Distribution and Abundance of Phoretic Mites (Astigmata, Mesostigmata) on Rhynchophorus ferrugineus (Coleoptera: Curculionidae)

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DISTRIBUTION AND ABUNDANCE OF PHORETIC MITES (ASTIGMATA, MESOSTIGMATA) ON *RHYNCHOPHORUS FERRUGINEUS* (COLEOPTERA: CURCULIONIDAE)

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ABSTRACT

The red palm weevil, *Rhynchophorus ferrugineus* Olivier, is the most important insect pest on date palms. Weevils were collected from randomly selected infested date palms in Al Ain, United Arab Emirates (UAE). Mites from three families were recorded. *Uroobovella* sp. (Acari: Urodinychidae) were the most common phoretic mites. *Curculanoetus* sp. (Acari: Histiostomatidae) were second in abundance. *Uropoda orbicularis* (Acari: Uropodidae) was recorded on one beetle. The abundance of phoretic mites varied among body parts of *R. ferrugineus*, and the maximum numbers occurred in the subelytral space. The mean intensity, mean abundance, and prevalence of *Uroobovella* and *Curculanoetus* mites did not differ between male and female weevils. *Uroobovella* had significantly greater mean intensity, abundance and prevalence compared to *Curculanoetus*. Most *Uroobovella* and *Curculanoetus* aggregated under the sublytral space, which presumably offers protection from the hot and dry environment. This study is the first to document the presence of phoretic mites on *R. ferrugineus* in UAE and will help to direct future research on their interactions.

Key Words: *Rhynchophorus ferrugineus*, Red palm weevil, *Uropoda orbicularis, Uroobovella*, *Curculanoetus*, phoresy

RESUMEN

El picudo rojo de la palma, *Rhynchophorus ferrugineus* Olivier, es el insecto plaga más importante de la palmera de dátiles. Picudos fueron colectados de palmas de dátiles infestadas seleccionadas al azar en Al Ain, Emiratos Árabes Unidos (EAU). Ácaros de tres familias fueron encontrados. Los ácaros foréticos más comunes fueron *Uroobovella* sp. (Acari: Urodinychidae). *Curculanoetus* sp. (Acari: Histiostomatidae) fueron segundos en orden de abundancia. *Uropoda orbicularis* (Acari: Uropodidae) se encontró en solo un picudo. La abundancia de ácaros foréticos en las diferentes partes del cuerpo de *R. ferrugineus* fue variable, los números más altos se encontraron el espacio subelitral. La intensidad promedio, abundancia promedio, y prevalencia de los ácaros *Uroobovella* y *Curculanoetus* no fue diferente entre picudos macho o hembra. *Uroobovella* tuvo una mayor intensidad promedio, abundancia y prevalencia que *Curculanoetus*. La mayoría de *Uroobovella* y *Curculanoetus* se agregaron en el espacio subelitrico, el cual presumiblemente ofrece protección de un ambiente caliente y seco. Este es el primer estudio que documenta la presencia de ácaros foréticos sobre *R. ferrugineus* en los EAU y servirá para direccionar futuras investigaciones sobre sus interacciones.

The red palm weevil (*Rhynchophorus ferrugineus* Olivier) is a pest of a broad range of palms (Palmae) in southern Asia (Murphy & Briscoe 1999). In the 1980s, the species expanded its range and invaded into the Middle East, North Africa (Abraham et al. 1998; Murphy & Briscoe 1999), and more recently into Western Europe (Conti et al. 2008). *Rhynchophorus ferrugineus* was first detected in the United Arab Emirates (UAE) in 1986 and has since been an important cause of decline in date production (Abraham et al. 1998). The date palm (*Phoenix dactylifera* L.) is the most commonly cultivated plant in the Middle East and North Africa, with hundreds of thousands of tons of dates being produced annually; amounting to millions of dollars worth of dates (Murphy & Briscoe 1999). Consequently, *R. ferrugineus* represents a major threat to the industry.

