SCANNING ELECTRON MICROSCOPY BASED MORPHOLOGY OF PAPAYA MEALYBUG[*Paracoccusmarginatus*Williams And Granara De Wilink(HEMIPTERA: PSEUDOCOCCIDAE)] ON MULBERRY PLANT OF ASSAM

Abstract: A scanning electron microscopy (SEM) study was conducted on the external morphology of the adult female Papaya mealybug, *Paracoccousmarginatus*Willam and Granara de Willink. This study aimed to enhance the understanding of the external morphology and wax-secreting pores of the papaya mealybug through SEM examination. The ultrastructure of the antenna, stylet fascicle, legs, cerarii, vulva, and wax-secreting pores was carefully examined. Despite the time-consuming and expensive nature of preparing specimens for SEM, the detailed information obtained is valuable for resolving challenging aspects of papaya mealybug taxonomy and enhancing our understanding of the role of wax in their biology.

Keywords: *SEM, Papaya mealybug, adult female, morphological characters.*

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Introduction:

"The Papaya mealybug (PMB) *Paracoccousmarginatus*Willam and Granara de Willink (Hemiptera: Pseudococcidae) is a troublesome insect pest known to infest various economically important plantssuch as papaya, mulberry, acalypha, hibiscus, plumeria, avocado, citrus, cotton, tomato, eggplant, pepper, beans and peas, sweet potato, mango, cherry and pomegranate" (Williams and Willink, 1992, Miller and Miller, 2002). This insect caused heavy infestation and yield reduction of mulberry throughout India recently. "It is an exotic polyphagous-sucking insect with high multiplication and spreading potential at the field level. It is native to Mexico and Central America, where it never acquires the status of a serious pest, probably due to the presence of an endemic natural enemy complex" (Tanwar*et al*., 2010). Though its presence was known since 1955, "it was only described in 1992" (Williams and Willink, 1992). "PMB became a pest when it invaded the Caribbean region. Since 1994 it has been recorded in 14 Caribbean countries. The pest was recorded in Bradenton, Florida in 1998 on *Hibiscus,* and by 2002 it spread to 18 different plant species in 30 different cities. The establishment of this pest in Guam in 2002" (Meyerdirk*et al*., 2004) and "Palau in 2003 resulted in further spread to neighboring Hawaiian Islands in the Pacific. In Asia, it was reported from Indonesia, India, and Sri Lanka in 2008" (Muniappan*et al.,* 2009), Maldives and Bangladesh in 2009 and Thailand, Cambodia and the Philippines in 2010. "It was also reported from Reunion Island in the Arabian Sea Ghana in West Africa in 2010 and Assam in 2013" (Sarma, 2013).

The SEM is a valuable tool for studying the detailed morphology of small structures on insect cuticles, and in the case of mealybugs, it helps in understanding the significance of waxes produced by different dermal pores in protecting developmental stages.The rigid exoskeleton of insects is especially suitable for electron microscopy because the specimen usually retains its shape under vacuum. "Wax on the body surface may prevent contamination by honeydew" (Broadbent, 1951; Cox and Pearce, 1983;

Pope, 1983) or “invasion of the body by bacteria, fungi, and other parasites” (Foldi and Pearce, 1985) or “prevent damage at the point where eggs touch, so facilitating hatching” (Hashimoto and Ueda, 1985). “The mealy wax covering on the integument of mealybugs differs from that of other insects in many ways, Its presence on the surface is well documented but there has been only limited speculation on its function or reasons for the variety of pores and ducts found within a single species” (Waku and Manable, 1981; Foldi and Cassier, 1985). “The structure of the wax-producing pores is complicated and can be elucidated better using SEM. However,only a few studies have reported on the ultrastructure and wax-secretion processes in the family Pseudococcidae” (Cox and Pearce, 1983; Kumar et al., 1997). Likewise, a few studies reported on the ultrastructure and wax-secretion processes in the family Pseudococcidae. There has not been any study of the structure of Papaya mealybug, hence, this study aimed to fill the gap in the understanding of the external morphology and wax-secreting glands of the Papaya mealybug through scanning electron microscopy.

