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Running title: *Leptus* parasitizing stingless bees

First record of *Leptus* spp. (Acari: Erythraeidae) parasitizing stingless bees (Apidae: Meliponini)

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Abstract

The first occurrence of *Leptus* mites parasitizing different species of stingless bees is reported. The samples were collected in the Argentinian province of Misiones on flowers and managed stingless bee colonies. Although the frequency registered was very low, the current report is relevant as it will drive research efforts on the understanding of parasite-host dynamics, consequences at community level, and disease management in wild and productive contexts.

Keywords. Argentina - Bee health - Mites - Wild bees

Introduction

Host-switching and geographical dispersal of parasites are common and complex phenomena. Nevertheless, research into host-parasite interactions mainly focuses on 'one host- one parasite' systems (Hellard *et al*., 2015) underestimating the influence of generalist parasites and its consequences in the ecology of the communities and epidemiology (Bonsall, 2010). Furthermore, species with a higher level of social organization, as bees forming colonies, usually impairs a deeper challenge to the understanding of the referred complexity.

Stingless bees (Meliponini) are social bees, native in several continents, including Central and South America, Australia, Asia and Africa (Rasmussen & Cameron 2010). More than 600 species were described worldwide and a total of 37 species are present in Argentina, mainly in the forests and jungles of the North of the country, where Misiones Province has the greatest richness and abundance of species (Zamudio & Alvarez, 2016). However, the knowledge about the diseases affecting stingless bees is still very restricted, specifically on the parasites hosted.

Introduced species, specifically bee species, can compete for food resources and are potential carriers of emerging parasites and infectious diseases (Paini, 2004; Strauss *et al*., 2012). A particular concern is the spill-over of pathogens from honey bee (*Apis mellifera* L.), a widely distributed bee species introduced in America more than 400 years ago and commonly sharing habitat with stingless bees. Its increasing population in the region, enhanced by food production activities, along with the introduction of other exotic pollinators (e.g. *Bombus terrestris* (L.)), may have potential impact on the native wild and managed bees (Nanetti *et al*., 2021).

The genus *Leptus* (Acari: Erythraeidae) parasitizes many arthropod groups worldwide (Salvatierra & Almeida, 2017). Currently, 281 species are described of which 227 are only known because of its larvae (Bernard *et al*., 2019). In Argentina, only few studies are available on Erythraeidae, including a report in psyllids (Baliotte *et al*., 2021) and orthoptera (De Santis *et al*., 1992). *Leptus* mites are only parasitic during the larval instar and are predators during post-larval stages. Larvae are characterized by presenting one pair of eyes set on each side of prodorsum, coxal field leg II and III with one seta, genu legs II and III each with less than 11 setae, trochanter legs I and II each with one setae, femur palpal genu with one setae; scutum prodorsal sclerite triangular usually wider than long and cheliceral base elongate distally with distal half less than one half width of basal width (flasklike) (Welbourn & Young, 1987). Furthermore, *Leptus* is divided in two subgenera: *Leptus* (*Leptus*) Latreille y *Leptus* (*Amaroptus*) Haitlinger, by the presence of two or three pairs of setae in the prodorsal shield, respectively (Haitlinger, 2000).

Until now, *Leptus* species have been reported as parasites in ten orders of arthropods (Haitlinger *et al.,* 2020), including several families of Hymenoptera: Formicidae, Vespidae, Apidae and Colletidae (Fletchmann, 1980; Southcott, 1989, 1993; Teixeira, 2008; Pereira *et al*., 2016). Although some mite species are associated with Meliponini (Fain & Fletchmann, 1985; Da-Costa *et al*., 2021), the goal of this work is to report the genus *Leptus* as a parasite in stingless bees.

