

Article

Erythraeid larvae of *Leptus* (Trombidiformes: Erythraeidae) parasitizing a harvestmen species in Atlantic Forest: biology and seasonality of host-parasite interactions (Opiliones: Gonyleptidae)

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ABSTRACT. Symbiotic relationships are associations where two or more species live closely associated, including parasitism, phoresis, mutualism, and others. In mites, one of the most common associations occurs between larvae of *Leptus* (Erythraeidae), a cosmopolitan genus that parasitizes several groups of arthropods. However, in Brazil, these reports are scarce. In the present study, we recorded the number of ectoparasitic erythraeid mite larvae of the genus *Leptus* found associated to Opiliones of the species *Gonyleptes fragilis* Mello-Leitão, 1923 (Gonyleptidae) in an Atlantic Forest fragment (Cubatão, São Paulo, Brazil), collected over one year, between the four seasons. We recorded 405 mite larvae associated with 152 opilionid individuals. The intensity and prevalence of mite's infection, seasonal variation, mite's preference for body parts and host sex were the parameters used to determine host-parasite relationship. Mean infestation intensity (number of mites per individual) ranged from 0.3 to 11.3 with maximum observed intensity of 20 mites/individual. There was no sexual host preference and regarding seasonality, there were differences between infestation intensity and season with infestation being higher in summer compared to the other seasons. As for location, most mites were found in the median region of the dorsal shield, near the median frame of area III and IV. This study provides the first description of annual and seasonal variation in mite infestation on Opiliones in the Brazilian Atlantic Forest.

KEYWORDS. *Gonyleptes fragilis*, Ectoparasitism, Symbiotic relationships, Neotropical, Laniatores.

RESUMO. Larvas de eritreídeos de *Leptus* (Trombidiformes: Erythraeidae) parasitando uma espécie de opilião na Mata Atlântica: biologia e sazonalidade das interações hospedeiro-parasita (Opiliones: Gonyleptidae). Relações simbióticas são associações onde duas ou mais espécies vivem intimamente associadas, incluindo parasitismo, forese, mutualismo e outros. Em ácaros, uma das associações mais comuns ocorre entre larvas de *Leptus* (Erythraeidae), gênero cosmopolita que parasita diversos grupos de artrópodes. No entanto, no Brasil, esses relatos são escassos. No presente estudo, registramos o número de larvas ectoparasitas de ácaros eritreídeos do gênero *Leptus* encontradas associadas a opiliões da espécie *Gonyleptes fragilis* Mello-Leitão, 1923 (Gonyleptidae) em um fragmento de Mata Atlântica (Cubatão, São Paulo, Brasil), coletadas ao longo de um ano, entre as quatro estações. Registramos 405 larvas de ácaros associadas a 152 indivíduos de opiliões. A intensidade e prevalência da infecção do ácaro, variação sazonal, preferência do ácaro por partes do corpo e sexo do hospedeiro foram os parâmetros usados para determinar a relação parasita-hospedeiro. A intensidade média de infestação (número de ácaros por indivíduo) variou de 0,3 a 11,3 com intensidade máxima observada de 20 ácaros/indivíduo. Não houve preferência sexual do hospedeiro e quanto à sazonalidade, houve diferenças entre intensidade de infestação e estação do ano, sendo a infestação maior no verão em relação às outras estações. Quanto à localização, a maioria dos ácaros foi encontrada na região mediana do escudo dorsal, próximo à região mediana das áreas III e IV. Este estudo fornece a primeira descrição da variação anual e sazonal na infestação de ácaros em opiliões na Mata Atlântica brasileira.

PALAVRAS-CHAVE. *Gonyleptes fragilis*, Ectoparasitismo, relações simbióticas, Neotropical, Laniatores.

