Acarina 22 (2): 92–99

ILLUSTRATION OF SOME MORPHOLOGICAL FEATURES IMPORTANT FOR IDENTIFICATION OF *RHYNCHOPOLIPUS RHYNCHOPHORI* (EWING) (ACARI: PODAPOLIPIDAE)

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ABSTRACT: *Rhynchopolipus rhynchophori* (Ewing) (Acari: Podapolipidae) is an ectoparasite of the palm weevils, *Rhynchophorus palmarum* L. and *Rhynchophorus ferrugineus* (Olivier), whose implications in the biological control of the weevils are acquiring more and more interest. Hence there is a need to discern this mite species and others species, which can be found on the palm weevils. In this paper, species-specific morphological features are illustrated using scanning electron microscopy, having in mind people inexperienced in mite morphology and systematics. Some morphological aspects of the male copulatory organ and its morphological relationship with the gnathosoma are illustrated here for the first time.

KEY WORDS: Palm Weevil, Red Palm Weevil, PW, RPW, scanning electron microscopy, light microscopy

INTRODUCTION

The family Podapolipidae comprises highly specialized but still poorly known species of internal and external arthropod parasites with some genera restricted to one family of host insects. Females of podapolipid mites often display reductions in the number of legs and modification of the body size and shape, while males exhibit modification of their copulatory organs and have three pairs of legs (Krantz and Walter 2009). Moreover the unusual behavioral of males mating with apparent pre-adult females is peculiar to members of this family.