The biology of *R. ferrugineus* has been studied in great detail in India on several palm species including date palms (Murphy & Briscoe 1999). Generally, *R. ferrugineus* assemble on damaged parts (and sometimes undamaged parts) of date palms. Larvae burrow through the soft tissue of the crown or the base of petioles forming extensive tunnels. In severely infested trees, these tunnels may cause the crown and trunk to weaken and eventually break, resulting in death of the tree. The larva develops in a cocoon and eventually emerges as an adult weevil (Abraham et al. 1998; Murphy & Briscoe 1999).

A number of species have been recorded as natural enemies of *R. ferrugineus* including viruses, bacteria, fungi, nematodes, insects and mites (Murphy & Briscoe 1999). However, limited information exists on the extent to which these species affect *R. ferrugineus* populations. The relationship between some of these species and *R. ferrugineus* ranges from phoretic to parasitic. Phoresy is a complex symbiotic association that has evolved in many organisms as a result of spatial and temporal isolation of their habitats (O'Connor 1982). It is defined as a form of commensalism facilitating the physical transport of one organism on the body of another, during which time no feeding or reproduction occurs in the phoretic organism (Binns 1982; O'Connor 1982). Although the term host is generally associated with parasitism, we use the term host to describe carriers of phoretics as well as hosts of parasites, following O'Connor (1982), Houck & O'Connor (1991) and Houck & Cohen (1995). In the Astigmata, only one of the life stages, the deutonymph (sometimes termed a hypopus), exhibits phoretic behavior. The hypopi have evolved morphological adaptations, such as attachment devices, to aid in phoresy. Phoretic associations are particularly diverse in astigmatid (Acari: Astigmata) and mesostigmatid mites (Acari: Mesostigmata) that have exploited many vertebrate and invertebrate hosts for movement between suitable habitats. Phoresy in the cohort Uropodina (Acari: Mesostigmata) also occurs in the deutonymphs although some species may not develop phoresy in certain habitats (O'Connor 1982; Houck & O'Connor 1991).

Although the primary function of phoresy is dispersal, phoretic associations may be complex (Binns 1982; O'Connor 1982; Houck & O'Connor 1991). Some apparently phoretic mites, for example, may use the association not only for dispersal, but in some examples may even derive nutrition from their host. The hypopi of the *Hemisarcoptes cooremani* (Acari: Hemisarcoptidae), formerly regarded as a phoretic species of the beetle *Chilocorus cacti*, has been shown to acquire fluids from the hemolymph of its beetle host suggesting a transition between a phoretic and a truly parasitic association (Houck & Cohen 1995). Although there is a large body of literature on phoretic mites and their hosts, a consensus on the functions and the adaptive significance of phoresy has not been reached.

Uropoda orbicularis (Acari: Uropodidae) is a widespread phoretic mite found in temperate and arid climates associated with a wide range of beetle species (Bajerlein & Bloszyk 2004). In temperate climates, *U. orbicularis* are found amongst forest leaf litter or on soil as adults but in relatively low densities, with about 30% immature individuals being present (Bloszyk et al. 2002). However, they preferentially live in unstable habitats such as compost heaps, manure, and nests of animals, where their populations are dominated by immature individuals (about 80%). They require phoretic dispersal between these habitats (Bloszyk et al. 2002). Over 25 species of beetles from several different families have been documented as host to *U. orbiculairs*, suggesting that this mite is a generalist without a particular preference for host species (Bajerlein & Bloszyk 2004). Similarly, different phoretic *Uroobovella* species were reported to be associated with a centipede (Bloszyk et al. 2006a) and in bird nests (Bloszyk et al. 2006b), demonstrating a range of acceptable hosts for this genus.

The distribution of *U. orbicularis* on specific regions of the host's body shows considerable variation (Bajerlein & Bloszyk 2004). A study by Atakan et al. (2009) reported the presence of *Uroobovella marginata* deutonymphs in the subelytral space on *R. ferrugineus* in Turkey. Bajerlein and Bloszyk (2004) found that *U. orbicularis* mites studied on 25 beetle species in Poland were mostly attached on the elytra, the ventral surface, and the third pair of legs, although the numbers on the first and second pair of legs and on the pronotum were also significant.

