

Diversity of sandflies (Psychodidae: Phlebotominae) captured in sandstone caves from Central Amazonia, Brazil

Veracilda Ribeiro Alves^{1/+}, Rui Alves de Freitas², Francisco Lima Santos², Toby Vincent Barrett¹

¹Laboratório de Triatominae, Phlebotominae e Fauna Nidícola, Coordenação de Pesquisas em Entomologia

²Laboratório de Leishmanioses, Coordenação de Pesquisas em Ciências da Saúde, Instituto Nacional de Pesquisas da Amazônia, Av. André Araújo 2936, 69083-970 Manaus, AM, Brasil

In the present paper we describe the diversity of phlebotomine sandflies collected in three sandstone caves in the municipality of Presidente Figueiredo, state of Amazonas, Brazil. The phlebotomines were captured during 2006 with CDC light traps. Guano samples from inside the Gruta Refúgio do Maruaga were collected to investigate the presence of immature specimens. A total of 2,160 adult phlebotomines representing 15 species were captured. Pinto-myia paca was the dominant species in Gruta dos Animais (1,723 specimens) and Gruta dos Lages (50 specimens) and Deanemyia maruaga new comb (280 specimens) was the dominant species in Gruta Refúgio do Maruaga. A total of 18 guano samples were collected and seven of these samples included immature specimens. A total of 507 immature specimens were captured; 495 of these specimens were larvae and 12 were pupae. The presence of paca (Agouti paca) footprints near Gruta dos Animais and Gruta dos Lages suggests the association of Pi. paca with this rodent. This finding may explain the abundance of Pi. paca in these locations, while the species is relatively rare in the forest. Deanemyia maruaga is a cave species that uses guano to breed during its immature stages. Adult specimens of this species are apparently parthenogenetic and autogenous and represent the second record of parthenogenesis for the subfamily Phlebotominae.

Key words: phlebotomine - cave fauna - parthenogenesis - immature - breeding site

Sandflies are small Diptera which can transmit bacteria, viruses and trypanosomatids to humans and animals. The transmission occurs during the bite of female insects. Among the trypanosomatids transmitted, the species of the genus *Leishmania* Ross, 1903 are responsible for causing visceral and cutaneous leishmaniasis (Rangel & Lainson 2003). In general, the period of activity of phlebotomine sandflies is from dusk to dawn and these insects remain most of the day at rest in natural shelters (Sherlock 2003).

Caves are places characterized by the absence of light in areas far from the entrance and present stable climate compared to the external environment (Poulson & White 1969). Fauna adapted to these conditions can be classified according to their level of adaptation: troglóbites (animals that present unique modifications to cave environments), troglófiles (adapted animals, but devoid of modifications that can also develop in the external environment) and troglóxenes (animals that use caves for shelter or refuge) (Holsinger & Culver 1988).

Although caves are apparently inhospitable environments for sandflies, some studies have shown that caves may harbour sandflies (Galati et al. 2003a, b, Alves et al. 2008); occurrence of morphological anomalies (Andrade-Filho et al. 2004) shows that insect diversity and density of sandflies in caves can be equal to or greater than those found in the forest (Galati et al. 2003c, 2006).

The municipality of Presidente Figueiredo, state of Amazonas (AM), Brazil, includes areas for ecotourism, such as waterfalls and caves. Among the latter, the Gruta Refúgio do Maruaga and Gruta dos Animais sandstone caves date back thousands of years and are regarded as rare relics of the Palaeozoic Era (Karmann 1986).

The aim of the present work was to study the diversity, period of activity and abundance of phlebotomine sandflies captured in three sandstones caves in the municipality of Presidente Figueiredo.

MATERIAL AND METHODS

Study area - Presidente Figueiredo municipality, where the caves are found, is located northern of municipality of Manaus at km 107 of BR-174 highway, connecting Manaus to Boa Vista, in state of Roraima. The Gruta Refúgio do Maruaga is located at km 6 of highway AM-240 (02°03'02"49S 59°57'48"85W) 600 m on the right side of the roadway. The cave Gruta dos Animais (02°03'02"64S 59°57'51"47W) is near Gruta Refúgio do Maruaga and the Gruta dos Lages (01°59'41"3S 60°01'36"5W) is located to the left side of 113 km of BR-174 highway.