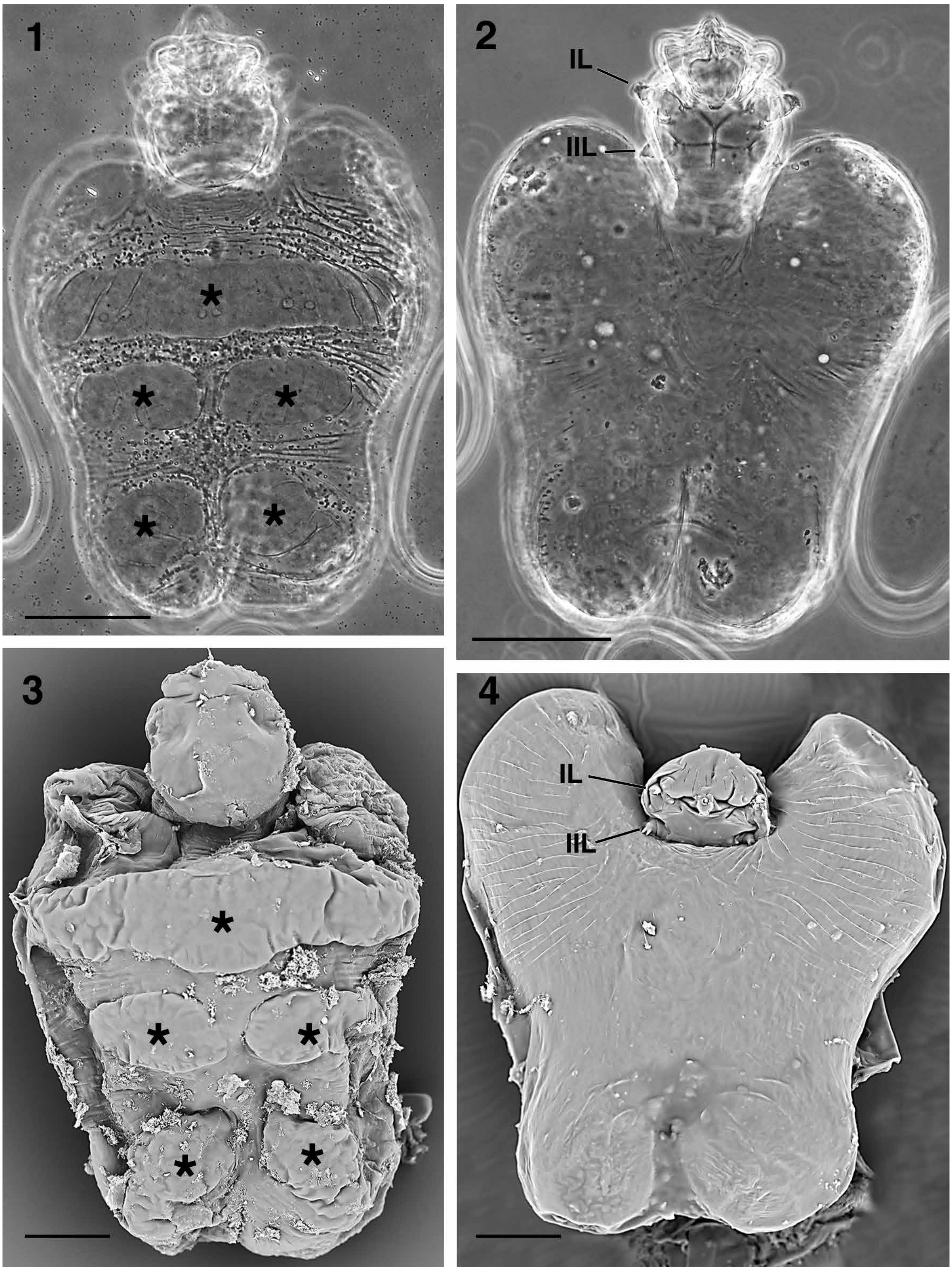
Rhynchopolipus rhynchophori (Ewing), belonging to the family Podapolipidae, feeds on many insects and other pests (Lindquist 1986) and is commonly found under the elytra of the coconut weevil *Rhynchophorus palmarum* L. (Husband and Fletchman 1972; Ochoa et al. 1995; Abdullah 2009). Actually both *Rh. rhynchophori* and *Tetrapolypus rhynchophori* (Ewing) infest adult palm weevils and are reported as possible bio-control agents for *Rhynchophorus ferrugineus* (Olivier) and *R. palmarum* L. (Abdullah 2009). The genus *Rhynchopolipus* was established by Husband and Fletchman (1972) on the basis of several peculiar morphological traits. Considering the economic importance of the palm weevils in all tropical and sub-tropical areas of the World and the increasing interest in using these parasite mites in their control, it is very important to correctly identify these mite species. Moreover most of people dealing with the management of palm weevils are not familiar with mite morphology and systematic. In the present paper morphological features specific of *Rh. rhynchophori* are illustrated by light and scanning electron microscopy, focusing on diagnostic traits given in the original description of Husband and Fletchman (1972). Moreover new details of the peculiar organization of the male copulatory organ are illustrated for the first time.

