The occurrence of ecto-parasitic *Leptus* sp. mites on Africanised honey bees

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Summary

Honey bee-mite-pathogen associations have led to the widespread collapse of *Apis mellifera* colonies in various parts of the world. The global trade in bees continues to expose honey bees to new pests and pathogens. Here we highlight to the beekeeping community a potential new mite-pathogen association. In South America ecto-parasitic *Leptus* mite larvae have been recorded parasitising adult honey bees and these mites are known to transmit *Spiroplasma* bacteria the causative agent of 'Mays disease' in bees. Here we provide new data and review past studies on *Leptus* mites and discuss the potential risk to *A. mellifera* this mite may pose in the future.

Key words: *Leptus*, mites, honey bees, *Spiroplasma* bacteria

Introduction

Although hundreds of mite species are associated with honey bee colonies, less than ten can be classed as parasitic, i.e. obtaining nourishment from the bee's haemolymph (De Jong, Morse, & Eickwort, 1982; Sammataro, Gerson, & Needham, 2000). These ten parasitic mites belong to just four groups (tracheal mites *Acarapis* spp; *Varroa* spp; *Euvarroa* spp and *Tropilaelaps* spp). In each case, a stable mite-honey bee relationship has evolved, so although many colonies may be infested the mite populations are maintained at a low level due to a suite of honey bee behaviours. The globalisation of beekeeping has resulted in the movement of *Apis mellifera* well outside their natural range. This has allowed new mite-honey bee associations to arise. For example, the *Varroa destructor* mite introduced a new viral transmission route for Deformed wing virus (Martin et al., 2012), with devastating results.

Therefore, we need to be aware of any new mite-pathogen-honeybee associations that under the right conditions may become another threat to honeybees.

During a research trip to Brazil, parasitic larvae of *Leptus* mites were seen infesting adult worker honey bees. The *Leptus* mites belong to the Trombidiidae family (large red velvet mites) those six-legged larval stage are parasitic on a wide range of adult invertebrates including harvestmen, moths, true bugs, aphids, flies and beetles (Welbourn & Jenning, 1983; Haltinger, 1992; Cokendolpher, 1993; Zhang, 1997; Pereira, Fadini, Pikart, Zanuncio, & Serrão, 2012). However, there are only a few reports from the Hymenoptera. The first was from sweat bees (Eickwort, 1979), followed by the first report on *A. mellifera* from Cerro de Pasco, Peru (Fletchtmann, 1980), then later from a single European honey bee colony in Guatemala (Wilson, Wooley, Nunamaker, & Rubink, 1987), which was later identified as *Leptus ariel* by Southcott (1989, 1992).

The basic life-cycle of all *Leptus* mites is similar with eggs laid in the soil or on vegetation. After hatching the parasitic larval attach themselves to their invertebrate host and gain access to the hosts blood supply by dissolving the hosts cuticle around the area of the mites mouthparts, which forms the attachment site. The larvae become engorged before dropping off their host and return to the soil to develop into an adult via a deutonymph stage. Both the adults and deutonymphs are predatory feeding on arthropods eggs within the vegetation or soil (Zhang, 1997).

The aim of this study is to bring new data about *Leptus* mites and evaluate the potential threat that this mite may poses to the beekeeping community.

Methods

After discovering *Leptus* larva attached to adult honey bees, a total of 30 Africanised honey bee colonies from four apiaries belonging to Federal University of the Reconcavo of Bahia (UFRB) were surveyed for the presence of the *Leptus* larvae during October 2014 and again in January 2015. The four apiaries where within 50km of Cruz das Almas, Bahia in North Eastern Brazil. Each brood frame was removed and the adult bees visually scanned for the mites and any infested bees collected. The blood red colour of the *Leptus* mites (Fig. 1) are very distinctive, resembling red pollen or propolis stuck to the bees body. During October a sample of 40-50 adults bees from each colony was collected from the brood comb and taken back to the laboratory. In the laboratory the attachment locations and numbers of mite infesting each bee were recorded. All mages were taken with a Leica camera attached to a Leica microscope.

Results

During October, visual sampling indicated that 26 (87%) of the 30 study colonies were infested with *Leptus* mites. Colony infestation levels were normally very low >>1%, which was confirmed by the lack of any infested honeybees in 28 out of the 30 bee samples collected via manual inspection. However, in one apiary two colonies had infestation rates between 3-6%, which was quickly apparent during the visual scanning. However, in January 2015 no mites could be found in any of the same 30 colonies. In October 2014 the mean infestation level was two mites per bee, although some bees were very heavily infested (Table 1, Fig. 1). *Leptus* mites were found attached to almost all parts of the bees body (Fig.2) even on the eyes and pollen basket. The mites were at all stages of engorgement (Fig. 1) and a small number of dead shivered mites, but still attached, were seen (Fig. 1). No

94 significant (p=0.7, Fisher's exact test) preference for the left or the right side of the bee was
95 detected.

96 A very close match between the morphology of our Brazilian specimens with the
97 images of *Leptus ariel* recorded parasitising a European honey bee colony in Guatemala
98 (Southcott, 1989) suggested it to be the same species, although this would need confirming
99 by an expert.

