

SCANNING ELECTRON MICROSCOPY OF THE RED PALM WEEVIL (RPW), *RHYNCHOPHORUS FERRUGINEUS* OLIVIER (COLEOPTERA: CURCULIONIDAE): A NEW INVASIVE PEST OF COCONUT PALMS IN TERENGGANU

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Scanning electron microscopy was used to study and describe some of morphological characters of the Red Palm Weevil, *Rhynchophorus ferrugineus* Olivier (Coleoptera: Curculionidae) adults from Terengganu, Malaysia. The Red Palm Weevil (RPW) is a major pest of cultivated palms (i.e. oil, date and coconut palms) in many parts of the world. Recently, the Department of Agriculture of Terengganu State reported that the RPW caused death of the crown of coconut palms in more than 800 locations in Terengganu. However, attack symptoms are very ambiguous and cannot be detected until the crown of the coconut palm had collapsed. This invasive beetle will be a threat to the coconut industry and, indeed, the survival of other palms (e.g. oil palm). In present study, the morphological structures of the male and female adults of *R. ferrugineus* were compared. Observations focused on head, antennae, mouthparts, snout, pronotum, foreleg, elytra and pygidium. This was the first time that morphometry analysis of the species have been reported from Peninsular Malaysia. Outcomes from this morphological study is hoped will give a better understanding on the taxonomy of this species, and could be one of the most considerable importance to develop new control strategy of this invasive pest.

Keywords: *Rhynchophorus ferrugineus*, Red Palm Weevil, morphological characters, coconut pest

INTRODUCTION

Rhynchophorus ferrugineus Olivier (Coleoptera: Curculionidae) or commonly known as the Red Palm Weevil (RPW) is one of the worst pests for cultivated palms (oil, date or coconut palms) in many parts of the world [1, 2]. It is a native of Southern Asia and Melanesia [1], and has a wide range, as it occurs from India and Ceylon eastwards throughout South East Asia, Australia and in Mediterranean countries such as Spain, Egypt, Turkey and Cyprus. Due to its wide distribution, *R. ferrugineus* is the most important and studied pest of palms throughout the world [2, 3, 4, 5].

In early 2007, severely infested coconut palms were observed in some small land holdings in Rhu Tapai, Setiu, Terengganu. Later a cryptic RPW (*Rhynchophorus* sp.) first reported in 2007 which caused the death of the crown of the 3 common cultivars of coconut palms; Malayan Tall, Mawa and Pandan, in almost 58 locations in Terengganu [6]. Recently, the Department of Agriculture of Terengganu State reported that the RPW was the cause of death for the coconut palms crowns in more than 800 locations in Terengganu [6].

Preliminary morphological assessments conducted were unable to confirm the identity of the RPW although remarkable similarities to *R. ferrugineus* were reported. This was the first time that the species have been reported on coconut palm in Peninsular Malaysia. Its origins are unknown but the theory surrounding its appearance is that the weevil could have been transported unwittingly in dates imported from the Middle East [7].

In the current study, some of the morphological characters of RPW adults were observed, including the antennae, rostrum, mouthpart, pronotum, elytra, fore leg and pygidium under Scanning of Electron Microscopy (SEM). In addition, certain morphological structures were measured as comparison between male and female RPW adults. This study reports the first morphometry analysis of this species on coconut (*Cocos nucifera*) from Terengganu, Peninsular Malaysia. It is hoped that information obtained from this study will give a better understanding on the taxonomy of this invasive species, which will be the first step in the development of effective integrated pest management of this weevil.

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MATERIALS AND METHODS

Sampling of Red Palm Weevils (RPW)

RPW adult samples were collected from coconut palm trees seriously infested by this pest in a coconut palm plantation in Rhu Tapai, Setiu, Terengganu. Adults captured were separated into male and female batches, placed under laboratory conditions at $26\pm0.2^{\circ}\text{C}$; 75–80% of relative humidity (RH) and photoperiod of 12L:12D hr; and fed with coconut shoots.

Measurements of morphological characters

Mean length of different morphological characters of each individual were measured to the nearest 0.01 mm with rulers, Vernier calipers or under a dissecting microscope. Body length of the adult was measured from the anterior end of the pronotum to the posterior end of the pygidium; whereas width of the body was measured at the base of the elytra across the humeri at their widest point. The length of the pronotum was measured dorsally from the middle of the base to the middle of the apical margin; and width of the pronotum, at the widest point on the pronotum. The length of the rostrum was measured dorsally from the ocular sulcus to the apex of the rostrum or the tip of epistoma. The length of the leg (fore leg) was measured dorsally from the base of femur to the tip of the tarsi where the two distal claws located, while the length of the antenna was measured dorsally from the deep scrobe or base of the scape to the tip of the antennal club.