The Histiostomatidae family is one of the largest families of the Astigmatina with about 500 species in 58 genera (O'Connor 2009). In this family dispersal is done through the deutonymphal instar (Evans 1992). Histiostomatid mites live in wet substrates such as decaying vegetable matter (rotting grain, plants, and mushrooms) (Hughes 1976; Fashing et al. 1996). Species of genera *Bonomoia* and *Histiostoma* may be found on decaying wood. Species in these genera live on the rotting wood itself and not restricted to insect galleries as some other taxa (O'Connor 1994). Deutonymphs of *Curculanoetus rhynchophorus* were found phoretic on the African palm weevil *Rhynchophorus phoenicis* (Coleoptera: Curculionidae) (Fain 1974).

Hypoaspis (Acari: Laelapidae) consists of a group of predatory mites which are found associated with many beetles, especially those in the family Scarabaeidae (Costa 1971). Mites in this genus have occasionally been observed eating eggs and larvae of scarabaeid beetles. *Hypoaspis* sp. and *Tetrapolypus rhynchophori* (Acari: Pyemotidae) have been found in association with *R. ferrugineus* (Peter 1989). *Tetrapolypus rhynchophori* are reported to be predators of *R. ferrugineus* (Lever 1969), but the frequency and extent to which these mites affect beetle populations remains unknown (Peter 1989). To date, there are no reports of phoretic organisms associated with *R. ferrugineus* in the UAE.

To this end, we examined the mites associated with *R. ferrugineus* collected from Al Ain, UAE. The objectives of this study were to i) determine the species composition and on-host distribution patterns of mites associated with *R. ferrugineus*; ii) examine patterns in the prevalence and abundance of mites on *R. ferrugineus*; and iii) discuss the nature of the association between the mites and their weevil hosts.

MATERIALS AND METHODS

Sample Collection and Preparation

Live adults and pupae (inside cocoons constructed from date palm tree fibers) of *R. ferrugineus* were collected from infested date palm plantations at Al-Ain (24° 11' N, 55° 45' E), UAE from Mar to Nov 2008 and 2009. Adults and pupae were individually collected by hand from infested trees and placed in plastic containers for transportation and were stored in a freezer at -20 °C in the laboratory. Twenty empty cocoons were placed in plastic containers and kept in an incubator 25 °C. Drops of distilled water were added every 2 days to maintain good moisture conditions. Containers were observed daily for 1 month.

For identification, mites were brushed off the weevils using a camel hair brush and stored in 70% ethanol until further processing. Mite specimens were cleared in lactophenol solution, mounted in Hoyer's medium on microscope slides (Krantz 1978; Evans 1992), and examined under a compound microscope. Scanning Electron Microscopy of selected specimens was done at the Central Laboratory Unit at UAE University. *Uropoda orbicularis* and *Uroobovella* sp. mites were identified by Durmus Ali Bal, Erzincan Education Faculty, Ataturk University, Erzincan, Turkey. *Curculanoetus* sp. mites were identified by Barry M. O'Connor, University of Michigan, USA. Mite samples were placed in the collection of the Biology Department of UAE University.

Quantification and Statistical Analysis

One hundred and five adult *R. ferrugineus* were collected from infested date palm trees. Individual *R. ferrugineus* were sexed and mites were counted on each weevil's head, thorax, legs, subelytral space, and abdomen under a dissecting microscope. The software Quantitative Parasitology 3.0, specifically developed to account for aggregated distributions and to allow distribution-free statistical tests, was used to compare loads (Reiczigel & Rózsa 2005). We quantified mean intensity (mean number of mites per weevil), mean abundance (mean number of mites per weevil) and prevalence (proportion of weevils that hosted mites) following Rózsa et al. (2000). Confidence intervals (at 95% confidence level) for mean intensities were computed using bootstrap techniques with 2000 replications (Rózsa et al. 2000). Exact confidence intervals (at 95% confidence level) were calculated for prevalence using the Clopper-Pearson method. Mean intensities and abundance of mites on male and female weevils were compared using bootstrap t-tests, and *P*-values were generated from 2000 replications (Rózsa et al. 2000). Prevalence of mites on males and females was compared using Fisher's Exact Test, with the exact *P*-value reported. Differences in the mean intensity, mean abundance and prevalence of different mite species found on *R. ferrugineus* were also compared using the same methods. The Index of Discrepancy was calculated to determine aggregated distributions of mites on *R. ferrugineus*.

