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Phlebotomine (Diptera: Psychodidae) fauna in a cavern containing cave paintings and its surrounding environment, Central-West Brazil

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ABSTRACT

The interior of caves usually presents climate, light, soil, flora and fauna that distinguish it from the external environment. Among the sandfly species found in caves, several may act as vectors of *Leishmania* spp. and others are a nuisance to humans due to their painful bites. This study sought to identify the phlebotomine fauna in a cavern with cave paintings ("Gruta do Pitoco") which attract tourists in Alcinopólis municipality, Mato Grosso do Sul state, Brazil. The sandflies were collected from March 2014 to September 2015, with automatic light traps, installed monthly, in the entrance and interior of the cave and outside it. A total of 7645 specimens belonging to 27 species were collected. *Lutzomyia dispar, Martinsmyia oliverai* and those of the *Lu. longipalpis* complex (*Lu. cruzi* and *Lu. longipalpis)* were the predominant species. Among the three environments sampled, no great difference between species richness was observed. Comparing these environments, the interior of the cave presented the lowest diversity index due to the high frequency of *Lu. dispar* (46%). For the first time, specimens (only females) of the genus *Deanemyia* were collected in Mato Grosso do Sul state. Moreover, the study revealed the sympatric occurrence of *Lu. cruzi* and *Lu. longipalpis*, both proven vectors of L. *infantum,* and also *Lu. dispar*, a highly anthropophilic species, which often causes annoyance to those who visit the cave.

1. Introduction

Phlebotomine sandflies are insects of medical significance in view of their transmitting etiological agents of leishmaniasis because of the bite of infected females. In addition to parasites of the *Leishmania* genus, sandflies may also transmit other pathogens such as *Bartonella* and arboviruses (Sherlock, 2003; Lainson and Shaw, 2005; Battisti et al., 2015).

Caves generally present different environments from the external ones, characterized by low luminosity, a large quantity of organic matter and adequate humidity and temperature, thus establishing favorable conditions for the proliferation of sandflies. Cave interiors also serve as habitats and / or shelters for several species of mammals, including bats, rodents, and cold-blooded animals, which are all blood-sources for sandflies (Galati et al., 2003).

According to the National Cave Census of Brazil, there are 7862 caves registered in the country, 172 of which are found in Mato Grosso do Sul state (CNC, 2019). Although there are few studies of insect fauna, especially sandflies, in caves, in recent years some sandfly species of cavernicolous behavior have been described in Brazil (Alves et al., 2008; Carvalho et al., 2010, 2011; Barata et al., 2012). Studies on sandflies in caves have demonstrated that some species are dominant in this habitat and their species diversity may be different from that of the external environment (Galati and Nunes, 1999; Galati et al., 2003, 2006).

In Brazil, there are several regions containing rock art, but based on their characteristics and location, the collection has been divided into "traditions": Agreste, Planalto, Nordeste, São Francisco, Litorânea, Geométrica, Meridional and Amazônica (Aguiar et al., 2012). The state of Mato Grosso do Sul has a remarkable collection of rock art dating