With more than 950 species and a very high rate of endemism, the Atlantic Forest has, probably, the greatest diversity of harvestmen in the world (NOGUEIRA *et al.*, 2019). However, relatively little is known about the ecology and symbiotic relationships of most species (MACHADO *et al.*, 2007). Factors including small body size, cryptical habits and nocturnal behavior have been contributed to the general paucity of information concerning the symbiotic relationships

of harvestmen with other organisms (COKENDOLPHER, 1993; COKENDOLPHER & MITOV 2007; GABRYŚ *et al.*, 2011). The most investigated interactions are those that involve the larval mites, especially erythraeid mites of the genus *Leptus* Latreille, 1796 (COKENDOLPHER, 1993). *Leptus* comprises 220 species (SABOORI *et al.*, 2020), the majority of those are known exclusively from larvae. To date, 54 larval species of the genus have been reported from America (HAITLINGER

et al., 2020) and fifteen larval species have been recorded from Brazil (JACINAVICIUS *et al.*, 2020).

Currently, there is some knowledge between the symbiotic relationships involving the larvae of erythraeid mites of the genus *Leptus* and harvestmen (ÂBRO, 1988; COKENDOLPHER, 1993; GUFFEY, 1998; MCALOON & DURDEN, 2000; COKENDOLPHER & MITOV, 2007; HAITLINGER *et al.*, 2020). It is assumed that there is a relationship of ectoparasitism, where the mites, that preferentially attach to the femur and tibia of the leg (but also attach to the ocularium and dorsal surface of the scutum) (MCALOON & DURDEN, 2000; TOWNSEND JR *et al.*, 2008), feed on hemolymph and adjacent tissues (ÂBRO, 1988) and little is known about the effects of the parasites on the survival, locomotion, or reproduction capacity of their hosts (GUFFEY, 1998).

The majority of studies that investigated interactions are those that involve ectoparasitic larval mites of *Leptus* that parasitize temperate species of the sclerosomatid harvestmen of the genus *Leiobunum* (ÂBRO, 1988; COKENDOLPHER, 1993; GUFFEY, 1998; MCALOON & DURDEN, 2000; COKENDOLPHER & MITOV, 2007). Little knowledge is available about larval mites that parasitize Neotropical harvestmen, especially Laniatores, although there are records of infestation for the Gonyleptidae and Cosmetidae (COKENDOLPHER, 1993; COKENDOLPHER & MITOV 2007; TOWNSEND JR, V. R. *et al.*, 2008 and HAITLINGER *et al.*, 2020).

Relatively few studies have examined other biotic and abiotic aspects in parasitism upon harvestmen and most

studies are restricted to sclerosomatid species (TOWNSEND JR *et al.*, 2006). To assess the host sexual preference, attachment site preference and seasonal variation in the intensity infestations of larval mite of the genus *Leptus* among a Neotropical harvestman, we studied a population of *Gonyleptes fragilis* Mello-Leitão, 1923 which occurs on a fragment of the Atlantic Forest, Brazil.

MATERIALS AND METHODS

Study area. The study was conducted in a forested area, one in the municipality of Cubatão located in the Baixada Santista, a coastal plain in the state of São Paulo (Fig. 1). The investigated site is on a slope of Serra do Mar ($23^{\circ}49'51.5''\text{S} - 46^{\circ}23'40.7''\text{W}$), which belongs to the company Copebrás – *Cia Petroquímica Brasileira* (currently controlled by CMOB Brasil). In this area, we sampled three different sites, located at about 100 m altitude, and these areas were at a distance of about 500 m from each other.

Sampling and identification. Sampling was performed through the nocturnal manual collection, a method widely used in arachnid collections (NOGUEIRA *et al.*, 2006; AZEVEDO *et al.*, 2013). In this method the collector, carrying a head flashlight, searches for arachnids from the ground up to the height he can reach, investigating the litter, shrub vegetation, logs, fallen trunks, and other microhabitats. Arachnids are captured with the aid of tweezers, vials, or by hand, and are immediately fixed in 70% ethanol. Each sample represents 1 hour of searching per collector along a