RESULTS

Deutonymphs of 2 mesostigmatid mite species, *Uropoda orbicularis* Müller (1776) and *Uroobovella* sp., were identified. Deutonymphs of *U. orbicularis* (Fig. 1) were encountered on 1 single young adult *R. ferrugineus* collected from a broken infested date palm trunk. The number of *Uroobovella* sp. deutonymphs (Fig. 2) on individual male and female weevils ranged from 0 to 381 and 0 to 598, respectively. Because the mean intensity (Bootstrap t-test, $P >> 0.05$), mean abundance (Bootstrap t-test, $P >> 0.05$), and prevalence (Fischer's Exact test, $P >> 0.05$) of the mites on males and females did not differ significantly, the data were pooled (Table 1). Another phoretic mite of the genus *Curculanoetus* (Astigmata: Histiostomatidae) was recorded mostly in the subelytral space, but very rarely in other areas (Fig. 4 A, D, E).

The prevalence of *Curculanoetus* (17.1) was significantly lower than that of *Uroobovella* (63.8) (Fischer's Exact test, $P < 0.001$). Similarly, the mean abundance (3.6) and mean intensity (21.2) of *Curculanoetus* was significantly lower than the mean abundance (40.2) and mean intensity (62.9)

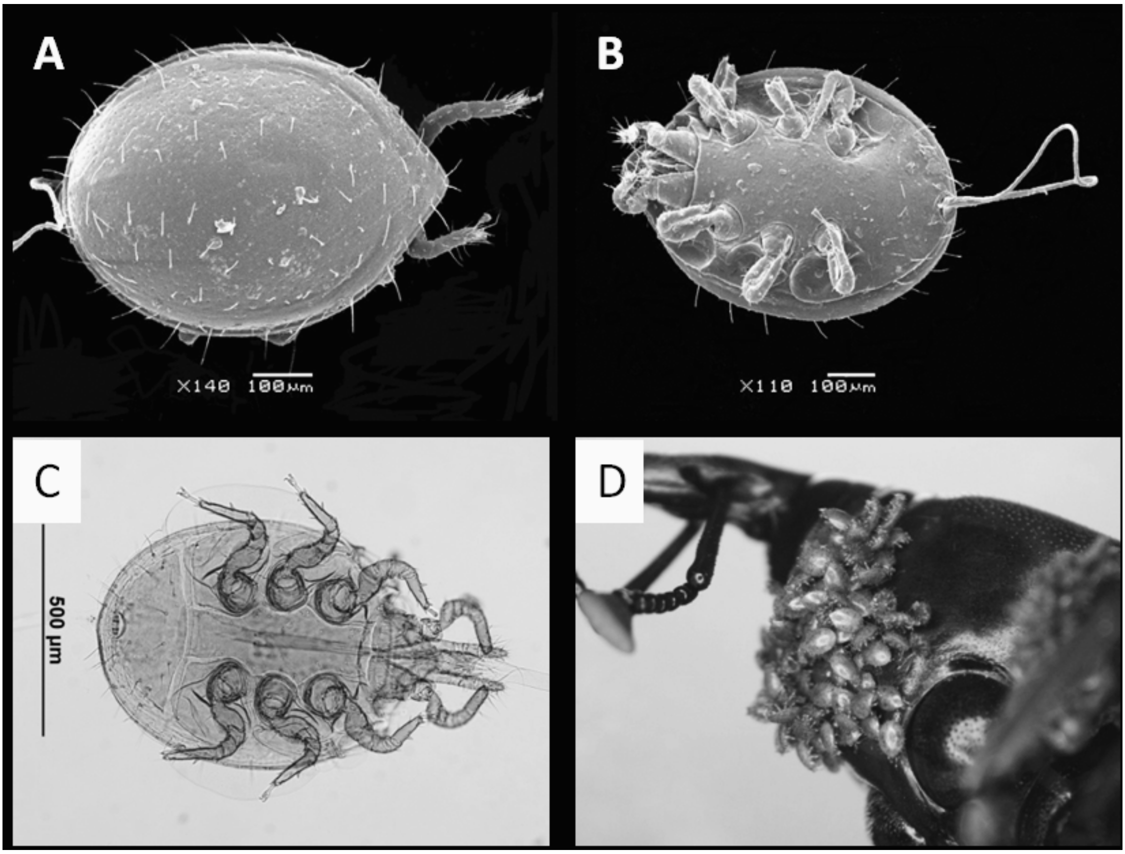

Fig. 1. *Uropoda orbicularis* deutonymph, (A) SEM dorsal view; (B) SEM ventral view showing anal pedicel; (C) under light microscope (×100); (D) deutonymphs aggregated on *R. ferrugineus* head and thorax.

of *Uroobovella* (Bootstrap t-test, P = 0.006 and P = 0.01, respectively). Index of Discrepancy values for *Curculanoetus* and *Uroobovella* were 0.929 and 0.802, respectively. Mite loads on different regions of the weevil also varied substantially (Fig. 3), with the greatest number of mites located in the subelytra in both sexes (Fig. 4 B, C). The membrane wings also had large numbers of mites, while the remaining regions of the