

Article

Phoretic mite infestations associated with *Rhynchophorus palmarum* (Coleoptera: Curculionidae) in southern California

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Abstract

The South American palm weevil, *Rhynchophorus palmarum* (Coleoptera: Curculionidae), established in San Diego County, California, USA sometime around 2014. Attached to the motile adults of this destructive palm pest, we identified three species of uropodine mites (Parasitiformes: Uropodina), *Centrouropoda* n. sp., *Dinychus* n. sp. and *Fuscuropoda marginata*. Two of these species, *Centrouropoda* n. sp. and *Dinychus* n. sp. are recorded for the first time in the USA and were likely introduced by *R. palmarum*. Several species of mites, primarily of Uropodina, have previously been recorded as phoretic on *Rhynchophorus* spp. In this study, we examined 3,035 adult *R. palmarum* trapped over a 2.5-year period, July 2016 to December 2018, and documented the presence of and species composition of phoretic mites and their relationship with weevil morphometrics (i.e., pronotum length and width). The presence and species composition of mites on weevil body parts changed over the survey period. No mites were found under weevil elytra in 2016 and mite prevalence under elytra increased over 2017–2018 due to an increased abundance of *Centrouropoda* n. sp. per individual beetle. Mite occurrence levels were significantly correlated with reduced pronotum widths of male weevils only. The significance of this finding on male weevil fitness is unknown. Potential implications of phoretic mites on aspects of the invasion biology of *R. palmarum* are discussed.

Keywords: invasive species, phoresy, South American palm weevil, species translocation

Introduction

Infiltration of new ecosystems by an invasive species may also result in the introduction of additional species—such as endosymbionts, pathogens, parasites and predators—associated with the invader (Thomas 2011). The South American palm weevil, *Rhynchophorus palmarum*, established in San Diego County, California, USA sometime around 2014, likely from populations that established in Tijuana, Baja California Norte, Mexico, which were first detected in 2010 (Hoddle & Hoddle 2017). Adult *R. palmarum* are highly vagile and capable of flying long distances in relatively short periods of time (Hoddle *et al.* 2020; Hoddle *et al.* 2021). These attributes may enable *R. palmarum* and associated symbionts, such as phoretic mites, to disperse naturally into new areas from infested regions.

Rhynchophorus palmarum is a notorious palm pest in its native range, which includes parts of Mexico, Central and South America, and the Caribbean (EPPO 2021). Larvae feed inside the palm crown, killing the apical growing region (Giblin-Davis 2001; Milosavljević *et al.* 2019). It is estimated that more than 10,000 ornamental *Phoenix canariensis* have been killed by *R. palmarum* in San Diego Co. (APC 2020). The risk posed to palms by *R. palmarum* is increased by its ability to vector a plant pathogenic nematode, *Bursaphelenchus cocophilus* (Cobb) (Aphelenchida: Parasitaphelenchidae), the causative agent of a lethal palm malady, red ring disease. This nematode

has not yet been detected in California, but in its native range it is a significant mortality agent in commercial oil palm and coconut plantations (Giblin-Davis 2001; Milosavljević *et al.* 2019).

Phoresy is a phenomenon by which an organism is actively carried to favorable habitats either on or in a host and for a limited period of time (OConnor 1982; Kaliszewski *et al.* 1995). Phoretic mite species that are adapted to their host insects are often specialists on the same resources used by their insect transporters (OConnor 1982; Wilson & Knollenberg 1987). Association with phoretic mites, primarily species belonging to the Uropodina (Acari: Mesostigmata), have been documented for several *Rhynchophorus* spp. in their native ranges including *R. phoenicis* (Fabricius) in Cameroon (Kontschán *et al.* 2012), *R. vulneratus* (Panzer) in Indonesia (Hoddle & Hoddle 2015), *R. ferrugineus* (Olivier) in the Philippines (Hoddle & Hoddle 2011) and Malaysia (Dilipkumar *et al.* 2015), *R. cruentatus* (Fabricius) in Florida (Wattanapongsiri 1966, Giblin-Davis 2001), and *R. palmarum* in Central and South America (Husband & Flechtmann 1972; Husband & OConnor 1999; Rodriguez-Morell *et al.* 2012). Additionally, phoretic mites have been recorded infesting invasive populations of *R. ferrugineus* in the Mediterranean basin (Gomaa 2006; Longo & Ragusa Di Chiara 2006; Atakan *et al.* 2009; Porcelli *et al.* 2009; El-Sharabasy 2010; Mazza *et al.* 2011), the Canary Islands, Spain (Abolafia & Ruiz-Cuenca 2020), the Middle East (Al-Deeb *et al.* 2011; Farahani *et al.* 2016), and Aruba and Curaçao Islands in the Caribbean (Amy Roda, USDA-APHIS pers. comm. 2021).

Phoretic mites may stimulate the onset of weevil flight and consequently colonization behavior which would favor the spread of mites and weevils (Porcelli *et al.* 2009). In contrast, secondary consequences of phoresy could result in deleterious effects on hosts. Heavy (i.e., hundreds of mites) mite loads on adult weevils may reduce the efficiency of foraging activities such as flying and increase susceptibility to predation and other stress-related causes that result in premature mortality (Bajerlein & Bloszyk 2004; Porcelli *et al.* 2009; Al-Deeb *et al.* 2011; Mazza *et al.* 2014). A possible parasitic interaction with hosts has been also suggested (Elzinga & Broce 1988; Houck & Cohen 1995; Holte *et al.* 2001; Cardoza *et al.* 2008; Al-Deeb *et al.* 2011;) and the life span of adult *R. ferrugineus* is reportedly reduced when weevils are infested with high numbers of phoretic mites (Mazza *et al.* 2011).

The life cycle and behavior of one phoretic mite species, *Centrouropoda almerodai* Hiramatsu & Hirschmann 1992 (Uropodina), found in association with *R. ferrugineus*, have been documented in some detail (Longo & Ragusa Di Chiara 2006; Porcelli *et al.* 2009). Female *C. almerodai* lay eggs on rotting palm fibers. Larvae that emerge from eggs and protonymphs feed on rotting ligneous palm material. Deutonymphs search for mature *R. ferrugineus* larvae, especially the last larval, or pre-pupal stage. Deutonymphs remain on pre-pupal weevil larvae as they build pupal cocoons from palm fibers within which they pupate. Prior to weevil emergence from the cocoon, deutonymphs cluster on different body parts of the teneral adult. Once attached to adult weevils, deutonymphs develop an anal pedicel which immobilizes and fixes mites onto hosts. This process occurs most commonly on the underside of the elytra. Stalks remain under elytra even after deutonymphs abandon hosts (Porcelli *et al.* 2009).

