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SHORT COMMUNICATION

A morphometric study of *Eutrombicula alfreddugesi* (Acari: Trombiculidae) infesting four sympatric species of *Tropidurus* (Squamata: Tropiduridae) in northeastern Brazil

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The mites of the trombiculid family, or "chiggers," are cosmopolitan and parasitic on a wide range of terrestrial vertebrates, including amphibians, reptiles, birds, and mammals. Only the larvae are parasites; the other stages are free-living in the soil (Wharton and Fuller 1952, Brennan and Goff 1977, Bush *et al*. 2001). Chiggers of the genus *Eutrombicula* Ewing, 1938, are thought not to have preferences for specific hosts (Brennan and Reed 1974) because they infest different taxa (Ewing 1944, Wharton 1952).

Eutrombicula alfreddugesi (Oudemans, 1910) is a chigger commonly found parasitizing different vertebrate host groups, such as reptiles, amphibians, birds, and mammals (e.g., Wharton and Fuller 1952, Daniel and Stekol'nikov 2004, Lareschi 2006, Rubio and Simonetti 2009). In North, Central, and South America, this is a common parasite of lizards (Wharton 1952, Loomis and Wrenn 1984). In Neotropical lizards, the mites often are densely clustered in the nuchal, axillar, inguinal, and post-femoral regions (Rodrigues 1987, Cunha-Barros and Rocha 1995, 2000). All chiggers living on lizards from southeastern Brazil have been identified as *E. alfreddugesi* (Cunha-Barros and Rocha 1995, 2000). However, more precise taxonomic studies for this group are scarce and, because of its wide host distribution, there is increasing doubt that all of these mites actually belong to the same species.

Some Brazilian studies have analyzed ecological parameters of the parasitism by *Eutrombicula alfreddugesi* in lizard populations and communities (Cunha-Barros and Rocha 1995, 2000, Vrcibradic *et al.* 2000, Carvalho *et al.* 2006). Lizards of the genus *Tropidurus*

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Wied-Neuwied, 1825 are often highly parasitized by these mites (Cunha-Barros and Rocha 1995, 2000, Carvalho *et al.* 2006), possibly because these lizards possess mite pockets. Apart from the presence of mite pockets, the imbricate scales of *Tropidurus* also may contribute to the intensity of the mite infestations (Cunha-Barros and Rocha 1995). Because *Tropidurus* is highly parasitized, lizards of this genus are useful models for studies regarding parasitism by chigger mites.

At Morro do Chapéu, Bahia State, in northeastern Brazil, four species of *Tropidurus* are sympatric—*T. hispidus* Spix, 1825; *T. cocorobensis* Rodrigues, 1987; *T. semitaeniatus* Spix, 1825; and *T. erythrocephalus* Rodrigues, 1987. These species differ in body size, in the number and distribution of mite pockets, and in behavioral traits, such as habitat use (Rodrigues 1987). Preliminary analysis indicates that these lizards are parasitized by *Eutrombicula alfreddugesi* in high prevalence (Rocha *et al.* 2008). Morphometric analyses of chitinous structures, plates, and setae of the mites have been proven to be an important tool in revealing the presence of cryptic species within complexes of polyxenous mites (Gettinger and Owen 2000, Gettinger *et al.* 2011). We undertook a sampling program to understand the extent of morphological variation in *Eutrombicula alfreddugesi* among the four host species of *Tropidurus* at Morro do Chapéu. We sought to assess the specificity of these mites to the species of the lizard hosts analyzed. Are these chigger mites cryptic species, each infesting a different set of the four hosts, or can *E. alfreddugesi* utilize all four lizard species as hosts? If *E. alfreddugesi* is polyxenous, infesting all four species of *Tropidurus*, are there differences in the prevalence and intensity of infestation that may represent host preferences? We used multivariate morphometric techniques to analyze some meristic characters of the chigger mites infesting these sympatric species of *Tropidurus*.