weevil harbored few mites in both sexes. *Curculanoetus* and *Uroobovella* mites were not recovered from the heads of weevils. *Uroobovella* deutonymphs were not always phoretic on *R. ferrugineus* adults (i.e. attached with anal pedicels to the weevil body for transportation). They were present in very large numbers on some pupae inside the cocoons (500-800/cocoon) and were noticed actively moving on the pupal exuvia.

In addition to uropodid mites Collembolla were observed on the fibers of the *R. ferrugineus* cocoons, which were incubated at 25 °C. The numbers of these microarthropods increased considerably with increasing stages of fiber decay.

Discussion

Deutonymph stages of 3 mite species (*U. orbicularis*, *Uroobovella* sp. and *Curculanoetus* sp.) were associated with *R. ferrugineus*. *Uropoda orbicularis* and *Uroobovella* sp. were attached to their weevil hosts with the aid of anal pedicels, consistent with other studies (Bajerlein & Bloszyk 2004). In contrast, *Curculanoetus* sp. uses sucker plates for attachment to the weevil hosts. Not all *R. ferrugineus* weevils were associated with phoretic mites and the Index of Discrepancy values for *Curculanoetus* and *Uroobovella* indicated highly aggregated distributions of these mites on their weevil hosts. *Uroobovella* deutonymphs were significantly more prevalent than *Curculanoetus*. This is likely because of food availability and environment conduciveness.

The large numbers of *Uroobovella* and *Curculanoetus*, especially in the subelytral space, suggest that this microhabitat offers protection to the mites as the *R. ferrugineus* walk on the rough date palm tree trunk or under the dense fibers be-

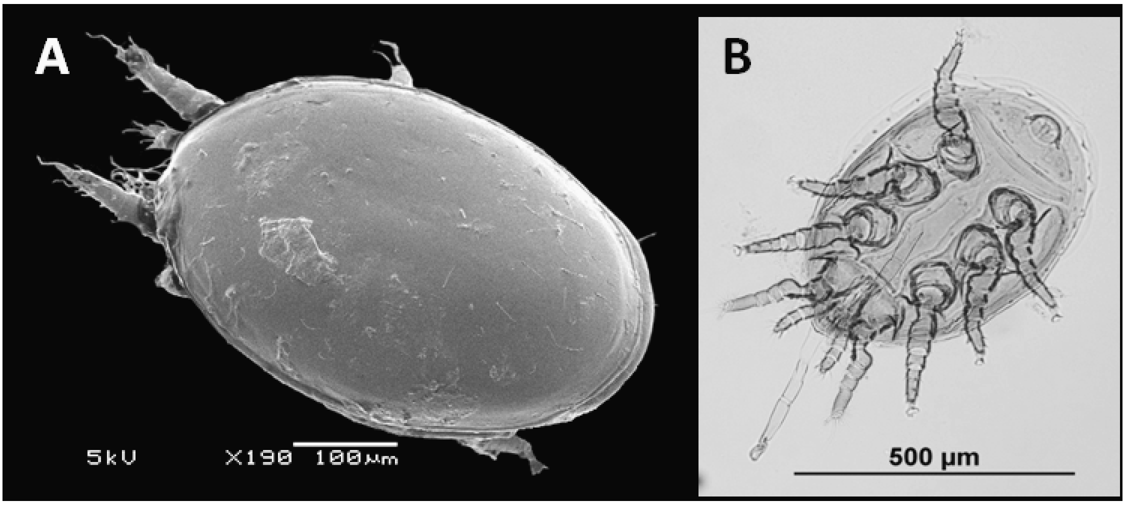

Fig. 2. Deutonymph of *Uroobovella* sp. (A) SEM dorsal view; (B) under light microscope (×100).