Centrouropoda almerodai has been reported in association with *R. ferrugineus* and from *R. palmarum* collected in Central America (Porcelli *et al.* 2009; Rodriguez-Morell *et al.* 2012). However, Kontschán *et al.* (2014) state that *C. almerodai* is not present in Central America and phoretic *Centrouropoda* mites associated with *R. palmarum* represent an undescribed species. Another species of phoretic mite associated with *R. ferrugineus* is the mycetophagous, *Fuscuropoda marginata* (Koch) 1839 (Uropodina: Urodinychidae) (often listed as *Uroobovella marginata*) (Bowman 2021). In contrast to *C. almerodai*, *F. marginata* preferentially attaches to exposed surfaces of the sternum, pygidium, head, and legs. In the context of uropodine-*Rhynchophorus*

associations, attachment site has been hypothesized as a good indicator of mite species (Porcelli *et al.* 2009). *Fuscuropoda* spp. have not previously been recorded in association with *R. palmarum*.

In 2016, a trapping program monitoring *R. palmarum* phenology in Bonita, San Diego County was initiated. As part of this effort, captured adult weevils were counted and sexed. During this handling and record keeping process it was noticed that numerous weevils were infested with phoretic mites which indicated the possible introduction of new mite species into California. Due to the presence of heavy mite loads on captured adult weevils, we hypothesized that the presence of heavy mite loads imposes detrimental fitness consequences on weevils. To test this hypothesis, the presence or absence and position of phoretic mites on individual captured weevils was recorded. Infestation levels were compared to an indicator of host size by measuring weevil thorax size (i.e., width and length). The prediction to be tested was that higher numbers of mites are correlated with smaller weevils as measured by thorax size. We also investigated the species diversity of phoretic mites associated with collected weevils and reported here on the chronological sequence of their appearance on *R. palmarum* in California.

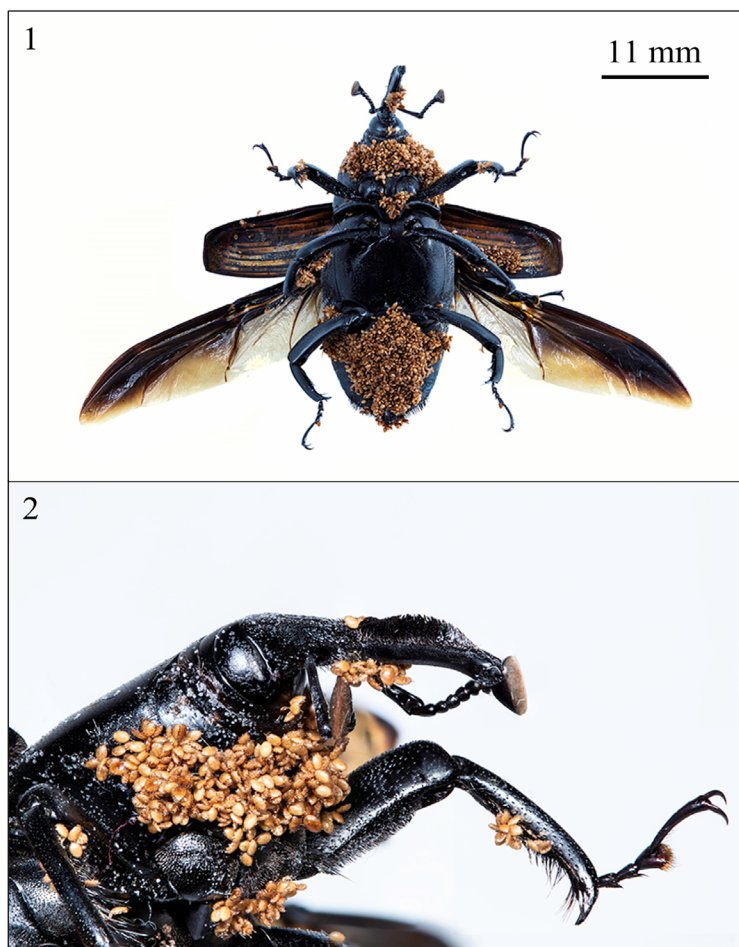
Material and Methods

Sampling and locations

From July 2016 to December 2018, *R. palmarum* populations were monitored monthly at the Sweetwater Recreational Reserve, Bonita, San Diego County in California, USA (N 32° 40' 57''; W 117° 00' 09''). This riparian area has more than 800 wilding *P. canariensis* many of which are infested with *R. palmarum*. Adult weevils were captured using traps made from white 7.5-liter paint buckets with lids (ULINE S-9941W, Pleasant Prairie, Wisconsin, USA). Buckets had four evenly spaced 5-cm circular holes cut into the sides of the upper half of the bucket to allow weevil entry (Milosavljević *et al.* 2020). Each bucket trap was wrapped with burlap fabric (ULINE S-14512, Pleasant Prairie, Wisconsin, USA) to enable adult weevils attracted to traps to climb the sides of the bucket to reach entry holes. Buckets were loaded with fermenting bait and commercially available aggregation pheromone to attract weevils. The fermenting bait was composed of dates, baker's yeast, and water held within containers (470 ml plastic containers Uline Inc., Pleasant Prairie, WI) fitted with perforated lids (~ten 0.3 mm diameter holes) to permit release of attractive fermentation volatiles. The commercial aggregation pheromone dispenser (ISCALure IT192, 700mg at 98% purity of [4S, 2E-6]-methyl-2-hepten-4-ol, ISCA Technologies, Riverside CA, USA) was suspended within the bucket from the lid. To increase the combined attractiveness of the pheromone and bait, a synergist, ethyl-acetate (20 ml, Grainger Industrial Supply, Jackson, MS, USA), contained within a 25 ml plastic vial (Thermo Fisher Scientific, Waltham, MA, USA) with a single perforation hole (~1–2 mm in diameter) in the screw cap, was hung inside the bucket from the lid. The bucket contained antifreeze (i.e., 1.5 L of 50% propylene glycol solution) to drown and preserve captured weevils. Bucket traps were suspended 1.5 m above the ground on tree branches. A total of 10 traps were hung in the reserve. Traps were cleared and lures and baits replaced monthly (i.e., approximately every 30 days).

Rhynchophorus palmarum populations and pronotum size, mite presence on body parts and species identification

Captured weevils were counted, sexed, preserved in 95% ethanol in labeled containers, and stored in a freezer at ~-5° C until examined. For this study, 3,035 weevils were sexed (sex ratio was calculated as the number of females divided by the total number of male and female weevils per trap) and pronotum width and length was measured in mm with a digital caliper (Digital Caliper-Fractional & Decimal Display, Neiko Tools USA) and recorded.



FIGURES 1–2. *Rhynchophorus palmarum* male infested with phoretic uropodine mites on (1) ventral side including underneath wing elytra and (2) lateral view of head, pronotum, and foreleg.