Electron Microscopy Analysis

Males and females of RPW were prepared to be free from dust or coconut fiber to avoid debris accumulation on the specimens. Head, body and legs of males and females were decapitated with fine scissors, immediately fixed in the Formalin Acetic Acid (FAA) and kept overnight in the vacuum oven. The samples then were washed and bathed in Sodium Cacodylate for about 10 minutes for 3 times. Samples were transferred in Osmium tetroxide for 2–3 hours, and washed again in Sodium Cacodylate for about 10 minutes for 3 times.

Finally, the samples were dehydrated in increasing alcohol baths 50%, 70%, 80%, 95% and 100% (twice for 5 minutes). Before the observation, samples were rinsed once, dried, fixed in the cylindrical stub and then sputtered-coated with gold. An electron microscope (JEOL JSM - 636 OLV) was used to observe the specimens. SEM photomicrographs were taken from different locations of each morphological part, for each RPW male and female.

RESULTS AND DISCUSSION

Comparison of different morphological characters between sexes

No significant differences were found on mean lengths and widths of the body and pronotum, as well as the lengths of the fore leg and antenna between males and females (Table 1). The males and females of RPW are usually about equal in size, reddish brown, sometimes orange to black with 2-7 black spots and markings of variable sizes and shapes on the pronotum. In males, the mean body lengths ranged 25.01 ± 0.58 mm and width ranged 11.01 ± 0.02 mm. In females, the mean body lengths and width were shorter as compared with males (24.68 ± 2.40 mm, 10.67 ± 0.88 mm respectively). Similarly, the mean lengths and widths of the pronotum and fore leg in males were longer than in females (Table 1). It was found that females have longer antenna (3.92 ± 0.97 mm) than males (3.83 ± 0.29 mm). However, the differences of these morphological characters between sexes were not statistically significant ($p > 0.05$).

Very few information on the morphometric of this weevil is available. Most of reports published only reported the body length and body width with mainly on male individuals, or perhaps there was no mention of the sex of the weevil. For example, Dutta et al. [8] reported that adults of RPW from Meghalaya, India ranged 33.2-34.05 mm (average 33.62 mm) in length and 11.9-13.1 mm (average 12.50 mm) in width. However, they did not compare between sexes and did not measure other characters. Based on the EPPO report [9], body length of adult male ranged from 19-42 mm and width of 8-16 mm; whereas adult female length was 26-40 mm with width of 10-16 mm. Another study done by Wattanapongsiri [10] found that mean length of adult male ranged 31.0-39.6 mm and width ranged 11.50-15.05 mm; whereas adult female length of 29.00-40.15 mm, with width of 12.0-14.7 mm. But, the origin of RPW samples collected by Wattanapongsiri (1966) were unknown, and might probably come from Thailand or other South East Asia countries. Based on the previous studies, the RPW adults from Terengganu were slightly smaller compared with those from Thailand [10], India [8] and Mediterranean countries [9].

The only structure that was significantly different between sexes was the length of the rostrum. Rostrum is the most important taxonomic character so far as it shows strong sexual dimorphism of both sexes. We found males have significantly shorter rostrum as compared with females; with 8.05 ± 0.58 mm in males and 9.68 ± 0.33 mm in females ($p < 0.05$). In males, a patch of brownish

'hairs' or rostral setae were present on the dorsal apical region of the rostrum. In contrast, the rostrum of female is bare (rostral setae absent), is shiny, curved, longer and slender than male. However, generally the variation in color and pronotal markings is similar in both sexes. The sexes of this species are always easily distinguished by the presence or absence of the setae on the rostrum and ventral side of the front femur and tibia, and shape of the pygidium. The comparative measurements of some morphological characters can be referred in Table 1.

Table 1. Measurement of some morphological characters between male and female of the RPW adults (Note: * $p > 0.05$, $n = 13$ individuals).

Morphological characters	Male (mm)	Female (mm)
1) Body length	25.01 ± 0.58	24.68 ± 2.40
2) Body width	11.01 ± 0.02	10.67 ± 0.88
3) Pronotum length	10.33 ± 0.67	9.33 ± 0.33
4) Pronotum width	9.05 ± 0.01	8.67 ± 0.88
5) Rostrum length	$8.05 \pm 0.58^*$	$9.68 \pm 0.33^*$
6) Fore leg length	14.33 ± 0.88	13.33 ± 1.20
7) Antennae length	3.83 ± 0.19	3.92 ± 0.17

Description of some morphological characters on both sexes

Antenna – Antennae of both sexes have the same shape and size. The antenna is laterally arising from deep scrobe at base of the pronotum. The antenna consists of a scape, a funicle and a club. The scape is elongated and longer than funicle and club. The mean length of the antennal scape and funicular segments varies slightly. The funicle comprised of 6 segments, whereas the antennal club is reddish-brown in colour and broadly triangular with several setae (antennal sensilla) dorsally and ventrally (Fig. 1).

