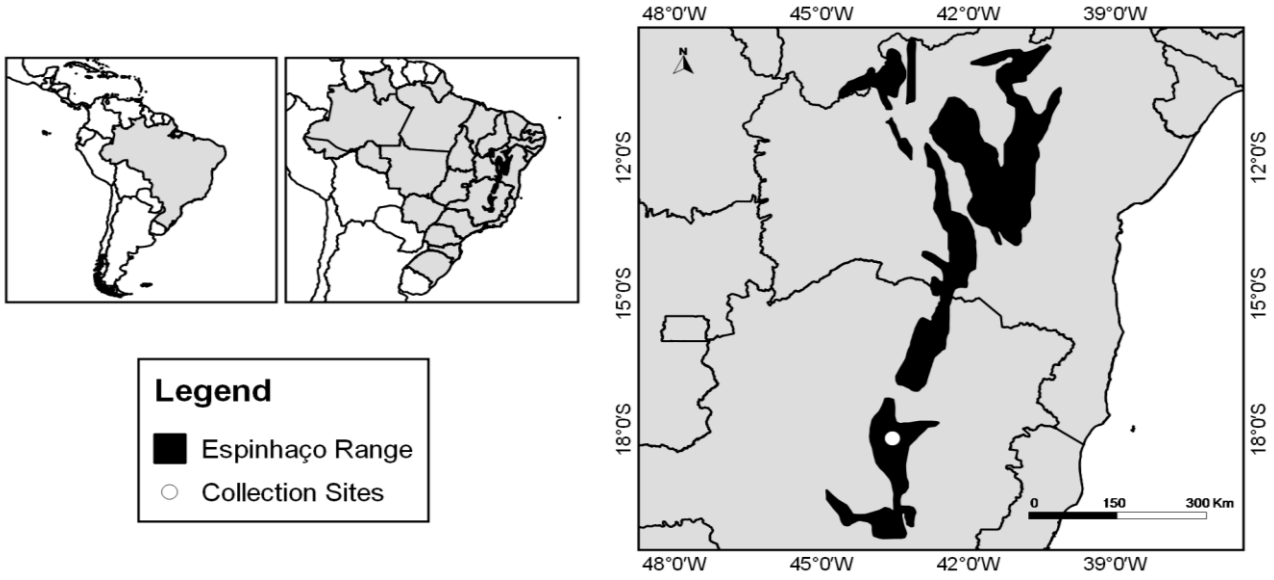

Fig. 1. Geographical locatization of the municipality of Diamantina in Minas Gerais state, Brazil.

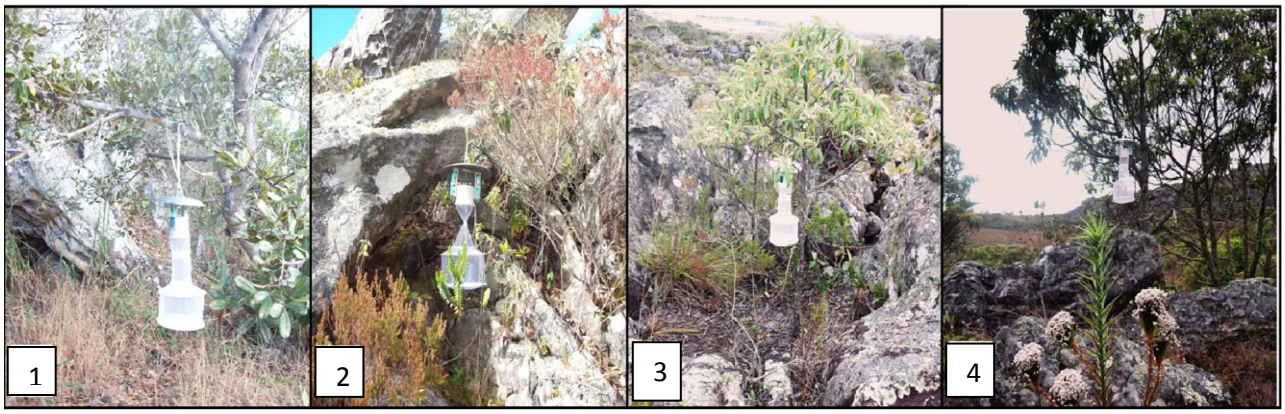

Fig. 2. Partial view of the collection sites: (1–4) inserted in Brazilian *Campo rupestre* vegetation of the Espinhaço Range, Minas Gerais state, Brazil.

technique of Langeron (1949). The taxonomic keys of Young and Duncan (1994) were used to identify sand fly species. Specimens with missing or damaged characters that impaired the identification at the specific level were considered as *Lutzomyia* spp. The specimens identified were deposited in the Coleção do Laboratório de Parasitologia, Universidade Federal dos Vales do Jequitinhonha e Mucuri.

