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Mass Trapping and Biological Control of *Rhynchophorus palmarum* L.: A hypothesis based on morphological evidences

Flávio Costa Miguens[1✉], Jorge André Sacramento de Magalhães[1], Livia Melo de Amorim[1], Viviane Rossi Goebel[1], Nicola Le Coustour[2], Marie Lummerzheim[2], José Inácio Lacerda Moura[3] & Rosane Motta Costa[1]

1. Universidade Estadual do Norte Fluminense Darci Ribeiro. Laboratório de Biologia Celular e Tecidual, Centro de Biociências e Biotecnologia, Campos dos Goytacazes, Rio de Janeiro, Brasil, e-mail: flavio.miguens@gmail.com (Autor para correspondência✉), jasm@uenf.br, livinha_amorin@yahoo.com.br, vivigoebels@yahoo.com.br, rmcosta@uenf.br. 2. Ecole Superiereure d' Agriculture Purpan, e-mail: lummerzheim@purpan.fr, marie.lummerzheim@purpan.fr. 3. Estação Experimental Lemos Maia CEPEC CEPLAC, e-mail: jinaciolacerda@yahoo.com.br.

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Abstract. Palm weevils have been reported as a pest and red ring nematode vectors for several palms of the Arecaceae family. *Rhynchophorus palmarum* L (Coleoptera: Curculionidae) is a pest for coconut crop and other palms. It is vector of *Bursaphelenchus cocophilus* (Cobb) Baujard (Nematoda) etiological agent of Red Ring disease and other nematodes. Current methods recommended use of enemies and parasites in integrated pest management of Rhynchophorinae. In addition, mass trap reduce environmental damage. The objectives of our study on coconut plantations were: (1) to determine the efficiency of low expensive kariomones traps and (2) low expensive kariomones and pheromones traps using adult males; and (3) to examine *R. palmarum* using light and scanning electron microscopy searching for ectoparasites which can be proposed in integrated pest management. Handmade kariomones mass traps were efficient to *R. palmarum* and other Curculionidae capture but kariomones plus adult male *R. palmarum* was maintained inside trap enhanced its attractiveness for this palm weevil and *Cosmopolites sordidus* (Germar) (Coleoptera: Curculionidae). *R. palmarum* presented occasionally fungal infection. In contrast, mites infested more than 50% of palm weevils. Infestation level was always high. Surface morphology of the mites and its interaction with *R. palmarum* were briefly described. All stages of ectoparasites life cycle were observed onto weevil elytra compartments. In this way, morphological evidences suggest the hypothesis of these mites as used as biological control agent in *R. palmarum* integrated pest management.

Keywords: Coconut palm; Curculionidae; Integrated Pest Management; Light and Electron Microscopy; Mites.

Coleta Massal e Controle Biológico de *Rhynchophorus palmarum* L.: Uma hipótese baseada me evidências morfológicas

Resumo. Coleópteros têm sido descritos como pragas e vetores de nematódeos causadores de Anel Vermelho em diversas palmeiras da família Arecaceae. *Rhynchophorus palmarum* L (Coleoptera: Curculionidae) é uma praga que afeta a cocoicultura e outras palmeiras; e, vetor de *Bursaphelenchus cocophilus* (Cobb) Baujard (Nematoda), agente etiológico de Anel Vermelho e de outros nematódeos. Atualmente, recomenda-se o emprego de inimigos naturais e parasitas no manejo integrado de pragas; dentre elas, Rhynchophorinae. Armadilhas de coleta massal são recomendadas no manejo integrado de pragas. Nosso estudo relata, na cocoicultura, a eficiência de armadilhas artesanais de baixo custo e a utilização cariomônios (toletes de cana-de-açúcar) e cariômonios mais feromônios (toletes de cana-de-açúcar e machos adultos de *R. palmarum*) como atrativos nas armadilhas. Ácaros ectoparasitas foram identificados nestes coleópteros, por microscopia, que podem ser propostos como parte do manejo integrado desta praga. Armadilhas de coleta massal com cariômonios foram eficientes na captura de *R. palmarum* e outros Curculionidae. No entanto, armadilhas de coleta massal com cariômonios e feromônios aumentaram a atratividade, em relação às primeiras, para este Coleoptera e *Cosmopolites sordidus* (Germar) (Coleoptera: Curculionidae). Eventualmente, exemplares de *R. palmarum* apresentavam infecção fúngica. Ácaros ectoparasitas infestavam mais de 50% dos exemplares de *R. palmarum*. A microanatomia destes ácaros e sua interação com *R. palmarum* foi preliminarmente descrita. Todos os estágios do ciclo de vida destes ácaros foram identificados no compartimento dos élitros. As evidências morfológicas suportam a hipótese de que estes ácaros podem ser empregados no controle biológico de *R. palmarum* em um programa de manejo integrado.