Rostrum – The rostrum in males is significantly shorter than females, appeared to be smooth and stout with a brownish hairy pad or rostral setae at the apex. In male, the rostrum is nearly four-fifths length of the pronotum. In high magnification of SEM micrograph, the rostral setae consists of a group of bristles (tooth-brush-like), which are stouter, ridged, thick-walled and almost similar in length (Fig. 2). It is suspected that the bristles are chemoreceptors and contain neuron sensory which important for mating, and possibly for respond to the odor stimulus. In contrast, the rostrum of female is slightly longer, bare, curved and slender than male. The apical tip of the female rostrum is more cylindrical with rounded ends, and slightly narrower at the anterior end (Fig. 3). Along the rostrum of both sexes, there are high densities of wall markings, but the function of the wall markings is unknown. It is

speculated that some kind of wax or fluid which is may be hydrocarbon based is emitted from the wall markings to act as lubricant for the rostrum to grind the soft tissue of palms.

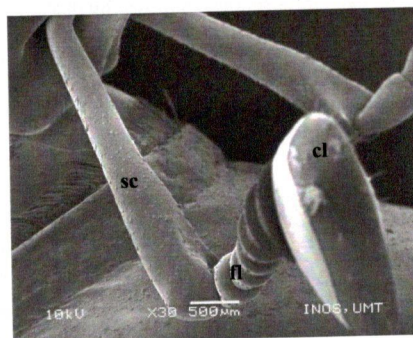


Fig. 1. The whole antenna of *R. ferrugineus* adults (sc: scape; fl: funicle; cl: club).

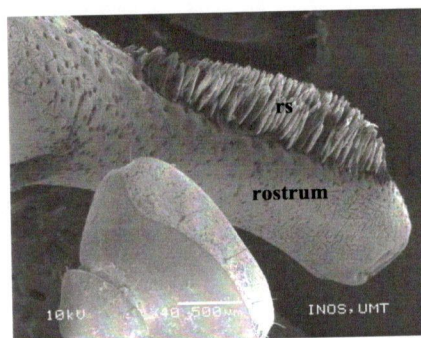


Fig. 2. Male rostrum – rostral setae (rs) present on the dorsal apical region which consists of a group of bristles.

Mouthpart – In general, the mouthparts such as mandible, maxilla and submentum of both sexes were similar in shape and size. The mandible of both sexes is narrowly rounded, deeply tridentate (Z shaped) with 3 sharply pointed teeth (Fig. 3). The distal half of the mandible is strongly depressed and submentum is oval distally. There is also a deep groove below the apical tip of the mouthpart. It is found that when the mandibles were opened for chewing or grinding the tissues of palms, the deep groove also opened and moved synchronously with the mandibles.

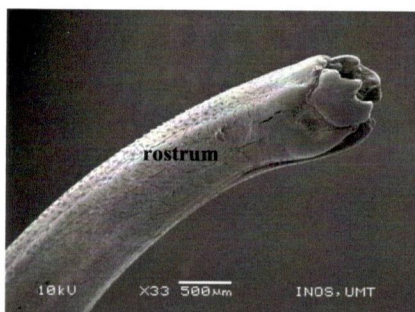


Fig. 3. Female rostrum – which is bare, curved, slender and more cylindrical with rounded ends.

Pronotum – Pronotum of both sexes is slightly pubescent to shiny, posterior margin nearly rounded and gradually narrowed anteriorly. The colour ranges from ferruginous to reddish brown and varying to orange, dark brown and deep black. Interestingly, we found variation in pronotal markings (black spots) while the shapes of pronotal markings is extremely variable. Attempts were made to point out such color variations in the descriptions and discussion along with the illustrations of each variation, which will be published soon in a paper. However, the use of markings on the pronotum in distinguishing species has caused much confusion. Therefore, it is suggested that the pronotal markings should not be relied alone to identify RPW and other morphological characters should be used whenever possible. Research is currently underway to investigate the genetic variation among the individuals of different coloration and pronotal markings as part of the taxonomical study of the RPW.

Elytra – Elytra of both sexes were dull to shiny, smooth or slightly velvety pubescent, almost rectangular, with punctuation along the outer edges. The colour of elytra varied from reddish brown to deep black. The length of each elytron is around two and one-third times its own width. The scutellum tapers broadly, varying from reddish brown to black, pointed posteriorly and the length is one-quarter to one fifth of the elytral.

