

SCANNING ELECTRON MICROSCOPY OF PAPAYA MEALYBUG, *PARACOCCLUS MARGINATUS* WILLIAMS AND GRANARA DE WILINK (HEMIPTERA: PSEUDOCOCCIDAE) ON MULBERRY PLANT OF ASSAM

Abstract: A scanning electron microscopy (SEM) was carried out in the external morphology of the adult female of Papaya mealybug *Paracoccocus marginatus* Willam and Granara de Willink. The aim of this study was to enrich the understanding of the external morphology and wax-secreting pores of papaya mealybug, using scanning electron microscopy. The ultra structure of the antenna, stylet fascicle, legs, cerarii, vulva and wax-secreting pores were examined. Although the preparation of specimens for viewing under the SEM is time-consuming and expensive, the effort is worthwhile because the detailed information obtained is useful to solve difficult aspects of papaya mealybug taxonomy and to improve understanding the role of wax in their biology.

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

Introduction:

The Papaya mealybug (PMB) *Paracoccocus marginatus* Willam and Granara de Willink (Hemiptera: Pseudococcidae) is an annoyance insect pest which attacks various economically important plants such as papaya, mulberry, acahypha, hibiscus, plumeria, avocado, citrus, cotton, tomato, eggplant, pepper, beans and peas, sweet potato, mango, cherry and pomegranate (Miller and Miller, 2002). This insect caused heavy infestation and yield reduction of papaya throughout India in the recent past. It is an exotic polyphagous sucking insect with high multiplication and spreading potentials at 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 neighbouring 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 the Reunion Island in the Arabian Sea and Ghana in West Africa in 2010 and Assam in 2013 (Sarma, 2013).

The scanning electron microscope (SEM) is extremely useful for studying the detailed morphology of very small structures on insect cuticle. The SEM is superior to a conventional compound light microscope in having higher resolution, three-dimensional clarity and great depth of field. These features make SEM superior to a conventional compound light microscope for such studies. The rigid exoskeleton of insects is especially suitable for electron microscopy because the specimen usually retains its shape under

vaccum. In mealybug, waxes produced by different dermal pores are important in protecting different developmental stages. 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 on 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 the SEM. However only a few studies have reported on the ultra structure and wax-secretion processes in the family Pseudococcidae (Cox and Pearce, 1983; Kumar et al., 1997). Likewise, a few studies reported on the ultra structure and wax-secretion processes in the family Pseudococcidae. There has not been any study of the ultra structure of Papaya mealybug, hence, this study was conducted to know the external morphology and wax secreting glands of this species using scanning electron microscopy.

Materials and methods:

Scanning electron microscopic studies were conducted with the adult female as the external micrographical characters of this stage is considered to be taxonomically vital (Plate 1). Adult female were kept in 100% acetone for 1 hour. Sample was kept overnight in 0.25% Gluteraldehyde fixative in refrigerator at 4°C. After overnight keeping in fixative, samples were than 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 putting 1 drop of Hexamethyldisilazane (HMD) with 1 drop of alcohol on the sample, it 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 sputter clouter for 90 minutes. To prevent the electron beam 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:

Scanning electron micrographic studies of external morphological characters of adult female *P. marginatus* 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. Separation between the apical (flagellum) and sub apical (scape and pedicel) segments was clear. Scape was a strong and stout segment followed by less stout pedicel. Pedicel had 4 recurvate setae (3 on the dorso-medial aspect and 1 on ventro-medial aspect). Total numbers of flagellar segment were eight, apical most flagellar segment is the longest and the ventro-lateral 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 ventro-lateral aspect (Fig. 1). All the antennal setae appeared to be trichoid sensillae 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 were erect 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 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, trochanter is of triangular

shape, ventro-medial seta in trochanter was the longest. Femur was the longest segment with 4 setae in ventro-medial and 3 setae in dorso-medial aspects. In tibia, 3 stout setae were present in ventro-medial aspect. Tarsus unisegmented as described (Fig. 3).