Materials and methods:

The scanning electron microscopic studies focused on the adult female, as the external micrographic characters of this stage are considered taxonomically vital.Adult females were kept in 100% acetone for 1 hour. The sample was kept overnight in 0.25% Glutaraldehyde fixative in the refrigerator at 4°C. After overnight keeping in fixative, samples were then washed three times in 0.1M phosphate buffer and were dehydrated by passing through a series of 30, 50, 70, 90, and 100% alcohol for 15 min in each concentration. After that 1 drop of Hexamethyldisilazane (HMD) with 1 drop of alcohol on the sample was open-dried. Each dehydrated specimen was then glued onto a standard SEM stub (diameter 12.5 mm, height 3.0 mm) using double-sided sticky tape. SEM stubs were then put in a sputter clouter for 90 minutes. To prevent the electron beam from burning a hole through the cuticle, each specimen was coated with a protective, reflective surface. Sputter coating covers the specimen with a very thin layer of heavy metal (gold-palladium mixture). Each specimen was placed in the sputter chamber, the air was pumped out and replaced with nitrogen gas, before which a plasma of metal molecules was released for 3.5-4.0 minutes to coat the specimen with metal. A fully computer-controlled scanning electron microscope ZEISS (EVO MA10) with a tungsten heated filament was used to scan each gold-palladium-coated specimen.

Results:

The scanning electron micrographic studies revealed detailed external morphological characters of the adult female Papaya mealybug *viz*., antenna, stylet fascicle, legs, cerarii, vulva, and wax-secreting pores revealed the following-

Antenna: Eight antennal segments were present in adult female papaya mealybug. The apical (flagellum) and sub-apical (scape and pedicel) segments clearly separated. Scape was a strong and stout segment followed by a less stout pedicel. Pedicel had 4 recurvate setae (3 on the dorsomedial aspect and 1 on the ventromedial aspect). The total number of the flagellar segment was eight, the apical most flagellar segment is the longest and the ventrolateral aspect of the flagellar segments had 10–12 flagellate setae, 3 fleshy setae, and 6 stiff setae near the apex. Two comparatively stouter and more pointed setae were present on the ventrolateral aspect (Fig. 1). All the antennal setae appeared to be trichoidsensillae for olfactory, gustatory, and thigmotactic functions.

Stylet fascicle: Stylet fascicle consisted of 4 distinct stylets (2 mandibular and 2 maxillary), labrum and labium were fused to form the proboscis. Setae on the proboscis was eract with curved tips These setae appeared to be chemosensillae for olfactory and gustatory response (Fig. 2).

Legs: Adult female mealybugs had only one tarsal segment and a single claw on the tarsus of each leg. At the expanded base of the claw, there was usually a pair of seta-like digitules (claw digitules), which were either shorter or longer than the claw, and either pointed or expanded distally. Coxa was the most robust segment with 2-3 recurvate setae, the trochanter is triangular, ventromedial seta in the trochanter was the longest. The femur was the longest segment with 4 setae in ventro-medial and 3 setae in dorso-medial aspects. In the tibia, 3 stout setae were present in the ventromedial aspect. Tarsus uni-segmented as described(Fig.3).

Cerarii: Each cerarius usually contains more than two conical setae and an aggregation of trilocular pores (Fig. 7).

Pygidium: Pygidium was heavy with an inwardly rounded periphery. Long and fleshy setae were present in pygidium apex of pygidium with, 4-5 inwardly curved long setae. Discoidal pores are present on the dorsal surface of the pygidial body (Fig. 4).

Vulva: The elevated, triangular vulva has a "T"-shaped slit opening with striations that radiate outward (Fig. 5).

Wax-secreting pores: The wax-secreting pores of adult female *P. marginatus* were as follows:

a. **Multilocular pores:**They are occasionally seen on the dorsum, although they are typically lacking on the venter, at least around the vulva. All of the pores were made up of an outer ring with 8–10 loculi and a circle with more than five holes. Each egg developed an attachment to the wax curls created by the multilocular pores surrounding the vulva.”The wax curls produced by the multilocular pores surrounding the vulva became attached to each egg as it left the genital opening” (Kumar *et al*., 1997).Wax produced by multilocular disc pores envelops the eggs in the ovisac, shielding them from natural enemies, precipitation, desiccation, and honeydew

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contamination. After their second instar, males also employ wax from the multilocular disc pores to build their cocoons (Fig. 6).

b. **Trilocular pores:**“Trilocular pores produce spiral wax filaments that fragment to form the fine, mealy body covering (Cox and Pearce, 1983). These pores were usually abundant and evenly distributed over both dorsum and venter; each consisted of a rounded-triangular, raised area having three elongate, narrow openings radiating from the center of the pore and slightly twisted, or swirled, about each other”(Cox and Pearce, 1983) (Fig. 7).

