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ECOLOGY OF *CAVERNICOLA PILOSA* BARBER, 1937 (HEMIPTERA: REDUVIIDAE: TRIATOMINAE) IN THE BOA ESPERANÇA CAVE, TOCANTINS, BRAZIL

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INTRODUCTION

Cavernicola pilosa (Hemiptera, Reduviidae, Triatominae) is a hematophagous insect (Barber 1937), invariably found closely related with bats inhabiting caves or tree cavities in either humid or dry tropical regions of Panama and South America (Brazil, Colombia, Ecuador, Peru, and Venezuela), at latitudes between 9°15' N and 23°18' S, and altitudes ranging from 140 to 1160 m a.s.l. (Barber 1937, Dias *et al.* 1942, Marinkelle 1966, Pipkin 1968, D'Alessandro *et al.* 1971, Carcavallo *et al.* 1976, Lent & Wygodzinsky 1979). There are reports of the presence of *C. pilosa* in human dwellings, inhabited or not (Pipkin 1968, Gomes & Pereira 1977, Barbosa *et al.* 2005), always associated with bats. Their close association with bats suggests that these are the only food source for *C. pilosa* in nature (Dias *et al.* 1942, Marinkelle 1966, D'Alessandro *et al.* 1971, Carcavallo *et al.* 1976), and according to these authors *C. pilosa* refuses any other blood source under laboratory conditions.

According to Dias *et al.* (1942), Marinkelle (1966), Pipkin (1968), D'Alessandro *et al.* (1971), Carcavallo *et al.* (1976), and Baker *et al.* (1978), *C. pilosa* is the vector of several *Schizotrypanum* species, kinetoplastid parasites of bats, including one that is morphologically identical to *Trypanosoma* (*Schizotrypanum*) *cruzi* (Chagas 1909) that causes a non-detectable infection in mice and guinea pigs. Neither do they cause a permanent infection in other *T. cruzi* triatomine vectors, except for *Rhodnus prolixus* under experimental conditions.

In order to provide further information on the natural environment of this triatomine species we describe in the present paper a cave that shelters thousands of bats together with a large triatomine colony. The maintenance of a colony in the lab supplied additionally useful information about the biology of the species.

MATERIAL AND METHODS

Study area. *C. pilosa* was collected in a cave located at Boa Esperança farm, Taquaruçu district, rural area of Palmas city, Tocantins state, Brazil, at 10°22.921'S 48°03.587'W, altitude 504 m. The climate in the area is characterized by a period with high rainfall (September to May) and another dry and cooler period, including up to two months with no rainfall. The annual pluviometric index is 1600 to 2100 mm, average annual temperature ranges from 24 to 26° C, and relative humidity is between 80 and 85% (Ministério

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das Minas e Energia 1981). The landscape is characterized by savanna formations known as *cerrado* fields in Central Brazil. Geologically, the region is located in the contact zone between Silurian-Devonian sediments of the Parnaíba basin, with gneissic and granitoid rocks in the regional silicate substrate (Ministério das Minas e Energia 1981). Ferruginous laterite fragments are widespread, and, after erosion, those iron minerals form the cavernous spaces which the bugs inhabit. A ferruginous canga substrate coats the Boa Esperança cave, characterized by fragments of various dimensions and shapes, linked by iron hydroxide. In the rainy season the cave walls are wet from water sources in parts of the ceiling.

Collections. *C. pilosa* was collected during the dry (July) and rainy (December) seasons. On the first visit, nine bats and 49 *C. pilosa* (11 females, 10 males, and 28 nymphs of different instars) were captured, and the insects were kept in receptacles with relative humidity maintained at about 80%. To assess the collected bats for trypanosomatid infection, xenodiagnosis was carried out in the field using third instar nymphs of *R. prolixus* (Bronfen *et al*. 1989), and a drop of blood was also collected for analysis of Giemsa-stained blood smears. Dead bats were transported to the laboratory immersed in alcohol for identification. On the second visit to the cave, 44 *C. pilosa* (14 females, seven males, and 23 nymphs) were collected.