100

101 **Discussion**

102 This study indicates that parasitism of adult worker honey bees by *Leptus* mites, can be
103 widespread and reach significantly high levels in some colonies e.g. 3-5% (this study) and
104 5% in the colony from Guatemala (Wilson et al., 1987). Mites do not appear to persist for
105 long periods of time in honey bee colonies, as found in studies of *Leptus* mites infesting
106 Harvestmen (Townsend, Mulholland, Bradford, Proud, & Parent, 2006). This may simply
107 reflect the mites' life-cycle. The heavy infestation of some individuals or colonies may reflect
108 foraging workers visiting plants or collecting water from areas infesting with many questing
109 *Leptus* larvae. The ability of the mite larva to dissolve the hosts cuticle to gain access to the
110 haemolymph means they can be found almost anywhere on the bees body (Fig. 2). Studies on
111 true bugs (Pereira et al., 2012) and Passalid beetles (Baker, 1982) indicated *Leptus* mites only
112 attached to the dorsal surface and none on the legs, suggesting that these places may be less
113 vulnerable to being removed. In honey bees this is not the case since many mites attached
114 themselves in very exposed and seemingly vulnerable positions. Although the mites are only
115 attached by their mouthparts, this is a firm attachment and even dead mites are difficult to
116 remove. However, the mites' soft body, which allows engorgement, should be vulnerable to
117 damage by the bees, but no damaged was seen.

Little is known about the impact of *Leptus* larva feeding on their host. Although *Leptus* larvae are able to transmit *Spiroplasma* bacteria to their host while feeding (DiBlasi Morse, Mayberry, Avila, Morando, & Dittmar, 2011). Furthermore, laboratory studies have shown that other ecto-parasitic mites (*Macrocheles subbadius*) can transfer *S. poulsonii* between different *Drosophila* species (Jaenike, Polak, Fiskin, Helou, & Minhas, 2007). *Spiroplasma* bacteria can be mutualistic or pathogentic (Gasparich, 2010) and in adult honey bees if *Spiroplasma* crosses the gut wall and enters the haemolymph it can kill the bee (Clark, 1977, 1978; Schwarz et al., 2014). In honey bees *Spiroplasma* bacteria are the causative agent of 'May disease' (Mouches, Bov, Albisetti, Clark, & Tully, 1982). A recent survey of *S. melliferum* and *S. apis* were detected in 54% and 33% of colonies surveyed in Brazil and USA respectively (Schwarz et al., 2014). They also found a strong seasonal fluctuation in *Sprioplasma* prevalence. Clark (1978) detected *Spiroplasma* bacteria on flowers suggesting a possible route of infection from the environment to the bees.

Therefore, *Sprioplasma* bacteria are widespread in *A. mellifera* colonies in the Americas and this can cause colony death in some cases. The feeding behaviour of the *Leptus* larvae will allow any *Spiroplasma* infections the mites are carrying, direct access to the host haemolymph, which in turn could lead to death of the bee. However, the impact on colony health appears to be small even in the two heavily infested colonies, which both remained healthy during the next year. This is because the adults infected will be foragers already at the end of their life. *Leptus* mites do not move between hosts and complete their life-cycle outside the hive. So currently the risk posed by *Leptus* mites is small. However, parasitism by *Leptus* larva is probably more common than currently recorded, at least in South America, which remains the only region where the mite has been recorded infesting honey bees.

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Table 1.The *Leptus* mite infestation levels of 86 individual adult honeybees.

Number of *Leptus* larvae per bee	1	2	3	4	5	7	9	10	19
Number of adult bees	58	13	5	2	3	1	2	1	1

Figure 1. Images showing *Leptus* mite larvae at different states of engorgement and at a variety of attachment sites. The lower right panel shows a yellow dead mite that still remains attached to the right hand side of the leg.

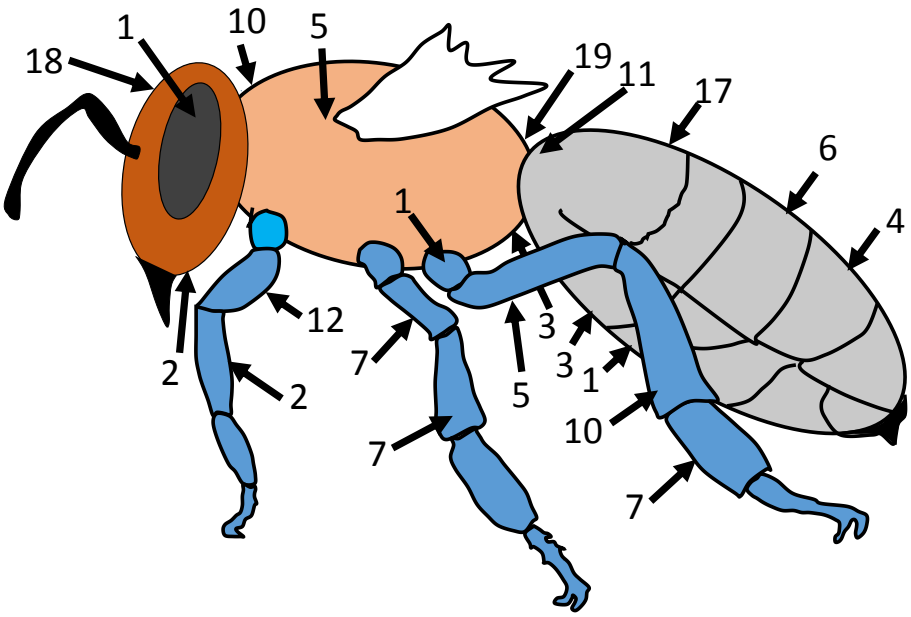

Figure 2. The various attachment locations of 152 *Leptus* mites on adult worker honey bees in this study.