



Scientific Note

Intranest mutualisms between the forest fire ant *Solenopsis virulens* (Fr. Smith) (Hymenoptera; Formicidae) and two families of Hemiptera (Membracidae, Monophlebidae) in Brazilian Atlantic Forest

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Abstract. Hemipterans and ants are frequently recorded in trophobiotic associations, generally mutualism. Nests of the fire ant *Solenopsis virulens* (Smith) found at the base of trees in the Atlantic Forest biome, Brazil, were investigated for their commensal fauna. The present study provides new information about the mutualism between the fire ant with hemipterans Membracidae and Monophlebidae found in their nests, this report is the first mention of this kind of relationships in South America. Such associations are very common between ants and treehoppers but have seldom been reported with the family Monophlebidae.

Keywords: Agroforests; Bahia; Endoiastinae; entomology; *Scytodepsa tricarinata*.

Numerous trophobiotic associations, generally mutualisms, have been reported between ants and hemipterans of the suborders Auchenorrhyncha and Sternorrhyncha (DELABIE 2001). This kind of relationships are very common between these two groups of organisms and a range of studies have been carried out to better understand their intimate relationships, including natural history (BLAIMER 2010; HAWKESWOOD & TAYLOR 2018; MARQUES *et al.* 2018), physiology (BLANCHARD *et al.* 2019), nutritional dependences (DETRAIN *et al.* 2010), as well as complex studies involving models of mutualistic interaction networks (FAGUNDES *et al.* 2016; FORNOFF *et al.* 2019).

Solenopsis virulens (Smith) (Figure 1A) (ANTWEB 2021) is a fire ant species with monomorphic workers (PITTS *et al.* 2018). This ant builds conspicuous earthen mounds at the base of trees and herbaceous plants, fallen tree trunks and below stones (PEREIRA *et al.* 2015). This species inhabits the entire Northeast of South America and is found in forests or agroforests from the base of the Andes in Southern Bolivia to the Atlantic Coast of Bahia, Brazil (TRAGER 1991). Until now, no study about mutualistic and other kinds of interactions between this ant and hemipterans is available, except the description a new species of Psychodidae found in colonies of *S. virulens* (PEREIRA *et al.* 2015). The present study provides new information and illustration about the protective behavior, and interaction between the ant *S. virulens* and hemipterans of the Membracidae and Monophlebidae families sampled in a remnant of the biome Atlantic Forest in Northeastern Brazil.

The hemipterans were collected from three nests of *S. virulens* in April to May 2013 in the Michelin Ecological Reserve (REM) - Ouro Verde (Figure 1B - collection trail), in both municipalities of Ituberá and Igrapiúna, state of Bahia, Brazil. This reserve incorporates discontinuous areas of primary and secondary forests (13°47'07.3"S 39°10'36.2"W), and the climate is considered wet equatorial (Köppen-Geiger climatic classification: Af). The Private Natural Heritage Reserve (RPPN) has an average annual rainfall close to 2,000 mm and temperatures that vary between 18° and 30°C, with rainfall distributed throughout the year (ICMBIO 2021).

The hemipteran specimens found interacting with *S. virulens* were actively collected with forceps, and are deposited in the Prof. Johann Becker Entomology Collection at the Zoological Museum of the Universidade Estadual de Feira de Santana, Bahia, Brazil (MZFS). The biological material was identified based on external morphology to species or family levels by specialists (Dr. Ana Lúcia B. G. Peronti, Universidade Estadual Paulista; Dr. Penelope Gullan, The Australian National University; and Dr. Olivia Evangelista, Australian National Collection).

Individuals of two families of Hemiptera were found in three fire ant colonies. One nest sheltered 18 imagos and nymphs of *Scytodepsa tricarinata* Funkhouser (Auchenorrhyncha, Membracidae, Endoiastinae) (Figure 1C, 2B - white circle), and the other two nests sheltered around 100 individuals of a single morphospecies of the Monophlebidae family

(Sternorrhyncha, Margarodidae *sensu lato* - probably a new genus and species) (Figure 1D, 2D, 3A-D). Species from these two families of hemipterans have already been reported living in mutualism with other ants, producing honeydew or wax and proteins in exchange for protection from predators. Such associations are very commonly reported for Membracidae (DELABIE 2001) but have seldom been recorded with the family Monophlebidae (as genus *Icerya* Signoret, in DELABIE 2001).