Palavras-chave: Ácaros; Coqueiro; Curculionidae; Manejo Integrado de Pragas; Microscopia de luz; Microscopia eletrônica de varredura.

ENTOMOLOGIA GERAL

The basic life cycles of *Rhynchophorus* species were similar. Adult female weevils are attracted to damaged, stressed, or healthy palms and oviposit 30 to 400 eggs on plants. Larvae bore into the palms and after several instars develop into adults in about 2 months (Watananpongsiri 1966, Giblin-Davis *et al.* 1989). *Rhynchophorus* species seek harborage in leaf axils of healthy palms (Weissling & Giblin-Davis 1993) *Rhynchophorus palmarum* L. and other palm weevils have crepuscular flight patterns (Rochat 1987).

Fermented sap exuding from dead or wounded palms is highly attractive to *Rhynchophorus cruentatus* (Fabricius) (Chittenden 1902). Moist fermenting tissues from various palm species, fruits, sugarcane and pineapple are similarly attractive to palm weevils (Diegado & Moreno 1986; Giblin-Davis *et al.* 1994). Early research provided evidence that general fermentation volatiles, such as ethanol, appeared attractive to *Rhynchophorus* weevils (Hagley 1965). Kairomones include ethyl acetate, ethyl propionate, ethyl butyrate, and ethyl isobutyrate for different weevil species (Gries *et al.* 1994). Studies with *Rhynchophorus obscurus* (Boisduval) provided the first evidence that males of palm weevils produce male and female-attracting aggregation pheromones (Chang & Curtis 1972). Subsequently, male-produced aggregation pheromones have been demonstrated for many species in the subfamily Rhynchophorinae, such as *R. palmarum* (Moura *et al.* 1989; Rochat *et al.* 1991a,b), *R. cruentatus* (Weissling *et al.* 1993), *Rhynchophorus phoenicis*

(Fabricius) (Gries *et al.* 1993), *Rhynchophorus ferrugineus* (Olivier) and *Rhynchophorus vulneratus* (Schoenherr) (Hallett *et al.* 1993), *Metamasius hemipterus* (Linneaus) (Giblin-Davis *et al.* 1994; Rochat *et al.* 1993), *Cosmopolites sordidus* (Germar) (Budenberg *et al.* 1993), *Sitophilus* spp. (Walgenbach *et al.* 1983). Ethyl acetate perceived by male *R. palmarum* also stimulates pheromone production (Jaffé *et al.* 1993). Lethal traps baited only with aggregation pheromones or host kairomones are not very attractive to palm weevils, but in combination synergize attractiveness 8-20 fold for *R. palmarum* (Oehlschlager *et al.* 1992) and other Rhyncophorinae (Giblin-Davis *et al.* 1994; Gries *et al.* 1993; Hallett *et al.* 1993; Oehlschlager *et al.* 1995; Perez *et al.* 1994; Perez *et al.* 1995; Rochat *et al.* 1995; Weissling *et al.* 1993, 1994).