C. pilosa rearing. The specimens were placed into acrylic containers with round-crumpled filter papers inside, in order to provide hiding places and oviposition substrate for the bugs. Swiss Webster mice (*Mus musculus*) were anesthetized, immobilized between two nylon screens, and then placed into the container for four hours. Blood feeding was offered three times per week. The containers were kept in closed plastic boxes, maintaining the humidity between 80 and 95% at 26 ± 1° C.

C. pilosa infection with Trypanosomatidae. Nine of the field-collected *C. pilosa* had their intestinal content analyzed for trypanosomatid infection using the same protocol utilized for the xenodiagnosis. Eight intestinal contents with positive results were cultured according to Chiari & Brener (1966), and maintained up to five months. Five mice (lineage Swiss Webster/ 25g) were inoculated intraperitoneally with metacyclic forms of one of the *C. pilosa* specimens. Parasitemia levels were followed from the third day up to the 35th day after inoculation. Another five C3H mice, of approximately 13 g weight, isogenic and more susceptible to *T. cruzi* than Swiss Webster mice (Bloom 1979, Tarleton *et al*. 1981), were inoculated with trypomastigote culture forms, and the parasitemia followed up according to Brener (1961). At 25 days of infection, the blood of all C3H mice was seeded into culture tubes containing NNN medium, analyzed weekly from the 2nd day after seeding, and maintained for two months using the same culture medium (cultures did not persist in LIT medium).

Characterization of the natural environment of C. pilosa. Measurements of altitude, longitude and latitude were taken, the cave area mapped by means of a sketch map (Fig. 1), and the fauna specimens were identified. Average annual temperatures and pluviometric indices in the region were provided by RADAMBRASIL Project (Ministério das Minas e Energia 1981). During the captures, temperature and air relative humidity were noted at several sites in the cave, as well as outside, using a digital Hygrotherm, maximum / minimum, –10° C to 60° C, 10% to 99%, resolution + 0,1° C and 1% UR, accuracy + 1° C and + 5%.

TABLE 1. Temperature and air relative humidity measurements taken inside the cave and in the outside environment.

	Inside environment		Outside environment	
Season	Temperature (°C) X ± sd	Humidity (%) X ± sd	Temperature (°C) X ± sd	Humidity (%) X ± sd
dry	30.7 ± 0.5	67.7 ± 6.0	29.5 ± 2.5	57.5 ± 7.5
rainy	31 ± 0.7	74.8 ± 0.8	30 ± 2.5	83 ± 3,4