tween frond bases. From our field observations, this habitat was also the preferred daytime location of the weevils. The subelytral space probably also gives the mites protection against desiccation in the extremely arid and hot desert climate of UAE. It can also provide phoretic mites a form of protection against predatory mites, since larger predatory mites such as *Hypoaspis* sp. usually remain on the surface of beetles as they seek prey (Costa 1971). Given that the subelytral space appears to be the best choice for phoretic mites, other areas of the host insect, such as membrane wings are likely secondarily populated as subelytral spaces become overcrowded. One additional possible explanation of the subelytra preference is that in Coleoptera the elytra are not used actively in flight, but the membrane wings vibrate strongly during flight. Our findings on mite distributions on *R. ferrugineus* body regions were also consistent with findings of Atakan et al. (2009) and Bajerlein and Bloszyk (2004). It is likely that the low numbers of mites on other body regions could also be due to overcrowding in the preferred subelytral space.

Curculanoetus deutonymphs were found aggregating in the subelytral space near the base of the elytron and inside a small groove in that region. Because they were minuscule compared to the size of *U. orbicularis* and *Uroobovella* they could easily go unnoticed. A clear spatial separation was noticed between the deutonymphs of *Curculanoetus* and *Uroobovella* on the elytron. This could be explained by territoriality or by the presence of specific elytral morphological advantages utilized by each mite group. Members of the family Histiostomatidae have been reported to be involved in phoretic associations with other insects: *Histiostoma* sp., on palm weevils of the genus *Rhynchophorus* (Curculionidae: Coleoptera) (Houck 1994), *H. formosana* on three subterranean termite species (Isoptera: Rhinotermitidae) (Wang et al. 2002), *Histiostoma* sp. on *Cambala reddelli* millipedes (Cokendolpher & Polyak 1996), and *C. rhynchophorus* on *R. phoenicis* (Fain 1974).

A limited number of phoretic uropodid and histiostomatid mites can reasonably disperse on a *R. ferrugineus* host without an obvious impact on the

TABLE 1. COMPARISON OF LOADS OF *CURCULANOETUS* SP. AND *UROOBOVELLA* SP. MITES ON *RHYNCHOPHORUS FERRUGINEUS*.

D	Mean Intensity	Mean Abundance	Prevalence	Range	Mean Intensity
0-93	17.1 (10.5-25.7)	3.6 (1.7-7.9)	21.2 (11.3-36.8)	0.929	*Curculanoetus*
0-598	63.8 (53.8-72.9)	40.2 (26.5-62.1)	62.9 (42.9-94.6)	0.802	*Uroobovella*

Values in parentheses indicate 95% confidence levels for mean abundance, mean intensity, and prevalence computed using 2000 replications. D = Index of Discrepancy, N = 105 adults of *R. ferrugineus*.

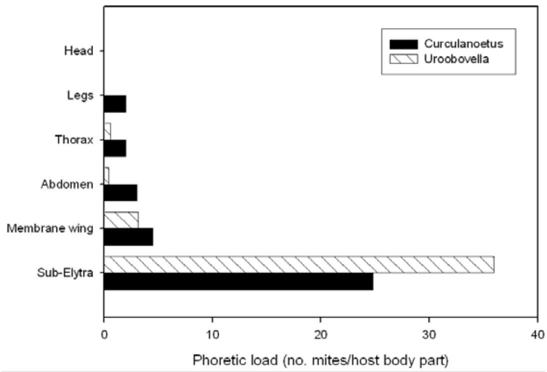

Fig. 3. The distribution of *Curculanoetus* sp. *Uroobovella* sp. deutonymphs on different regions of male and female *R. ferrugineus*.