It has been demonstrated that many species of the subfamily Rhynchophorinae are pests of crop and ornamental palms. *R. ferrugineus* has been infested in Mediterranean area (Murphy & Briscoe 1999; Barranco *et al.* 2000). Integrated pest management (IPM) based on pheromone traps, chemical controls and entomopathogenic nematodes have been used to save infested date palms. Despite good results of these techniques in the laboratory, they were not efficient in the field to succeed in eliminating red palm weevil (Ferry & Gomes 2002). Since 1921, palm weevils have been reported as red ring nematode vectors (Ashby 1921). The coconut palm weevil, *R. palmarum* is a pest for *Cocos nucifera* L (Bondar 1940; Franco 1964) and several plants of the Arecaceae family and sugarcane. In Brazil, larvae and adult *R. palmarum* have been found in nineteen species from Arecaceae family (SILVA *et al.* 1968). In addition, *R. palmarum* has been reported as vector of *Bursaphelenchus cocophilus* (Cobb) Baujard (Nematoda), causal agent of Red Ring disease (Hagley 1963; Griffith 1987). Observations of *R. palmarum* fecal material made by LM and SEM showed three species cohabiting these samples, being also present in fresh juice and fragments of infected coconut tree: *B. cocophilus*, *Teratorhabditis palmarum* (Gerber & Giblin-Davis) (Nematoda: Rhabditidae) and *Diplogasteritus* sp (Nematoda: Diplogasteridae) (Magalhães *et al.* 2008).

The term biological control has been applied in a broad sense to cover the use of any organism to control a pest or pathogen and classical biological control when any organism has been used to control a pest. Pests almost invariably have natural enemies or parasites somewhere in their geographic area and introduction of such enemies or parasites can reduce the size of the pest population (Lucas 1998). Parasitoid is an organism that is parasite early in its development but that finally kills the host during or at the completion of development; often used in reference to many insect parasites of other parasites (Schmidt & Roberts 1989; Eggleton & Gaston 1992). Current methods recommended for the management of *Rhynchophorus* species have focused on integrated pest management (IPM) involving surveillance, pheromone lures, cultural control and chemical treatments (Murphy & Briscoe 1999).

Few studies have been conducted on the natural enemies of *R. ferrugineus* or other *Rhynchophorus* species within their native ranges has been identified virus, bacteria, nematodes, insects and mites (Murphy & Briscoe 1999). Two mite species, *Hypoaspis* sp. and *Tetrapolypus rhynchophori* Ewing (Pymotidae) have been recorded infesting the adult weevil, but the status of these species as parasites is uncertain (Peter 1989). Researches on the natural enemies of *R. palmarum* in the Neotropical Region have only revealed that members of the parasitic dipterans family, Tachinidae, attack this weevil; *Paratheresia menezesi* (Townsend) palm (Moura *et al.* 1993, 1995) and *Paratheresia rhynchophorae* (Blanchard) (Guimarães *et al.* 1977) have been also identified in Brazil as ectoparasites. Three species of endoparasitic nematodes have been reported but their pathogenicity is unclear (Gerber & Giblin-Davis 1990). In addition, it has been suggested that various potential fungal and bacterial plant and weevil pathogens may contaminate and affect behavior and population dynamics of palm weevils (Griffith 1987).

The objectives of our study on coconut plantations were: (1) to determine the efficiency of low expensive kariomones traps and (2) low expensive kariomones and pheromones traps using adult males; and (3) to examine *R. palmarum* using light and scanning electron microscopy searching for ectoparasites which can be proposed in integrated pest management.

MATERIALS AND METHODS

Study site and field experiments. The study was done near Conceição de Macabu municipality (22º05'07"S - 41º52'06"W) on the northern of Rio de Janeiro state, Brazil. Experiments were carried out on three commercial farms, during 2005 and 2006. Phoenix farm total area was 775 ha, 80 ha to coconut plantations, approximately 11,800 plants divided in 3 areas. Saint Anthony farm had 6,500 coconut trees occupying 45 ha divided in 3 areas and its total area were 300 ha. In Saint Mary farm, 16,000 plants were distributed in four areas occupying 107 ha of 350 ha total area. *C. nucifera* phenotype Green Draft were planted at low density, 8.5 x 8.5 m spacing, approximately 140 plants/ha. Plants age varied between 1 to 15 years old. The vegetation around coconut plantations was predominantly grasses but fragments of Atlantic Rain Forest occurred. The soil was clayey. The climate in this region is warm tropical subhumid with relatively low rainfall and high atmospheric temperatures. The total annual precipitation was 796.6 mm (2005) and 672.9 mm (2005) and the mean annual air temperature was about 26oC in 2005.