X ± sd = average ± standard deviation

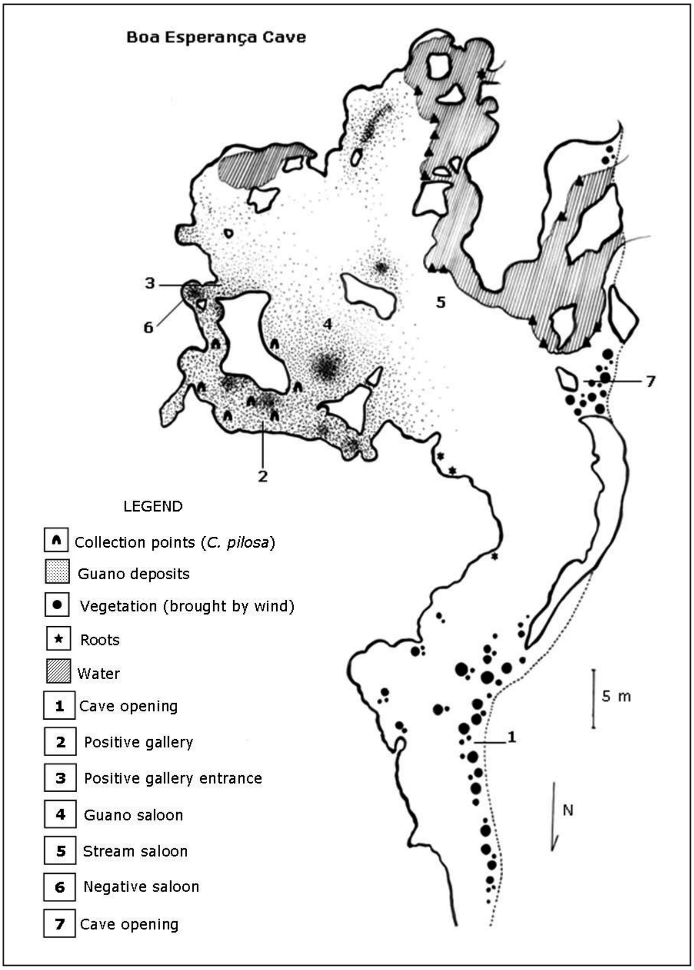

FIG. 1. Sketch map of the Boa Esperança Cave (10°20.192'S, 48°03.587'W, Palmas, TO), where *C. pilosa* was collected.

The visits to the cave, collections, and procedures were performed in accordance with biosafety regulations determined by the Technical Committee of Biosafety of Fundação Oswaldo Cruz (FIOCRUZ 1998) and under IBAMA (Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis) license number 049/2005.

RESULTS

Characterization of C. pilosa natural environment. The temperature in both dry and rainy seasons revealed higher averages inside the cave than outside, with smaller variations inside the cave. Average air humidity in the cave during the dry season was higher than in the outer environment, but during the rainy season it was lower inside the cave than outside (Table 1). The climate inside the cave remained stable, with slight variations compared with the outside climate ranges. The cave's greatest extent along its north-south length is 78.94 m and its maximum width is 38.24 m. There is some light until the beginning of the stream (point 5 in the sketch map, Fig. 1). From this point on there is no noticeable light, and the ground is covered with a guano layer of approximately 2 m depth below the numerous bat colonies. The smaller shaded area on the sketch map corresponds to a rainfall accumulation (8.82 m long), only ob-

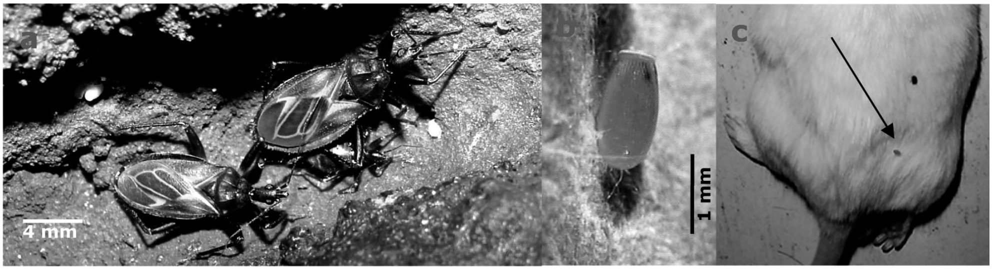

FIG. 2. (a) *C. pilosa*: eggs fixed on the cave wall, female, male, and nymph; (b) *C. pilosa* egg fixed on paper; (c) *C. pilosa* egg fixed on mouse.

served in the rainy season. The larger shaded area corresponds to a stream coming from the cave ceiling in a waterfall in the extreme south, running approximately 50 m until it reaches the southwest opening of the cave. The capture sites of *C. pilosa* correspond to areas with a high concentration of guano and bats with no noticeable light, where average temperatures ranged from 24 to 31°C and relative humidity from 62 to 80% (points 2 and 4). In places where there was guano but with high humidity (above 95%) no *C. pilosa* colony was found. All individuals were found above one meter high, occupying the entire wall until the ceiling, where the bat colonies were. Four species of bats were identified: *Anoura geoffroyi* Gray, 1838, *Phyllostomus hastatus* (Pallas, 1767), *Carollia perspicillata* (Linnaeus, 1758), all Phyllostomidae, and *Pteronotus parnellii* (Gray, 1843), Mormoopidae. The cave also contained about 50 arthropod species belonging to 20 orders, spread throughout the cave.