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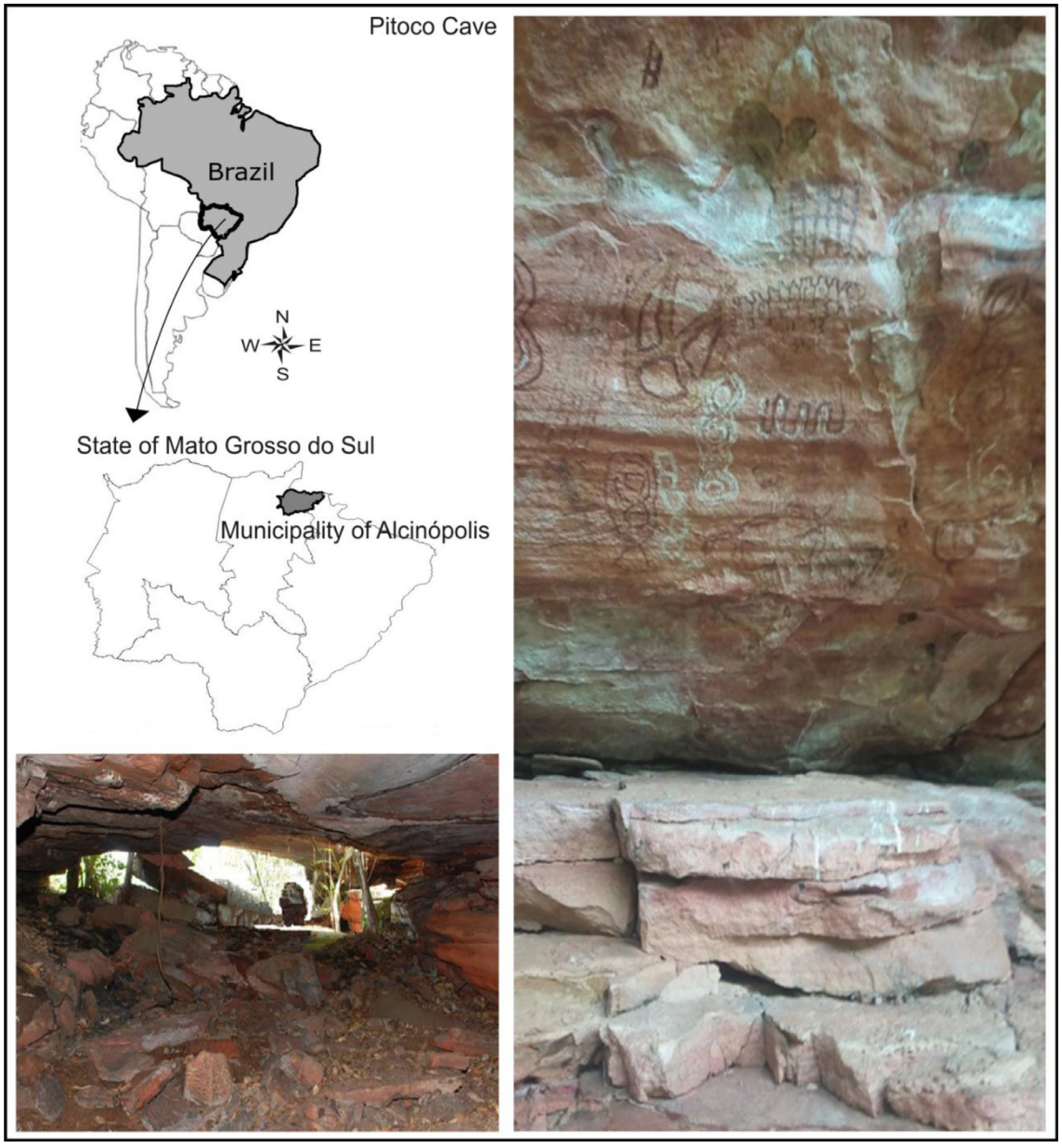

Fig. 1. Map of the location of Alcinópolis municipality, and photographs of cave paintings in the Gruta do Pitoco (Pitoco Cave), Alcinópolis municipality, Mato Grosso do Sul, Brazil (photos by Almeida and Brilhante, 2015).

back 12 thousand years, and the municipality of Alcinópolis holds a third of all the state's rock art, characteristically belonging to the plateau (Planalto) tradition, being thus considered the state's rock art capital (Aguiar et al., 2016), and is thus frequently visited by tourists.

In the light of the medical interest in sandflies and the touristic potential of the region, the present study sought to identify the phlebotomine fauna in a cavern with cave paintings and its surrounding area and compare the diversity of species in the different ecotopes sampled.