Sampling of adults - Samples were collected during 2006 inside the caves with CDC traps placed 10 m equidistant from each one another, with the first at a distance of 10 m from the entrance, the traps operated for 48 h continuously and the samples were collected every 12 h and separated in night-time (18:00 pm-6:00 am) and day-time (6:00 am-18:00 pm) periods. The period of capture and sampling effort of the sandflies were not the same in all areas due to the distance between Gruta dos Lages and the other caves. Measures of instantaneous temperature and relative humidity were performed us-

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+ Corresponding author: verabioufnt@yahoo.com.br

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ing a digital thermo-hygrometer (Minipa/MTH-1361). In Gruta dos Animais it was not possible to assess measures of temperature and humidity due to saturation of local humidity. Inside Gruta Refúgio do Maruaga measures were taken at five points, from entrance into the final chambers and the average temperature and humidity were calculated.

The nomenclature of species follows Galati (2003) and the abbreviation of the genera follows Marcondes (2007).

Gruta dos Animais - The specimen collections were conducted during the months of February-April using five traps. The first trap was 10 m away from the entrance of the cave, the second and third traps were separated by approximately 10 m and the fourth and fifth traps were at a distance of 80 m and 120 m, respectively.

Gruta dos Lages - The collection was made in May. Eight traps were randomly distributed during the night (3 nights); luminosity inside the cave prevented the use of the light traps during day-time.

Gruta Refúgio do Maruaga - The collections were made during four months in the period from February-May. A total of 14 traps (February) and 20 traps (March-May) were used, the increase in the number of traps was due to the higher abundance of sandflies at the end of the cave. All captured specimens were identified after clarification process (Young & Duncan 1994). For *Deaneomyia maruaga*, other techniques were used to clarify and are discussed in Alves et al. (2008), as well as a description of the biology of adults.

Statistical analysis - The frequency of the insects that were captured during the simultaneous periods that occurred during nocturnal or diurnal periods in March, April and May 2006 were obtained using Williams' geometric mean (Haddow 1960). To determine whether there was a significant difference in the distances between the collection points 2-5 in Gruta dos Animais for *Pintomyia paca*, between the collection points for *De. maruaga*, and for periods day-time and night-time in Gruta Refúgio do Maruaga, nonparametric Kruskal-Wallis test were performed by using PAST software, version 1.92 (Hammer et al. 2001).

Sampling of immature - Guano collections were made in 12 points of the guano deposit at the final chambers of the Gruta Refúgio do Maruaga during the months of March-May and September. Each guano sample weighed approximately 300-500 g of guano. A total of 15 samples from March-May and three from September were taken. The remaining samples were analyzed by flotation technique (Hanson 1961). Larvae and pupae were reared in the laboratory, according to the methods of Killick-Kendrick et al. (1977) with supplementation of the medium with autoclaved guano.

RESULTS

In Gruta dos Animais, 1,770 specimens were captured and belonged to 12 species, in Gruta Refúgio do Maruaga, 298 specimens of seven species and in Gruta dos Lages, 91 specimens of seven species (Table I).

In Gruta dos Animais, 1,426 and 297 individuals of *Pi. paca* were collected during night-time and day-time periods, respectively. At night, the specimens of this species were captured along the entire length of the cave while during the day the insects were collected near the entrance of the cave (Table II). *Pi. paca* was the dominant species in both periods.

In Gruta dos Lages, of the 91 specimens captured the most representative species were *Pi. paca* (50), *Evanromyia georgii* (18) and *Sciopemyia sordellii* (11).

In Gruta Refúgio do Maruaga, 280 specimens were captured and identified as *De. maruaga*, of which, 176 were collected during night-time and 104 during day-time. During the night, this species was captured along nearly the entire length of the cave. In the day-time, it was only collected before 20 and after 120 m from the cave entrance (Table III).

The areas of greatest concentration of *De. maruaga* in Gruta Refúgio do Maruaga were between 120-200 m from the cave entrance, with a higher peak during the night, between 190-200 m, at sites located at the end of the cave. This result is confirmed by comparison between the Williams' geometric mean of the gradient of the absolute frequency in Gruta Refúgio do Maruaga during the simultaneous period of capture between March-May 2006 (Figure).