MATERIAL AND METHODS

Mites were found under the elytra of the coconut weevil *Rhynchophorus palmarum* L. (Linnaeus, 1758) collected in October near a coconut tree (*Cocos nucifera* L.) plantation in Feliz Deserto (Alagoas State, North East Brazil). The weevils were collected by pheromone and apneumone bucket-traps lured by Rincoforol and sugarcane pieces. Dry specimens of coconut weevil were sent to Italy and observed under a stereo-microscope for mites. Since dried mites were useless for our purposes because of cuticle shrinkage, to get back as much as possible the natural body shape, mites were left for one week in Hoyer's fluid (Krantz and Walter 2009) and then mounted on slides with Hoyer's medium for light microscopy (LM) observations. Observations, identification and light images were obtained using an Olympus BX51 microscope equipped with an Olympus E330 camera. Identification of the mites followed Husband and Fletchman (1972).

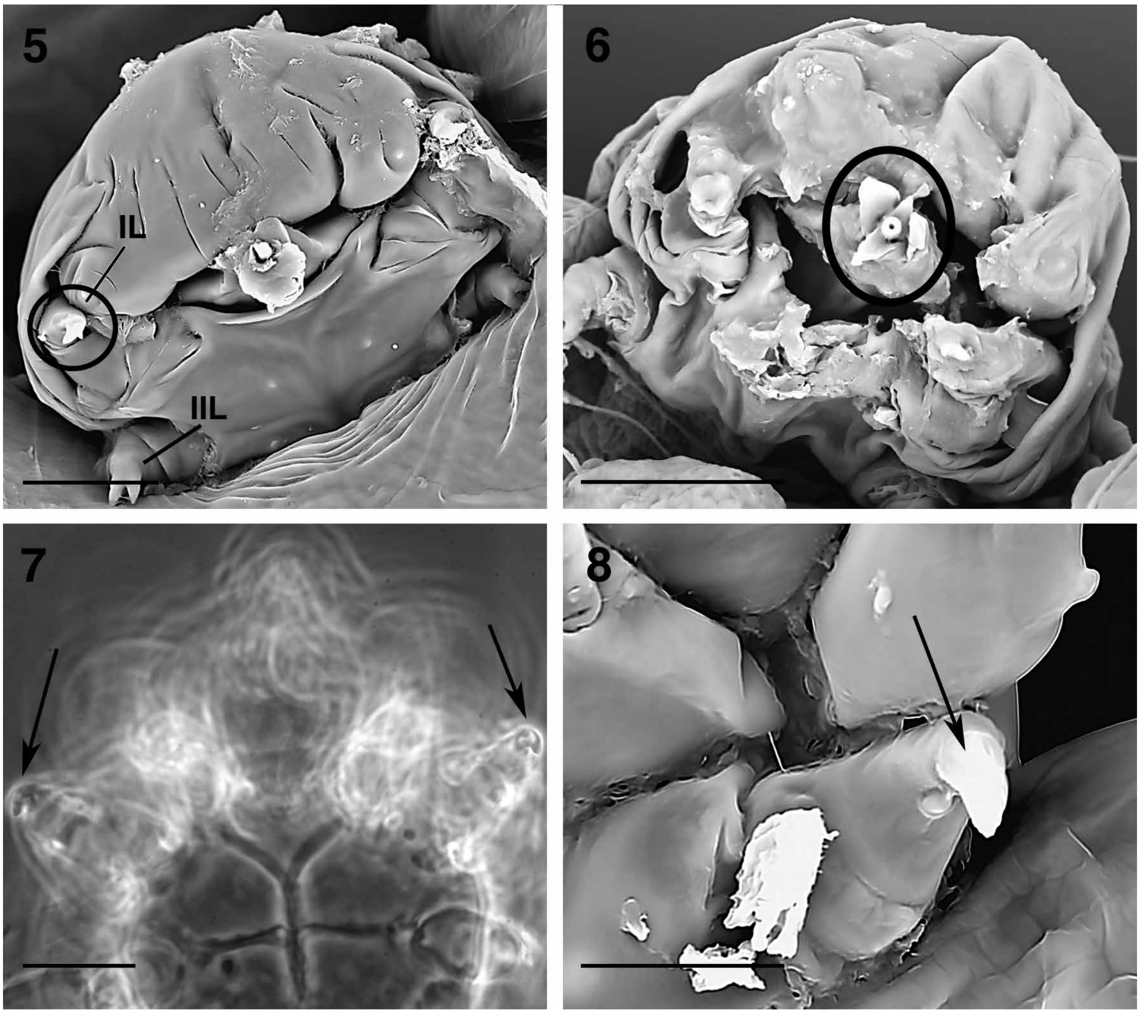
Some mite specimens were collected from the beetles and transferred directly to 70% ethanol and performed for scanning electron microscope

Illustration of some morphological features important for identification of *Rhynchopolipus rhynchophori*



Figs 1–4. *Rhynchopolipus rhynchophori* female. 1) LM, dorsal view: propodosoma with legs, enlarged body of the female with two anterio-lateral lobes, metapodosoma and opisthosoma (no evident separation line) with 5 plates (asterisks). 2) LM, ventral view: propodosoma bearing two pairs of legs and the enlarged posterior region of the body (metapodosoma and opisthosoma). 3) SEM, dorsal view as in fig. 1 showing the 5 plates (asterisks). 4) SEM, ventral view: enlarged body with two lobes embedding the propodosoma bearing two pairs of legs. Abbr.: IL, first leg; IIL, II leg. Scale bars: 100 µm (1–2); 50 µm (3–4).

(SEM) photography. They were dehydrated through a graded ethanol series, dried using a Leica EM CPD300 critical point dryer, mounted on SEM stubs using conductive carbon adhesive tabs and sputter coated using an Edwards S150A sputter coater apparatus. Specimens were observed and photographed with a Hitachi TM3030 tabletop scanning electron microscope.