2. Material and methods

2.1. Study area

The municipality of Alcinópolis (18° 19′ 26 "S; 53 ° 42′ 21″ W) is located in the south of the West-Central region of Brazil, in the north of Mato Grosso do Sul, in the Alto do Taquari Microregion, and borders on the state of Mato Grosso (Fig. 1). Alcinopólis has a tropical climate, with two well-defined seasons: hot and humid, with a rainy period extending from October to May, and a dry period from June to September. The average annual temperature is 26 °C (IBGE, 2019; Prefeitura Municipal de Alcinópolis, 2019).

The local economy is based on agriculture, with plantations of pineapple, cotton, rice, banana, rubber, cocoa, bean, sunflower, orange, manioc, corn, soybean, sorghum and wheat. In the livestock sector is based on the breeding of cattle, pigs, horses, sheep and poultry (Prefeitura Municipal de Alcinópolis, 2019).

Alcinópolis has recently been considered the state capital of rock art, attracting researchers and tourists from all over the world. The region has as its main rock art sites: Casa de Pedra, Barro Branco, Templo dos Pilares, Pata da Onça, Arco de Pedra and Gruta do Pitoco (Aguiar et al., 2012). This latter was selected as the area for this present study.

The Gruta do Pitoco (Pitoco Cave) (18° 15′ 72″ S; 53° 38′ 16″ W) has natural architecture and mysterious subterranean passages, and is full of cave paintings (Fig. 1). It is easily accessible and is one of the most

attractive sites in the municipality. Its surroundings consist of areas of dense and well-preserved forest, a factor that guarantees its protected status. The area contains several vestiges of its ancient inhabitants, including paintings made on the inner walls with animal blood and vegetable products that date back 11–12 thousand years (Aguiar et al., 2016; Prefeitura Municipal de Alcinópolis, 2019).

2.2. Phlebotomine collections

The phlebotomine collections were undertaken monthly from March 2014 to September 2015 inclusive, using automatic CDC type light traps installed from 6.0 pm to 6.0 am. The sites sampled consisted of one inside the cave, another at the entrance of the cave, the distance between them being of about 100 m, and the third in the forest area outside the cave about 400 m away from its entrance.

The insects were clarified following the technique described by Forattini (1973) with one modification that consisted in the replacement of the creosote de Faya by eugenol. For the identification, the insects were mounted on slides and observed under the optical microscope. The specimens were identified according to the classification proposed by Galati (2003, 2018) and the abbreviation of generic names follows Marcondes (2007).

2.3. Statistical analyses

To compare the variation in abundance of the most frequent species in the three ecotopes (inside, at the entrance and outside the cave) the Mann-Whitney test was applied.

Sample adequacy was evaluated by plotting sample-based and individual-based species accumulation curves and the total richness was estimated by the Chao 1 method. In both cases 1000 randomizations without any replacement and estimated 95% confidence intervals were implemented. The variation in the diversity between the three ecotopes was measured by the Shannon diversity index (H), considering 1000 random resamplings of 100 individuals in each ecotope and estimated 95% confidence intervals. The species composition similarity between the different ecotopes was evaluated by the Sorensen similarity index. The software EstimateS version 9.1 (Colwell, 2013) was used to make these analyses.

3. Results

The phlebotomine fauna of the Gruta do Pitoco and its external environment consisted of 32 species. *Lutzomyia dispar, Martinsmyia oliverai, Lu. longipalpis* complex (*Lu. cruzi* and *Lu. longipalpis*) were the predominant taxa. A total of 7645 (3912 male and 3733 female) specimens were collected, the highest sandfly density and species richness being obtained in the interior of the cave (Table 1).

Lutzomyia dispar was significantly more abundant inside the cave than at the entrance ($U = 21$; $p = 0.0002$) or outside it ($U = 1$; $p = 0.0001$).

Fig. 2 shows the species accumulation curve per sample for the three environments. Apparently, there is little overlap between the ecotope inside the cave and that outside it. In all three places, there is a tendency to reach asymptote.