The presence or absence of mites in four areas of the body: head and pronotum, abdomen, legs, and the underside of the elytra (Figures 1–2) were recorded. Weevils with missing body parts (e.g., heads) were excluded from the study. A subsample of mites attached to the four body areas examined were removed and preserved in 95% ethanol in labeled vials for species-level identification. The discovery of deutonymphs of three species of Uropodina led to a secondary investigation of attachment site preference by each species and possible sequence of species appearances in California. To this end, a limited sample consisting of three weevils each, collected in Fall of 2016, 2017 and 2018, was examined for presence and, if present, attachment site by different mite species. A subsample of 50–150 mites / year for both external and subelytral sites (if available) were cleared and slide mounted (see Walter & Krantz 2009 for mounting details) and identified to genus or species using available taxonomic keys (Karg 1989; Krantz & Ainscough 1990; Hirschmann 1993). Images were prepared using the automated Z-stacking feature of the Nikon NIS Elements package on a Nikon Eclipse 90i (Melville, NY) compound microscope with a PC controlled Ds-5M-U1 digital camera.

Statistical Analyses

Generalized linear models were used to compare sex ratios of *R. palmarum* captures between months and years. Quasibinomial error variance was used in model fittings as over-dispersion in the sex ratio data was detected (Crawley 2007). A generalized linear model was used to analyze the presence/absence of mites by sex and month. To analyze whether *R. palmarum* pronotum size (i.e., width and length) was affected by sex and/or mite presence on sampled weevils repeated measures generalized mixed-effects models were used to analyze data for two complete years, 2017 and 2018 (2016 was excluded from this analysis as only six months of data were available for this year). The factors for the repeated measures generalized mixed-effects model were sex, mite presence/absence, their interactions, and month as a random factor. The sample size was large ($n=2,957$ of the 3,035 weevils were used for analyses [see below]) and data were normally distributed. The level of significance for all the analyses was set at < 0.05 . Analytical packages “stats”, “glm” and “nlm” in R (3.6.2. version) (R Core Team 2021) were used for analyses.

Results

Rhynchophorus palmarum populations

Bucket traps deployed at the Sweetwater Reserve captured a total number of 3,035 *R. palmarum* from July 2016 to December 2018. A total of 2,957 weevils were undamaged (i.e., not missing heads or legs) and used for data collection. The maximum total number of weevils captured in all 10 traps in a single month was 245 in April of 2018. Female sex ratio averaged 0.623 ± 0.188 (Figure 2). There were no significant differences in female sex ratio between months across years (GLM: Month, $F_{1,116}=0.497$, $P=0.931$; Year, $F_{1,130}=0.794$, $P=0.375$) (Figure 2).

Mite presence on body parts

The infestation levels of mites under the elytra increased over the three years of this survey. Of the 2,957 adult *R. palmarum* examined for mite presence and from which pronotum measurements were taken, 91.5% of captured adults were infested with phoretic mites on some part of the body, with 68% of weevils harboring mites under the elytra. Half of the weevils were infested with mites in summer of 2016 ($n=136$ weevils) and only one weevil had mites under the elytra during this period (Figure 3). From January 2017 until the end of the survey in December 2018, more than 85% of captured weevils were infested with mites in any given period (i.e., season). Specifically, 99% of weevils captured over winter (January–March) and spring (April–June) of 2018 were infested with mites (Figure 3). In comparison with 2016, weevils captured in summer (July–September) and fall (October–December) of 2017 exhibited 95% and 94% levels of infestation, respectively. The maximum prevalence of mites under elytra reached 79% of captured weevils and was observed in winter 2018 (Figure 3). The percentage of weevils infested with mites differed significantly ($F_{1,256}=5.72$, $P=0.017$) between the sexes and averaged $86.6\% \pm 1.9$ and $92.5\% \pm 1.5$ for females and males, respectively.

Rhynchophorus palmarum pronotum size

For weevils captured over 2017 and 2018, weevil sex and the presence of mites were correlated with reduced pronotum width in males only. A similar relationship was not observed for female weevils (Table 1 and Figure 4). The pronotum width of males infested with mites averaged 10.74 ± 0.02 (mm $\pm$ SE) and was significantly smaller than the average pronotum width (11.08 ± 0.1) for males with no mites on any body part. However, the average size difference is small being only a 3% difference in width (Table 1). Greater differences in the average pronotum width between males and

females were found in May 2018 [10.776 ± 0.779 and 10.294 ± 0.116 for females ($n=77$) and males ($n=67$) respectively] and in June 2018 [10.356 ± 0.108 and 9.913 ± 0.124 for females ($n=88$) and males ($n=53$) respectively]. Pronotum length was not affected by sex, mite presence, and/or their interaction (Table 1 and Figure 5).

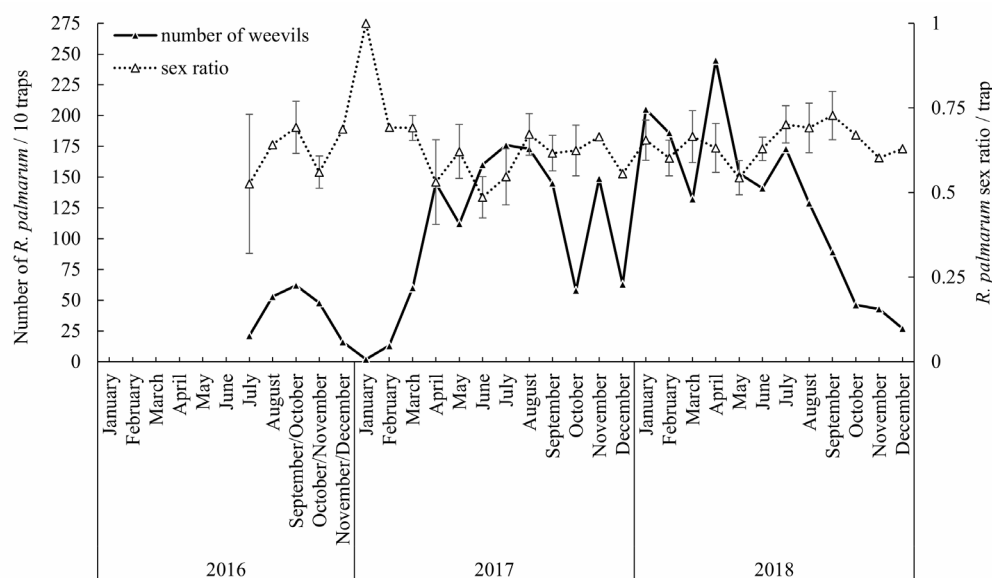


FIGURE 3. *Rhynchophorus palmarum* population dynamics and proportion of captured females (sex ratio) in the Sweetwater Recreational Reserve, Bonita, San Diego County, California, USA from July 2016 to December 2018. The total number of weevils was calculated as the sum of 10 traps per month and the average sex ratio was calculated (females/total number of male and female weevils captured) for each trap with weevils.