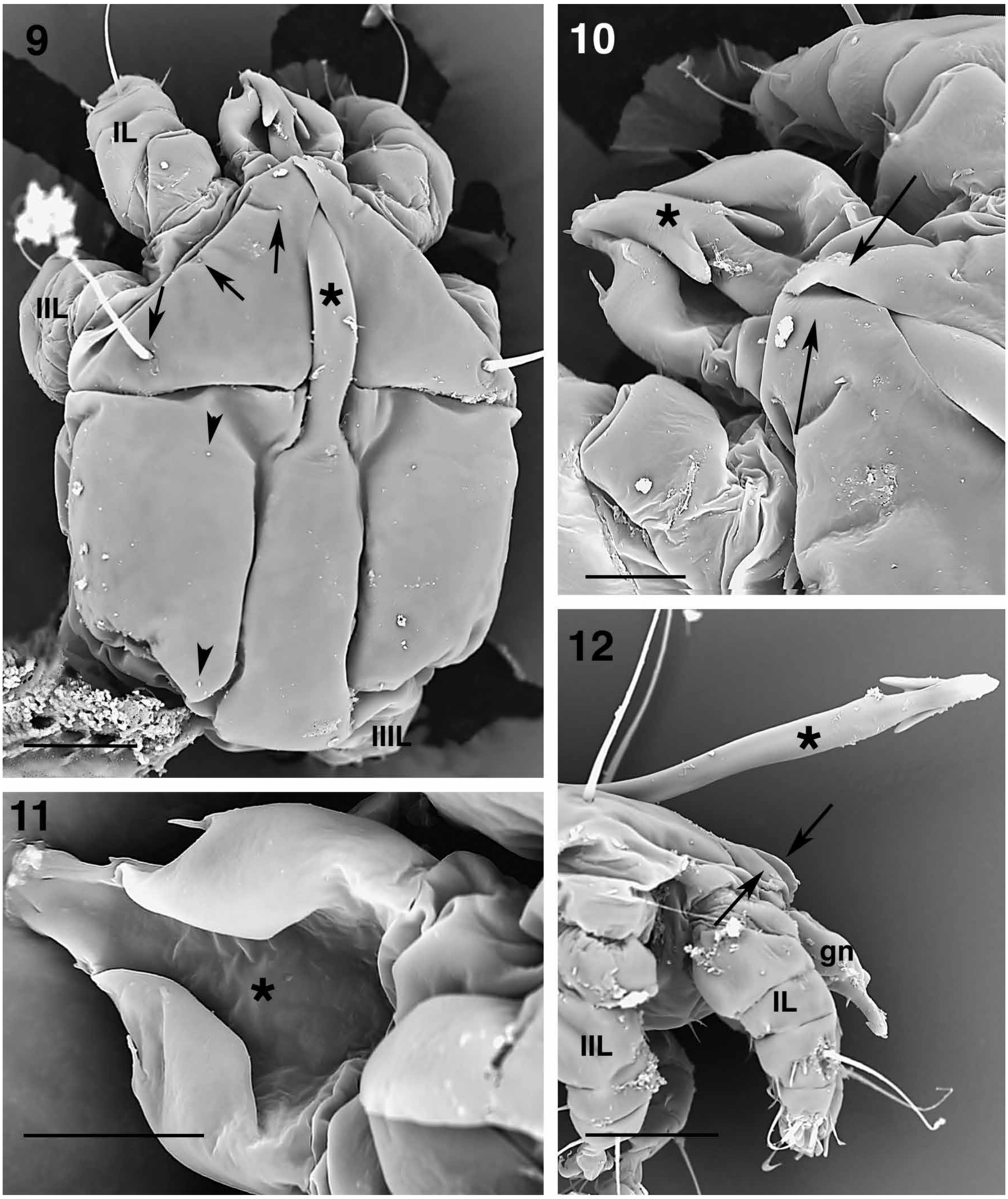
Figs 5–8. *Rhynchopolipus rhynchophori* female. 5) SEM, detail of fig. 4 showing an enlargement of the gnathosoma and the propodosoma where the two pairs of legs are evident. The first pair of legs shows a large terminal hook (encircled). 6) SEM, detail of the propodosoma and gnathosoma showing the apical region with the preoral groove surrounded by petal like lateral lips (encircled). 7) LM, ventral view: detail of the propodosoma showing the first pair of legs with the terminal hook (arrows). 8) SEM, detail of the terminal hook of the first pair of legs (arrow). Abbr.: IL, first leg; IIL, II leg.
Scale bars: 25 µm (5,7); 30 µm (6); 10 µm (8).

DESCRIPTION AND DISCUSSION

The adult female shows an enlarged body (about 450 µm long) where no separation between metapodosoma and opisthosoma is visible (Figs 1–4), while five dorsal plates are present (Figs 1, 3). The gnathosoma is as broad as long (Figs 5–6), with a peculiar, petal like lateral lips surrounding the pre-oral groove (where the stylets slide) (Fig. 6) and assuring the sealing of the mouthparts to the host surface. Females have only two pairs of legs, with leg I lacking the sucker-like pulvillus and provided with a large terminal hook (Figs 5, 7–8).