In the curve of species accumulation by number of individuals collected (Fig. 3), the maximum simulated number of individuals per environment was 631, which represents the total of sandflies collected in the 'outside' environment, where the lowest abundance was observed. This graphical analysis attempts to simulate the number of species in each environment if the same number of specimens had been collected at all three sites. The graph reveals that, apparently, no great difference has been noted between species richness in the three environments (all curves and intervals are superimposed).

Fig. 4 shows the observed and estimated richness in each of the three ecotopes. The richness observed at the three sites is within the confidence interval for the estimated richness (although the 'inside' point is at the lower limit of the CI). This analysis shows that apparently almost all the species present at the study sites were sampled.

According to the analysis of the diversity index, the interior of the cave presents the highest richness though its diversity is the lowest of the three sites. This is due to the fact that *Lu. dispar* contributed with 46.29% of the specimens collected at this site, resulting in low uniformity and high dominance (Fig. 5).

Regarding the similarity in species composition (based on the Sorensen index) between the environments, it was verified that there is a greater similarity between the interior and the entrance (0.92) of the cave than between the interior and the external environment (0.81).

4. Discussion

The ecological indices analyzed allow the differences in the composition, diversity and richness of the fauna in the different environments to be verified, since most of the species collected occur in all the three environments sampled. Thus, in the analyses of ecotopes, the interior and the entrance of the cave showed a higher population density, being related to the fact that the environment in caves presents less variation than the external ones, which favors the adaptation of the insects to this type of environment, as observed by Carvalho et al. (2013). The similarity indices of the species collected in the environments sampled are coherent with the other results presented above.

During the study period, a high richness of phlebotomine species was found; however, the highest population density occurred with the *Lutzomyia* species, a fact which had also been observed in other caves, in the states of Minas Gerais (Barata et al., 2008; Barata and Apolinário, 2012; Carvalho et al., 2013; Campos et al., 2017) and Mato Grosso do Sul (Galati et al., 2003). In this latter state, the second most frequent species was *Mt. oliverai*, which also presented high frequencies in the Bodoquena caves (Galati et al., 2003).

The high abundance of *Lu. dispar* in the interior and cave entrance environments was also noted. Some studies have shown that this species presents highly anthropophilic behavior, being also associated with rocky environments, attracted by the black Shannon trap (Infran et al., 2017) and its type-locality is, further, a cave region (Williams and Carvalho, 1979). Infran et al. (2017) carried out collections with Shannon traps in a transitional zone of Paleozoic highlands where a predominance of *Lu. dispar* females was observed. However, the Shannon traps tend to attract more females. These authors also verified that this species has nocturnal habits. In our study, we used CDC traps, males and females collected in approximately the same proportion. Furthermore, in the municipality of Camapuã, the type-locality of *Lu. dispar*, in the state of Mato Grosso do Sul (Williams and Carvalho, 1979), these authors reported the capture of females of this species biting humans, horses and dogs, thus they considered them eclectic blood-feeders. Despite its anthropophily and habit of biting other *Leishmania* hosts, a behavior which could classify it as a potential *Leishmania* vector, there are no records of its natural infection by any *Leishmania*.

The caves are a favorable environment for sandflies, as regards both shelter and food, usually related to the presence of bats, rodents and cold-blooded animals such as frogs, snakes and lizards (Galati et al., 2006). Although there are species living exclusively in cave environments (troglobiontes) as reported for *Deanemyia maruaga* (Alves et al., 2011), for some sandfly species the environment around the caves also offers favorable conditions for cave species (troglophyles). On the other hand, the caves may often provide shelter or sources of food for sandfly species that have their habitats outside the caves, trogloxenes (Carvalho et al., 2013). The presence in the spelological environment of troglobionte, troglophilous and trogoxenic species can result in a highly diverse cave fauna of which new species have frequently been described (Galati and Nunes, 1999; Alves et al., 2008; Carvalho et al., 2012; Vilela

Table 1
Phlebotomine fauna collected in the Pitoco Cave and its external environment, Alcinópolis municipality, Mato Grosso do Sul state, Brazil, from March 2014 to September 2015.