weevil's ability to fly. However, excessively large numbers on an individual insect could be a burden and may limit the insect's flight performance. It is unknown if phoretic *U. orbicularis*, *Uroobovella,* and *Curculanoetus* could have a detrimental impact to the host, if, by virtue of their presence in extremely large numbers. Such potential negative impacts may include reduction in the efficiency of foraging activities or of movement, making the weevils more susceptible to predation. Therefore, behavioral experiments need to be performed to illustrate if high mite loads are detrimental to *R. ferrugineus*. The negative impacts of phoresy could lead to parasitism and is often regarded as a transition between phoresy and parasitism (Houck & Cohen 1995). Parasitism generally implies some sort of transfer of resources and an associated negative effect on reproductive success of the host. Many mites live on hosts without seemingly harming them (Krantz 1978; Houck & O'Connor 1991). However, quantitative estimates with regard to this aspect of phoresy are limited. For example, Houck and Cohen (1995) documented a transfer of fluid from the hemolymph of the host beetle into the 'phoretic' mite, suggesting a first step toward parasitism. It has been demonstrated that *Rhyzopertha dominica* (Coleoptera: Bostrichidae) adult take-off ability was significantly decreased in the presence of high mite loads (>7 mites per host) of phoretic mites (Rocha et al. 2009). Elzinga and Broce (1988) mentioned that house flies (Diptera: Muscidae) were so burdened with histiostomatid hypopi that they were unable to fly or exhibit normal behavior.

In this study it was noticed that phoresy was not a mandatory choice for every *Uoorobovella* deutonymph. Many of these deutonymphs were observed in large numbers inside and outside cocoons moving freely on the fibers and on the pupal cast skin while adult weevils emerged from these cocoons carrying phoretic deutonymphs (Al-Deeb, unpublished data). Finding a large number of deutonymphs near and on the non-flying stage (pupa) could be explained by shelter- and/or moisture-seeking inside the cocoon. This could also be an attempt of deutonymphs to be as near to the newly emerging adult weevil as possible in order to secure an attachment point on its body.

In addition to *Uoorobovella* deutonymphs, collembolans were observed on the fibers of some of the cocoons of *R. ferrugineus*. Numbers of mites and collembolans increased considerably with increasing stages of decay at 25 °C in the laboratory. Eventually fibers dried out and resembled sawdust. Decaying plant matter can provide food directly or indirectly for many invertebrates (Lasebikan 1977). In our study, soil is the likely source of microarthropod (mites and collembolans) colonization of the cocoons. Our laboratory observations suggest that mites and collembolans progressively build up their populations as decomposition progresses. Additionally, we observed few uropodid adults on and in the dry remains of decaying dead bodies of *R. ferrugineus* adults.

This study did not investigate what mechanism attracted the phoretic mites to the *R. ferrugineus* weevils. However, it is known that *R. ferrugineus* males produce aggregation pheromones (Oehlschlager et al. 1995) to attract individuals of the same species. *Uropoda orbicularis*, *Uroobovella*, and *Curculanoetus* mites probably use these pheromones to find potential hosts. Niogret et al. (2006) showed that the phoretic mite *Macrocheles saceri* (Acari: Mesostigmata) used semiochemicals of the cuticle as kairomonal signals to differentiate and locate *Scarabaeus* dung beetle hosts (Coleoptera: Scarabaeidae). Behavioral experiments are warranted to elucidate the mite attraction process to *R. ferrugineus*.

In conclusion, this study documented preferential distribution of phoretic *U. orbicularis* and *Uroobovella* sp. on *R. ferrugineus* weevils in palm trees. It also documented the presence of phoretic *Curculanoetus* sp. deutonymphs on this insect pest and the presence of collembolans on the cocoon fibers. The interactions between these species are not necessarily straightforward and need further careful studies. Collectively, *Curculanoetus* and *Uroobovella* were numerically abundant on *R. ferrugineus* and the effects of this association may be more detrimental to the host than suggested by mere phoresy. The possible detrimental role of excessive phoretic mites on the weevils needs further investigation. Large field surveys on microarthropods in date palm plantations is warranted as there is the possibility of finding local natural enemies, especially on eggs or neonates, which could be used in successful biological control of *R. ferrugineus*. To our knowledge, this study is the first record of *U. orbicularis*, *Uroobovella* sp., and *Curculanoetus* sp. mites in UAE.