TABLE 1. Results of repeated measures generalized mixed-effects model examining the effects of sex and mite occurrence (presence vs absence) on pronotum width and length of *Rhynchophorus palmarum* captured in bucket traps at the Sweetwater Recreational Reserve, San Diego, California in 2017 and 2018.

	df	pronotum width		pronotum length	
		F	P	F	P
Sex (S)	2752	18.562	< 0.001	3.389	0.066
mite presence (MP)		5.015	0.025	0.040	0.842
S x MP		2.132	0.144	3.807	0.051

Mite species identification

Mites infesting *R. palmarum* were identified as *Centrouropoda* n. sp., *Fuscuropoda marginata*, and *Dinychus* n. sp. (Figures 6–11). Kotschan *et al.* (2014) noted that the *Centrouropoda* deutonymphs from *R. palmarum* in Panama identified as *C. almerodai* by Rodriguez-Morell *et al.* (2012) differed from typical *C. almerodai* by the lack of a fourth pair of setae on the ventrianal shield and by the presence of a pair of long ventrals caudal (JV5?) setae. Specimens collected in this study share these characters (Figure 7), suggesting that the *Centrouropoda* specimens associated with *R. palmarum* throughout the Americas represents a new species. The identification of *Dinychus* n. sp. is tentative, as this species may represent a new genus (Figures 10, 11). Voucher specimens are deposited in the Ohio State University Acarology Collection, with respective accession numbers OSAL 0153163-5, OSAL 0153166-7, and OSAL 0153737-41.

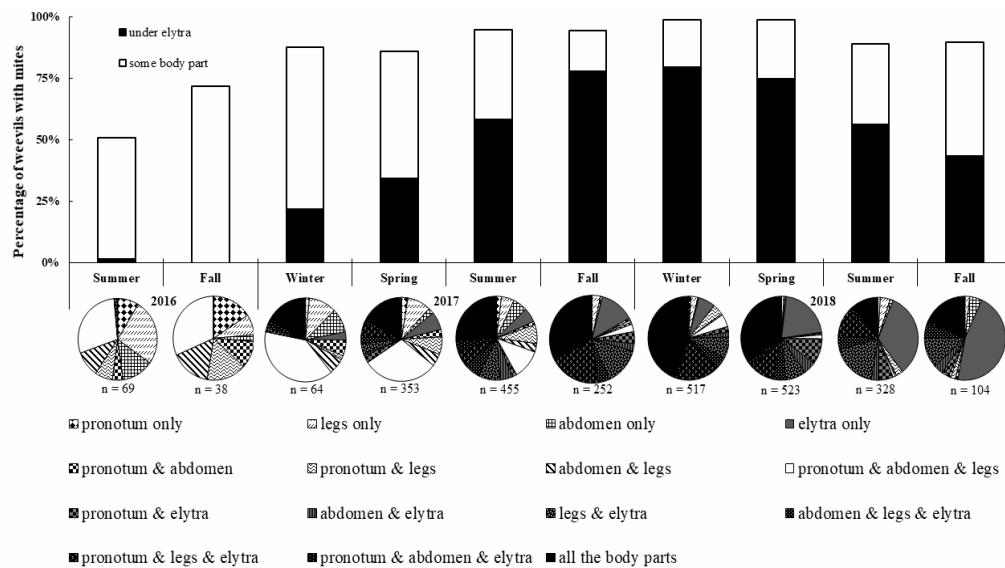


FIGURE 4. Percentage of *Rhynchophorus palmarum* infested with mites, and proportion of mites on each body part. Bars show the percentage of weevils with mites detected on some part of the body (excluding elytra) and black areas correspond to the percentage of weevils with (one or more) mites under elytra (other body parts not excluded). Pie charts represent the percentage of weevils infested with mites on different parts of the body (“n” indicates the number of weevils with mites within each season by year).

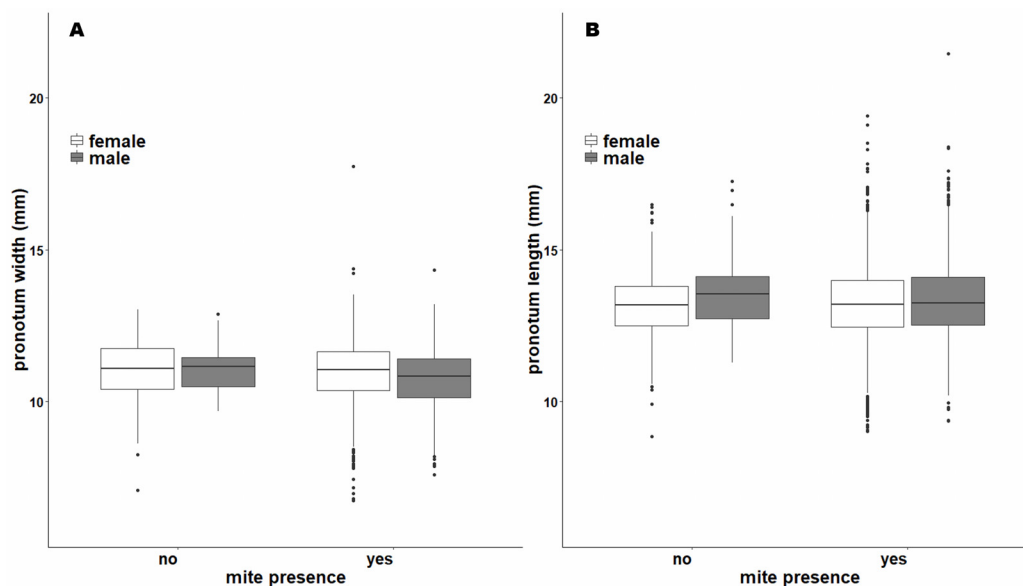


FIGURE 5. *Rhynchophorus palmarum* A) pronotum width and B) pronotum length (mm $\pm$ SE) for females and males that were infested and not infested with phoretic mites in 2017 and 2018 (uninfested females n=121 and uninfested males n=50, infested females n=1595 and infested males n=1001).

Centrouropoda n. sp. was commonly found under the elytra but was also found on the venter and legs of weevils. Its occurrence on the legs and venter seemed to happen most commonly as the numbers and subsequent densities of *Centrouropoda* n. sp. increased, suggesting mites may “spill-

over” from preferred attachment sites to occupy less preferred attachment sites on adult weevils. As noted previously for *R. ferrugineus* (Porcelli *et al.* 2009), *F. marginata* attaches to a variety of external locations on weevils (i.e., head, coxal region, ventral abdomen, around the anus, femora and tibiae). *Fuscuropoda marginata* does not attach to sites under the elytra. Among the “external” attachment sites there was no clear site preference by either *Centrouropoda* n. sp. or *F. marginata*. Nearly all recovered specimens of *Dinychus* n. sp. (n=43) were located under the elytra, with only a few (n=3) individuals found among removed specimens of the other, externally attached, mite species. Deutonymphs of *Centrouropoda* n. sp. and *F. marginata* attach to weevil hosts via stalks secreted by anal glands. Our observations on *Dinychus* n. sp. suggest that this species does not generate stalks for host attachment.