Material and methods

Worker stingless bees, representing several species, were collected in the frame of ecological and meliponiculture sanitary studies, performed in Misiones Province, Argentina. The individuals were collected over flowers, with entomological nets, bait traps or at the entrance of managed colonies. Although the sampling efforts in the monitoring campaigns, performed in a period of eight years, were abroad and included a wide range of the territory of the Misiones Province, we focus the present report on a reduced number of specimens and four specific sampling dates (Table 1, Figure 1). One sampling campaign, performed in 2019 in Capioví city, Misiones, achieved the sample collection on 12 managed stingless bee colonies of *Scaptotrigona depilis*. Newly collected specimens of stingless bees (2019), or specimens collected in different previous campaigns (2011, 2016, 2017) and later deposited in Museo de La Plata, Argentina (MLP), were identified with the aid of a stereoscopic microscope Nikon SMZ 745T. During the search for infested bees in the Museum collection, 1769 individuals from 12 different stingless bee species were visualized.

The number of individuals collected was variable depending on the methodological needs for each study; however, when the information was available, the parasitism frequency was estimated (Table I). After the identification of potential parasitic mites, the individuals were photographed on its host, recording its position on the bee body. Then, the mites were removed with entomological forceps from the fixation site and preserved in 70% alcohol for taxonomic identification according to the keys proposed by Welbourn & Young (1987).

Results

From the sampling campaign performed in 2019 in Capioví city, only one *Leptus* mite was detected in one bee from a pool of 60 worker bees, collected from a managed colony of *Scaptotrigona depilis* (Moure) (Figure 2 and 3). The mite was easily visualized over the thorax because of its relatively big size and bright red-colored cuticle. Details of the larva collected on *S. depilis* in 2019 are shown in Figures 2 and 3. More mites were obtained from stingless bee individuals deposited in the museum (MLP, Argentina). From this sample set, *Leptus* mites were found in other three stingless bee species identified as *Melipona torrida* Friese, *Schwarziana quadripunctata* (Lepeletier) and *Trigona spinipes* (Fabricius). Each parasitized insect had only one attached *Leptus* mite larva, placed on different parts of all tagmata (Table 1 and Figure 2). Despite the collected mite specimens were not identified at a specific level, morphological differences were found indicating that parasitic mites might belong to more than one species.

Prevalence of parasitism, estimated from the total number of stingless bees collected was variable (Table I).

Discussion

Beyond the recognized economic and cultural importance that Meliponini represents for rural communities (Zamudio & Hilgert, 2015), it is important to sustain with scientific approaches the understanding of the delicate forest ecosystem, either to reinforce conservation actions as well as for the assessment of sustainable meliponiculture production. In this work, we report for the first time the occurrence of larvae of *Leptus* parasitizing four species of stingless bees, a fact which we consider of interest because of the scarcity of knowledge on the health status of managed and wild stingless bee populations.

Although in many cases, the proportion of parasitism with *Leptus* larvae in relation with the total amount of bees collected in one sampling was higher than 5% (i.e. 6,25% in *T. spinipes* or 14% in *S. depilis*), the deep search in the museum collection and the large quantity of colonies sampled in different studies without findings of mites, should be interpreted as a commonly low prevalence of these genus. Furthermore, the neighbor stingless beekeepers interviewed during our field work had no record of the presence of parasites of this kind in their colonies (personal communication). It is worth understanding if the few specimens found answer to a low natural frequency that it is stable over time or if, on the contrary, it is a phenomenon that is possibly increasing its intensity with stingless bees' rationalization. Furthermore, it could be relevant to address the potential danger associated with pathogens dispersed from managed *A. mellifera* colonies, which are commonly bred and multiplied in the region for productive purposes. In the last five years an increasing diversity of parasites and pathogens of *A. mellifera* have been reported in stingless bees (Ueira-Vieira *et al.*, 2015; Guimarães-Cestaro 2016; Nunes-Silva *et al.*, 2016; Caesar *et al.*, 2019; Porrini *et al.*, 2017; Alvarez *et al.*, 2018; Teixeira *et al.*, 2018; Yañez *et al.*, 2020). Therefore, the infestation of stingless bees with *Leptus* coming from honey bee populations is probable since both species usually share the same floral resources. Moreover, it is known that honey bee rationalization (from the wild nests to a system of rational-based frames and hives with mobile parts) has led to the development and spread of honey bee diseases (Fontana *et al*., 2018) and therefore, the intensification of this process in stingless bees may enhance pathogens and parasites spread, posing a threat to both managed and wild stingless bee populations. For this reason, it is necessary to be alert to these first detections of parasitism, which could involve spillover and spillback phenomena in detriment of native bee populations (Daszak *et al*., 2000). Further studies are required to analyze the degree of parasitism in the system *Leptus*-stingless bees due to the feeding habit of *Leptus* larvae, which actively consume the hemolymph of their host by perforating its cuticle, and could act as vector of viral particles or other microorganisms (Martin & Correia-Oliveira, 2016).