The study was carried out in Morro do Chapéu Municipality (11°29' S, 41°07' W), Bahia State, in northeastern Brazil (Figure 1). The area

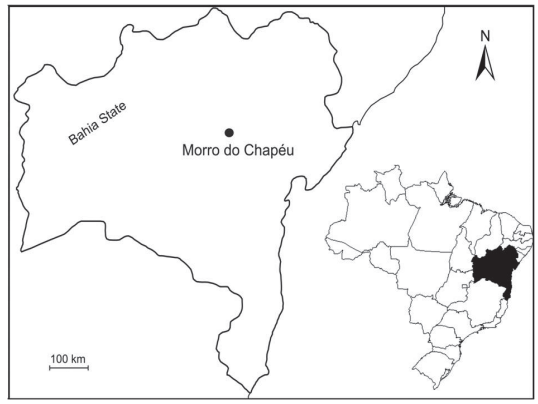

Figure 1 - Location map from study area at Morro do Chapéu Municipality, Bahia State, in northeastern Brazil (11°29' S, 41°07' W).

is transitional between caatinga vegetation (Brazilian ecosystem of thorny shrubs and stunted trees) and "campos rupestres" (rocky fields) covered by open vegetation with a predominance of herbaceous and shrubby plants on rock and sandy soils. The climate is warm and dry. The mean air temperature during the study period was 29.3ºC and the relative air humidity was 48.9%. Lizards (n = 20 *T. hispidus,* 13 *T. erythrocephalus,* 34 *T. semitaeniatus,* and 13 *T. cocorobensis*) were collected in November and December 2000 with rubber bands and nooses; specimens were labeled and immediately fixed in 10% formalin and later preserved in 70% alcohol. In the laboratory, we checked them for the presence of mites under a stereomicroscope. We carefully sampled each mite attachment site (i.e., mite pockets), and mounted and identified individual mites from different individual hosts, to assure that we included a wide range of possibly variable taxa. The mites found were cleared in lactophenol and mounted on permanent slides with Hoyer's medium for identification and morphometric study under a microscope. Mites were identified based on the taxonomic literature (Wharton and Fuller 1952, Brennan and Goff 1977).

We chose six scutal characters to measure with a filar micrometer eyepiece (to the nearest 1 µm): (1) distance between sensillary bases (SB); (2) anterior width (AW); (3) posterior width (PW); (4) distance from anterolateral setal base to posterolateral setal base (AP); (5) length of anterolateral seta (AL); and (6) length of posterolateral seta (PL). We measured ten chigger mites from each of five different individuals (2 mites per individual) of each lizard species, totaling 40 chigger mites from 20 lizards. We used principal components analysis (NTSYS®) to evaluate the morphological variation of these mites.

All lizards were infested by *Eutrombicula alfreddugesi*, except one individual of *T. semitaeniatus* (SVL = 31.7 mm), but each species had different infestation rates (Table 1). Although mite prevalence was high in all species studied, *Tropidurus erythrocephalus* was most heavily infested and *T. semitaeniatus* the least infested. We did not find significant morphological between-group difference in the mites infesting these lizard species (Table 2). Eigenvalues of the three first axes were 11.54, 5.53, and 3.6, respectively, and the total variation explained was 64.6% (36.1%, 27.0%, and 11.2%; Figure 2). The mites on *T. semitaeniatus* had the highest values for all morphometric characters, except AP. In contrast, the mites on *T. cocorobensis* had the smallest meristic values for most of the metric characters (Table 2).

Although mite prevalence was high in all species studied, the differences observed in intensity of infestations can be correlated with morphological differences among the species of lizards. According to Rocha *et al*. (2008), they differ with respect to their ecology and the location and depth of mite pockets. *Tropidurus erythrocephalus, T. hispidus,* and *T. cocorobensis* are microhabitat generalists, with deep mite pockets in the gular, axillary, and inguinal regions. *Tropidurus semitaeniatus* is exclusively saxicolous and has a dorsoventrally flattened habitus; unlike the other three taxa, this species has smooth, juxtaposed dorsal scales (vs. keeled and imbricate). Moreover, *T. semitaeniatus* lacks "true" mite pockets; instead, it has undifferentiated skin folds in the gular, axillary, and inguinal regions (Vanzolini *et al*. 1980, Rodrigues 1987, Frost *et al*. 2001).

Table 1 - Prevalence and mean intensity of infestation by *Eutrombicula alfreddugesi* in sympatric species of *Tropidurus* from Morro do Chapéu, Bahia State, Brazil. Sample sizes (*n*) in parentheses.