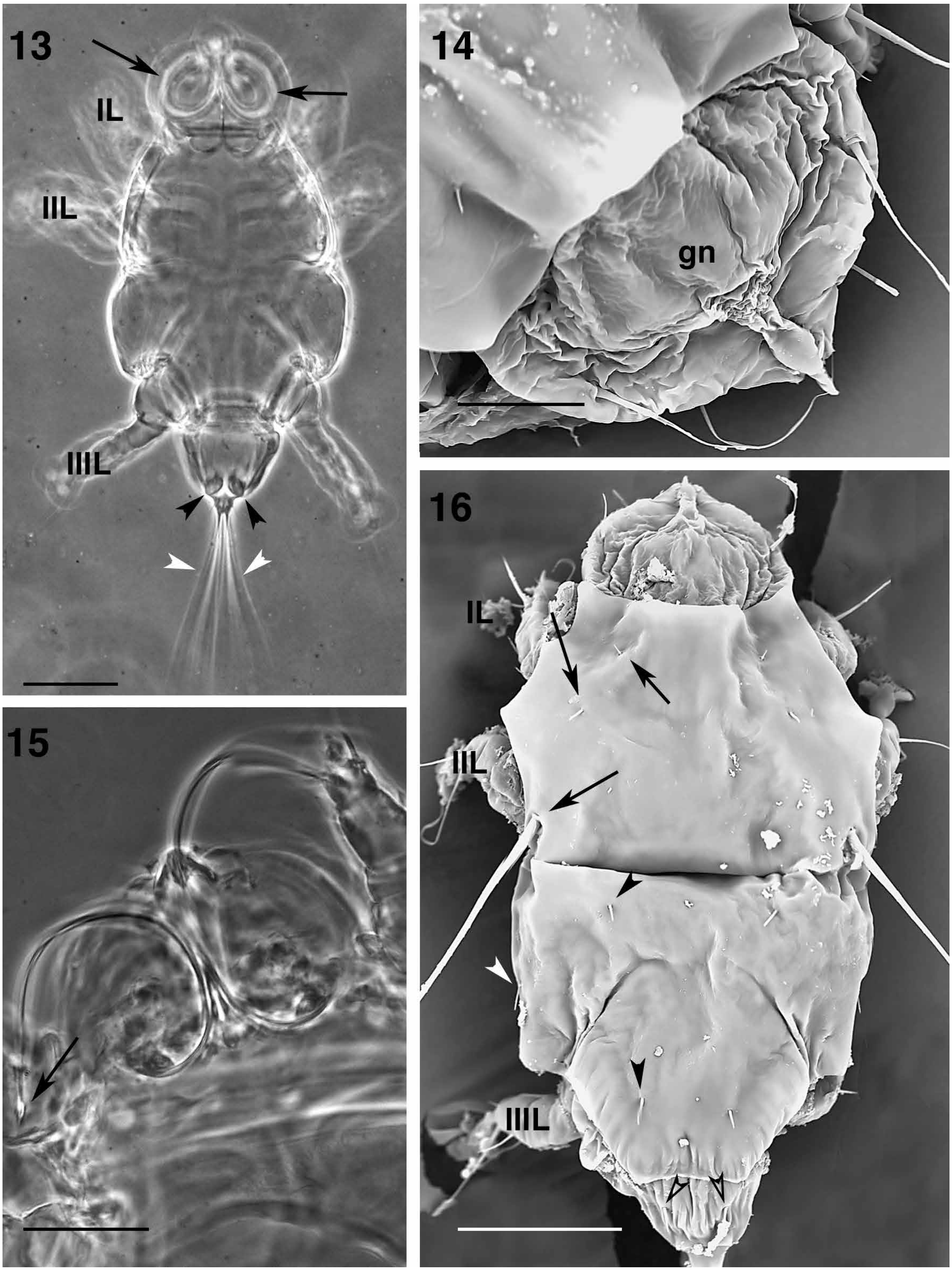
The male is much smaller (about 150 µm long) than adult females and have three pairs of legs (Fig. 9). The propodosoma has 2 pairs of short setae and 1 pair of long setae (Fig. 9); the metapodosoma has 2 plates and each plate with 3 very short setae while the opisthosomal plates and setae are lacking (Fig. 9). The most peculiar feature is the aedeagus, a bizarre structure, which is arrow-shaped distally, originates on the posterior-dorsal surface of the mite and extends over the gnathosoma (Husband 1969; Husband and Fletchman 1972) (Figs 9–10). The aedeagus appears as a projection of the dorsal region of the male body but, as shown in fig. 20, a slit is only present in the proximal region, suggesting that the aedeagus starts as a projection and distally becomes a tube due to the fusion on the lateral walls of this projection that are bent and fused.