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

Pygidium: Pygidium was heavy with 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 dorsal surface of the pygidial body (Fig. 4).

Vulva: Vulva triangular and raised; 'T' shaped slit opening with radiating striations (Fig. 5).

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

Multilocular pores: Multilocular pores are usually absent on the venter, at least around the vulva; sometimes they were present on the dorsum. Each pore consisted of a circle of more than five openings and the outer ring contained 8–10 loculi. The wax curls produced by the multilocular pores surrounding the vulva became attached to each egg as it leaves the genital opening (Kumar *et al.*, 1997). Multilocular disc pores produce wax that surrounds the eggs in the ovisac, and the wax protects the eggs from rain, desiccation, honeydew contamination, and natural enemies. Wax from the multilocular disc pores also is used by males to construct their cocoons after their second instar (Fig. 6).

Trilocular pores: Trilocular pores produced 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 centre of the pore and slightly twisted, or swirled, about each other (Fig. 7).

Discussion:

Papaya mealybug is a small polyphagous sucking insect, infested the veins of older leaves and all parts of young leaves and fruits. From the experiment micrographs of external morphological characters of papaya mealybug (antenna, stylet fascicle, legs, cerarii, vulva and wax-secreting pores) were till not done by other but similar work was done on six selected mealybug species of Sri Lanka (Sirisena *et al.*, 2015). Early workers used the number of antennal setae as primary character to separate species (Maskell, 1894; Essig, 1909 and Hollinger, 1917). The overall length of the antenna, as well as the length of the individual segments, were recorded in details by numerous workers until Ferris (1918) decided that variation made is 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 (McKenzie, 1967; Cox and Pearce, 1985) most of which produce various types of wax for different purposes. 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 leaves 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 secretion of the pores and their significant in the biology of the pest.

Conclusion:

The Scanning Electron Microscope (SEM) is extremely useful for studying the detailed morphology of very small structures on insect cuticle, enabling 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. Although the preparation of specimens for viewing under the SEM is time-consuming and expensive, the effort is worthwhile because the detailed information obtained is useful to solve difficult aspects of papaya mealybug taxonomy and to improve understanding the role of wax in their biology.

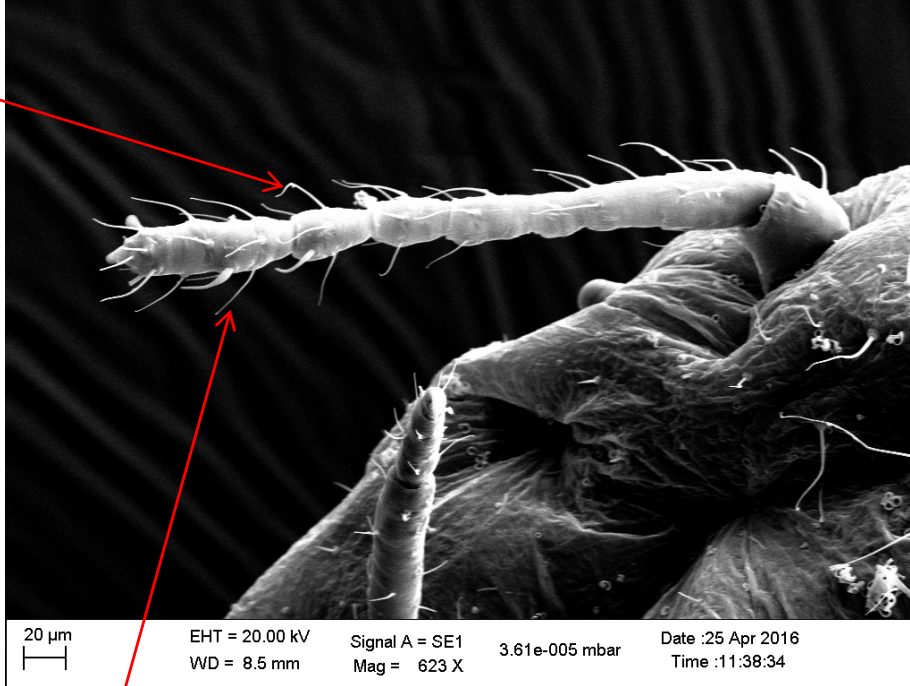
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Recurvate
Seta