Ecotopes	Inside the cave				Cave entrance				Area outside the cave				Total			
Species/Sex	M	F	ST	%	M	F	ST	%	M	F	ST	%	M	F	ST	%
Br. avellari	102	64	166	2.75	44	27	71	2.58	66	74	140	22.20	212	165	377	4.00
Br. brumpti	2		2	0.03									2		2	0.02
Br. pintoi	16	11	27	0.44	5		5	0.19	3	2	5	0.80	24	13	37	0.40
Brumptomyia sp.		9	9	0.15										9	9	0.09
Deanemyia sp.		10	10	0.16		4	4	0.14						14	14	0.14
Ev. lenti	174	101	275	4.54	67	45	112	4.06	38	38	76	12.04	279	184	463	4.90
Ev. saulensis	1	102	103	1.70	6	55	61	2.21		5	5	0.80	7	162	169	1.80
Ev. teratodes						3	3	0.10		2	2	0.31		5	5	0.05
Lu. longipalpis complex		286	286	4.73		458	458	16.60		17	17	2.70		761	761	8.06
Lu. cruzi	228		228	3.77	375		375	13.60	28		28	4.43	631		631	6.70
Lu. dispar	1330	1468	2798	46.29	305	330	635	23.01	11	22	33	5.23	1646	1820	3466	36.73
Lu. longipalpis	247		247	4.10	260		260	9.42	15		15	2.38	522		522	5.53
Mi. acanthopharynx	3	23	26	0.43		5	5	0.19		1	1	0.15	3	29	32	0.34
Mi. echinatopharynx		5	5	0.08		1	1	0.03						6	6	0.06
Mi. peresi		1	1	0.02										1	1	0.01
Mi. quinquefer	174	284	458	7.58	76	115	191	6.92	9	18	27	4.30	259	417	676	7.16
Mi. vonatzingeni	14	40	54	0.90	2	34	36	1.30		1	1	0.15	16	75	91	0.97
Mt. oliveirai	411	395	806	13.33	103	125	228	8.26	16	31	47	7.45	530	551	1081	11.46
Ny. whitmani	93	75	168	2.77	69	55	124	4.50	3		3	0.47	165	130	295	3.12
Pa. abonnenci	1		1	0.02	1		1	0.03					2		2	0.02
Pa. aragaoi	39	33	72	1.20	34	45	79	2.87	20	22	42	6.65	93	100	193	2.04
Pa. bigeniculata	20	17	37	0.61	10	8	18	0.65	12	11	23	3.64	42	36	78	0.82
Pa. brasiliensis	9	9	18	0.30	11	5	16	0.59	1	2	3	0.48	21	16	37	0.40
Pa. campograndensis	20	11	31	0.51	5	1	6	0.21		1	1	0.15	25	13	38	0.41
Pa. punctigeniculata	2		2	0.03		1	1	0.03					2	1	3	0.03
Pi. christenseni		4	4	0.06		4	4	0.14	13	11	24	3.80	13	19	32	0.34
Pi. kuscheli									1	5	6	0.95	1	5	6	0.06
Ps. davisi	1	11	12	0.20	14	7	21	0.77	87	40	127	20.12	102	58	160	1.70
Sc. sordellii	57	143	200	3.30	21	23	44	1.60		5	5	0.80	78	171	249	2.64
TOTAL	2943	3102	6045	100	1408	1351	2759	100	323	308	631	100	4674	4761	9435	100
H		2.01				2. 36				2.40				2. 27		
J		0.60				0.73				0.77				0.67		