Discussion:

The results of the study contribute to the understanding of the external morphology of the Papaya mealybug, providing valuable information for taxonomic identification and differentiation from related species."From the experiment, micrographs of the papaya mealybug's external morphological characters (antenna, stylet fascicle, legs, cerarii, vulva, and pores that secrete wax) were not made by anyone else, but comparable research was conducted on six specific mealybug species found in Sri Lanka" (Sirisena et. al., 2015).“Early workers used the number of antennal setae as primary characters to separate species” (Maskell, 1894; Essig, 1909 Hollinger, 1917). “The overall length of the antenna, as well as the length of the individual segments, were recorded in detail by numerous workers until Ferris (1918) decided that the variation made was unreliable as a taxonomic character”. “Differences in wax pore ultrastructure may be taxonomically useful. Such differences have been used to revise the generic placement of some scale insect species into families, such as Coccidae, Diaspididae, and Monophlebidae” (Takagi, 1990; Ülgentürk and Wilhem, 2001; Unruh, 2008; Unruh and Gullan, 2008). “Adult female mealybugs are covered with hundreds of pores most of which produce various types of wax for different purposes”(McKenzie, 1967; Cox and Pearce, 1985). “These pores are distinct sclerotized structures that act as molds to produce structurally different forms of wax in different areas of the body”(Cox and Pearce, 1985). “The wax curls produced by the multilocular pores surrounding the vulva became attached to each egg as it left the genital opening” (Kumar *et al*., 1997). The details of the setae and pores recorded in the present study would be useful in ascertaining the identity of the species and differentiating it from other related species. Moreover, it will provide a basis for the secretion of the pores and their significance in the biology of the pest.

Conclusion:

The scanning electron microscope (SEM) is a crucial tool for studying the detailed morphology of very small structures on insect cuticles, providing quick and accurate representation.SEM is a very useful tool for observing minute, three-dimensional structures of papaya mealybug, and some of these details can only seen under the SEM. Despite the time-consuming and expensive nature of preparing specimens for SEM, the detailed information obtained is essential for resolving challenging aspects of papaya mealybug taxonomy and improving understanding of the role of wax in their biology.

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Disclaimer: Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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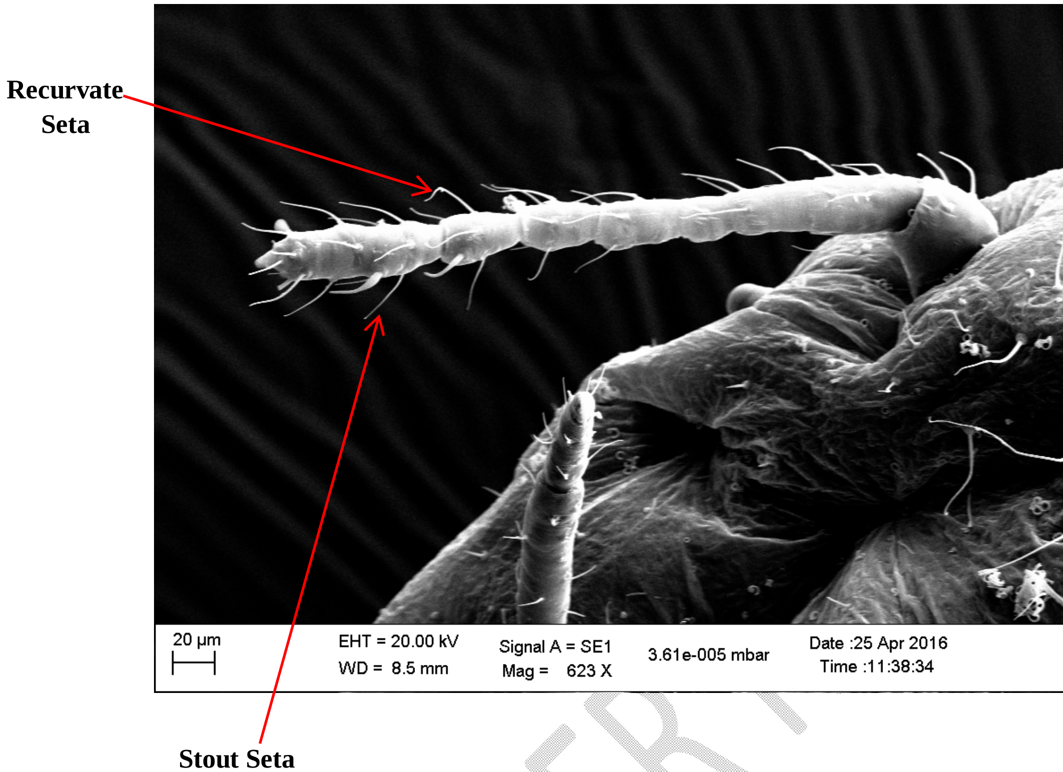