FIGURES 6–11. Phoretic mites (uropodine deutonymphs) found on *Rhynchophorus palmarum* collected in Fall of 2016, 2017, and 2018 in Sweetwater Recreational Reserve, Bonita, San Diego County in California, USA. Dorsal (6, 8, 10) and ventral (7, 9, 11) views of *Centrouropoda* n. sp. (6, 7), *Fuscuropoda marginata* (8, 9), and *Dinychus* n. sp. (10, 11) (this species may represent a new genus). Scale bars are 100 μ m.

Invasion sequence and species interactions

Based on the limited sampling of weevils inspected for mites in Fall of 2016, 2017, and 2018, *F. marginata* was present and common in 2016, but *Centrouropoda* n. sp. and *Dinychus* n. sp. were not recovered until 2017. When recovered, these latter two species were initially found predominantly under the elytra. In 2018, *Centrouropoda* n. sp. was dominant not only under the elytra, but also externally (Table 2). These observations are consistent with the observation that the 2016 collections seldom included subelytral mites. Observed numbers of subelytral mites were large

(>200 / elytron), occupying nearly all the space under elytra. External mites can be equally numerous and were often attached in clusters on body parts (Figures 1, 2). Members of *Centrouropoda* n. sp. and *F. marginata* were found in mixed species clusters, often with intertwined anal stalks.

TABLE 2. Relative abundance of uropodine deutonymphs on *Rhynchophorus palmarum* by attachment site, based on subsamples of mites identified and taken from weevils collected during Fall of 2016, 2017 and 2018. Format: total number of mites on the subsampled weevils (percentage of total for *Centrouropoda* n. sp. and *F. marginata*).

Site	External			Sub-elytral		
Year	<i>Centrouropoda</i> n. sp.	<i>Fuscuropoda marginata</i>	<i>Dinychus</i> n. sp.	<i>Centrouropoda</i> n. sp.	<i>Fuscuropoda marginata</i>	<i>Dinychus</i> n. sp.
2016	0	73 (100%)	0	0	0	0
2017	15 (25%)	45 (75%)	0	74	0	39
2018	123 (94%)	8 (6%)	3	55	0	4

Discussion

This study documents the association between *R. palmarum* and phoretic mites in southern California for the first time. Three species of phoretic mite were detected from weevil trapping surveys conducted over a 2.5-year period: *Centrouropoda* n. sp., *Fuscuropoda marginata*, and *Dinychus* n. sp. (this species may actually represent a new genus). The percentage of *R. palmarum* infested with mites ranged from 51% (in 2016) to 99% (in 2018). Previous studies examining relationships between *R. palmarum* and phoretic mites did not specify the percentage of weevils infested. However, Mazza *et al.* (2011) found similar infestation levels of phoretic mites on *R. ferrugineus* in Italy.

The composition of the phoretic mite fauna of California collected on *R. palmarum* is comparable to that of *R. ferrugineus* in the Mediterranean region where *C. almerodai* and *F. marginata* typically infest weevils (Porcelli *et al.* 2009; Farahani *et al.* 2016). The detection of *Centrouropoda* n. sp. and *Dinychus* n. sp. in this study may be the first official record of these species in the U.S. Notably, phoretic mites have been observed infesting *R. cruentatus* in Florida (R. Giblin-Davis, Univ. Florida, pers. comm. June 2021). This palm weevil is native to the southeast U.S. but the identities of associated phoretic mites have not been determined/published and warrant investigation. With respect to the association of *Dinychus* n. sp. with a *Rhynchophorus* sp., this is an unusual finding given that other members of this genus are rarely recorded in association with beetles (Wisniewski & Hirschmann 1993).

Each phoretic mite species observed in this study appears to have site attachment preferences on *R. palmarum*. However, *Centrouropoda* n. sp. appear to “spill over” to attach to external sites if its preferred subelytral sites are occupied. If this hypothesis is correct it suggests that this mite may exhibit flexibility in attachment site selection. Data presented here also suggest that the three species of phoretic mites associated with *R. palmarum* in California invaded sequentially, specifically *Centrouropoda* n. sp. arrived later than *F. marginata*. The situation for *Dinychus* n. sp. is less clear as the abundance of this species is substantially lower than the other two species and its relative rarity, especially at invasion onset, may have made detection difficult. Interestingly, our data suggests that the increase in numbers of *Centrouropoda* n. sp. on weevils may have led to the competitive exclusion of *F. marginata* in 2018 (Table 2). The putative mechanism for such preferred attachment site changes (from subelytral to external sites) and the subsequent exclusion mechanism

of mites from preferred weevil body parts is unclear for two reasons. First, mites rarely occupied all available external sites, and second, the observation of mixed species clusters suggests some inter-species tolerance. Consequently, these hypotheses of attachment site preferences and exclusion mechanisms between species of phoretic mites infesting *R. palmarum* are tentative given the relatively small sample size examined and would greatly benefit from additional investigation.

Finally, it is important to note that Uropodina are not the only mites, or even the only mesostigmatid mites, associated with *Rhynchophorus* spp. Phoretic mites of the family Diplogyniidae (Mesostigmata: Trigynaspida) were found attached to *R. palmarum* adults in Brazil (Negrisoli Junior *et al.* 2011). Halliday (2019) suggested that these mites may be *Crenamargo binuseta* Hicks (Diplogyniidae) which has been previously found on *R. palmarum* in Nicaragua (Hicks 1958) and Brazil (Flechtman 1981). Collections from California have not yet generated any confirmed cases of Diplogyniidae on *R. palmarum*, but these mites, when present, typically occur in small numbers and may be overlooked. It is possible that diplogyniid mites may have invaded California with *R. palmarum*, and if so, they may be detected with additional sampling efforts.

The infestation severity of mites on male *R. palmarum* was slightly higher than those observed for female weevils. This finding may indicate a minor preference of phoretic mites for female weevils. This finding is tentatively supported by previous studies which found male biased associations of phoretic mites on some species of beetles (Grossman & Smith 2008). In this study, the presence of mites on *R. palmarum* males was associated with a significant, but relatively small, 3% reduction in pronotum widths (but not lengths) in male weevils only. This finding may suggest that the presence of phoretic mites, assuming that they are associated with pre-pupal and pupal weevils prior to infesting their respective adult hosts, could impose a fitness cost in terms of resulting male size as measured by pronotum width. The exact nature of that possible fitness cost on male weevils is unknown.