Stout Seta

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

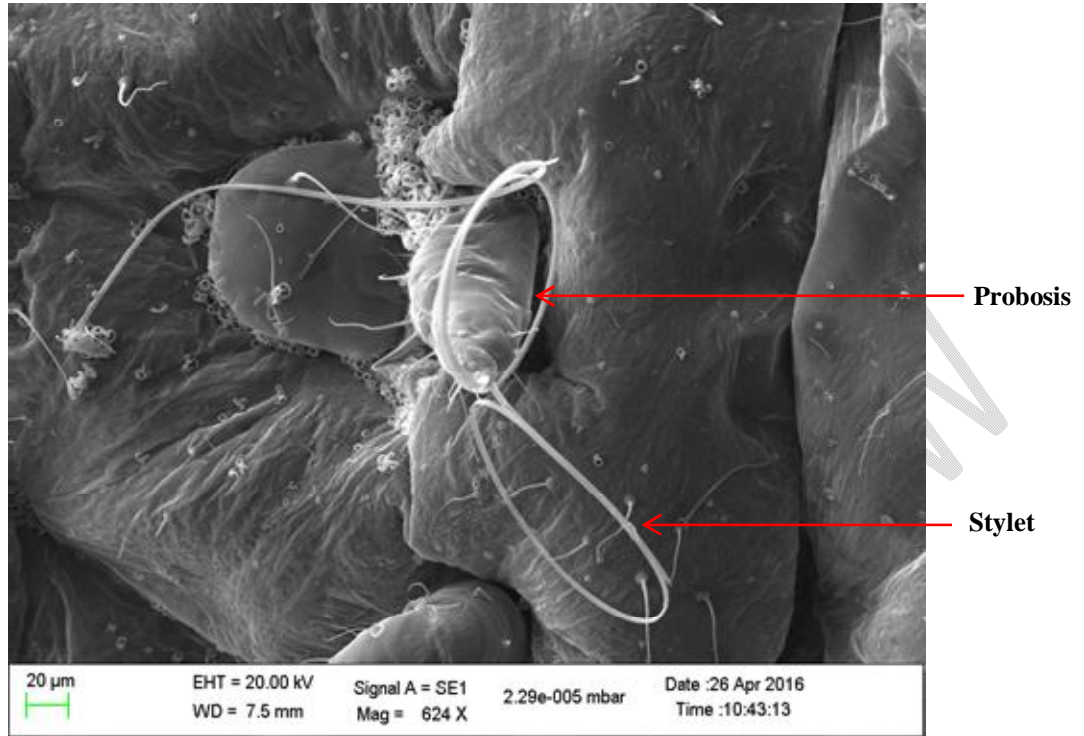
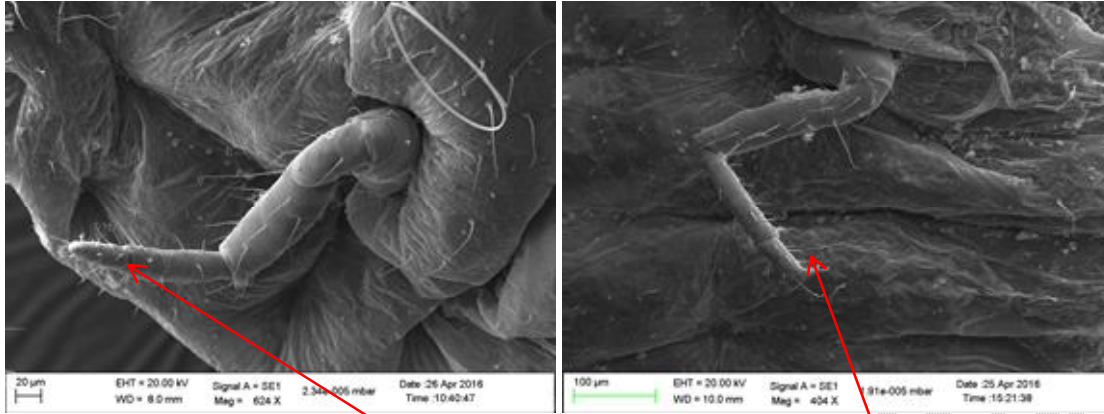
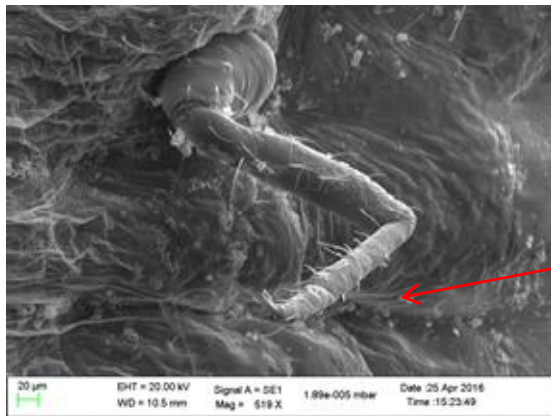