Host	Prevalence (%) (*n*)	Mean intensity ± SD (range)
T. hispidus	100 (20)	146.16 ± 114.2 (31–483)
T. cocorobensis	100 (16)	70.06 ± 41.7 (21–186)
T. erythrocephalus	100 (13)	165.85 ± 126.0 (40–520)
T. semitaeniatus	97.1 (34)	52.32 ± 42.4 (3–162)

We did not find significant morphological between-group differences in the mites infesting these lizard species. There are relatively few studies about lizards as hosts for *Eutrombicula alfreddugesi* in Brazil (Cunha-Barros and Rocha 1995, 2000, Carvalho *et al.* 2006, Rocha *et al*. 2008). One remarkable aspect is the intense infestation of *Tropidurus* by *E. alfreddugesi* (Cunha-Barros and Rocha 1995, 2000, Carvalho *et al.* 2006, Rocha *et al*. 2008). These lizards have imbricate scales and mite-pockets (Rodrigues 1987) that are suitable habitats for ectoparasites. At Morro do Chapéu, all individuals were heavily infested by mites, except for a juvenile. Possibly, this is because the adults had more time for contact with the parasites than did the juvenile (Aho 1990, Fontes *et al.* 2003).

Proximate factors related to the ecology and physiology of *Tropidurus* clearly are involved with these host-mite relationships. The observed

differences in mite abundance may be correlated with: (1) host body size; however, there was no evidence of distinct size classes of lizards, and samples included unequal numbers of different ages and sexes; (2) size and development of mite pockets—e.g., *T. semitaeniatus* lacks well-developed pockets and had lowest prevalence and intensities of infestation; and (3) microhabitat-use patterns by the hosts. Further study will be necessary to evaluate these host influences.

Eutrombicula alfreddugesi parasitizes different groups of vertebrates from North America to Central and South America, with no apparent species-specific relationships (e.g., Wharton and Fuller 1952, Daniel and Stekol'nikov 2004). Our data support the contention that *E. alfreddugesi* has a low specificity for its lizard hosts, and can parasitize different hosts at both high prevalence and intensities (Cunha-Barros and Rocha 2000). On the one hand, the apparent lack of differences among populations of this mite from the different species of lizards supports our initial contention that all of the lizards are infested by the same species. Moreover, our results suggest that all chiggers measured are indeed the same species—one that has low host specificity. However, based on the present status of knowledge of the group, we cautiously accept this conclusion, because it is possible that we may be dealing with a morphologically cryptic species, the existence of which could be revealed by analysis of genomic data in the future.

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Table 2 - Morphological attributes (mean ± 1 SD, to the nearest 1 µm) of chigger mites in an area of caatinga at Morro do Chapéu municipality, Bahia State, in northeastern Brazil (SB = distance between sensillary bases; AW = anterior width; PW = posterior width; AP = distance from anterolateral setal base to posteriorlateral setal base; AL = length of anterolateral seta; PL = length of posterolateral seta). Ten mites were measured from five lizards of each species of *Tropidurus*.

	Morphological attributes					
Species	**SB** ± 1 SD (range)	**AW** ± 1 SD (range)	**PW** ± 1 SD (range)	**AP** ± 1 SD (range)	**AL** ± 1 SD (range)	**PL** ± 1 SD (range)
All species	42.7 ± 2.4 (37.5–48.8)	77.9 ± 3.4 (70.0–85.0)	89.9 ± 3.5 (82.5–98.8)	26.9 ± 1.5 (25.0–30.0)	27.9 ± 2.0 (23.8–32.5)	34.6 ± 3.0 (27.5–40.0)
T. semitaeniatus	44.1 ± 3.4 (37.5–48.8)	78.9 ± 4.9 (70.0–85.0)	91.2 ± 5.5 (82.5–98.8)	26.1 ± 1.1 (25.0–27.5)	28.5 ± 2.9 (23.8–32.5)	35.8 ± 4.8 (27.5–40.0)
T. erythrocephalus	42.4 ± 1.5 (41.3–45.0)	78.1 ± 1.9 (73.8–80.0)	89.9 ± 2.2 (85.0–92.5)	27.1 ± 1.7 (25.0–30.0)	27.4 ± 1.9 (23.8–30.0)	33.3 ± 2.2 (28.8–36.3)
T. hispidus	42.4 ± 2.5 (37.5–46.3)	77.4 ± 3.2 (72.5–83.8)	89.6 ± 2.5 (87.5–95.0)	27.1 ± 1.8 (25.0–30.0)	28.1 ± 1.1 (27.5–30.0)	34.9 ± 1.7 32.5–37.5)
T. cocorobensis	42.0 ± 1.1 (40.0–43.8)	77.4 ± 3.2 (72.5–81.3)	89.1 ± 2.8 (83.8–93.8)	27.0 ± 1.6 (25.0–30.0)	27.6 ± 1.8 (25.0–30.0)	34.4 ± 2.1 (31.3–37.5)