For *Pi. paca*, the Kruskal-Wallis test showed no significant difference for the values between points P2-P5 in Gruta dos Animais ($H = 4.003$, $df = 4$, $p = 0.406$). Similarly, the analysis of the distance between sampling points and the day-time and night-time periods in Gruta Refúgio do Maruaga for the species *De. maruaga* was not significant ($H = 0.1538$, $df = 4$, $p = 0.6949$).

The search for immature sandflies from samples collected in Gruta Refúgio do Maruaga resulted in a total of 507 specimens of which 495 (97.6%) were larvae, mostly at third and fourth instars, and 12 (2.4%) were pupae. Of the 18 samples, seven were positive for immature *De. maruaga* (39%). The average time between the recovery of the larvae to adult emergence was approximately 70 days. Approximately 30 adult females were separated in rearing pots to lay eggs. Each female laid an average of eight eggs, but batches of 12 and 23 eggs were also observed. Some eggs were found stranded on the abdomen of the females after laying. The eggs took an average of 21 days to hatch. Approximately 98% of larvae died during the first 15 days (1st instar). Only one completed the life-cycle from egg to adult in 90 days, in laboratory conditions.

DISCUSSION

The sandflies species collected in the caves reflect the known fauna of continuous forest in the municipalities of Presidente Figueiredo and Balbina (Barrett 1993), except for *De. maruaga*. However, *Pi. paca* is rarely captured in the forest showing a low frequency in captures with CDC traps (Barrett 1993, Dias-Lima et al. 2003). On the other hand, *Pi. paca* was the dominant species in Gruta dos Animais and Gruta dos Lages. This species' presence in these caves might be associated with the presence of paca (*Agouti paca*), which might act as a

TABLE II
Phlebotomine captured in Gruta dos Animais, Presidente Figueiredo, Amazonas, Brazil,
by collection site in day-time (DT) and night-time (NT) periods from February-April 2006

Species	P1		P2		P3		P4		P5	
	NT	DT	NT	DT	NT	DT	NT	DT	NT	DT
<i>Nyssomyia anduzei</i>	-	-	1	-	-	-	1	-	-	-
<i>Psychodopygus davisi</i>	-	-	-	-	-	-	1	-	-	-
<i>Psathyromyia cuzquena</i>	1	-	-	-	-	-	-	-	-	-
<i>Bichromomyia flaviscutellata</i>	1	-	-	-	-	-	-	-	-	-
<i>Evandromyia inpai</i>	-	-	-	-	-	-	-	-	-	1
<i>Evandromyia monstrosa</i>	-	-	3	-	-	-	-	-	-	-
<i>Pintomyia pacaе</i>	1,285	297	41	-	49	-	31	-	20	-
<i>Evandromyia sericea</i>	-	-	1	-	-	-	-	-	-	-
<i>Micropygomyia pilosa</i>	-	-	1	-	-	-	-	-	-	-
<i>Sciopemyia pennyi</i>	25	-	1	-	-	1	-	-	-	-
<i>Sciopemyia sordellii</i>	4	1	2	-	-	-	-	-	1	-
<i>Nyssomyia umbratilis</i>	-	-	1	-	-	-	-	-	-	-
Total	1,316	298	51	-	49	1	33	-	21	1

distance of the light traps from the mouth Gruta dos Animais: P1: 10 m; P2: 20 m; P3: 30 m; P4: 80 m; P5: 120 m.

TABLE III
Abundance of sandflies species collected in Gruta Refúgio do Maruaga, Presidente Figueiredo, Amazonas, Brazil,
during night-time and day-time periods, from February-May 2006