Insect traps were located in the periphery of coconut plantation areas. They were divided in two groups. Trap type A, white plastic 100L bucket presenting 2.5 cm in aperture and containing 4 kg of sugarcane 25 cm long tablets or 4 kg of sugarcane 25 cm long tablets plus adult male of *R. palmarum*. Trap type B, transparent plastic 6L pet bottle for soft drink presenting 2.2 cm in aperture and containing 250 g of sugarcane 10 cm long tablets or 250g of sugarcane 10 cm long tablets plus adult male of *R. palmarum*. Pheromone and insecticides were not used. Intervals of collect were 15 days. Controls were the same number of empty traps.

Photographic data was carried out using a SONY DSC-P93®Cyber-shot (Sony Corporation, Tokyo, Japan) and images were done at 3.2 megapixels/inch^2.

Light microscopy and scanning electron microscopy. For light microscopy, insects were anesthetized at 4 C, during 20 minutes, and fixed by immersion for 2h at room temperature in 2.5% glutaraldehyde, 4% formaldehyde in 0.1 M cacodylate buffer, pH 7.2, washed in the same buffer during 2 hours. Then, insects were dissected and observed in an Axioplan Zeiss microscope. Images were digitized with a CCD camera ZVS Zeiss (Germany) controlled by an image analysis system AnalySIS® (Soft Imaging System, Zeiss, Germany). Similar samples were used for light and electron microscopy.

For scanning electron microscopy, insects were anesthetized as described above and fixed by perfusion for 2h at room temperature in 2.5% glutaraldehyde, 4% formaldehyde in 0.1 M cacodylate buffer, pH 7.2, washed in the same buffer, post fixed in a buffered 1% OsO_4 solution for 1h, washed, dehydrated in acetone series. Then, samples were critical point dried in a Balzer's apparatus (CPD 030) and covered with gold - 20 nm - in a Balzer's apparatus (SCD 050). Data were carried out at 25 kV in a Zeiss DSM 962 or ZEISS EVO 40 scanning electron microscope.

Acarology terms were used in according to Glossary of Terms in Acarology of University of Illinois at Urbana-Chanpaingn (CVM 2005).

RESULTS

Curculionidae were predominant in mass trapping capture. They were represented by *R. palmarum* and *C. sordidus*. Kariomones traps presented low effectiveness in *R. palmarum* capture (Figure 1), when compared to traps in which kariomones plus adult male *R. palmarum* was maintained inside trap (Figure 2). Seasonal differences in weevil capture were not identified. Traps disposed close to Atlantic Forest fragments were the most effectives. Apparently, color trap did not affect capture level. Male and female *R. palmarum* distribution in captures was haphazard. *C. sordidus* were copiously captured and their number was most expressive in traps close to Atlantic Forest fragment border. Non-Curculionidae insects were extremely diversified and were not identified (Figures 1 and 2).

Male and female *R. palmarum* presented eventually fungal infection mainly on its thorax and abdomen articulations (Figure 3). Mites infested more than 50% of *R. palmarum*. Ectoparasites occurred occasionally on weevil body but preponderantly in elytra compartment. Adult mites presented yellow-brown color (Figure 4) while eggs and nymph instars were translucent (Figures 8 to 11).

Egg, nymph instars and adult mites were observed under elytra (Figures 5 and 7), in both sides of hind wings (Figures 8 to 11) and on dorsal surface of the coleopterans abdomen. In elytra external surfaces mites were absent (Figure 6). Adult mites and nymph instars were approximately 3 mm long and were found mainly in elytra internal surface (Figures 4 and 5). Elliptical eggs measurements were 30 μm in length and 15 μm in width and were found adhered to both sides of hind wings (Figures 8 and 9). Some images suggest nymph feeding (Figure 10). Ecdysis could be detected due exoskeleton ghost presence on hind wings (Figure 11).