Infection with Trypanosomatidae. No trypanosomatid was found in the analysis of bat blood smears. Of the nine bats submitted to xenodiagnosis, eight (89%), of all species identified, showed infection with trypanosomatids. Also 89% of *C. pilosa* specimens showed trypanosomatids in their feces. Cultures of these trypanosomatids, and those isolated from the bats through xenodiagnosis, showed, at first, an exponential growth, then a decreasing growth rate after two months and, after five months, no trypanosomatid was found in the culture tubes. Inoculated mice, both Swiss Webster and C3H, showed no patent parasitemia through fresh blood analysis and all of them survived the infection. Hemoculture of C3H mice blood showed them to be positive for all culture tubes for two months, being negative after that. The isolated parasites were morphologically similar to *T.* (*S.*) *cruzi*. Considering the behavior of such trypanosomatids under culture conditions, the absence of parasitemia and mortality in the inoculated mice (despite being infected, as shown by hemoculture), the parasite detected is likely to be *T.* (*S.*) *cruzi marinkellei*, according Baker *et al.* (1978).

C. pilosa rearing. *C. pilosa* rearing was successful under laboratory conditions only when mice were anesthetized, immobilized, and placed into the container (so that the bugs did not have to feed through nylon cloths or cotton); more hiding places for oviposition were provided and air relative humidity was kept high, between 80 and 95%. It was not possible to obtain precise data on the *C. pilosa* biological cycle because individuals tended to die after manipulation or isolation, but it was possible to observe the rapidness of the egg-to-egg cycle, which could be completed in less than two months.

C. pilosa eggs were found fixed to the substrate of paper or wood (in the laboratory), and the cave walls (Fig. 2). In the laboratory, we also found eggs fixed on the dorsal line of an anesthetized mouse on which bugs were taking blood, near some drops of the triatomine feces.

DISCUSSION

The medium-grain-size laterita that constitutes the Boa Esperança cave provides shelter and oviposition sites for *C. pilosa*. The stream running across the cave, along the north-south length, which ran even in the dry season, ensures high humidity inside the cave and, together with guano, a rich organic matter source. Temperature ranges measured inside the cave were close to the annual average temperature outside, making the cave environment more stable than the outside environment, (Ferreira *et al.* 2000). The climate at the cave entrances is much more under the influence of the outside environment. *C. pilosa* colonies were found in sites far from the cave entrances, where temperature and air relative humidity were more stable, apparently in darkness. It seems likely that bats are the only organisms associated with the presence of *C. pilosa*. However, spiders (Segestridae) were found in trap-holes in the same area as *C. pilosa*, and *Loxosceles puortoi* (Sicariidae) spiders, that built their residential webs close to *C. pilosa* colonies, were observed preying on these bugs. Air humidity and the great amount of organic matter (vegetal and mainly guano) ensure ideal conditions for the survival of the arthropod species found all over the cave. The occurrence of *C. pilosa* is restricted to microclimates such as those in caves, basements, and tree cavities, usually warm and wet with low or no light at all, always associated with the presence of bats. *C. pilosa* colonies tend to be very numerous, and when a small number are found it may be considered an accidental finding, such as those reported in human dwellings in Panama (Pipkin 1968). Such data suggest that *C. pilosa* is extremely specialized, and its limiting factors are temperature, humidity, and food source. *Cavernicola pilosa* has always been found associated with Chiroptera species, which led to the belief that the bug feeds only on bats (Carcavallo *et al.* 1976), making breeding of the insect under laboratory conditions difficult. However, successful *C. pilosa* colonization in our laboratory, where only mice were provided as a blood-feeding source, suggests that climate is the major limiting factor for survival, at least under laboratory conditions. Our findings revealed that it is possible to rear *C. pilosa* by feeding them exclusively on mice blood, which makes their colonization feasible and enables further laboratory studies on this insect species.

Despite being widespread, *C. pilosa* tends to occur focally. Its occurrence in sheltered habitats, protected against climate and light variation, suggests passive dispersal. The finding of *C. pilosa* eggs fixed on a mouse in the laboratory supports the hypothesis that *C. pilosa* dispersal occurs mainly through eggs fixed on bat hairs, with hatching and development taking place in another favorable environment.

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