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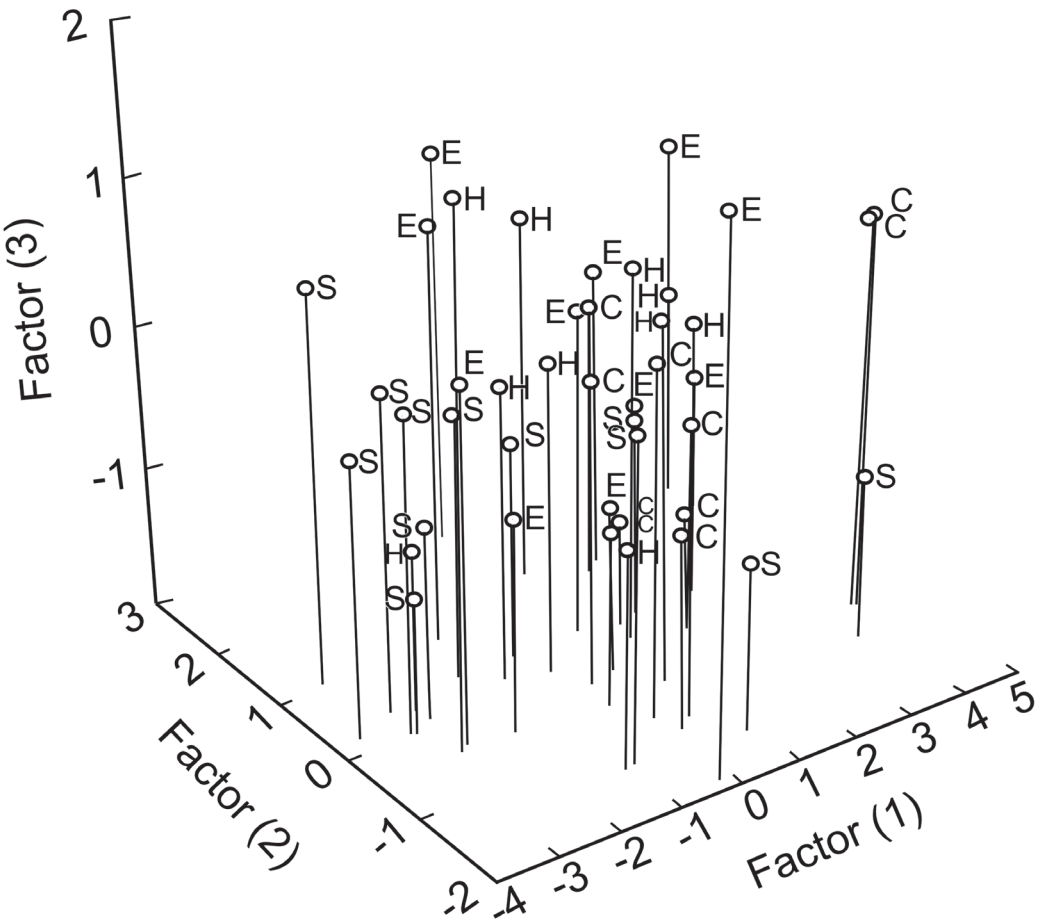

Figure 2 - PCA graph showing the *Eutrombicula alfreddugesi* measured in each lizard species (S = *Tropidurus semitaeniatus*, C = *Tropidurus cocorobensis*, H = *Tropidurus hispidus*, E = *Tropidurus erythrocephalus*).

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