Ecological data

The frequency of each species was calculated via the constance index (CI) (Dajoz 1973). The species were categorised as constant (species present in 50% or more of the catches), accessory (found in 25–50% of the catches) and accidental (present in less than 25% of the catches).

Results

The sand fly fauna of the Brazilian *Campo rupestre* vegetation included 9 species: *Lutzomyia cipoensis* Martins, Falcão & Silva 1964, *Lutzomyia diamantinensis* Barata, Serra-e-Meira & Carvalho 2012, *Lutzomyia evandroi* (Costa Lima & Antunes 1936), *Lutzomyia goiana* Martins, Falcão & Silva 1962, *Lutzomyia ischyracantha* Martins, Falcão & Silva 1962, *Lutzomyia lenti* (Mangabeira 1938), *Lutzomyia pessoai* (Coutinho & Barreto 1940), *Lutzomyia quinquefer* (Dyar 1929) and *Lutzomyia whitmani* (Antunes & Coutinho 1939), totaling 295 specimens, from which 184 were females (62.4%) and 111 males (37.6%) (Table 1).

Table 2 shows the monthly distribution and constancy index of phlebotomine captured in Brazilian *Campo rupestre* vegetation of the Espinhaço Range, Minas Gerais. The period with the highest density of sand flies was December 2013, with 53 specimens captured, coinciding with the peak of the rainy season (Table 3). According to the constancy index, *Lu. ischyracantha* and *Lu. lenti* were categorized as constant species. However, only *Lu. ischyracantha* was found in all months of capture. The category of accessory species included *Lu. cipoensis*, *Lu. goiana* and *Lu. quinquefer*, while the category of accidental species included *Lu. diamantinensis*, *Lu. evandroi*, *Lu. pessoai* and *Lu. whitmani* (Table 2).

Table 1. Number of phlebotomine species collected per sex in Brazilian *Campo rupestre* vegetation of the Espinhaço Range, Minas Gerais State, from October 2012 and September 2013.

Species	Male	Female	Total	%
Lutzomyia cipoensis	7	23	30	10.2
Lu. diamantinensis	0	1	1	0.3
Lu. evandroi	2	1	3	1.0
Lu. goiana	1	3	4	1.4
Lu. ischyracantha	79	126	205	69.5
Lu. lenti	18	24	42	14.2
Lu. pessoai	0	1	1	0.3
Lu. quinquefer	0	5	5	1.7
Lu. whitmani	1	0	1	0.3
Lutzomyia spp.	3	0	3	1.0
Total	111	184	295	100

Table 2. Monthly distribution and constancy index (CI) of phlebotomine species collected in Brazilian *Campo rupestre* vegetation of the Espinhaço Range, Minas Gerais State, from October 2012 and September 2013.

Species	2012			2013							IC (%)	Classification
	Oct	Nov	Dec	Jan	Feb	Apr	Jun	Jul	Aug	Sep		
Lutzomyia cipoensis	0	0	0	0	0	3	18	9	0	0	30	Accessory
Lu. diamantinensis	0	0	0	0	1	0	0	0	0	0	10	Accidental
Lu. evandroi	0	0	0	0	2	1	0	0	0	0	20	Accidental
Lu. goiana	0	1	0	0	0	2	1	0	0	0	30	Accessory
Lu. ischyracantha	16	22	40	3	26	29	17	14	7	31	100	Constant
Lu. lenti	6	12	12	2	0	1	0	0	0	9	60	Constant
Lu. pessoai	1	0	0	0	0	0	0	0	0	0	10	Accidental
Lu. quinquefer	0	1	1	0	0	0	2	1	0	0	40	Accessory
Lu. whitmani	0	0	0	1	0	0	0	0	0	0	10	Accidental
Lutzomyia spp.	1	0	0	0	1	0	1	0	0	0	-	-
Total	24	36	53	6	30	36	39	24	7	40	-	-

Discussion

The phlebotomine sand fly fauna registered in Brazilian *Campo rupestre* vegetation of the Espinhaço Range, Minas Gerais resembles that found in other typical areas of cerrado (Cutolo & Von Zuben 2008; Rebêlo *et al.* 1999), including the presence of *Lutzomyia whitmani*, considered to be a vector species of tegumentary leishmaniasis in Brazil (Azevedo & Rangel 1991; Cuba-Cuba *et al.* 1985).