The morphological organization of the male body is modified in order to manage this long cop-



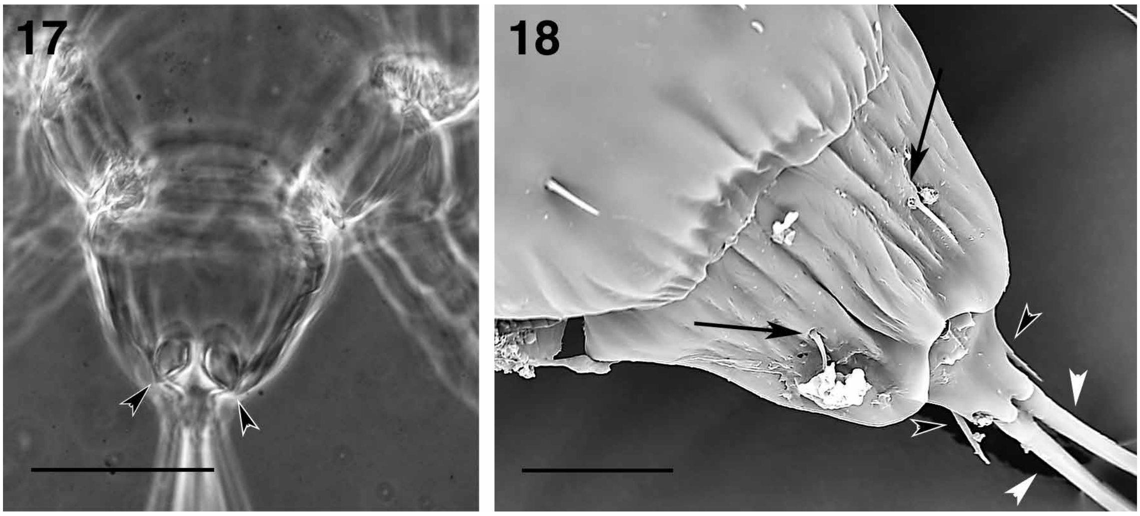
Figs 9–12. *Rhynchopolipus rhynchophori* male. 9) SEM, dorsal view of the male with three pairs of legs. Note the peculiar arrow-shaped aedeagus (asterisk) projecting from the posterior region of the body and reaching over the gnathosoma. Two overlapping flaps arise from the propodosoma to keep in place the aedeagus. The propodosoma shows two pairs of short setae and one pair of long ones (arrows). Metapodosoma with two plates (one on each side of the aedeagus) bearing each three very short setae (arrowheads: only two of the three setae are visible in the picture). 10) SEM, detail of the previous picture showing the two overlapping flaps (arrows) to keep in place the aedeagus with the terminal arrow (asterisk) housed in a dorsal depression of the gnathosoma. 11) SEM, detail of the dorsal region of the gnathosoma showing a peculiar gutter (asterisk) shaped to house the distal arrow-shaped region of the aedeagus (removed). 12) SEM, lateral view of the male showing the long aedeagus (asterisk) freed from the overlapping flaps (arrows) and the gnathosoma. Abbr.: gn, gnathosoma; IL, I leg; IIL, II leg; IIIL, III leg. Scale bars: 25 µm (9, 12); 10 µm (10–11).

ulatory organ. The propodosoma shows two dorsal overlapping flaps to keep in place the long aedeagus (Figs 9–10); moreover the dorsal region of the gnathosoma has a congruous gutter with purposely shaped walls to accommodate the distal arrow-shaped region of the aedeagus (Figs 10–11). These morphological adaptations to such long structure have the purpose to avoid the misplacing