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Disclosure statement

The authors report there are no competing interests to declare.

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Table 1

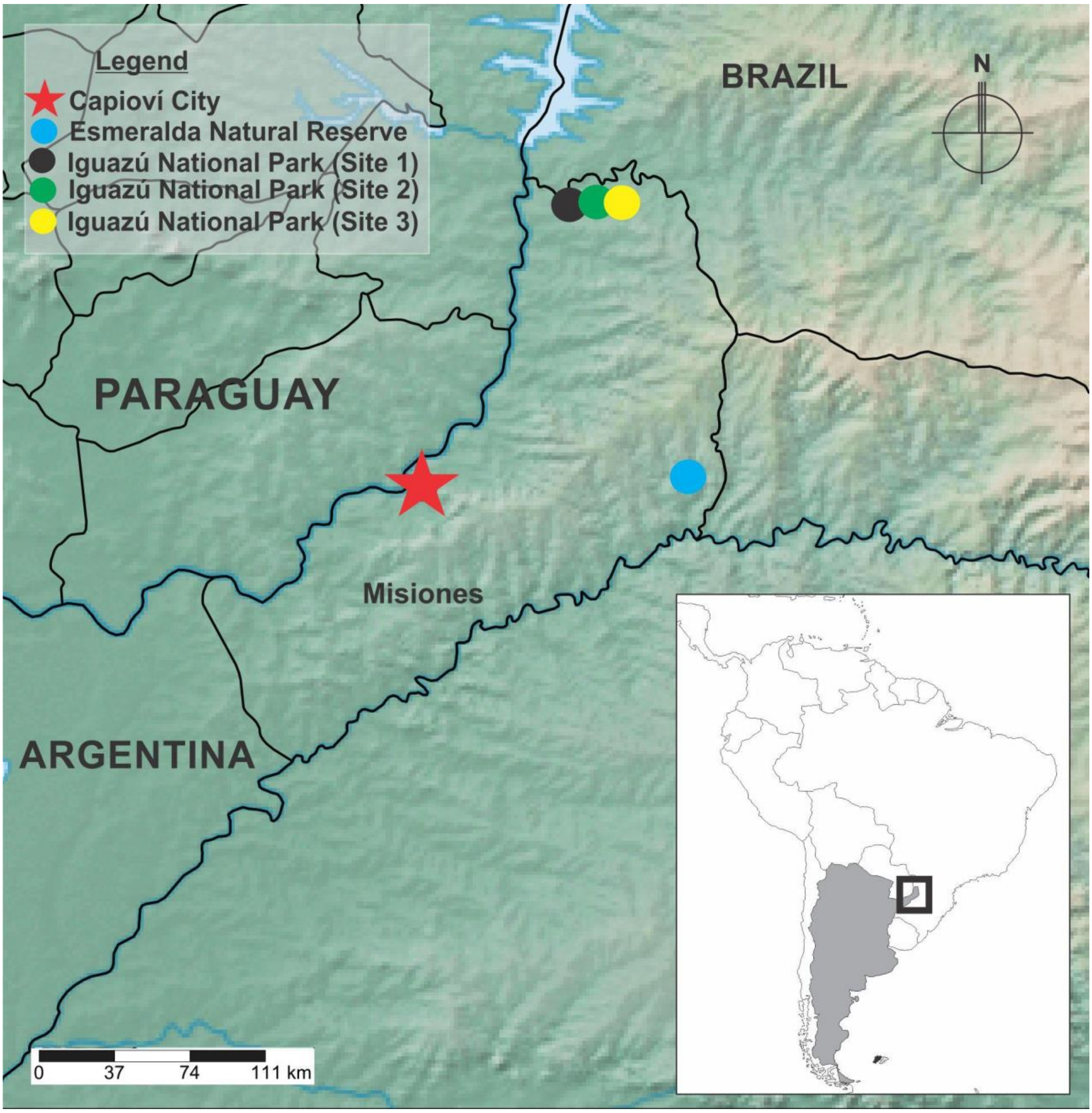
Stingless bee species	Collection date	Collection place	Capture method and relative frequency	Mite position in the worker bee body
Melipona torrida (Friese, 1916)	01/10/2016	Iguazú National Park, Iguazú (Site 1)	Forager, from flower. **(3/5)**	Right gena (Fig. 2A)
Melipona torrida (Friese, 1916)	01/10/2016	Iguazú National Park, Iguazú (Site 1)	Forager, from flower. **(3/5)****	Distal portion of the anterior surface of the left midfemur (Fig. 2B)
Melipona torrida (Friese, 1916)	01/10/2016	Iguazú National Park, Iguazú (Site 2	Forager, from flower. **(3/5)****	Right side of the dorsal surface of pronoto
Scaptotrigona depilis (Moure, 1942)	08/12/2017	Iguazú National Park, Iguazú (Site 3)	Forager, captured with bait traps **(1/7)****	Right lateroinferior surface of propodeum (Fig. 2C, Fig 3)
Scaptotrigona depilis (Moure, 1942)	01/09/2019	Capioví	Forager from rational hive **(1/60)***	Center of propodeum (Fig. 2D)
Schwarziana quadripunctata (Lepeletier, 1836)	15/12/2011	Esmeralda Natural Reserve, San Pedro	Forager, from flower **(1/19)****	Vertical portion of T1(Fig. 2E)