Although restricted to only one occurrence, the record of *Lu. whitmani* in Brazilian *Campo rupestre* vegetation deserves special attention for suggesting their participation in the enzootic cycle of *Leishmania* sp. in this area. Present in all five regions of the country, in various habitats, this species has been found in wild environments and also in disturbed areas, demonstrating a wide ecological plasticity (Peterson & Shaw 2003; Costa *et al.* 2007).

Table 3. Climatic conditions in Brazilian *Campo rupestre* vegetation of the Espinhaço Range, Minas Gerais State, between October 2012 and September 2013.

Period	Rainfall (mm^3)	Sand flies
Oct/12	76.0	24
Nov/12	402.4	36
Dec/12	108.6	53
Jan/13	434.6	6
Feb/13	8.8	30
Apr/13	159.4	36
Jun/13	18.6	39
Jul/13	2.2	24
Aug/13	4.2	7
Sep/13	35.0	40
Total	-	295

Lutzomyia pessoai, also captured in this study, have been identified in other areas as a species suspect of transmitting tegumentary leishmaniasis (Forattini *et al.* 1972; Pessoa & Coutinho 1940). However, unlike *Lu. whitmani*, this species has not presented the same degree of adaptability to urban environments, and many studies over the years, show its presence restricted to wild environments, such as fragments and remnants of forests (Forattini 1954; Teodoro *et al.* 1998).

The species most often collected was *Lu. ischyracantha*. Until now, there is no record of this species transmitting leishmaniasis. Its high frequency in the study area confirms other findings that demonstrate their strong presence in wild environments (Barata & Apolinário 2012; Barata *et al.* 2012a).

Lutzomyia lenti was the second most frequently collected species, with previous records in native cerrado areas and residences peridomicile (Galati *et al.* 1996). Although this species has not been described as a vector, further research is necessary in this regard, since it was found naturally infected with promastigotes of *Leishmania* sp. and *Leishmania* (*V.*) *braziliensis* (Paiva *et al.* 2010; Sherlock 1996). In addition, its capture is always associated with *Lu. longipalpis*, which may suggest its participation as a secondary vector of leishmaniasis in other Brazilian states.

Only two species were trapped throughout the study: *Lu. lenti* and *Lu. ischyracantha*. Analyzing

the collection sites, we realized that all are located at elevations above 1300 m. Several authors have shown that sand fly populations are directly affected by altitude, influencing the dynamics of distribution of these insects (Ferreira *et al.* 2001; Gebre-Michael *et al.* 2004). Therefore, it is likely that the environmental conditions found in the *Campo rupestre* region may have affected the richness and abundance of some less resistant species, since collections carried out at lower elevations of the municipality found a large number of sand flies (data not shown).

A higher peak sand fly density was observed in the rainy season of the study area (Table 3). In Brazil, the rainy season covers the months from November to February. Chaniotis *et al.* (1971) suggesting that the seasonal variation is related to the rainfall distribution patterns, which would help the sand flies when moderate, and harm these insects when excessive, i.e. flooding the ground and reducing the opportunity to breed and killing the pupae in the soil. This hypothesis may explain the low number of specimens captured in January.

Barata *et al.* (2012b) described the species *Lu. diamantinensis* from quartzite caves near the study area. Surprisingly, this species was also found in non-cave areas, demonstrating an ecological valence and wider distribution than previously expected. Despite its accidental occurrence, this new record in the Espinhaço Range may be evidence of endemism, since this species was not found in any other region, after its description.

Finally, it is important to emphasize that this is the first report on the sand fly fauna in the Brazilian *Campo rupestre* vegetation of the Espinhaço Range. The presence of tegumentary leishmaniasis vector species reinforces the necessity of conducting studies involving the research of infection by *Leishmania* in sand flies and possible reservoirs in order to better understand the distribution and participation of these insects regarding the epidemiology of leishmaniasis in this Brazilian phytophysiognomy.

Conclusions

The results showed that the fauna phlebotomine in the region is quite diverse. It is likely that the environmental conditions found in the *Campo rupestre* region may have affected the richness and abundance of some less resistant species. The presence of tegumentary leishmaniasis vector species reinforces the necessity of conducting studies involving the research of infection by *Leishmania* in sand flies and possible reservoirs in order to better understand the distribution and participation of these insects regarding the epidemiology of leishmaniasis in this Brazilian phytophysiognomy.

Acknowledgments

We would like to thank Gilmara Nunes de Miranda who kindly revised the English manuscript. This work was supported by the Fundação de Amparo à Pesquisa do Estado de Minas Gerais (APQ-0098/10).

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(Received on 01.4.2015 and accepted after revisions, on 04.08.2015)

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