Fig. 1. Antennae of female *P. marginatus*

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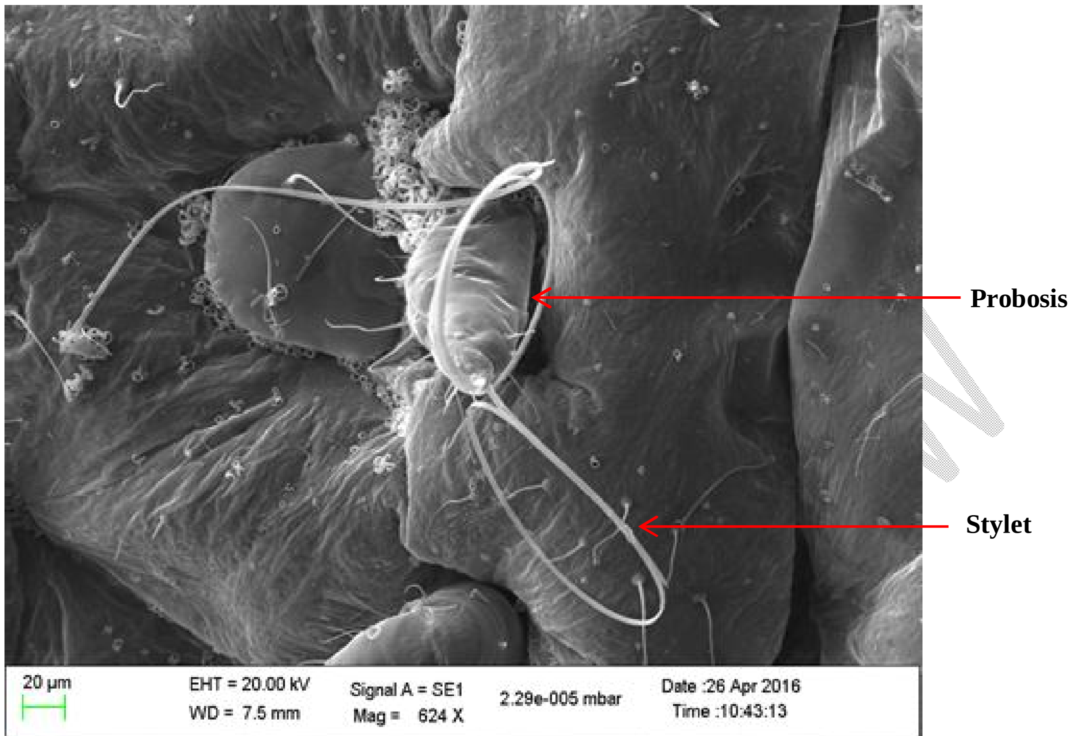