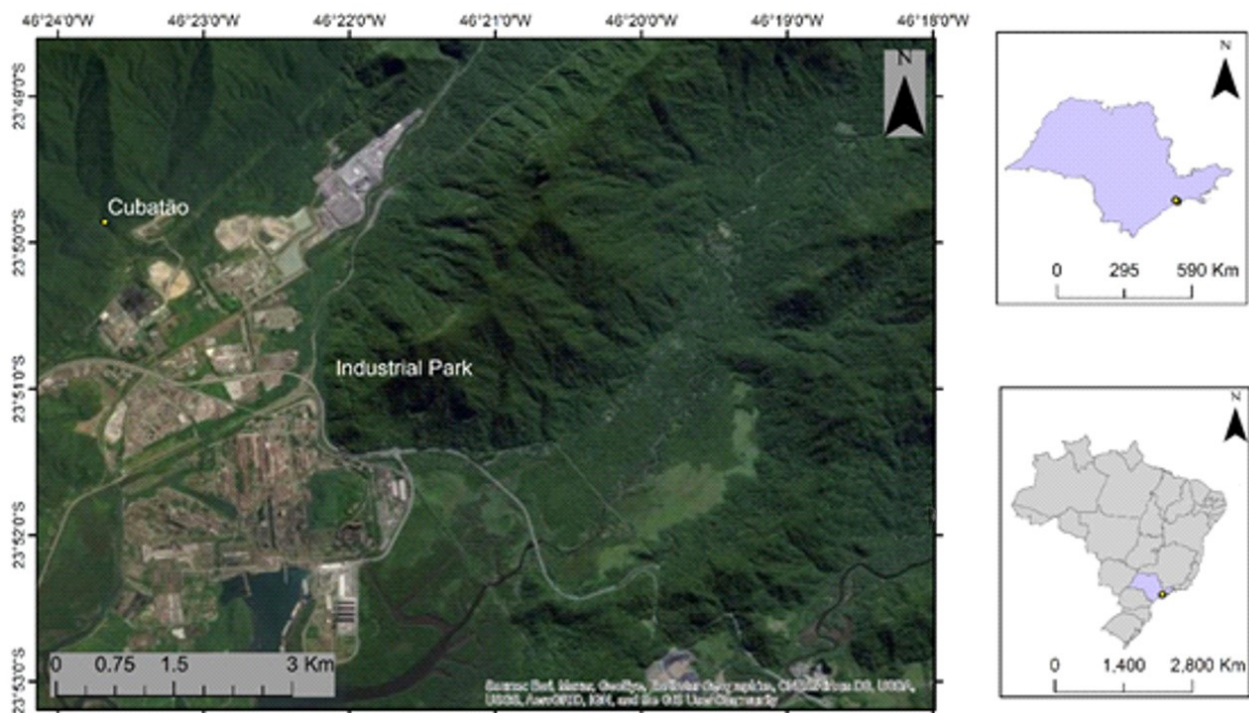


Fig. 1. Location of the study area in the Municipality of Cubatão, São Paulo, Brazil.

30 m long transect, which can be moved about 5 meters each way, totaling approximately 300 m² of the investigated area.

Collections were carried out at night, between 7:30 pm and 11:00 pm, by a team of three collectors. The areas in Cubatão were sampled in all seasons, between November 2004 and August 2005 (summer: collected conducted in February; autumn: collected conducted in April; winter: collected conducted in July; *Spring*: collected conducted in November). In each season, one collecting trip was made and we spent one night sampling each of the three sites investigated, resulting in 12 samples for each season, which yielded a total of 48 samples. However, for the analysis, only samples containing specimens of *Gonyleptes fragilis* were considered (22 samples, see Tab. I).

All collected material was identified using a stereomicroscope and is deposited in the arachnology collection of Museu de Zoologia da Universidade de São Paulo (curator: Ricardo Pinto-da-Rocha).

Data analysis. To study the seasonal intensity and prevalence of parasites we used one-way analysis of variance (one-way ANOVA), followed by Tukey's HSD (Honest Significant Difference) Post Hoc Test. To verify the differences of intensity of parasites related to the sexes, we performed a Student T-test. Homogeneity of variance among

categories was tested using Bartlett's test and, if necessary, data were rank-transformed.

