Original Paper

Ultra structural study on different sensory structure and some associated body parts of mango mealy bug, *Drosicha mangiferae* (Stebbing, 1903) by scanning electron microscopy

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Observation on the ultra- structure of both mouth parts and sensory apparatus of mango mealy bug, *Drosicha mangiferae* (Stebbing, 1903) (Hemiptera: Coccoidea) was carried out. Seven segments of different size in each antenna have been identified in *D. mangiferae* in the present observation. Out of the all segments, the terminal segment was the longest trailed by third and second flagellomere in downward order. Sensilla with flexible socket was noted in almost every body parts in the present observation. Abundant wax pores on the dorsal and ventral surface on the body surface of mealy bug secrete spiral wax filaments. Two types of wax pores namely trilocular and quinquelocular were observed. Leg consisted of basal coxa, trochanter, femur, tibia and tarsus which ended with curved claw and two claw digitules. Hair like sensilla trichoidea was mostly abundant in antenna and was of four subtypes. All of these function as mechanoreceptor or gustatory receptor. The labium of second instar nymphs of *D. mangiferae* had three segments with a median labial groove for housing stylet fascicle. Ten pairs of trichoid sensilla were found in the labium that also function as mechanoreceptor sensilla with flexible sockets, sensilla were found having inflexible sockets and pores with probable olfactory function.

Keywords: Drosicha mangiferae, antenna, sensilla, mechanoreceptor, labium, stylet fascicle, wax pores

1 Introduction

Mango mealy bug, Drosicha mangiferae (Stebbing, 1903) belonging to family Margarodidae and suborder Sternorrhyncha (Hemiptera: Coccoidea) is an important insect pest to mango orchards rendering huge damage to the growing mango fruits and makes it unfit for human consumption. Recurrent infestation renders massive fruit loss at economic scale nearing 50% (Karar et al., 2006). Damage to mango fruits by mango mealy bug is fruit growth stage specific for which a steady and definite food searching by the pest is required (Peregrine, 1972). Mango mealy bug for the most part depend on various kinds of sensilla that are disseminated on various body parts like mouthparts, legs, antenna, wings, genitalia to distinguish legitimate food source (Catala, 1997). Out of all the tangible structures, antennary and labial sensilla are the for

the most part conveyed structures that are used by Sternorrhyncha for investigating plant surface.

Both and chemical and physical 'external' cues changes the food searching behaviour of insect for host plant (Backus, 1988).The 'pre-feeding' detection of plant by an insect consists of recurring 'rubbing' of leaf exterior by and labial tips and antenna. During such ,recognition' antenna throbs however labial tip consistently stays in vicinity with the food source (Calatayud and Le Rü, 2006). Therefore, sensillum of mouth parts of mango mealy bug is very important in 'host-choice'. Notwithstanding that mango mealy bug mouth parts additionally helps to choose appropriate food source. (Blanke et al., 2015; Whietefield et al., 2015). Antennary sensory structure mealy bugs belonging to Pseodococcidae is like that of *Planococcus citri* (Risso, 1813; Salama, 1971) or *Phenacoccus aceris* (Signoret, 1875; Alliaumeet al., 2018).

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Calatayud and Le Rü (2006) had recorded the role of labial and antennary sensilla for food choice in *Phenacoccus manihoti* (Matile-Ferrero, 1977) Ahamad et al. (2012) then again, had noticed that mouthparts of lac bug, *Kerria lacca* (Kerr, 1782) had likeness with that of other hemipteran bugs. Beside that, external 'wax coat' released from the wax pores in nymphs and adults of mealy bug as as a protective case for the insect. Body wax further stalls honeydew buildup (Pope, 1983) and also furthermore guards from the intrusion of microscopic organisms and parasites (Foldi and Lambdin, 1995). Additionally, hydrophobic wax threads avoid the eggs inside the ovisac from contact of insecticide spray (Manjunatha et al., 1993).

However, still now, ultra-structure of mealy bug mouth parts sensory apparatus have been poorly documented without proper descriptions (Pesson, 1944;Vahedi and Mahfar, 2010). Further, there are only a few references regarding the study on the ultra-structure of wax pores of mealy bug (Vahedi and Mahfar, 2010; Jansen, 2001). Works so far done on the sensory structure of mealy bug was based on the study of other mealy bugs but there is no published report of sensory structures of mango mealy bug (*D. mangiferae*) concerned with conclusive detail structural representation.