Fig. 2. Stylet fascicle of female *P. marginatus*

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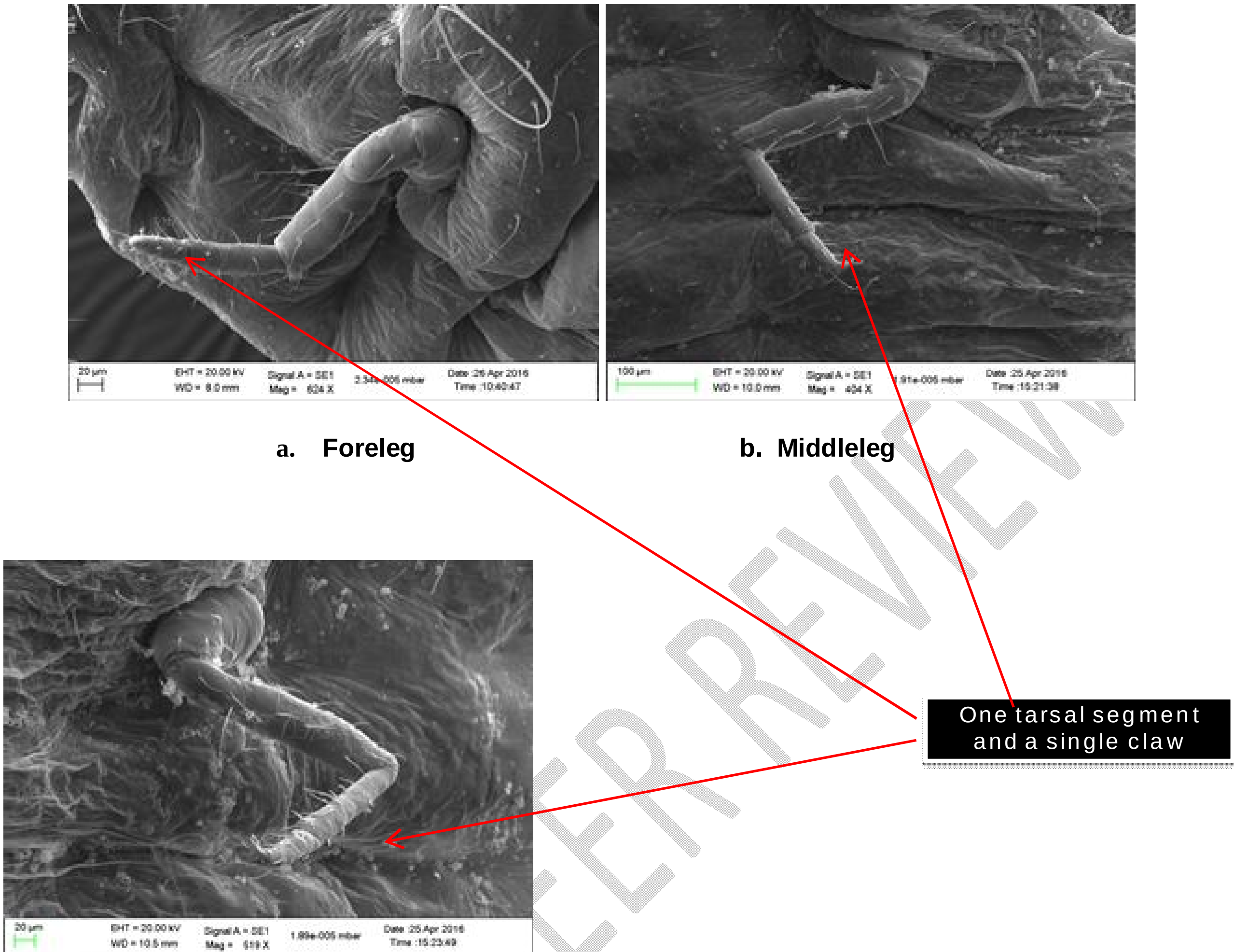

a. **Foreleg**

b. **Middleleg**

c. **Hindleg**

Fig. 3. a. Foreleg, b. middleleg, c. hindleg of female *P. marginatus*

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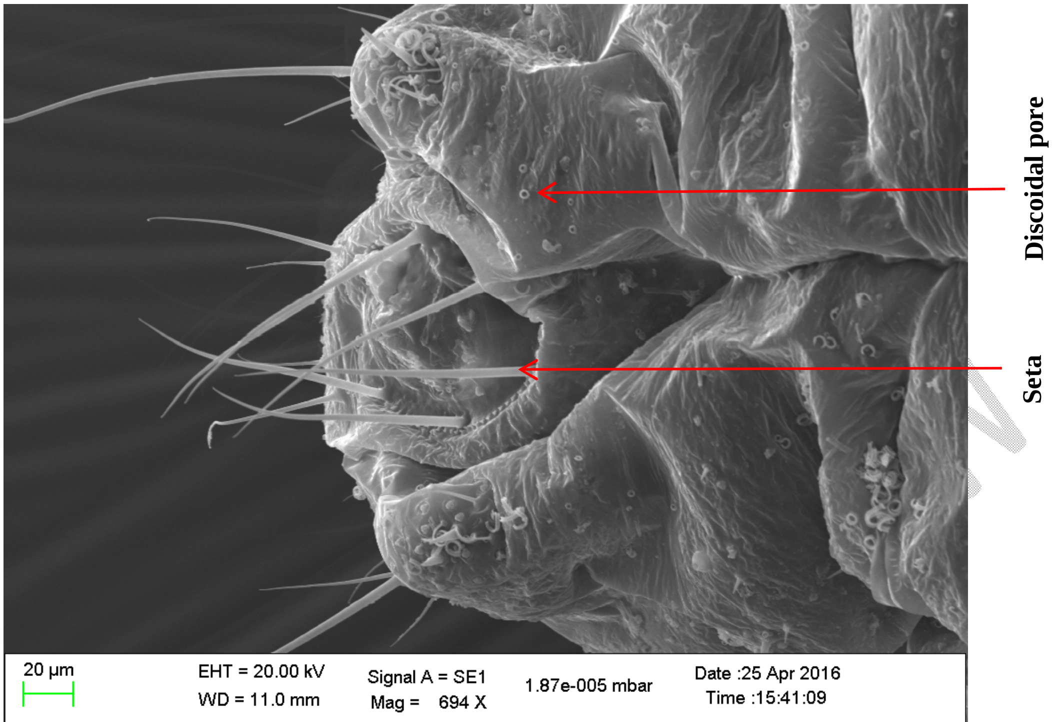