Trigona spinipes (Fabricius, 1793)	29/09/2016	Iguazú National Park, Iguazú (Site 3)	Forager, captured with bait traps **(2/32)****	Vertical portion of T1
Trigona spinipes (Fabricius, 1793)	29/09/2016	Iguazú National Park, Iguazú (Site 3)	Forager, captured with bait traps **(2/32)****	On the right portion of T2 (Fig. 2F)

Caption Table 1.

Parasitized stingless bee species in Misiones, Argentina. Date, collection place, capture method, and position of the mite on the bee. The frequency was estimated based on the number of infested individual stingless bees over the total quantity collected in one hive, trap or sampled on flowers. * One mite was detected over one bee out of 60 worker bees sampled from one managed rational hive (out of 12 managed colonies of the same species present in the meliponary). ** During the search for infested bees,1769 individuals deposited in the Museum of La Plata (Argentina) were visualized under a stereoscopic magnifying glass, representing 12 different stingless bee species.

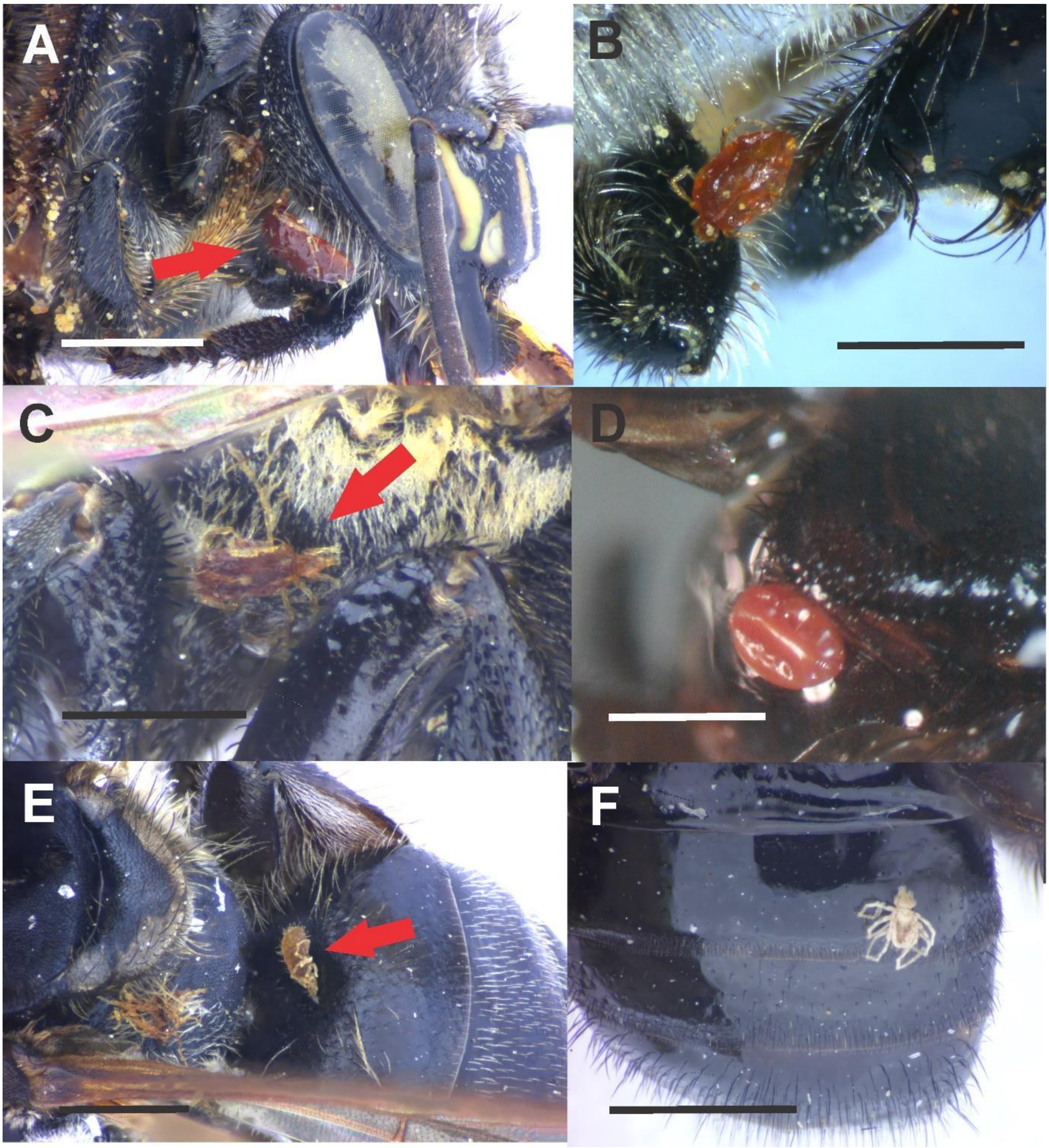
Figure 1



Caption Figure 1.

Map showing the localities where the *Leptus*-stingless bees association was registered. Star: Sample collected on managed stingless bee colony. Circles: Sample points of ecological studies where foraging bees were collected with nets or traps. Capioví City: -26.930344, -55.049297 (Red); Esmeralda Natural Reserve: -26.894167, -53.874363 (Blue) and localities in Iguazú National Park [Site 1: -25.689694 -54.322000 (Black), Site 2: -25.673806, -54.275778 (Green) and Site 3: -25.675906 -54.230741(Yellow)]. The map was generated using SimpleMappr (https://www.simplemappr.net/).