M: Male; F: Female; ST: Subtotal; H: Shannon index; J: Pielou index;%: percentage. *Br.: Brumptomyia; Ev.: Evandromyia; Lu.: Lutzomyia; Mi.: Micropygomyia; Mt.: Martinsmyia; Ny.: Nyssomyia; Pa.: Psathyromyia; Pi.: Pintomyia; Ps.: Psychodopygus; Sc.: Sciopemyia.*

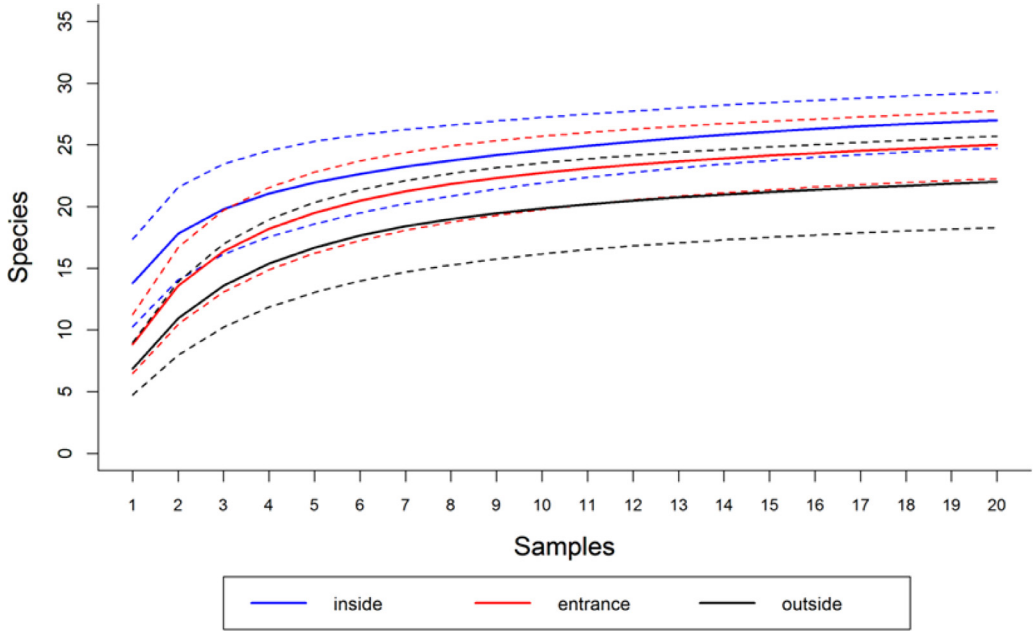

Fig. 2. Sample-based species accumulation curves with a 95% confidence interval for phlebotomine sandflies in ecotopes inside, at the entrance and outside of the cave, Alcinópolis municipality, Mato Grosso do Sul State, Brazil, 2014–2015.