Fig. 4. Pygidium of female *P. marginatus*

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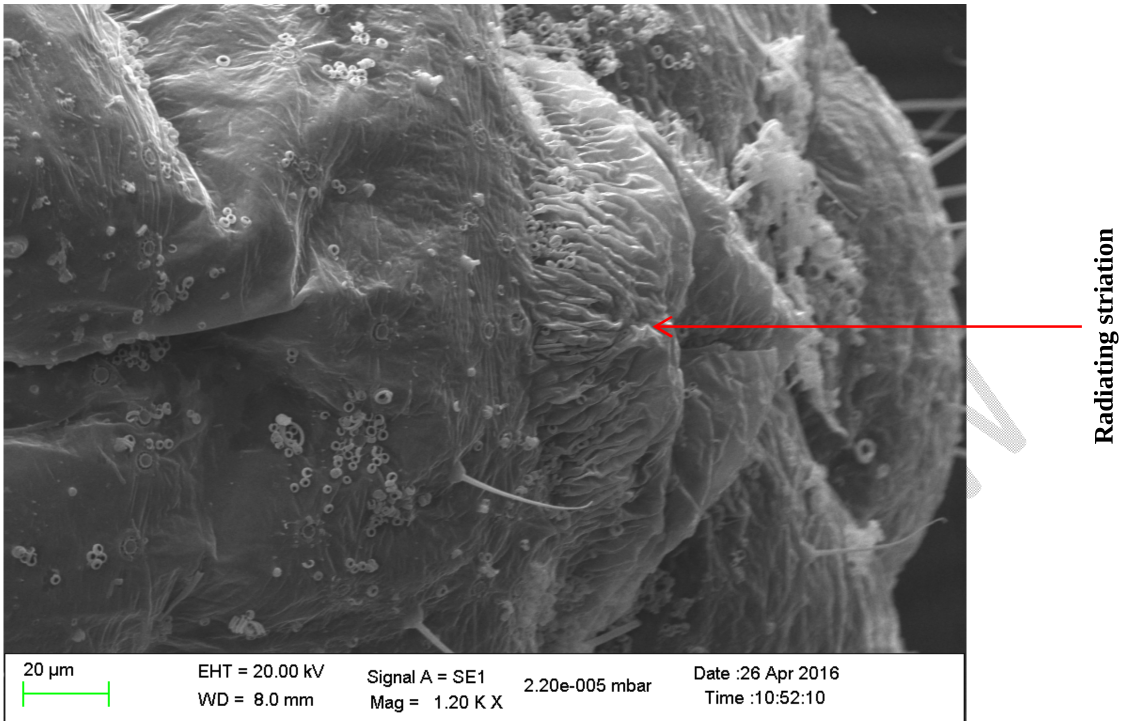