External morphology was similar in nymph and adults mites. They presented bilateral symmetry and body shape was oblong slightly reduced in anterior region (Figures 12 to 14). Capitulum consisted of pedipalps and hypostome (Figures 12 to 15). Pedipalps present six segments that emerged of basis capituli (Figure 12) and long setae were present in segment 6. They were visualized extended, partially (Figures 12, 14 and 15) or completely retracted (Figure 13). In dorsal view, a pair of setae was observed close to anterior extremity, four setae laterally disposed and a row of three setae in anterior middle body (Figure 14). Hyposteme was frequently hidden and disposed medially (Figure 15). Legs had six segments ending in a claw (Figures 14 and 16). Nymph instars were identified. Pedipalps were not fully developed in nymph (Figure 16). This was the main difference between adults and nymph. They were adhered to insect surface by stalks that were disconnected during development (Figure 16).

DISCUSSION

Rhynchophorinae weevils are suitable for mass trapping because of their long life cycle and adult longevity, low fecundity, and reliance on aggregation pheromones and host kariomones (Oehlschlager *et al.* 2002). In coconut plantations, *R. palmarum* and *C. sordidus* were copiously captured mainly in traps using kariomones plus adult male *R. palmarum* inside trap, probably due pheromone liberation. These results confirm previous reports that sugarcane fermentation volatiles are attractive to palm weevils (Diegado & Moreno 1986, Giblin-Davis *et al.* 1994) and according to Jaffe *et al.* (1993) kariomones perceived by this weevil stimulate pheromone production. In addition, traps baited only with aggregation pheromones or kairomones are not very attractive to palm weevils, but in combination synergize attractiveness 8-20 fold to *R. palmarum* and other Rhynchophorinae (Moura *et al.* 1989; Rochat et al, 1991a; Oehlschlager *et al.* 2002). *C. sordidus* capture probably is relates to *R. palmarum* male-produced aggregation pheromones is effective for many species in the subfamily Rhynchophorinae as well as *C. sordidus* (Budenberg *et al.* 1993).

The abundance of adult palm weevils is affected by seasonal changes. *R. palmarum* populations appear to peak at the end of the rainy season and throughout most of the dry season in coconut plantations in Trinidad (Hagley 1963), and in the dry season in oil palm plantations in Brazil (Schuiling & Dinther 1981). In Florida, *R. cruentatus* (Weissling *et al.* 1994) is more abundant in spring, before the onset of the rainy season. Seasonal differences were not identified of trap capture level of *R. palmarum* in coconut plantations in Rio de Janeiro state, Brazil. In contrast, the effectiveness of capture was increased when traps disposed close to Atlantic Forest fragments.

Trap silhouette and color as potential visual cues for foraging palm weevils. Different trap designs have been tested to optimize capture of palm weevils (Giblin-Davis *et al.* 1994; Oehlschlager *et al.* 1992; 1993; Weissling *et al.* 1992; 1993). Insect traps baited with 100 L white plastic bucket or baited with 6 L transparent plastic pet bottle for soft drink presented similar results suggesting that design and color could not interfere in capture features. These results are coherent with palm weevil habits previously reported no color in the human perceived spectrum is a critical parameter for *R. palmarum* (Oehlschlager *et al.*, 1993). *R. palmarum* has been captured equally well in ground traps and suspended traps (Giblin-Davis *et al.* 1996). Similar results were obtained in this work.

Continuous mass trapping reduced *R. palmarum* trap counts over time and lowered Red Ring disease (RRD) incidence (Chinchilla *et al.* 1993; Oehlschlager *et al.* 1995). There was only one confirmed case of RRD during these experiments suggesting that low expensive traps using recycled pet bottles and kariomones plus adult *R. palmarum* are effective in weevil capture reducing RRD in coconut plantations and must be considered to facilitate pest management decisions.