Keeping these in mind a study mostly on the ultra structure of antennary, legs and mouth parts of 2nd instar nymphs of mango mealy bug was done in the present study.

2 Materials and method

The processing and preparation of sample

Collection of sample

Mango mealy bug 2nd instar nymphs were gathered from mango plantations at Kaligram, Malda, West Bengal and then 2–3 nymphs were handled further for ultramicroscopic observation.

Sample dehydration and dewaxing

2nd instar nymph wax were taken away by a paintbrush and then immersed in a blend of 25% alcohol + 1% triton X for 30 minutes followed by a series of dipping in 70%, 80%, 90% and absolute alcohol in mounting order. Lastly, the insect sample was soaked again in 100% alcohol to guarantee total expulsion of water.

Metal coating of the insect sample by a sputter coater

The test sample was then dried in air by keeping them in a paper and under a bulb of 100 watt for around 10 minutes (Figure 1a). The sample was platinum covered after that within a φ 150TES sputter coater. Thereafter, coating of the sample was achieved by specimen mounting in an aluminum stab and maintenance of the specimen within the stab in 10⁻² mbar pressure for 40 minutes. Around 50 nm coating was achieved by setting a coating speed of 10 nm min⁻¹ (Figure 1b and 1c).

Scanning electron microscopic imaging of the test sample

The sample was then kept inside the SEM chamber and the air was emptied utilizing high vacuum. Examining was finished with the assistance of electron beam at 15 kV from various edges. Examining was done in a completely PC controlled scanning electron microscope (Carl Zeiss EVO 18). The tungsten warmed fiber was utilized to examine for every platinum covered specimen (Figure 1d and 1e).

External anatomy of antenna and mouth parts as recorded by scanning electron microscopy from 2nd instar nymphs of mealy bug was consequently noted.

3 Results and discussion

The mango mealy bug ultrastructural details of antenna mouth parts, leg and wax pores are portrayed underneath.



Figure 1

Specimen coating and imaging in scanning electron microscope (Carl Zeiss EVO 18) a – drying; b – mounting and fixing of 2nd instar nymphs in aluminium stab; c and d – platinum coating in sputter coater; e – imaging in scanning electron microscope

3.1 Antennary ultra and sensory structure

Every antenna is seven sectioned. The ultra structure had demonstrated that the terminal antennal portion is the longest (mean length of 244.66 μ M ±1.15) trailed by third (mean length 196.26 μ M ±0.64) and second fragment (85.54 µM ±1.15). In each fragment 2-5 trichoid sensilla were found to be present. These sensilla were aporous that gets tapered from base to the tip and work as mechano receptors. Four diverse sort of sensilla were found in the antenna to be specific they were sensilla trichoidea (St), sensilla chaetica (SCh), sensilla basiconica (Sb)and sensilla Coeloconica (SCo). The grouping is done based on connection with sixe, shape, attachment pattern with cuticle and availability of pores according to standard accessible writing (Altner and Prillinger, 1980; Shields and Hildebrand, 1999; Hallberg et al., 2003). Following sort of sensilla were recorded:

1. *Sensilla basiconica* (Sb): These were conical in shape and straight. They came up from a flexible socket and found to be present sparsely in the most terminal flagellomere of antennae (Figure 2c). These sensilla possessed a grooved surface indicating pores and as such it can be said that they serve as an olfactory receptor.

- 2. Sensilla coeloconica (SCo): These sesilla looked very much like a peg and remain installed in a cuticular depression but still a part of it remains outside as well. Two such kind of sensilla were recognized in the apical most segment and probably they act as thermo/ hygroreceptor (Figure 2c).
- 3. Sensilla chaetica (SCh): This particular type were biggest amongst the lot and had stiff hair like appearance having a length of 190–120 μ M (Figure 2b and 2f). In all antennary segments these kind of sensilla were identified. They had a pointed tip and broad base and did not bear any pores. They also performed as mechanoreceptors.
- 4. Sensilla trichoidea (St): Sensilla trichoidea was also hair like in shape and emerged from either flexible or rigid sockets. (Figure 2a).These kind of sensilla were actually the most diverse and plentiful. Sensilla length of this particular type differed greatly and altogether four subtypes were identified. In all antennary segments they were identified. Four subtypes of trichoid sensilla have been recognized.