Fore leg – Generally, the length of fore legs in both sexes was not distinct at all. However, the most striking differences in the fore legs are the presence of the reddish brown setae on the ventral side of the front tibia in males. While in females, setae on front tibia are much shorter and strongly curved than in males. In both sexes, the first tarsal segment is twice as long as the second segment. The second segment is slightly shorter than the third segment and the fourth segment is as long as 3

segments combined. There is also two distal claws on the apical tip of the fourth tarsal segment.

Pygidium – Pygidium is one of the useful characters to distinguish between sexes. The male pygidium is flat, smooth, sparsely and minutely punctured posteriorly and dorsolaterally. It is convex in shape, with the dorsal cleft narrowly convergent toward the posterior end. In females, the pygidium consists of a vaginal base, spiculum ventrale, genital styli, and vaginal chamber [10]. The female pygidium is narrower and fringed with longer setae than in males, and the vaginal base is triangular in shape, pointed posteriorly and sclerotized (Fig. 4).

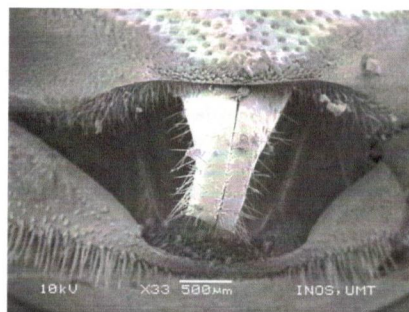


Fig. 4. Female pygidium which is triangular in shape, pointed posteriorly and sclerotized.

CONCLUSIONS

Generally, it can be concluded that RPW is morphologically similar, but there were some structures showed strong sexual dimorphisms between sexes. No significant differences in length and width, type or shape of body, pronotum, elytra, antennae and mouthpart between males and females were observed. However, the most important taxonomic characters to distinguish between sexes, so far as the adult is concerned, are found on the rostrum, front tibia, and shape of the pygidium. There were marked morphological differences on the pronotal markings of RPW, revision of the taxonomic status utilizing molecular techniques will be applied for further investigation. Outcomes from this morphometric study are hopefully will provide information for a basis of further formulation to control the attack of RPW particularly in Terengganu.

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- [10] Wattanapongsiri, A. (1966). A revision of the genera *Rhynchophorus* and *Dynamis* (Coleoptera: Curculionidae). (Report of Department of Agriculture Science Bulletin, Vol. 1., Bangkok, Thailand).

REFERENCES

- [1] Kaakeh, W.A.A., Khamis A., Aboul-Nour, M.M. (2001). *The Red Palm Weevil: The Most Dangerous Agricultural Pest*. UAE University Printing Press, pp. 90-165
- [2] Faleiro, J.R. (2006). A review of the issues and management of the red palm weevil *Rhynchophorus ferrugineus* (Coleoptera: Rhynchophoridae) in coconut and date palm during the last one hundred years. *Int. J. Trop. Insect Sci.* 26, 135-154
- [3] Gadelhak, G.G. Enan, M. R. (2005). Genetic Diversity Among Populations of Red Palm Weevil, *Rhynchophorus ferrugineus* Olivier (Coleoptera: Curculionidae), Determined by Random Amplified Polymorphic DNA-Polymerase Chain Reaction (RAPD-PCR). *Int. J. Agric. Biol.* 7(3), 395-399
- [4] Salama, H.S., Saker, M.M. (2002). DNA fingerprints of three different forms of red palm weevil collected from Egyptian date palm orchards. *Arch. Phytopathology Pl. Prot.* 35, 299-306
- [5] Prabhu, S.T., Pathil, R.S. (2009). Studies on the biological aspects of red palm weevil, *Rhynchophorus ferrugineus* (Oliv.). *J. Agric. Sci.* 22, 732-733
- [6] Department of Agriculture (2011). Report on current status of attack of the Red Palm Weevil, *Rhynchophorus ferrugineus* in Terengganu. (Report of DOA, Government Press, Malaysia).
- [7] Zazali, C. (2010). Personal Communication. (Department of Agriculture, Terengganu, Malaysia).
- [8] Dutta, R., Thakur, N.S.A., Bag, T.K., Anita, N., Chandra, S., Ngachan, S.V. (2010). New Record of Red Palm Weevil, *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae) on Arecanut (*Areca catechu*) from Meghalaya, India. *Fla. Entomol.* 93(3), 446-448
- [9] EPPO (2007) *Rhynchophorus ferrugineus* and *Rhynchophorus palmarum*. *European and Mediterranean Plant Protection Organization Bulletin* 37, 571-579