To verify the attachment site preference of mites, we analyzed the intensity of the ectoparasite related to the different parts of the harvestman's body. For this, we first separated the harvestman's body into seven different segments and then we counted the presence and number of mites attached in each of that segment. The seven body segments are: ocularium, scutal area I, scutal area II, scutal areas III/IV, lateral borders of dorsal scutum, free tergites, and legs IV.

RESULTS

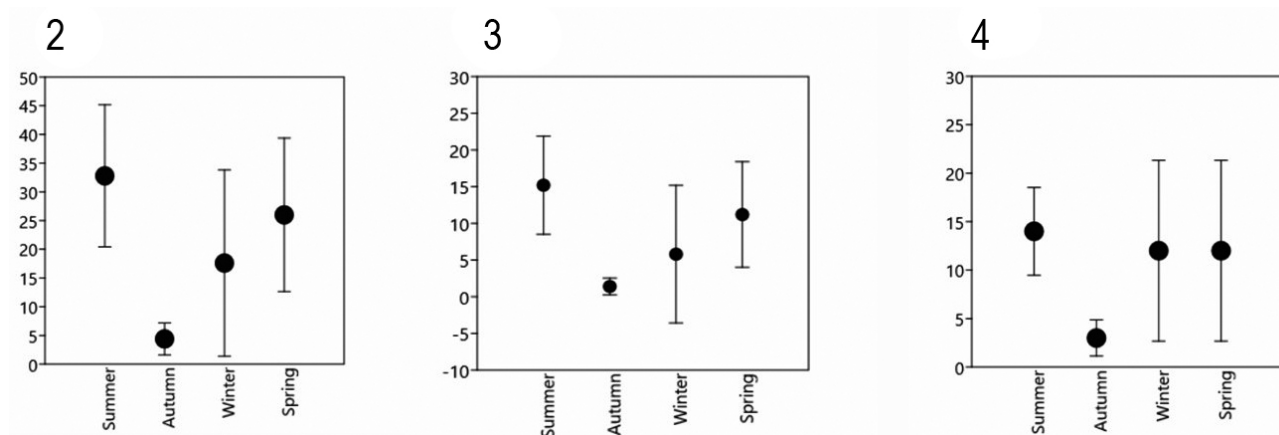
A total of 152 adult harvestmen *Gonyleptes fragilis* were collected, present in 22 of 48 samples, with a total of 408 associated mites (Tab. I). Overall, the prevalence of infestation for larval mites upon adult harvestmen (N= 143) was 67.9%. The intensity of mite infestation varied from 1 to 20 mites per host.

Regarding the different seasons, we found that there was a significant difference in the number of parasites in the different seasons, with a higher intensity of mites in summer compared to autumn, while between spring and autumn we noticed a marginally significant difference Tab. I; Fig. 2). If the analysis is performed separately by sexes, as well as for general data, there were differences between the summer and autumn (p=0.025 for males and p=0.01 for females) (Figs 3, 4, respectively). The prevalence of infestation also significantly varied across the four seasons (F=4.94; p<0.01), with harvestmen from summer exhibiting the highest overall prevalence of infestation at 97.1% and the autumn exhibiting the lowest, with only 38.3%.

There was also no host sexual preference. The prevalence of infestation was not related to the sexes (t= 0.66; p=0.51) and there was no difference between the

Table I. Number of mites, prevalence, and intensity of *Leptus* larvae.

	Number of individuals of <i>Gonyleptes fragilis</i>	number of mites	prevalence (%)	intensity range
total	153	408	67.9	1 - 20
male	54	188	74.1	1 - 20
female	99	220	64.6	1 - 16
summer	35	34	97.1	1 - 18
autumn	47	18	38.3	1 - 3
winter	34	21	61.7	1 - 19
spring	37	31	83.8	1 - 20



Figs 2-4. Difference in mite infestation in harvestmen between the different seasons of the year. Bars indicate standard deviation. 2: total data, including males and females; 3: only males; 4: only females.

intensity of mites and the sexes ($t = 0.13$, with $p = 0.71$; Fig. 5). Finally, regarding the attachment site preference, the results showed that the median region of the dorsal shield, near the median frame of area III and IV had the highest concentration

of associated mites (Fig. 6), in both sexes (Figs 7, 8). This region of the body was responsible for the presence of 266 of the 408 larvae, which represents more than 65% of the mites found in this study.