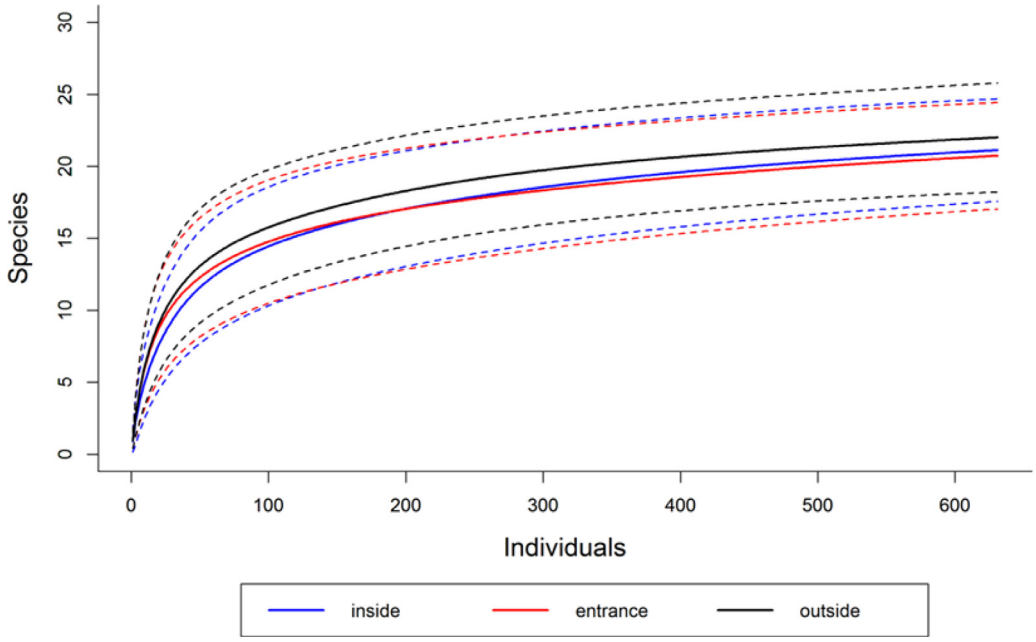

Fig. 3. Individual-based species accumulation curves with a 95% confidence interval for phlebotomine sandflies in the ecotopes inside, at the entrance and outside of the cave, Alcinópolis municipality, Mato Grosso do Sul State, Brazil, 2014–2015.

et al., 2015).

The species of the *Lu. longipalpis* complex (*Lu. cruzi* and *Lu. longipalpis*) occurred in sympatry in the interior of and entrance to the cave; they are species of great epidemiological importance because they are proven vectors of *Leishmania infantum* in Mato Grosso do Sul state, and they may also be permissive in the transmission of L. *amazonensis* (Pita-Pereira et al., 2008; Savani et al., 2009; Dorval et al., 2016; Oliveira et al., 2017a; 2017b).

The species *Mt. oliveirai* and *Sc. sordellii* were frequent in this research. In regard to *Mt. oliveirai,* little is known about their food habits, some authors suggesting they are associated with rodents (Galati et al., 2003). However, *Sc. sordellii* is reported to prefer cold-blooded animals, this species having been reported naturally infected by trypanosomatids from frogs (Ferreira et al., 2008). For neither species is there any record of natural infection by *Leishmania*.

Mi. quinquefer was also found in our collections, this species has also been associated with cold-blooded animals. In Mato Grosso do Sul it was found to be positive by molecular techniques for *Leishmania* DNA (Paiva et al., 2010), suggesting that this species may be seeking different blood sources. Among the other species of *Micropygomyia* collected in Pitoco cave, deserve attention, *Mi. echinathopharynx* for its first register in Mato Grosso do Sul.

Species of *Brumptomyia,* a genus frequently found in burrows of Dasipodidae, are common to the cavernicolous environments (Galati et al., 2010).

Other species collected in lesser proportion, though important vectors or permissive vectors of etiological *Leishmania* spp. were those of the genera *Psychodopygus* and *Ny. whitmani*. According to information from the epidemiological surveillance service of the municipality, Alcinópolis has two human cases of visceral leishmaniasis (VL), 10