In the Mesostigmata, the phoretic stage is usually either the last immature instar (the deutonymph) of both sexes, or the adult female (Athias-Binche 1993; Walter & Proctor 1998). The other stages (i.e., eggs, protonymphs) of phoretic mites are usually free-living forms. In this study, the free-living forms share the same ecological niche, rotting plant material in the interior of palm trees, with *R. palmarum* larvae. Uropodid mites are generally considered mycophagous (O'Connor 1984). However, some species of the Uropodidae have been described as predators or parasitic haemolymph feeders (Walter & Proctor 1998). Weevil fitness could be adversely affected by phoretic mites if those mites feed on immature hosts to obtain protein (Longo & Ragusa Di Chiara 2006; Mazza *et al.* 2011). Consequently, mites may not only exploit weevils for dispersal, but they could also engage in a parasitic relationship by using hosts as a protein source as suggested by Mazza *et al.* (2011). For example, Holte *et al.* (2001) reported that the phoretic (heteromorphic) deutonymphs of *Hemisarcoptes cooremani* (Astigmata: Hemisarcoptidae) extract materials from adult coccinellid hosts, suggesting a parasitic relationship between mites and beetle hosts. A similar process may explain why adult *R. ferrugineus* infested with phoretic mites exhibit reduced longevity (by ~33%) when compared to uninfested weevils (Mazza *et al.* 2011).

Substantial loads of phoretic mites on adult weevils may impede flight capabilities (Atakan *et al.* 2009). However, mites might not impede completely flight capacity as mite-infested weevils are readily captured in traps (Mazza *et al.* 2011). Similarly, in this study, 91.5% of weevils that flew to bucket traps and were captured hosted mites on some part of the body. However, the distances flown to reach traps are unknown and substantial mite loads on weevils might have a strong negative effect on long-distance flight capacities. Interestingly, phoretic mites may stimulate weevils to abandon palms of declining quality to search for healthy palms to infest, which promotes the spread of both mites and weevils (Porcelli *et al.* 2009). Flight mill studies are ideally suited to test these hypotheses regarding the effects of infestations of varying densities of single or mixed species loads of phoretic mites on the flight capabilities of male and female *R. palmarum* (Hoddle *et al.* 2020, 2021).

Mazza *et al.* (2011) suggested that the chronological spread of invasive palm weevils into new areas may be reconstructed by tracking the spread of accompanying species that have dependent interspecific relationships with the host or vector. When surveys of *R. palmarum* in southern California commenced in 2016, phoretic mites were found primarily on the pronotum, legs, and abdomen and the only identified species was *F. marginata* (Figures 6 and 7). Over the course of this 2.5-year study, increasing densities of mites were found on the underside of elytra while mite infestation levels on other body parts remained relatively consistent. Mite species composition changed with subsequent detections of *Centrouropoda* n. sp. (one and three weevils with mites under elytra in September/October 2016 and February 2017, respectively) (Figure 3). This observation of sequential additions of phoretic mite species associated with *R. palmarum* over time, if confirmed, could be used to determine the relative age of local weevil infestations as this pest spreads into new areas of California. The strength of the relationship and its utility for reconstructing invasion timelines could be investigated by examining mite infestation loads on weevils captured at the leading edges of the invasion and comparing mite loads (i.e., proportions of weevils infested with phoretic mites, and mite species compositions and densities) to populations with known residency times in infested areas.

Additionally, the identification of the phoretic mite fauna associated with specific *Rhynchophorus* species could be studied to determine the competitive interactions amongst different mite species. For example, the phoretic mite fauna on *R. cruentatus* (native to Florida) has not been studied and needs documentation. It is possible that at some future time *R. palmarum*, *R. ferrugineus* (established in the Caribbean), and *R. cruentatus* could become sympatric in southeast USA. Baseline data on the phoretic mite fauna for *R. cruentatus* while in allopatry would be essential for determining mite interactions after congeneric weevil species become sympatric. Similarly, *R. palmarum* (native) and *R. ferrugineus* (invasive and introduced from Egypt) co-exist in Aruba and Curaçao islands, in the Caribbean (A. Roda, USDA-APHIS, pers. comm. June 2021). The phoretic mite fauna associated with these two weevil species has not been studied in the Caribbean but might exhibit mixed species compositions comprised of mite species from the Caribbean, the Middle East, and/or possibly from the original native range of *R. ferrugineus* in tropical Asia. This possibility could be determined by characterizing the phoretic mite fauna for each weevil species in allopatry and then comparing mite species compositions to weevil populations that occur in sympatry.

In conclusion, work presented here suggests that two species, *Centrouropoda* n. sp. and *Dinychus* n. sp., of phoretic mites have sequentially invaded California with *R. palmarum*. *Fuscuropoda marginata* is considered cosmopolitan and was likely already present in California and may not have invaded in association with *R. palmarum*. Data presented here documented that mite densities and potentially species compositions changed over the course of this ~2.5-year study. Phoretic mites may extract a fitness cost on adult male *R. palmarum* as infested males, on average, tend to exhibit reduced (~3%) pronotum widths when compared to uninfested males. However, the fitness effects of possible size reduction in males are unclear. Manipulative experiments that assess the effects of varying phoretic mite loads associated with prepupal larvae, pupae, and adults have on adult male and female size (e.g., flight mill studies to quantify dispersal capabilities), or on longevity could be undertaken to better understand the potential fitness costs of phoretic mites on *R. palmarum*.