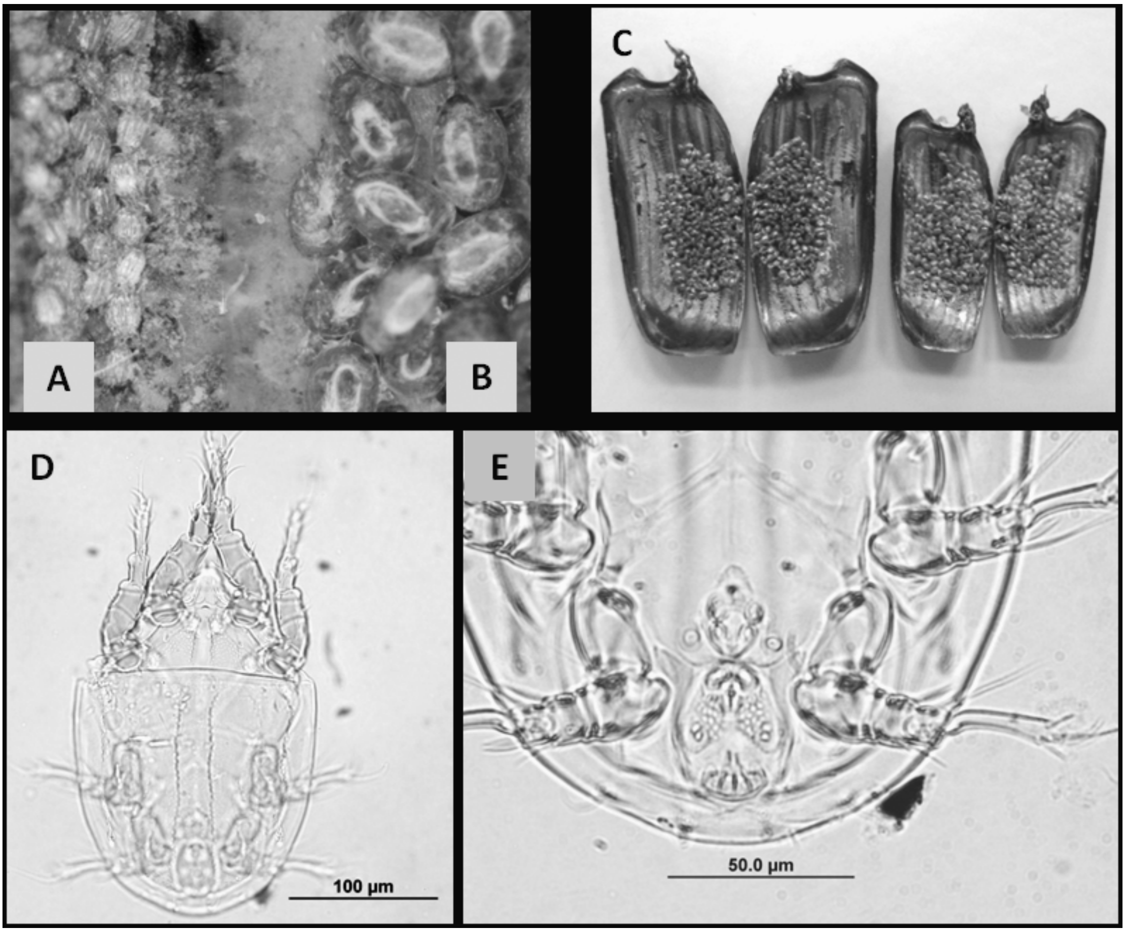

Fig. 4. (A) *Curculanoetus* sp. deutonymphs on subelytron; (B, C) *Uroobovella* sp. deutonymphs on subelytron under dissecting stereoscope; (D) *Curculanoetus* sp. dorsal view (×400); (E) *Curculanoetus* sp. ventral view showing sucker plate (×1000) under light microscope.

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