- a) Sensilla trichoidea type 1 (St-1): These were thicker and comparatively shorter measuring 60–70 μM. These kind were not porous and possessed a rounded tip (Figure 2c).They were mostly noticed in the most apical segment and emerged from a bendable socket. It work as mechanoreceptors.
- b) Sensilla trichoidea type 2 (St-2): These sensilla had bent and pointed tip could grow up to $15-70 \mu$ M (Figure 2c) and emerged from a supple socket. These were not\porous andwere heavily distributed in the apical, second and third segment of antenna. It functions as mechanoreceptors.
- c) Sensilla trichoidea type 3 (St-3): These tiny trichoid sensilla and measured in the range of $10-25 \,\mu$ M (Figure 9d). They were regularly found in large number at the in ventral division of second and third segment of antenna. This type of sensilla play mechanoreceptor role (Figure 2d).
- d) Sensilla trichoidea type 4 (St-4): These were pointed hair like and measured around $60-90 \,\mu$ M (Figure 2e). These arose from a rigid socket. The range of it's length differed from 20–60 μ M. These were also found to be concentrated in second and third antennary segment and act as gustatory receptor.

3.2 Labial sensory structure

Mouth parts comprised of a three segmented labium with a median labial food groove and various kinds of sensilla (Figure 3a). The average length of second instar labium was found to be 163.3 μ M \pm 0.9 ($n = 3,160.2-162.2 \mu$ M) and width was calculated to be around about 105.1 μ M \pm 0.9. The base of the labium was broad and the most apical segment was narrowest and conical in shape but every segment had a groove for accommodating stylet fascicle (Figure 3b).

diverse sorts of sensilla were identified in labium. Ten pairs of trichoid sensilla (Sst) were noted found in LB1 labial segment (Figure 3c). These were straight hair like in appearance emerging from stretchy sockets and found to be evenly distributed on the two sides of cental groove (Figure 3c). These sensilla also act as mechanoreceptors. Ten comparitively longer sensilla chaetica (SCh) having a pointed tip were noted in LB1, LB2 and LB3 segment (Figure 3c). Other sensilla were also located coming out from a rigid socket and fprobably function as olfactory receptor (Figure 3d).

3.3 Stylet fascicle fine structure

A stylet fascicle looking very much similar to spine arose from the most terminal labial segment in continuity with labial groove (Figure 4a). It was made out of interlocking separate maxillary and mandibular stylets. The average diameter of stylet fascicle was calculated to be around 4.1 μ M ±0.10 (n = 03, range 3.98–4.18 μ M). A food canal was plainly recognizable in the middle of two stylets (Figure 4b).

3.4 Ultrastructure and sensory parts present in the leg

Legs of mango mealy bug (*D. mangiferae*) was made up of of proximal coxa trailed by by trochanter, femur, tibia and tarsus (Figure 5 a). But as opposed to several tarsal sections as found in other species of mealy bugs there is just one segment found in tarsus (Figure 5a). At the end of every tarsus there was only one arched claw. In addition to claw, two claw digitules were also visible and these two were compritively long than original claw (Figure 5b).

Trichoid hairs were abundant throghout the different parts of leg (Figure 5d). While a small and blunt basiconic sensilla emerging from a rigid socket was also located in the most proximal coxal segment (Figure 5e). These trichoid sensilla act as mechanoreceptor while probable function of basiconic sensilla is to perform the role of gustatory receptor.