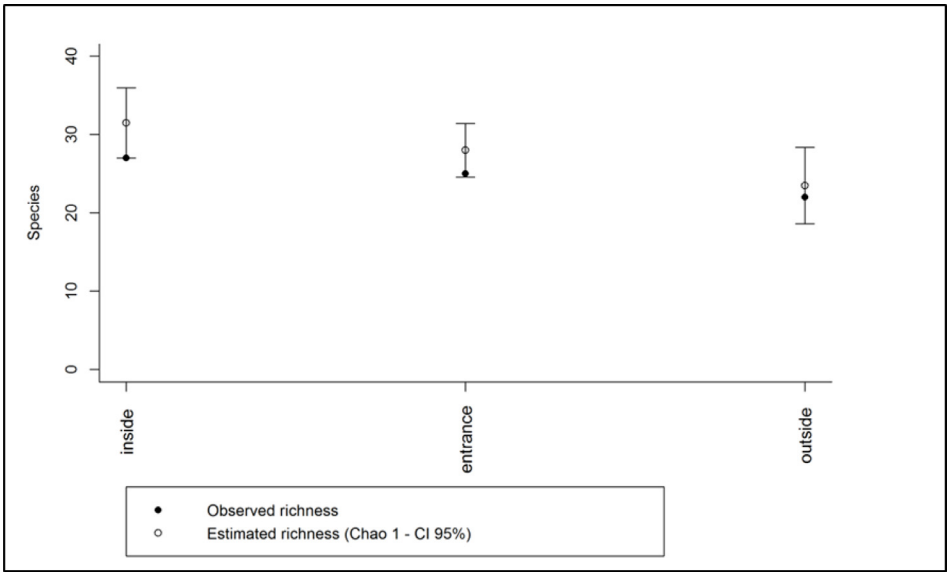

Fig. 4. Observed and estimated species richness of phlebotomine sandflies collected in the three ecotopes: inside, at the entrance and outside of the cave, Alcinópolis municipality, Mato Grosso do Sul State, Brazil, 2014–2015.

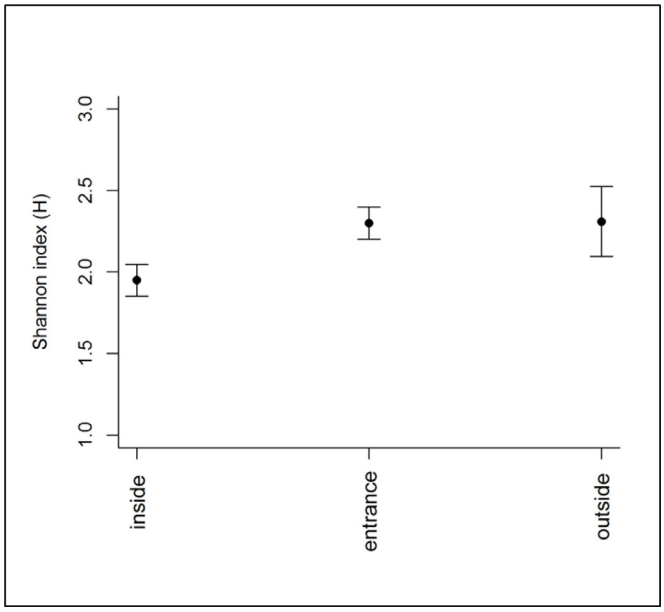

Fig. 5. Comparison of Shannon diversity index (H) for phlebotomine sandflies collected in the three ecotopes: inside, at the entrance and outside of the cave, Alcinópolis municipality, Mato Grosso do Sul State, Brazil, 2014–2015.

cases of cutaneous leishmaniasis and various canine cases of VL, so these findings deserve the attention of the health authorities, given the occurrence of these vectors.

In this study, we report the presence of *Deanemyia* sp. for the first time in Mato Grosso do Sul. Species of this genus have been collected in caves or xeric environments (Freitas and Barret, 1999; Alves et al., 2011; 2008). Of the five species that constitute this genus, *De. maruaga* is considered parthenogenetic and autogenous (Alves et al., 2011) and *Deanemiya appendiculata* is described only on the basis of the male (Galati, 2018). Therefore, as only females were collected it was impossible to identify them at the species level.

5. Conclusion

Considering the role of sandflies in the transmission cycles of leishmaniasis, the occurrence of vectors and permissive species, and the tourist potential of the region that attracts people from all over the world, the surveillance and monitoring of these areas are necessary to prevent risks to visitors. The high prevalence of *Lu dispar*, a highly anthropophilic species, in the cave can represent a serious annoyance to the visitors to the area. In addition, this study contributes to the knowledge of the diversity of phlebotomine fauna in cave ecosystems, because there are few studies of these types of environment.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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