The investigated *S. virulens* nests were found as conspicuous ground mounds in a remnant of native forest. The trees where the nests were found were close to a hiking trail which crosses the reserve. Two nests (10-20 cm in height) were leaning at the base of trunks. One nest had aboveground shelters reaching approximately one meter above the soil level (Figure 2C, selected area). The hemipterans were collected feeding directly on the trunk, inside the ant nest, near the tree basis. Most specimens were in contact with roots.

Although the passive and active defense behavior of ants to hemipterans in mutualisms is very common, we report and illustrate such a relationship for *S. virulens* for the first time. Their passive defense behavior involved building a structure from a layer of soil to hide/defend the hemipterans from potential predators or parasitoids, such as a protection shelter as does a range of other ants, in general, arboreal species. Figures 2A-B (A - before and B - after soil removed) and figures 2C-D (C - before and D - after soil removed) show

the contrasting soil volume used by the ants to cover the hemipterans.

The active protection behavior observed between *S. virulens* and their hemipterans was no different from what has already been reported in the literature (STADLER & DIXON 2008). The ants defended themselves against the forceps in all colonies and actively tried to protect individuals of both families of Hemiptera with stings and bites. Also, the ants inspected the hemipterans with their antennae and mandibles (Figure 3A-D). It is well known that the presence of ants effectively reduces the populations of Hemiptera natural enemies (HÖLLDOBLER & WILSON 1990; DELABIE 2001; STADLER & DIXON 2008), e.g., Hymenoptera endoparasitoids of *Phenacoccus solenopsis* Tinsley, the cotton mealybug (XU et al. 2020).

The shrub from the genus *Piper* (Piperaceae) hosted the mutualism between *S. tricarinata* x *S. virulens*. This is the first record of membracids of the genus *Scytodepsa* associated with ants. *Scytodepsa* belongs to the subfamily Endoistastinae, which shows parental care behavior (DIETRICH et al. 2017) and have now been reported being assisted by ants, characteristics that are common in other membracids with reported close relationships with ants. Although aboveground carton shelters have frequently been described ("cowsheds" in ANDERSON & MCSHEA 2000 - review) for arboreal ants (eg., *Crematogaster*, MCKAMEY 1992), they are seldom described for ground mounds and membracids (KLIMES et al. 2018), and this report is then the first mention of this kind of relationships