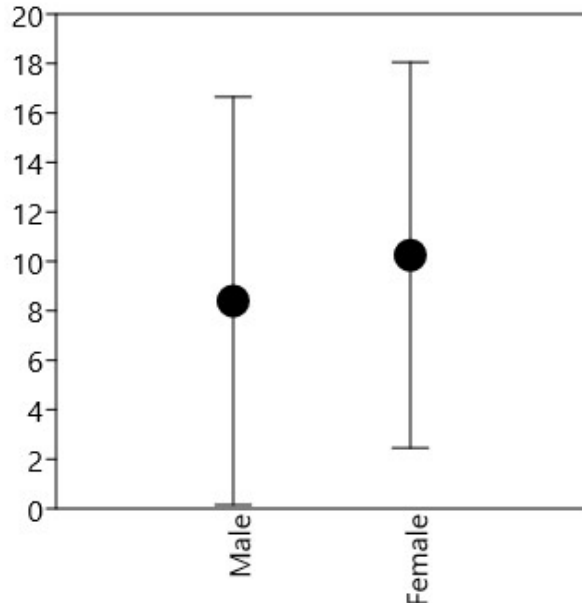


Fig. 5. Difference in mite infestation in relation to gender in harvestmen *Gonyleptes fragilis* Mello-Leitão, 1923.

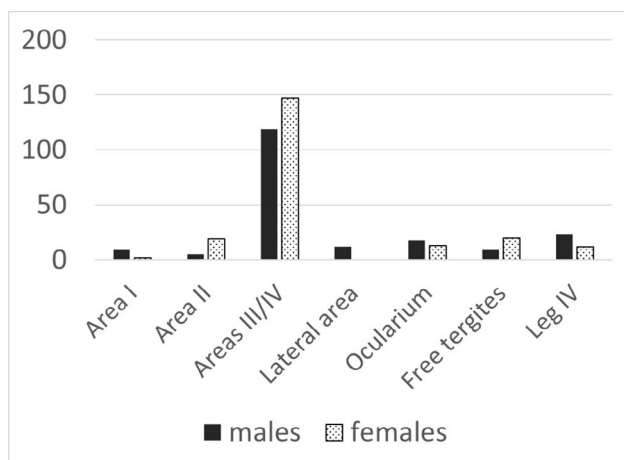
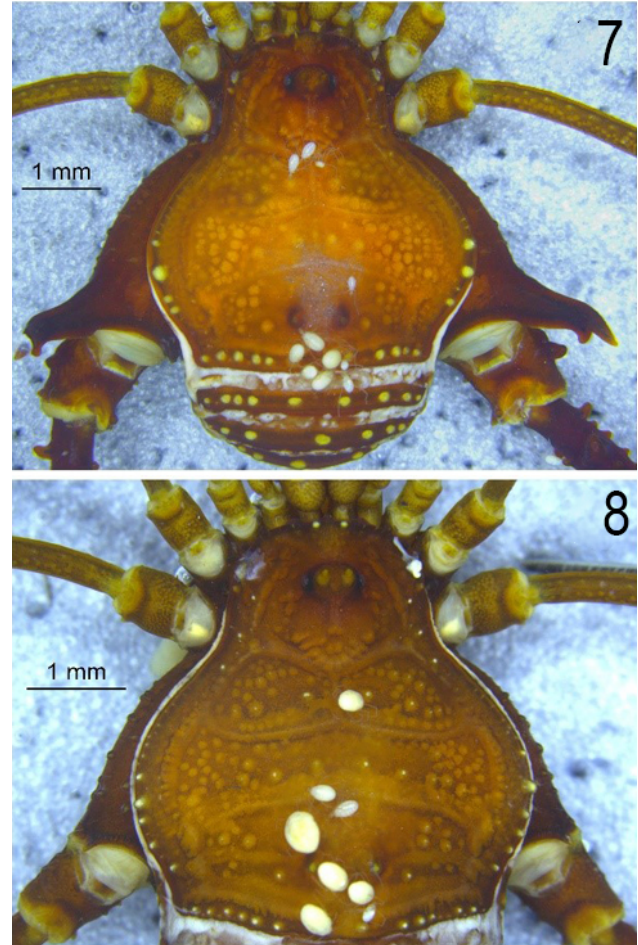


Fig. 6. Location of *Leptus* larvae in different parts of the body of *Gonyleptes fragilis* Mello-Leitão, 1923.