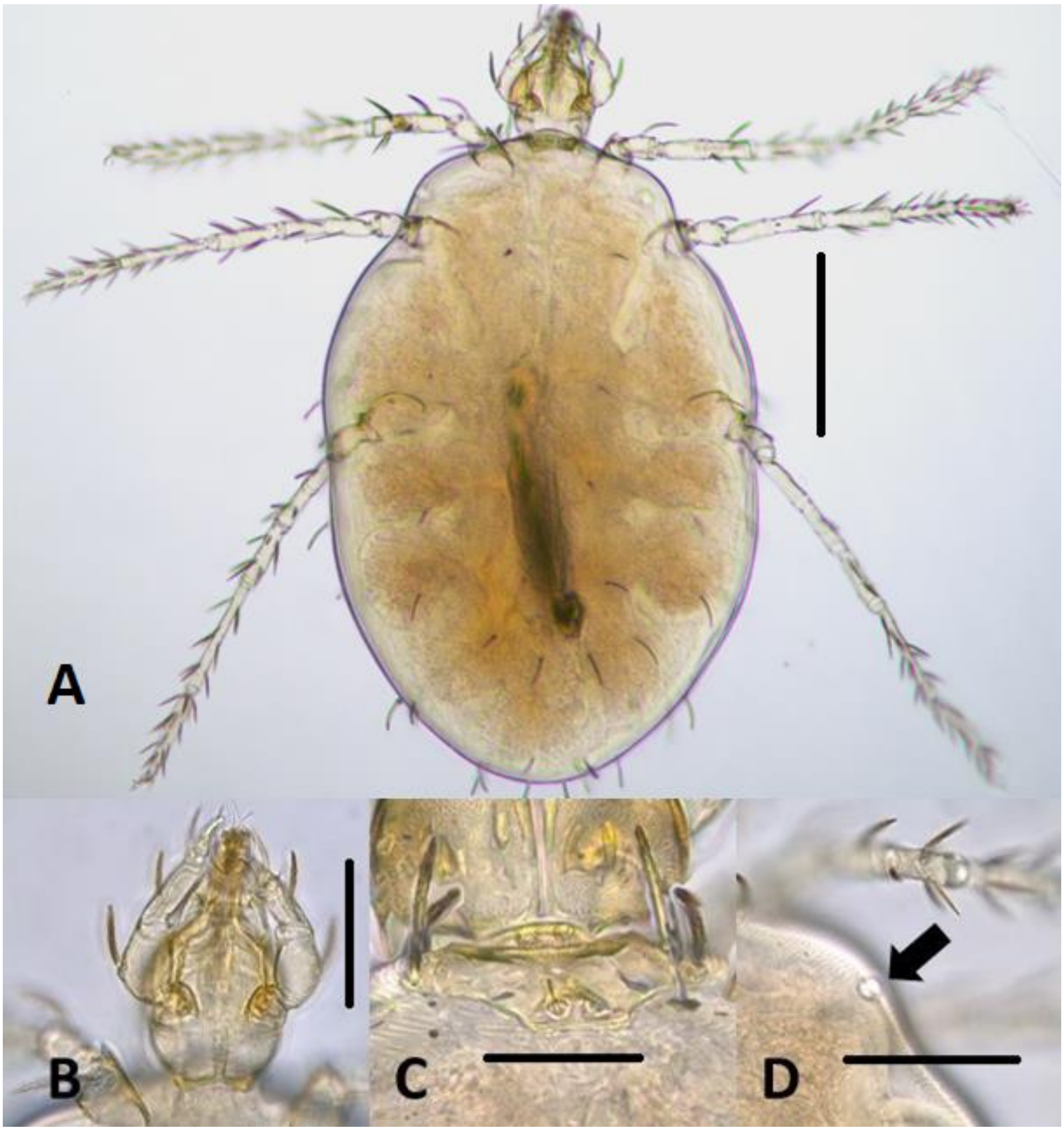
Figure 2



Caption Figure 2.

Position of *Leptus* larvae on stingless bees. A. On right gena in *M. torrida*. B. On left midfemur in *M. torrida*. C. On right lateroinferior surface of propodeum in *S. depilis.* D. On the center of propodeum in *S. depilis*. E. On vertical portion of T1 in *S. quadripunctata*. F. On the right portion of T2 in *T. spinipes*. Red arrow indicates the mite. Scale bars: A-F= 1mm.

Figure 3



Caption Figure 3.

Leptus sp. larva collected from *S. depilis* in 2019. Also shown in Fig. 2D. A) Ventral view; B) Detail of the gnathosoma, ventral view; C) Prodorsal shield; D) Humeral region, the arrow indicates the eye. Scale bars: A= 200µm, B and D= 100µm, C= 50µm.