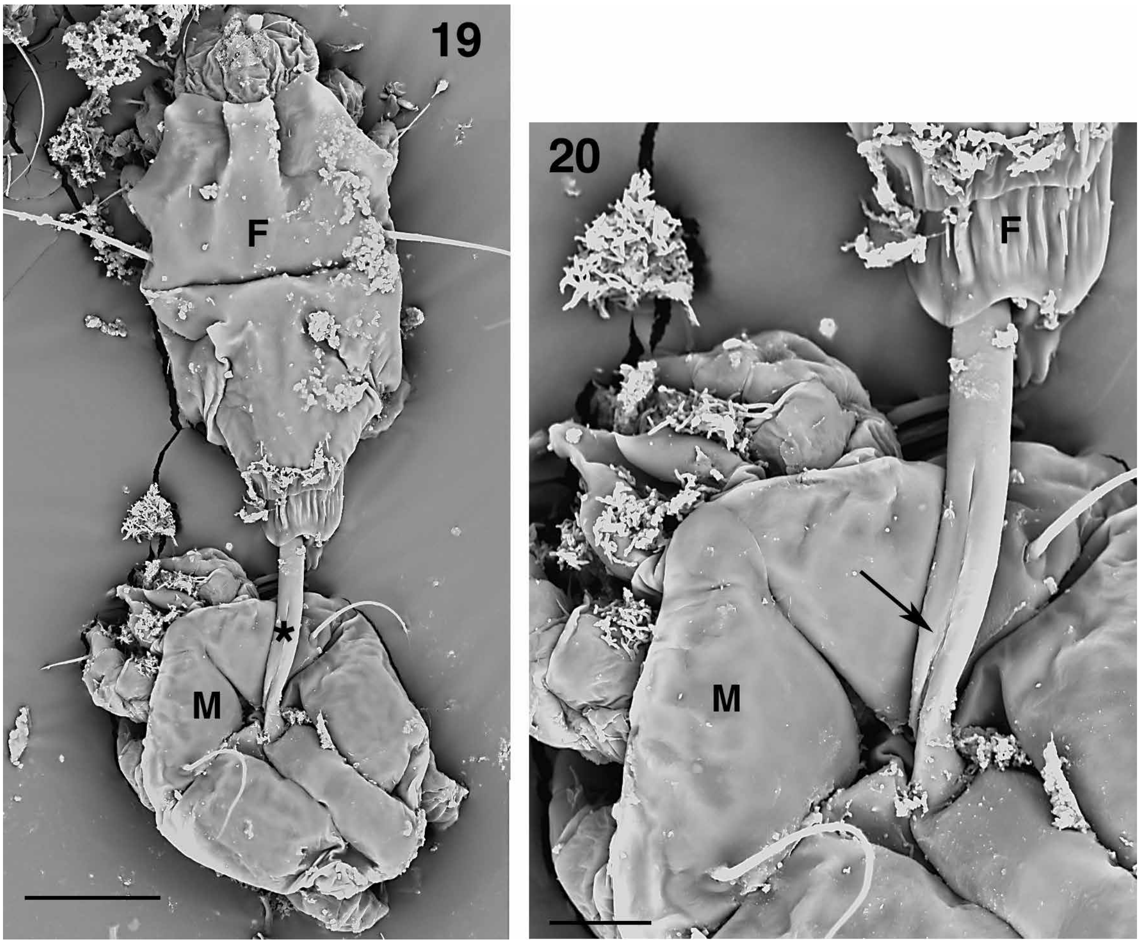


Figs 13–16. *Rhynchopolipus rhynchophori* larviform female. 13) LM, ventral overview showing the three pairs of legs, the long stylets (curved when withdrawn) (arrows), the peculiar circular structures (arrowheads) and the long terminal setae (white arrowheads). 14) SEM, detail of the circular gnathosomal capsule. 15) LM, detail of the stylets forced out of the gnathosoma during preparation. Note the serrate terminal apex (arrow). 16) SEM, dorsal overview showing setae. Two pairs of short setae and one pair of long setae on the propodosomal plate (arrows), three pairs of short setae on the metapososomal plate (arrowheads), one pair of short setae on the opistosomal plate 1 (empty arrowheads). Abbr.: gn, gnathosoma; IL, first leg; IIL, II leg; IIIL, third pair of leg. Scale bars: 50 µm (13); 25 µm (15); 15 µm (14, 16).

the aedeagus during usual mite activities, but in a few specimens, during preparation, the structure was misplaced (Fig. 12) even though the male was not mating.



Figs 17–18. *Rhynchopolipus rhynchophori* larviform female. 17) LM, ventral view: detail of the peculiar circular structures (arrowhead). 18) SEM, dorsal view: detail the pair of setae on opisthosomal plate 1 (arrows), the two short setae on opisthosomal plate 2 (black arrowheads) and the terminal pair of long setae (white arrowheads). Scale bars: 50 µm (17); 15 µm (18).



Figs 19–20. *Rhynchopolipus rhynchophori* mating. 19) SEM, male and female during copulation. It is evident that the aeadeagus (asterisk) is inserted into the female. 20) SEM, detail showing the twisted aedeagus with the ventral groove (arrow).
Abbr.: F, female; M, male. Scale bars: 50 µm (19); 15 µm (20).