DISCUSSION

Relatively few quantitative studies have examined the mite-harvestmen interactions. This study represents the first investigation of the prevalence and intensity of mite infestation related to seasonal and sexes for a Neotropical harvestman species. In comparison to other studies in harvestmen, our investigation revealed the highest intensity of mite infestation



Figs 7, 8. Location of *Leptus* larvae in the body of *Gonyleptes fragilis* Mello-Leitão, 1923: 7, male; 8, female.

in Laniatores, with the maximum of 20 mites (median 2.92). Prior to our study, the highest intensity of *Leptus* mite infestation had been found in a cosmetid being parasitized by 17 larvae (TOWNSEND JR *et al.*, 2008). In Eupnoi, GABRYŚ *et al.* (2011) found 26 mites in *Palangium opilio* (Linnaeus, 1758), followed by MCALOON & DURDEN (2000) that founded 14 mites in *Leiobunum formosum* (Wood, 1868). The prevalence of infestation (the percentage of individuals that are parasitized) was 67.9%, the highest reported for harvestmen until now. MCALOON & DURDEN (2000) has been reported 61% for *L. formosum*. The ecological significance

of this variation is difficult to assess because no adverse effects of ectoparasitic mites upon harvestmen physiology or reproductive success have yet been empirically demonstrated (GUFFEY, 1998). Our field observation and previous studies on living and preserved harvestmen infested with mites indicate that hosts are generally not in poor condition, nor do they have difficulty with their locomotion (MCALOON & DURDEN, 2000; TOWNSEND JR *et al.*, 2008), indicating they do not seem to suffer any immediate detrimental effects of mite infestation. However, most harvestmen previously studied were only infested with relatively few mites. In our investigation, we found several individuals with more than 10 larvae, with the maximum of the 20 larvae. For these individuals, the short and long-term effects of heavy mite infestations upon the survivorship, locomotion, and fecundity, remain to be determined. For dipterans, POLAK & MARKOW (1995) found that, ectoparasitic mites can severely impair the reproductive activities of individual flies and thus, high intensity mite infections can have serious consequences for individual fitness. On the other side, CORDERO-RIVERA *et al.* (2018) indicates that mites did not affect the survival of the partenogenetics damselflies (Odonata: Coenagrionidae). Therefore, further research on the physiological consequences of mite intensity of infestation upon harvestmen is required.

In the present study, we did not find relationship between the intensity of prevalence of mites and the sexes. In mosquitoes, a strong preference was found for females' attachment, but the reproductive biology, where female mosquito individuals returning to habitat multiple times to oviposit, could have increased chances of mites to attach to female individuals (ATWA *et al.*, 2017). The other parameters used to determine host-parasite relationship, as seasonal variation, and mite's preference for body parts, proved to be more informative and consistent with harvestmen biology.

Differences in the intensity and prevalence of mite infestation could reflect variation in the life history of the parasite. In erythraeid mites, only the larvae are parasitic (SOUTHCOTT, 1992; COKENDOLPHER, 1993). Thus, the marked seasonal variation found in our study, which was also found by TOWNSEND JR *et al.* (2006) in a temperate environment, should reflect the reproductive biology of the mite, i.e. periods in the year when larval mites are particularly common or relatively rare. Furthermore, different habitats may be more or less favorable for the erythraeid mite development and reproduction, regardless of season or other abiotic characteristics. We must point out that adult erythraeid mites inhabit the litter community and deposit eggs in the soil (SOUTHCOTT, 1992). So, the harvestmen should find mite larvae while moving through leaf litter or when seeking refuge in logs or rocks (TOWNSEND JR *et al.*, 2006, 2008). According to TOWNSEND JR *et al.* (2008), harvestmen species that live in proximity to the forest floor are more likely to be parasitized and with greater intensities than individuals that occupy higher microhabitats, e.g., arboreal species or

species than lives on bromeliads in the canopy. *Gonyleptes fragilis* occurs in the low understory, always close to the ground, in small trunks and low leaves, but rarely buried in the litter (C. Bragagnolo, pers. obs.). This habitat may be favorable for erythraeid mite infestation.