Various potential fungal and bacterial plant and weevil pathogens may contaminate and affect behavior and population dynamics of palm weevils (Griffith 1987). It has been demonstrated that *Beauveria bassiana* (Balsamo) Vuillemin cause 100% of mortality in *R. palmarum* (Santana & Lima 1994). In this way, fungal colonization of palm weevil surface from Rio de Janeiro might represent a potential agent for natural biocontrol. However, research efforts must be made in this way.

Integrated pest management has been proposed to control pests and pathogens in coconut crop (Moura *et al.* 2002). Biological control and classical biological control have been proposed to control plant pathogens and pests, respectively. Natural enemies or parasites can reduce the size of the pest population (Lucas 1998). Parasitoid and cleptoparasitoid habit might be evolved in host disease or dead (Eggleton & Gaston 1990; Eggleton & Belshaw 1992; 1993). Studies have been conducted on the natural enemies of Rhynchophorinae species within their native ranges has been identified virus, bacteria, nematodes, insects and mites (Murphy & Briscoe 1999). It has been suggested that association of *R. palmarum* with red ring nematode and three species of endoparasitic nematodes may affect the population dynamics of the weevils but their pathogenicity is unclear (Gerber & Giblin-Davis 1990; Giblin-Davis 1993). Two mite species, *Hypoaspis* sp. and *T. rhynchophori* (Pymotidae) have been recorded infesting the adult weevil, but the status of these species as parasites is uncertain (Peter 1989), although Acari have been proposed as a biocontrol agent against insects (Welbourn & Young 1988). The studies on the natural enemies of *R. palmarum* in the Neotropic have only revealed that this weevil is attacked by members of the parasitic dipterans family, Tachinidae; *P. Menezesi* palm (Moura *et al.* 1993; 1995) and P. rhynchophorae (Guimaraes *et al.* 1977) have been also identified in Brazil as ectoparasites. Association of *R. palmarum* with mites may affect the population dynamics of the weevils and/or its behavior. In addition, mite life cycle is apparently completed in elytra compartment of *R. palmarum*. In this way, morphological evidences presented here suggest mites as weevil ectoparasites and its use a possible biological control agent for *R. palmarum* in integrated pest management.

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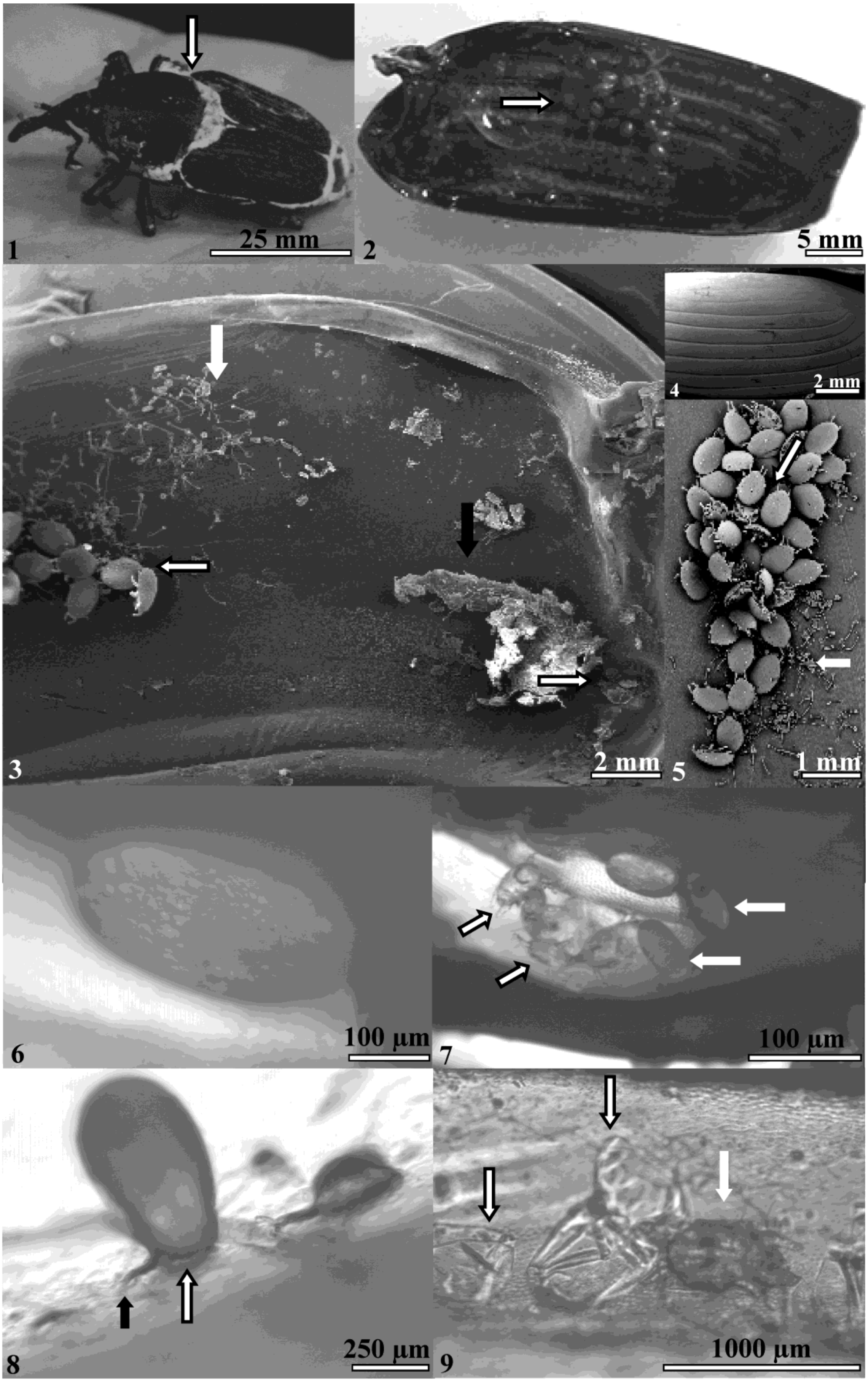