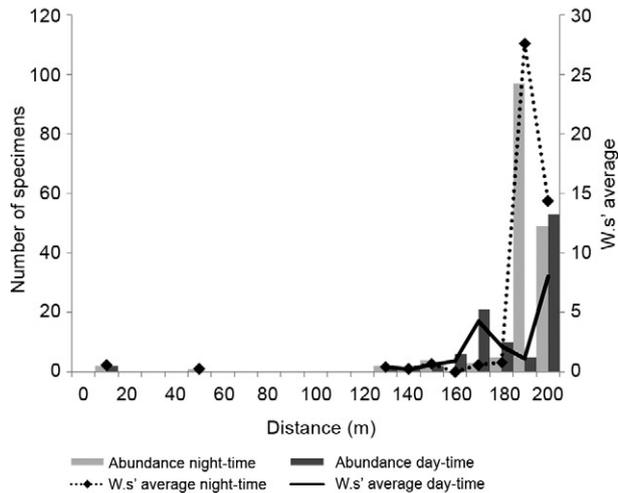
Species	Distance (m)											
	10	20	50	120	130	140	150	160	170	180	190	200
Night-time												
<i>Nyssomyia anduzei</i>	1	-	-	1	-	-	-	-	-	-	-	-
<i>Bichromomyia flaviscutellata</i>	1	5	-	-	-	-	-	-	-	-	-	-
<i>Pintomyia pacaе</i>	2	1	-	-	-	-	-	-	-	-	-	-
<i>Micropygomyia pilosa</i>	-	1	-	-	-	-	-	-	-	-	-	-
<i>Evandromyia sericea</i>	1	-	-	-	-	-	-	-	-	-	-	-
<i>Sciopemyia sordellii</i>	3	2	-	-	-	-	-	-	-	-	-	-
<i>Deanemyia maruaga</i>	6	4	1	1	2	4	4	-	3	5	97	49
Day-time												
<i>Deanemyia maruaga</i>	2	-	-	-	2	3	2	6	21	10	5	53

source of food for this sandfly. In the two caves, recent footprints of this rodent were found, suggesting that the rodents use these caves as shelter explaining the abundance of *Pi. pacaе* in these environments.

De. maruaga is a species restricted to Gruta Refúgio do Maruaga, being the areas of greatest occurrence is 160-200 m from the cave entrance, where the main deposit of guano and breeding site are also located. A study (Barrett 1993) carried out in this region recorded

68 species of sandflies, but no records of the *De. maruaga* were found. Furthermore, in three days of night capture in February 2006 outside entrance of the cave, no specimen of *De. maruaga* was collected implying that it is restricted to the inside in Gruta Refúgio do Maruaga.

De. maruaga is the first sandfly species restricted to caves described from Brazil. Immature and adult specimens collected in this study developed in the aphotic zone of Gruta Refúgio do Maruaga, with a high humid-



Monthly Williams' (W.s.) average and abundance of *Deanemyia maruaga* captured in Gruta Refúgio do Maruaga, Presidente Figueiredo, Amazonas, Brazil, during day-time and night-time periods in March, April and May 2006.

ity (about 95%) and an average temperature of 26°C and used guano as breeding site. The observation of immature specimens of *De. maruaga* breeding in guano in Gruta Refúgio do Maruaga results in the first record of a natural breeding site under in this conditions for Brazil. Galati et al. (2003c), who studied sandflies in the Lago Azul cave (state of Mato Grosso do Sul), detected three third instar larvae of *Lutzomyia almerioi* on the floor of the base of the cave wall suggesting that the breeding site could be found at this location. Most records of breeding sites referred to the bases of trees as potential sites for the development of immature because they are rich in organic matter (Hanson 1961, Sherlock 1962, Feliciangeli 2004). Of the 18 guano samples analyzed, 39% were positive for immature sandflies; this number being higher than that found by Hanson (1961) in Panama (20.4%) and by Ghosh and Bhattacharya (1991) in India (11.45%). This is probably due to the inherent characteristics of the micro-habitat and specificity of the larva. Thus, the number of immature collected and the frequency of positive samples collected are relevant for establishing the natural breeding site of this species.

De. maruaga has several morphological and physiological peculiarities regarding the egg, larva, adult, and its life cycle. The egg colour is gray, whereas the eggs of other sandfly species are dark coloured (Feliciangeli et al. 1993, Young & Duncan 1994). This difference in colour may be due to the fact that this species evolved in caves in the aphotic zone with no need for protection from sunlight for the development of the larva. In fact, hours before the eggs hatch the larvae can be observed inside the egg. In the laboratory, the number of eggs per clutch for each female of *De. maruaga* was variable and was generally between 6-12 eggs; however, there were instances of 23 eggs. Presumably, the reduced number of eggs is caused by artificial rearing and feeding condi-

tions. In other species of sandflies, the number of eggs per clutch ranges from 40-70 eggs, depending on various factors, such as the size of the species, the acquisition of a prior blood meal and the diet (Young & Duncan 1994). The incubation period for the eggs of *De. maruaga* was approximately 21 days. Young and Duncan (1994) argue that the normal incubation period for sandflies species is approximately 10 days. However, this period may extend up to 30 days or more if the optimal conditions are not present. Brazil and Oliveira (1999) obtained an average of 11 days for the egg development of *Pintomyia mamedei* and a fecundity of 28 eggs per female. This value was fewer than the values reported by Young and Duncan (1994) but was similar to that for *De. maruaga* if we consider the number of eggs that are retained in the abdomen, as these eggs would likely develop fully in natural conditions.