Several studies are dedicated to understanding the mode and sites of attachment of erythraeid larvae to hosts (ÂBRO, 1988; MCALOON & DURDEN, 2000; TOWNSEND JR *et al.*, 2008; MARTIN & CORREIA-OLIVEIRA, 2016; BERNARD *et al.*, 2019). Regarding to the mode of attachment, according to ÂBRO (1988), the larvae of *Leptus* deposit at the attachment site a cementing substance forming a superficial cone that concurrently with the distension of the distal portions of the inserted chelicerae promotes firm anchorage in order to ensure the long attachment of the larvae to the host and so suck up hemolymph plasma and tissue fluids (ÂBRO, 1988; see a good picture in TOWNSEND JR *et al.*, 2008, Fig. 1). Although the present study did not verify whether the relationship between the species is in fact ectoparasitism (or just phoresy, as an alternative hypothesis), the fact that *Leptus* larvae remained attached to the harvestman's exoskeleton even after immersion in alcohol, in moment of fixation, indicates that there are fixation structures between the parasite and the host, therefore it is a relationship of parasitism.

Most studies in invertebrates found that the mites show attachment sites preferences, varying to thorax and the ventral surface of the body and/or the legs (in insects, YOUNG & WELBOURN, 1988; PEREIRA *et al.*, 2012, MARTIN & CORREIA-OLIVEIRA, 2016; ATWA *et al.*, 2017; BERNARD *et al.*, 2019; and in harvestmen, MCALOON & DURDEN, 2000; TOWNSEND JR *et al.*, 2008). In insects, the mites preferentially attached sites along moult suture lines, such as the thorax (ATWA *et al.*, 2017) or the ventral surface (BERNARD *et al.*, 2019), sites that have the softest and most flexible cuticle. The only study that verified the location of the parasites in Laniatores species found 175 of the 178 larvae on the tibia-femur of the legs. The other three mites were observed on the dorsal scutum, near the spines (TOWNSEND JR *et al.*, 2008). The same preference was found for Eupnoi species, as *Leiobunum indianensis* (MCALOON & DURDEN, 2000). In a general way, the mites attached to smooth areas of the host integument between rows of setae and/or spines (MCALOON & DURDEN, 2000). These findings in harvestmen could reflect a response to agonistic behaviour by the harvestmen attempting to deter or dislodge the ectoparasites (MCALOON *et al.*, 2000). However, it is important to note that no studies to date have found agonistic behavior in the hosts. PEREIRA *et al.* (2012) noted that all parasitized reduviid predators brought to the laboratory fed normally with no distinct differences in its behavior. No defensive attempts were also observed in harvestmen by ÂBRO (1987). However, it should be noted that even if harvestmen ignore mite larvae, this does not prevent them from behaving defensively against other possible harm.

Laniatores have one of their main protection strategies against predation the release of odoriferous substances from glands presents in the latero-anterior region of the body (PINTO-DA-ROCHA *et al.*, 2007) and when threatened, some groups squirt this substance, whereas in other groups, as many Gonyleptidae species (C. Bragagnolo, pers. comm.), this substance runs down the entire side of the animal's body.

Laniatores also present a frequent grooming, which could prevent or make it difficult for the parasites to attach to the appendages. Therefore, it is possible to postulate that the location of the ectoparasites in the middle region of the scutal area III/IV is a possibility for the erythraeid larvae to be, at the same time, not under the effect of grooming or of the repugnant substance. Such preferences could explain adaptive mechanisms that allow larval mites to co-evolve successfully and parallel to their hosts.

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