Fig. 5. ‘T’ shaped slit of vulva of female *P. marginatus*

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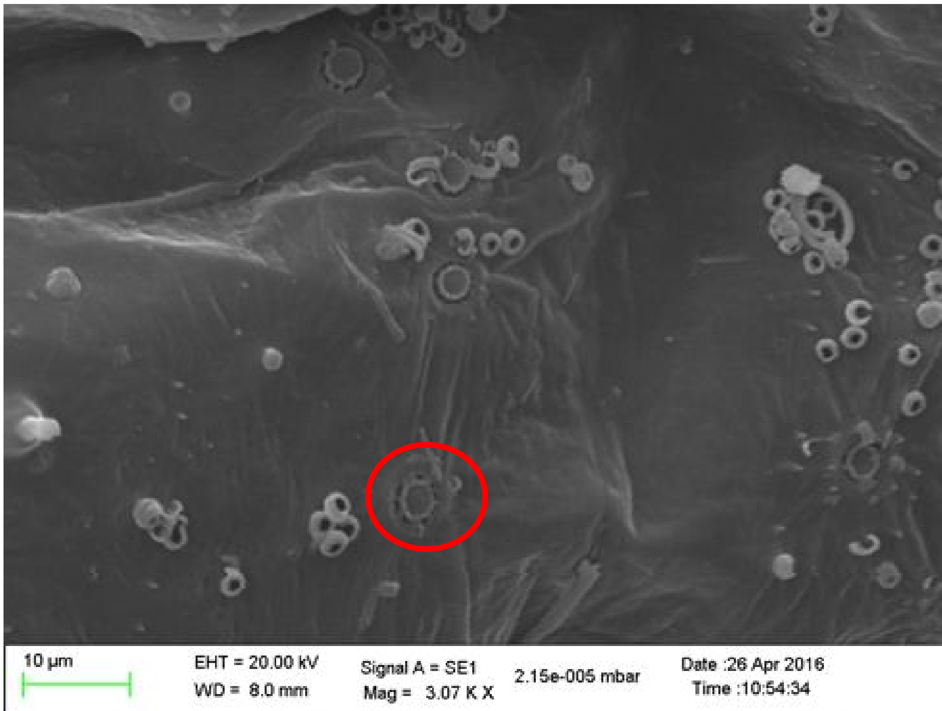

Fig. 6. Multilocular pore of female *P. marginatus*

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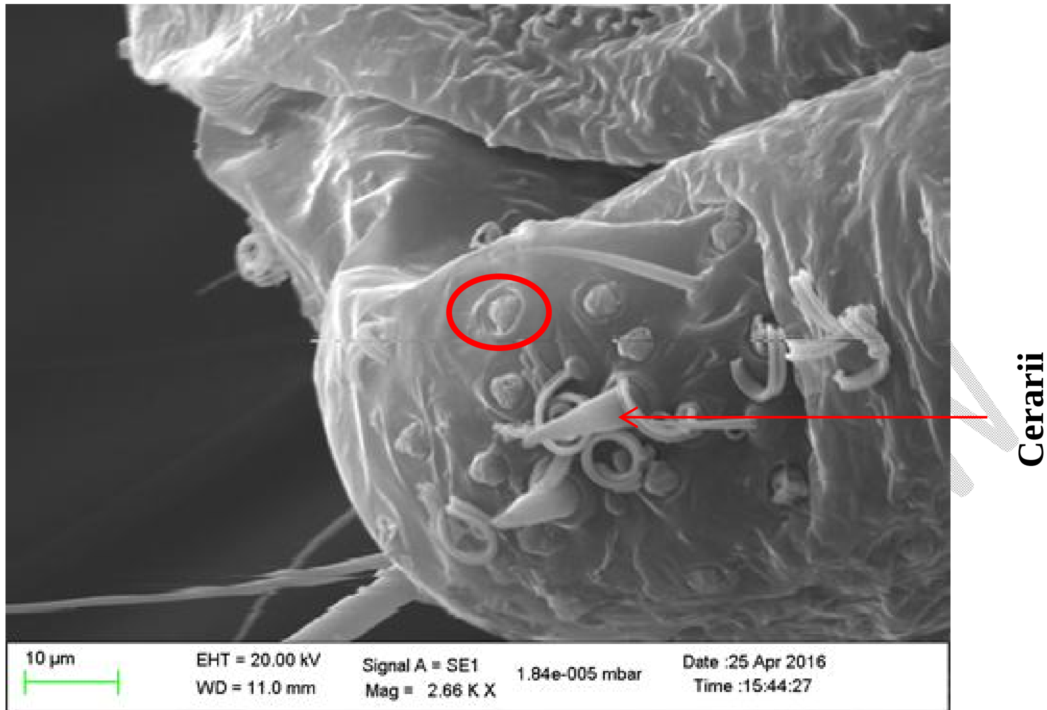

Fig. 7. Trilocular pore of female *P. marginatus*

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