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References

- Abolafia, J. & Ruiz-Cuenca, A.N. (2020) Phoretic invertebrates associated with *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae) in Canarian date palm from southern Spain. *Journal of Natural History*, 54, 2265–2284. <https://doi.org/10.1080/00222933.2020.1842930>
- Al-Deeb, M.A., Muzaffar, S.B., Abuagla, A.M. & Sharif, E.M. (2011) Distribution and abundance of phoretic mites (Astigmata: Mesostigmata) on *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae). *The Florida Entomologist*, 94, 748–755. <https://doi.org/10.1653/024.094.0403>
- APC (2020) "South American palm weevil in San Diego." Available from <https://aguilarplantcare.com/south-american-palm-weevil/>. (Accessed 10 May 2020)
- Atakan, E., Çobanoğlu, S., Yüksel, O. & Bal, D.A. (2009) Phoretic uropodid mites (Acarina: Uropodidae) on the red palm weevil *Rhynchophorus ferrugineus* (Oliver, 1790) (Coleoptera: Curculionidae)]. *Türkiye Entomoloji Dergisi*, 33, 93–105.
- Athias-Binche, F. (1993) Dispersal in varying environments: The case of phoretic uropodid mites. *Canadian Journal of Zoology*, 71, 1793–1798. <https://doi.org/10.1139/z93-255>
- Bajerlein, D. & Błoszyk, J. (2004) Phoresy of *Uropoda orbicularis* (Acari: Mesostigmata) by beetles (Coleoptera) associated with cattle dung in Poland. *European Journal of Entomology*, 101, 185–188. <https://doi.org/10.14411/eje.2004.022>
- Bowman, C.E. (2021) Feeding design in free-living mesostigmatid chelicerae (Acari: Anactinotrichida). *Experimental and Applied Acarology*, 84, 1–119. <https://doi.org/10.1007/s10493-021-00612-8>
- Cardoza, Y.J., Moser, J.C., Klepzig, K.D. & Raffa, K.F. (2008) Multipartite symbioses among fungi, mites, nematodes, and the Spruce Beetle, *Dendroctonus rufipennis*. *Environmental Entomology*, 37, 956–963. <https://doi.org/10.1093/ee/37.4.956>
- Crawley, M.J. (2007) *The R book*. John Wiley & Sons, Chichester, United Kingdom, 1080 pp.
- Dilipkumar, M., Ahadiyat, A., Maśán, P. & Chuah, T.S. (2015) Mites (Acari) associated with *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae) in Malaysia, with a revised list of the mites found on this weevil. *Journal of Asia-Pacific Entomology*, 18, 169–174. <https://doi.org/10.1016/j.aspen.2014.12.010>
- El-Sharabasy, H.M. (2010) A survey of mite species associated with the red palm weevil, *Rhynchophorus ferrugineus* (Olivier) in Egypt. *Egyptian Journal of Biological Pest Control*, 20, 67.
- Elzinga, R.J. & Broce, A.B. (1988) Hypopi (Acari: Histiogastromatidae) on House Flies (Diptera: Muscidae): a case of detrimental phoresy. *Journal of the Kansas Entomological Society*, 61, 208–213.
- European and Mediterranean Plant Protection Organization (EPPO). (2020) *Rhynchophorus palmarum*. EPPO datasheets on pests recommended for regulation. Available from <https://gd.eppo.int/taxon/RHYCPA/datasheet> (last accessed 26 Feb. 2021).
- Farahani, V.F., Ahadiyat, A., Maśán, P. & Dehvari, M. (2016) Phoretic uropodine mites (Acari: Mesostigmata) associated with the red palm weevil, *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae) in Iran. *Journal of Entomological and Acarological Research*, 48, 317–322. <https://doi.org/10.4081/jeur.2016.5853>
- Flechtmann, C. (1981) New records of mites from Brazil with description of two new species in the genus *Oligonychus* Berlese (Acari, Tetranychidae). *Revista Brasileira de biologia*, 41, 861–866.
- Giblin-Davis, R.M. (2001) Borers of palms. In: Howard, F.W., Moore, D., Giblin-Davis, R.M. & Abad, R.G. (Eds.), *Insects on palms*. Wallingford, United Kingdom, International C.A.B.C., pp. 267–304. <https://doi.org/10.1079/9780851993263.0267>
- Gomaa, W.O. (2006) Three mites species associated with the red palm weevil, *Rhynchophorus ferrugineus* (Oliv.), in Egypt. *Bulletin of Faculty of Agriculture, Cairo University*, 57, 543–548.

- Grossman, J.D. & Smith, R.J. (2008) Phoretic mite discrimination among male burying beetle (*Nicrophorus investigator*) hosts. *Annals of the Entomological Society of America*, 101, 266–271.
[https://doi.org/10.1603/0013-8746\(2008\)101\[266:PMDAMB\]2.0.CO;2](https://doi.org/10.1603/0013-8746(2008)101[266:PMDAMB]2.0.CO;2)
- Halliday, B. (2019) The enemy of my parasite is my friend: the possible role of predatory mites as biological control agents of pest beetles in soil. *International Journal of Acarology*, 45, 189–196.
<https://doi.org/10.1080/01647954.2019.1574895>
- Hicks, E.A. (1958) A new genus and species of diplogyniid from Nicaragua (Order Acarina, Family Diplogyniidae). *Iowa State College Journal of Science*, 33, 103–110.
- Hirschmann, W. (1993) Gangsystematik der Parasitiformes. Teil 550, Bestimmungstabellen der Uropodiden der Erde, Atlas der Ganggattungen der Atrichopygidiina. *Acarologie, Schriftenreihe für vergleichende Milbenkunde, Nürnberg*, 40, 292–370.
- Hoddle, M.S. & Hoddle, C.D. (2011) Evaluation of three trapping strategies for red palm weevil, *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae) in the Philippines. *Pakistan Entomologist*, 33, 77–80.
- Hoddle, M.S. & Hoddle, C.D. (2015) Evaluation of three trapping strategies for the palm weevil, *Rhynchophorus vulneratus* (Coleoptera: Curculionidae) in Sumatra, Indonesia. *Pakistan Entomologist*, 37, 73–77.
- Hoddle, M.S. & Hoddle, C.D. (2017) Palmageddon: the invasion of California by the South American palm weevil is underway. *CAPCA Adviser*, 20, 40–44.
- Hoddle, M.S., Hoddle, C.D. & Milosavljević, I. (2020) How far can *Rhynchophorus palmarum* (Coleoptera: Curculionidae) fly? *Journal of Economic Entomology*, 113, 1786–1795.
<https://doi.org/10.1093/jee/toaa115>
- Hoddle, M.S., Hoddle, C.D. & Milosavljević, I. (2021) Quantification of the lifetime flight capabilities of the South American palm weevil, *Rhynchophorus palmarum* (L.) (Coleoptera: Curculionidae). *Insects*, 12, 126.
<https://doi.org/10.3390/insects12020126>
- Holte, A.E., Houck, M.A. & Collie, N.L. (2001) Potential role of parasitism in the evolution of mutualism in astigmatid mites: *Hemisarcptes cooremani* as a model. *Experimental & Applied Acarology*, 25, 97–107.
<https://doi.org/10.1023/A:1010655610575>
- Houck, M.A. & Cohen, A.C. (1995) The potential role of phoresy in the evolution of parasitism: radiolabelling (tritium) evidence from an astigmatid mite. *Experimental & Applied Acarology*, 19, 677–694.
<https://doi.org/10.1007/BF00052079>
- Husband, R.W. & Flechtmann, C.H. (1972) New genus of mite, *Rhynchopolipus*, associated with the palm weevil in Central and South America (Acarina, Podapolipodidae). *Revista Brasileira de biologia*, 32, 519–522.
- Husband, R.W. & Oconnor, B.M. (1999) Two new ectoparasitic mites (Acari: Podapolipidae) of *Rhynchophorus* spp. (Coleoptera: Curculionidae) from Indonesia, Malaysia, the Philippines and West Africa. *International Journal of Acarology*, 25, 101–110.
<https://doi.org/10.1080/01647959908683621>
- Kaliszewski, M., Athias-Binche, F. & Lindquist, E.E. (1995) Parasitism and parasitoidism in Tarsonemina (Acari: Heterostigmata) and evolutionary considerations. In: Baker, J.R., Muller, R. & Rollinson, D. (Eds.), *Advances in Parasitology*. Academic Press, pp. 335–367.
[https://doi.org/10.1016/S0065-308X\(08\)60074-3](https://doi.org/10.1016/S0065-308X(08)60074-3)
- Karg, W. (1989) Acari (Acarina), Milben, Unterordnung Parasitiformes (Anactinochaeta), Uropodina Kramer, Schildkrötenmilben. *Die Tierwelt Deutschlands*, 67, 1–203.
- Kontschán, J., Tambe, J.T. & Riolo, P. (2012) *Uroobovella phoenicicola* sp. n., a new Uropodina mite (Acari: Mesostigmata) associated with the African palm weevil (*Rhynchophorus phoenicis* Fabricius, 1801) from Cameroon. *African Invertebrates*, 53, 593–600.
<https://doi.org/10.5733/afin.053.0205>
- Kontschán, J., Mazza, G., Nannelli, R. & Roversi, P.F. (2014) The true identity of the red palm weevil associated Uropodina mite, *Centrouropoda almerodai* Hiramatsu & Hirschmann, 1992. *Redia-Giornale Di Zoologia*, 97, 83–88.
- Krantz, G.W. & Ainscough, B.D. (1990) Acarina: Mesostigmata (Gamasida). In: Dindal, D.L. (Ed.), *Soil Biology Guide*. John Wiley & Sons, Inc., New York, pp. 583–665.
- Longo, S. & Ragusa Di Chiara, S. (2006) Presenza e diffusione in Italia dell'acaro *Centrouropoda almerodai* (Uroactiniinae, Uropodina). *Bolletino di Zoologia Agraria e di Bachicoltura*, 38, 265–269.
- Mazza, G., Cini, A., Cervo, R. & Longo, S. (2011) Just phoresy? Reduced lifespan in red palm weevils *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae) infested by the mite *Centrouropoda almerodai* (Uroactiniinae: Uropodina). *Italian Journal of Zoology*, 78, 101–105.
<https://doi.org/10.1080/11250003.2010.509135>