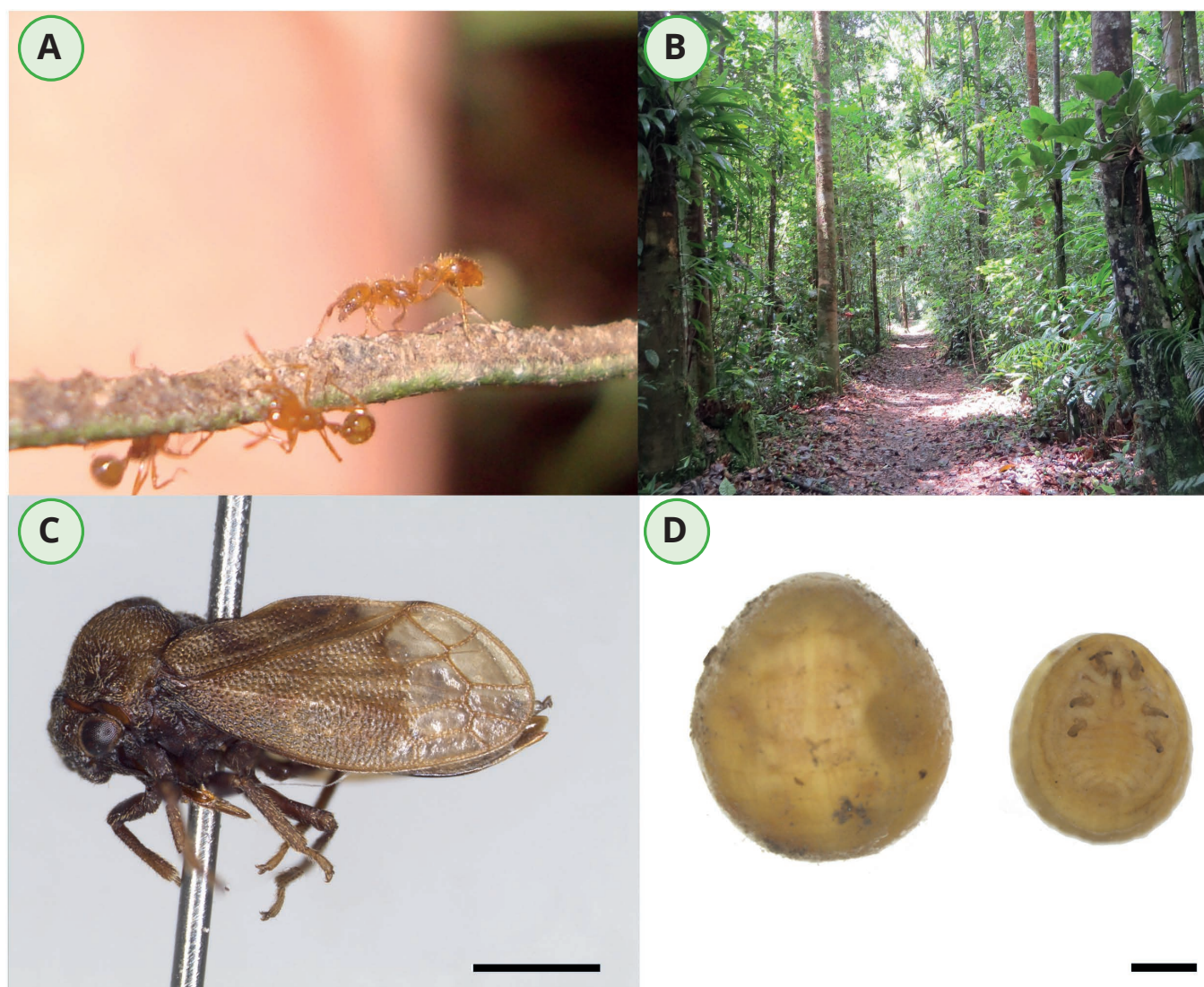


Figure 1. A. *Solenopsis virulens* (Smith) (Hymenoptera: Formicidae: Myrmicinae); B. Collection trail - Michelin Ecological Reserve (REM) - Ouro Verde RPPN - Bahia - Brazil - C. *Scytodepsa tricarinata* Funkhouser (Hemiptera: Auchenorrhyncha: Membracidae); D. Unidentified Monophlebidae (Hemiptera: Sternorrhyncha), dorsal (left) and ventral (right) view. Scale bars: C-D = 1 mm.



Figure 2. *Solenopsis virulens* (Smith) (Hymenoptera: Formicidae: Myrmicinae) nests. A.B. Nest on *Piper* (Piperaceae) shrub. A. Before soil removal B. After soil removal, white circles = *Scytodepsa tricarinata* Funkhouser (Hemiptera: Auchenorrhyncha: Membracidae) nymph detail. C.D. Nest on tree C. Before soil removal, selected area = nest. D. After soil removal, white circle = unidentified Monophlebidae in detail.



Figure 3. Interaction between *Solenopsis virulens* (Smith) (Hymenoptera: Formicidae: Myrmicinae) and unidentified Monophlebidae (Hemiptera: Sternorrhyncha). A.B.C.D. *S. virulens* interacting with the Monophlebidae sp. White circles = interaction in detail.

in South America.

The interaction between the family Monophlebidae (Margarodidae *sensu lato*) and the fire ant *S. virulens* is being reported herein for the first time. Reports of Monophlebidae interacting with ants (MARICONI & ZAMITH 1960; HAWKESWOOD & TAYLOR 2018) and protected by an ant-constructed shelter have already been mentioned but were not illustrated nor detailed, such as in *Mimosicerya schraderae* (Vayssière) in Panama (VAYSSIÈRE & HUGHES-SCHRADER 1948 - unidentified ant).

Other studies described fire ants sheltering mealybugs (Pseudococcidae) (HELMS & VINSON 2002) and plant nectaries (ÁVILA-NÚÑEZ & OTERO 2018). Behavioral similarities between other fire ants were pointed out by HELMS & VINSON (2002): *Solenopsis invicta* Buren builds shelters in the ground housing

the mealybug *Antonina graminis* (Maskell) and aboveground shelter maintains aphid colonies (Hemiptera: Aphididae).

It is important to highlight that Monophlebidae, Margarodidae *sensu stricto* and Margarodidae *sensu lato* had suffered taxonomic important changes in the past few decades (FOLDI 2005; BEN-DOV 2011; FOLDI & GULLAN 2014), and former information about the mutualisms between Margarodidae/Monophlebidae and ants needs to be checked.

The discovery of a putative new genus of Monophlebidae in the Atlantic Forest only reinforces the need to know more about the fauna diversity and preserve the Biome. Finally, understanding more about the mutualisms between ants and hemipterans could help reaffirm evolutionary hypothesis about these two groups of organisms (eg. when

this interaction first occurred in the phylogeny of the groups (DIETRICH et al. 2017)), and the results found herein fill an information gap about the biology of these insects.

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