Figure 1 to 9. 1 - *Rhynchophorus palmarum* highly infected by fungus (arrow) mainly in its articulations. Digital photograph.; 2 - *R. palmarum* internal surface of elytron. Note adult mites (arrow) grouped on elytron. Digital photograph; 3 - *R. palmarum* internal surface of elytron showing mites nymphae (black/white arrows), several stalks (white arrow) and mucus (black arrow). SEM; 4 - *R. palmarum* external surface of elytron showing parallel striae. Note surface free of mites. SEM; 5 - *R. palmarum* internal surface of elytron showing mites nymphae (black/white arrows), several stalks (white arrow) on surface. SEM; 6 – Mite egg measuring approximately 30 µm in length and 15 µm in width on hind wings. DIC microscopy; 7 – Hind wing of *R. palmarum* showing mites nymphae (black/white arrows), and eggs on its internal surface (white arrows). DIC microscopy; 8 – Hind wing surface of *R. palmarum* showing mite nymph (black/white arrow) with pedipalps adhered in wing surface (black arrows). DIC microscopy; 9 – Hind wing of *R. palmarum* showing mites nymphae exoskeletal ghost (black/white arrow) and nymph on its internal surface (white arrows). DIC microscopy.

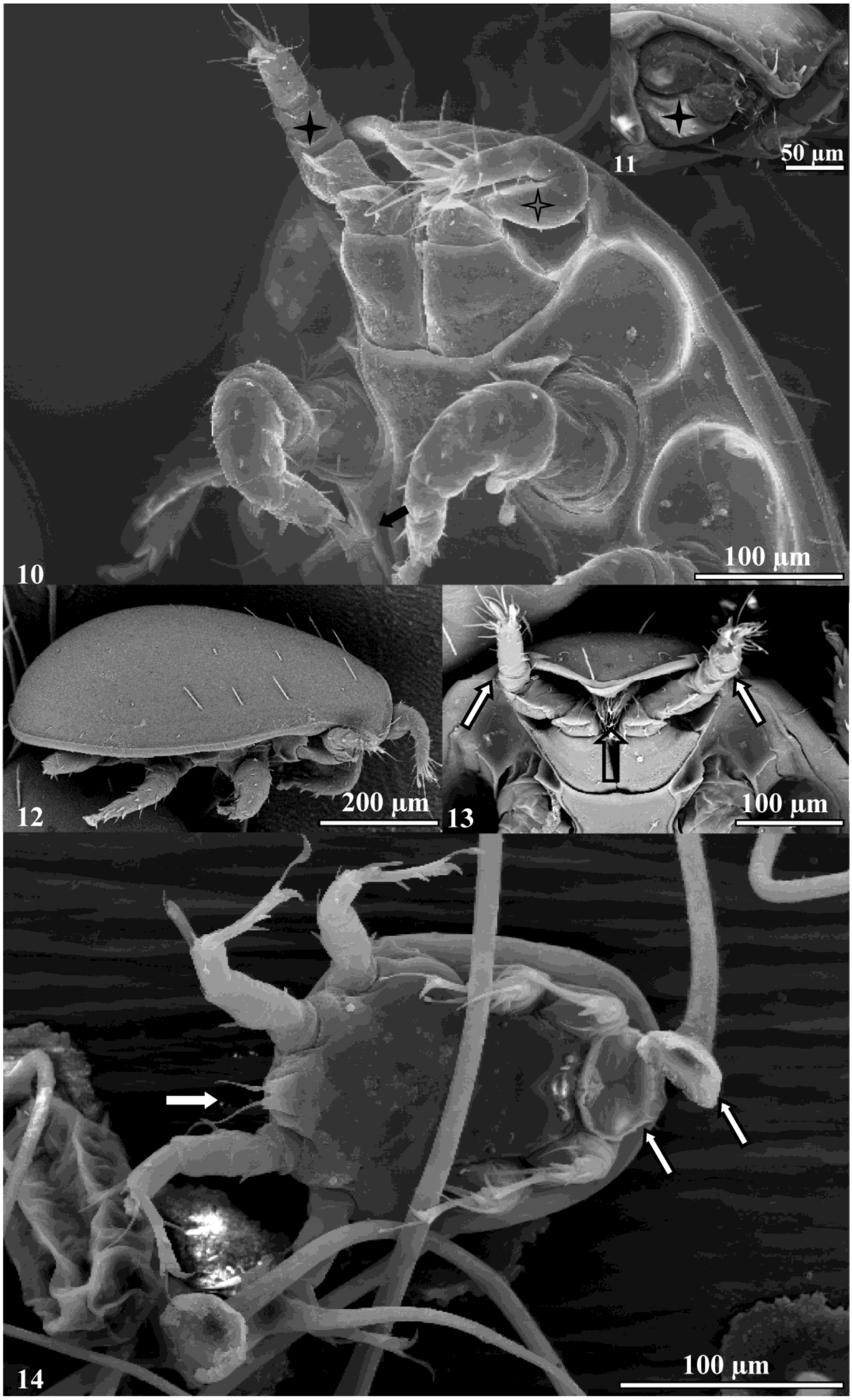

Figure 10 to 14. 10 – Frontal view of mite anterior region on *R. palmarum* elytron. Capitulum and leg pair 1 can be observed. Note pedipalp completely extended (star) and partially articulated (open star). Setae can be observed in last pedipalp segment. SEM; 11 – Lateral view of mite anterior region on *R. palmarum* elytron. Capitulum showing pedipalp completely retracted (star). SEM; 12 – Lateral view of mite nymph on *R. palmarum* hind wing. Mite body present elliptical feature. A pair of setae can be observed in anterior dorsum, two rolls with four setae disposed laterally and one central roll. SE/BSE, SEM; 13 – Frontal view of mite on *R. palmarum* hind wing. Capitulum is clearly observed showing extended pedipalps (arrow) and hyposteme (open arrow). SE/BSE, SEM; 14 – Ventral view of mite nymph on *R. palmarum* elytron. Capitulum (white arrow) showing differences compared to adult – Figure 13. Note adhesion stalk (black/white arrow) in posterior region of the nymph. SEM.

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