- Mazza, G., Francardi, V., Simoni, S., Benvenuti, C., Cervo, R., Faleiro, J.R., Ll  cer, E., Longo, S., Nannelli, R., Tarasco, E. & Roversi, P.F. (2014) An overview on the natural enemies of *Rhynchophorus* palm weevils, with focus on *R. ferrugineus*. *Biological Control*, 77, 83–92.
<https://doi.org/10.1016/j.biocontrol.2014.06.010>
- Milosavljevi  , I., El-Shafie, H.A.F., Faleiro, J.R., Hoddle, C.D., Lewis, M. & Hoddle, M.S. (2019) Palmageddon: the wasting of ornamental palms by invasive palm weevils, *Rhynchophorus* spp. *Journal of Pest Science*, 92, 143–156.
<https://doi.org/10.1007/s10340-018-1044-3>
- Milosavljevi  , I., Hoddle, C.D., Mafra-Neto, A., G  mez-Marco, F. & Hoddle, M.S. (2020) Effects of food bait and trap type on captures of *Rhynchophorus palmarum* (Coleoptera: Curculionidae) and trap bycatch in Southern California. *Journal of Economic Entomology*, 113, 2407–2417.
<https://doi.org/10.1093/jee/toaa175>
- Negrisol Junior, A.S., Silva, E.S., Negrisol, C.R.d.C.B., dos Santos, N.L. & Guzzo, E.C. (2011) Cria  o em labor  rio da broca-do-olho-do-coqueiro *Rhynchophorus palmarum* L. (Coleoptera: Curculionidae) visando pesquisas para o controle das suas larvas. *Embrapa Tabuleiros Costeiros-Comunicado T  cnico* (INFOTECA-E).
- OConnor, B.M. (1982) Evolutionary ecology of astigmatid mites. *Annual Review of Entomology*, 27, 385–409.
<https://doi.org/10.1146/annurev.en.27.010182.002125>
- OConnor, B.M. (1984) Acarine–fungal relationships: the evolution of symbiotic associations. In: Wheeler, Q. & Blackwell, M. (Eds.), *Fungus–Insect relationships, perspectives in ecology and evolution*. New York, Columbia University Press, pp. 354–381.
- Porcelli, F., Ragusa, E., D’onghia, A.M., Mizzi, S. & Mifsud, D. (2009) Occurrence of *Centrouropoda almerodai* and *Uroobovella marginata* (Acari: Uropodina) phoretic on the red palm weevil in Malta. *Bulletin of the Entomological Society of Malta*, 2, 61–66.
- R Core Team (2021) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available from <https://www.R-project.org/>.
- Rodr  guez-Morell, H., Quir  s-Mc Intire, E.I., Domingo-Quir  s, A.E., Chico-Morej  n, R. & Porcelli, F. (2012) Presencia de *Centrouropoda almerodai* y *Glyptolaspis* sp. (Acari: Uropodina, Macrochelidae) sobre el picudo negro del cocotero (*Rhynchophorus palmarum*) (Coleoptera: Curculionidae) en Panam  . *M  todos en Ecolog   y Sistem  tica*, 7, 1–7.
- Thomas, C.D. (2011) Translocation of species, climate change, and the end of trying to recreate past ecological communities. *Trends in Ecology & Evolution*, 26, 216–221.
<https://doi.org/10.1016/j.tree.2011.02.006>
- Walter, D.E. & Proctor, H.C. (1998) Feeding behavior and phylogeny: observations on early derivative Acari. *Experimental & Applied Acarology*, 22, 39–50.
<https://doi.org/10.1023/A:1006033407957>
- Walter, D.E. & Krantz, G.W. (2009) Collecting, rearing and preparing specimens. In: Krantz, G.W. & Walter, D.E. (Eds.), *A manual of Acarology*, Third Edition. Lubbock, Texas Tech University Press, pp. 83–95.
- Wattanapongsiri, A. (1966) “A revision of the genera *Rhynchophorus* and *Dynamis* (Coleoptera: Curculionidae)”. PhD. Oregon State University, Corvallis, US, pp. 1–185.
- Wilson, D.S. & Knollenberg, W.G. (1987) Adaptive indirect effects: the fitness of burying beetles with and without their phoretic mites. *Evolutionary Ecology*, 1, 139–159.
<https://doi.org/10.1007/BF02067397>
- Wisniewski, J. & Hirschmann, W. (1993) Gangsystematik der Parasitifomes. Teil 548, Katalog der Ganggattungen, Untergattungen, Gruppen und Arten der Uropodiden der Erde (Taxonomie, Literatur, Gr  sse, Verbreitung, Vorkommen. *Acarologie, Schriftenreihe f  r vergleichende Milbenkunde*, N  rnberg, 40, 1–220.

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