The larviform females (about 190 µm long) have three pairs of legs (Figs 13, 16), a large evident gnathosomal capsule (Figs 13–14) with very long, needle-like chelicerae (Fig. 13). The chelicerae have a serrate distal margin (Fig. 15) and, when withdrawn in the gnathosoma, appears to be curved (Fig. 13). There are 2 pairs of short setae and 1 pair of long setae on the propodosomal plate; metapodosomal plates 1 and 2 are fused mesally and bear 3 pairs of short setae; while opisthosomal plate 1 carries 1 pair of short setae (Fig. 16). Ventrally one pair of peculiar circular structures with pointed postero-lateral projections is visible (Figs 13, 17) immediately anterior to the pair of short setae on opisthosomal plate 2 and to the pair of very long terminal setae (Fig. 18).

In consideration of the dorsally directed copulatory organ, the mating position of this species is affected. As common for this family, males copulate with pre-adult female (or larviform female) and males during copulation are firmly attached to the larviform females as proved by specimens collected during mating and still interlocked even after being mounted for microscopic observations (Fig. 19). It is likely that the arrow-shaped distal region of the aedeagus is responsible for keeping the couple firmly attached; moreover during copulation the male is reported as being right side up while the female up side down (Husband 1969; Husband and Fletchman 1972). This is supported by our observations as well as where both partners are right side up but, since it is evident that the aedeagus is twisted (Fig. 20 showing the aedeagus ventral groove), it means that during preparation the female was turned and it was actually up side down.

CONCLUSIONS

The aim of this paper is to illustrate important identifying characters for the *Rhynchopolipus rhyncophori*. Several species of podapolipid mites have been noted as potential biological control agents against insect pests (Krantz and Walter 2009) On palm weevils at least two species have been detected (*Rh. rhynchophori* and *Tetrapolypus rhynchophori*) (Husband 1969; Husband and Fletchman 1972; Ochoa et al. 1995; Abdullah 2009). In this respect a gallery of the key characters can greatly help to avoid ambiguities during identification process.

In particular the presence of the following characters are crucial to distinguish the *Rhynchopolipus* genus from the *Tetrapolypus*: five dorsal plates in the adult female (0–2 in *Tetrapolypus*) (Figs 1, 3); leg I lacking the sucker-like pulvillus (present in *Tetrapolypus*) and provided with a large terminal hook in adult female (Figs 5, 7–8); the unique long (extending over the entire propodosoma), arrow-shaped distally aedeagus of the male (short and with no terminal arrow in *Tetrapolypus)* (Figs 9–10); the peculiar ventral circular structures with pointed postero-lateral projections in larviform females (absent in *Tetrapolypus*) (Figs 13, 17) (Husband 1973).

The long stylets of the females can support the idea that these mites act as parasites of the palm weevils. What is very interesting is the presence of such a long copulatory organ in the male, which is accompanied by a modification of the gnathosoma. It is questionable whether males are able to feed given the presence of the deep groove in the center of the gnathosoma to house the distal arrow-shaped aedeagus (Figs 10–11). Moreover in SEM observations it looks like there is no opening of the preoral groove at the tip of the gnathosoma. The preoral groove is the grove that takes the food to the pharingeal pump and hence, if there is not an opening for such groove it is difficult to image how they can feed. Moreover stylets, which should be used to pierce the host, are barely visible in LM observations. Of course a study of transmission electron microscopy sections is necessary to solve the question.

Husband and Fletchman (1972) suggest that larviform females can firmly attach themselves to the host elytra thanks to the slytets and obviously that the peculiarly shaped aedeagus can fit in the opisthosomal structures of the female. On the other hand it is evident that mating couples are difficult to separate so it looks like the shape and length of the aedeagus is an adaptation to assure a firm attachment of the male to the female and this makes even more sense considering that males have stylets much shorter than the larviform females and so are not able to somehow fix themselves to a host that usually moves around flying. In our samples the presence of males was abundant but less than the larviform females (males were more or less 60% of the larviform females), and this might support the idea that males able to attach themselves to the females, and hence to the host, during copulation can have an advantage over the males who are not copulating and can be accidentally removed from the host.

ACKNOWLEDGEMENTS

We thank Dr Leticia Duarte Martinez, Entomology department, UFLA, Lavras, MG, Brasil for collecting and sending *Rhynchophorus palmarum* L. specimens.

AUTHORS' CONTRIBUTIONS

AD conceived the study, collected and identified the mite specimens, prepared them for electron and light microscopy and carried out the light and SEM observations, organized the photo gallery and drafted the manuscript. FP received the samples and reviewed the manuscript. Both authors read and approved the final version of the manuscript.

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