Fig. 2. Stylet fascicle of female *P. marginatus*



a. Foreleg

b. Middleleg



c. Hindleg

One tarsal segment
and a single claw

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

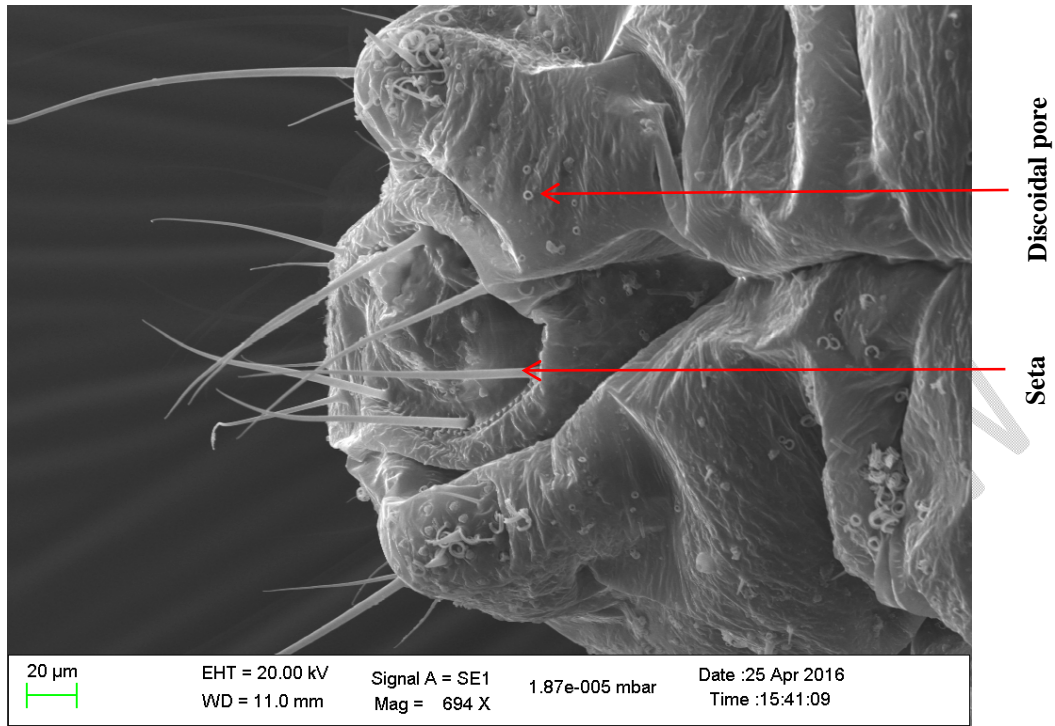


Fig. 4. Pygidium of female *P. marginatus*

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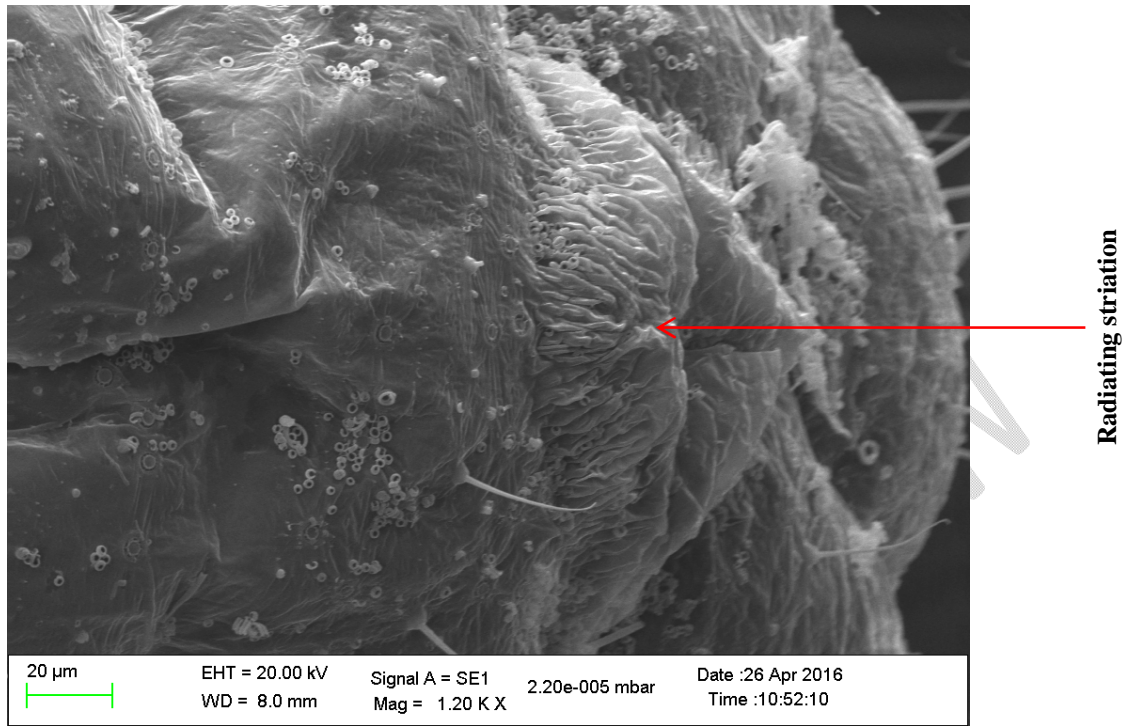