The larva has a pair of caudal setae on the first instar and two pairs of setae on the following instars of different lengths. The upper pair of setae is approximately twice the length of the lower, which is a distinct characteristic for the larvae of the known sandfly species that have two pairs of caudal setae of similar size (Young & Duncan 1994). The sandfly species that were studied in caves in Africa exhibited similar characteristics. For example, *Phlebotomus mirabilis*, which breeds its immature stages in guano in the deep, dark areas of caves, has a lower pair of long caudal setae that are short in the first instar (Vattier-Bernard 1971a). The pattern of the body setae of the larvae of *De. maruaga* is also different from that of the other described American species, resembling the setae of *Sergentomyia hamoni* (Trouillet 1979). In the present study, the first instar larva had a period of development of approximately three weeks, the second, third and fourth instar had a developmental period of approximately 15 days each and the pupa had a developmental period of approximately 15 days. According to Young and Duncan (1994), the length of the larval period of several species of sandflies may depend on the weather conditions (cold or dry) and may range from 18 days to several months. In these species, the pupal period varies from seven to 12 days. Brazil and Oliveira (1999) reported an average of 27.6 days for the larval and pupal stages for *Pi. mamedei*, with an average of 10.8 days (6-21 days).

The females of *De. maruaga* have a visible reserve of fat in the abdomen, which is not commonly observed in other sandfly species. A few days (\pm 5-6 days) after the emergence of the adult, the development of eggs and a decrease in the amount of the fat reserves was observed. Many insects do not feed as adults; they depend on fat, protein and glycogen that have been deposited in the fat body during their development. In addition, these glycogen deposits can be quickly mobilised to release glucose in response to hormones (Rupert et al. 2005).

Autogeny has been observed in several species of Culicidae (O'Meara & Kranisck 1970, O'Meara & Evans 1977), Tabanidae (Charlwood & Rafael 1980) and sandflies (Feliciangeli et al. 1993, Brazil & Oliveira 1999). This is a phenomenon that occurs in haematophagous

insects that allows for the development of eggs without a prior blood meal, depending on the insect's diet during the larval period (O'Meara & Kranisck 1970, Perondini et al. 1975). The autogenic behaviour observed in *De. maruaga* may be explained by the fact that guano is rich in nutrients, enabling the larva to feed adequately and to produce fat reserves that are sufficient to maintain the adult and to enable reproduction.

De. maruaga presented a life span that exceeded 90 days in the laboratory. For another species of sandfly, *Nyssomyia umbratilis*, Justiniano et al. (2004) obtained an average of 77 days for this species' life cycle from two populations in AM. However, the life cycle of other Neotropical species ranged from 34-54 days (Sherlock & Sherlock 1959, Killick-Kendrick et al. 1977, Ward 1977, Ferro et al. 1998, Brazil & Oliveira 1999). For *P. mirabilis*, an African cave species, the average life cycle duration was 110.6 days in the laboratory that was erected inside of the cave, which is lower than the average that was obtained from the colony of wild females (133.7) (Vattier-Bernard 1971a). This author also suggests that the life cycle is longer in authentic cave species (troglobites) than in other species (Vattier-Bernard 1971b). The environment in which *P. mirabilis* was found has abiotic characteristics (temperature of approximately 25°C and relative humidity between 96-100%) that are similar to the natural habitat of *De. maruaga*.

The findings about the breeding site of *De. maruaga*, this species' life cycle inside the Gruta Refúgio do Maruaga and the morphological and physiological changes of this species indicate that this specimen can be classified as a troglobite. There is no known record of other Neotropical sandfly species with their life cycle restricted to the inside of a cave. The development of eggs in the absence of males and without a prior blood meal in this species is the second record of parthenogenesis in conjunction with autogeny within the subfamily Phlebotominae; the first instance was described by Brazil and Oliveira (1999) for *Pi. mamedei*.

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