Fig. 5. 'T' shaped slit of vulva of female *P. marginatus*

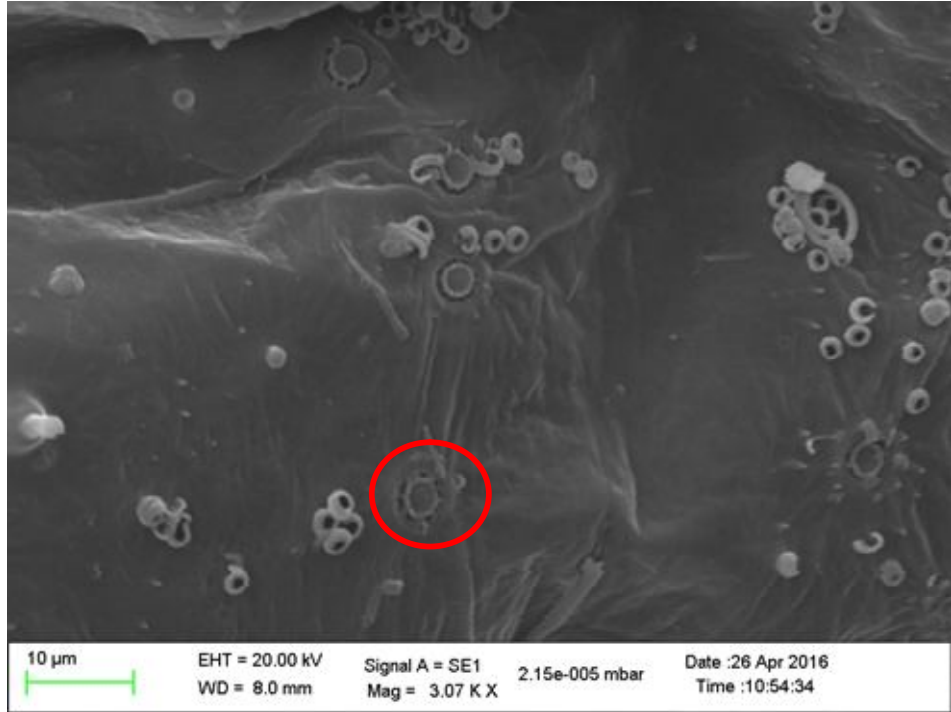


Fig. 6. Multilocular pore of female *P. marginatus*

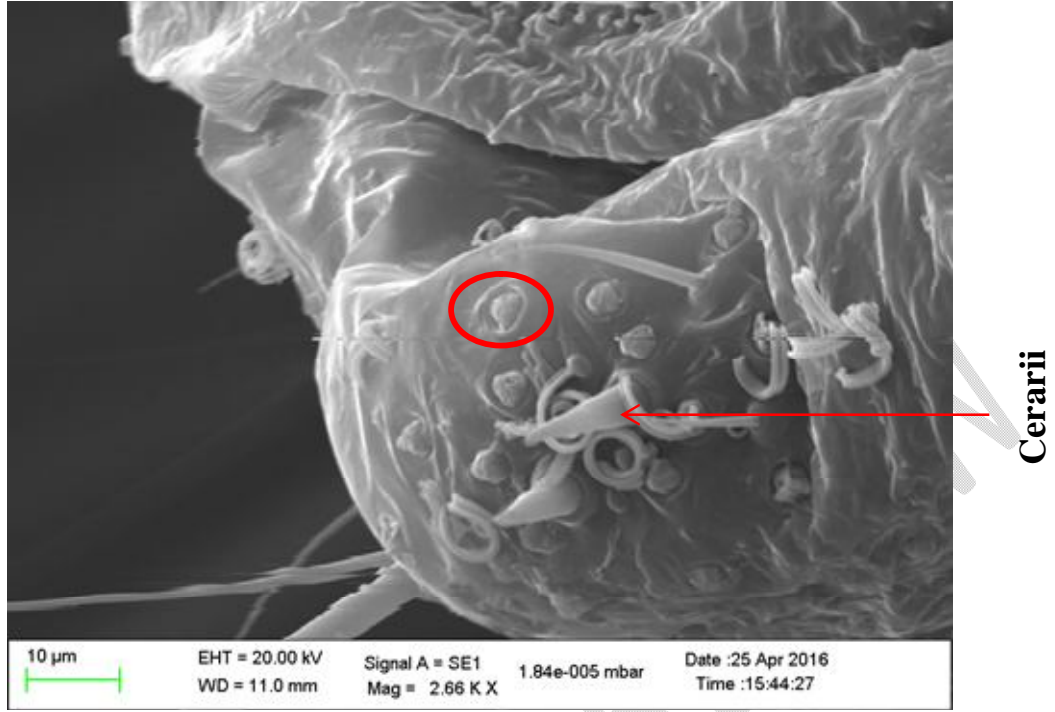


Fig. 7. Trilocular pore of female *P. marginatus*

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