Figure 3 Ultra-micrograph of *D. mangiferae* labium a – three segmented labium (LB 1 – red arrow, LB 2 – green arrow and LB 3 – white arrow); b – labial groove in the ventral midline of labial segment (red arrow); c – trichoid sensilla – St (red arrow) *Sensilla chaetica* (SCh – star marks); d – grooved (porous) sensilla from inflexible socket in the labium (red arrow)



Figure 4 Ultramicrograph of labial stylet fascicle a – stylet fascicle arising from apical segment of labium (red arrow) and labial groove (red arrow); b – maxillary stylet (red arrow) and mandibular stylet (right arrow) fusing together to make food canal (red arrow)



Figure 5

Ultrastucture of leg of D. mangiferae

a – various parts of leg, coxa (white arrow), trochanter (yellow arrow), femur (green arrow), tibia (red arrow) and tarsus (indigo arrow); b – distal portion of the leg showing single tarsal segment (arrow); c – tarsus with a curved claw (right arrow) and two claw digitules (left arrow); d – trichoid sensilla in most of the segments (arrows); e – short, blunt basiconic sensilla in the basal coxa (circle)

3.5 Anatomical detail of wax pores and associated structures

Wax pores were found abundantly throughout the mango mealy bug's body parts. These wax pores discharge coiled wax threads that structures a coating on the surface of mango mealy bug after being fragmented. Every wax pore comprised of elevated round area with three slender openings from centre of the pore. The secreted wax works as a protective gear for these pests and prevents any kinds of desiccation and sticking of eggs within ovisac. The ultra structural details have been depicted in Figure 6. The identified wax pores were of two types, trilocular and quinquelocular.

- a) Trilocular wax pores: The distribution of these pores was even on both dorsal and ventral parts of the body. These pores consisted of triangular round elevated area housing three slender perforations that exude from centre of the pore (Figure 6a and 6b). A coiled wax thread is generally released from these wax pores and a wax covering is formed from these released wax on the mealy bug's body surface.
- b) **Quinquelocular pores:** Quinquelocular pores were also found in addition to trilocular pores and were dense around venter close to vulva. These are cuticular elevated pentagonal area housing five loculi or bowshaped openings (Figure 6c and 6d). Wax filaments released from these pores can be noticed beside pores. In vulvar region and all over the body these wax

threads forms a protective shield. The secreted wax also covers up the egg during coming out from the opening of genitalia.

- c) Wax pore associated sensory structure:
 - **Ostioles:** Ostiole, a cuticular structure looking like a lip which is found in most mealy bug and trademark of mealy bug belonging to Pseudococcidae family was absent in mango mealy bug (Figure 7 a).
 - **Cerarii:** At the edge of the body a group of pores and setae are generally noticed in other mealy bugswere missing in mango mealy bug (Figure 7b and 7c).
 - **Circulus:** A button-shaped "circulus helps mealy bug's adherence to any surface and generally found in the middle of ventral part of other mealy bug. But it was not present in mango mealy bug midline of venter (Figure 7b).

The present study elaborates the ultra structure of different types of antennary sensilla with various kinds of sensory structure including wax pores of mango mealy bug. On the whole, seven fragments in every antenna have been distinguished in *D. mangiferae* in present study. Amongst all the segments the most apical segment was found to be the longest trailed by third and second antennomere. Presence of sockets at the base of each sensilla makes it more flexible (McIver, 1975). Such





Ultramicrograph of wax pores of *D. mangiferae* a – trilocular pores distributed on the ventral part of the body (arrow); b – magnified image of one trilocular pore (arrow); c – quinquelocular pore distributed densely adjacent to vulva (red arrows); d – magnified image of one quinquelocular pore (arrow)



Figure 7 Ultra structure of dorsum and margin of *D. mangiferae* a – absence of anterior and posterior ostioles (circle); b – margin of the body showing absence of ceracius (white arrows) and ventral part showing absence of circulus (green arrow); c – magnified view of the margin showing absence of cerarii (white arrow)

structure offers micro-perception to stimuli even under mild touch or even under docile air movement (Shields and Hildebrand, 1999). Present study corroborates with the observation of Sirisena et al. (2015) who have also reported somewhat similar variation of the size of antennal segments when working with six different mealy bug species. Sensilla of mango mealy bug that are present in antenna helps to perceive different external stimuli and accordingly facilitates to orient their behaviors (Hu et al., 2009). In parity to the observation of McIver (1975), four types of sensilla trichoidea and a single type of sensilla chaetica having mechano-receptive function have been identified in the current study. By several studies reported elsewhere it has been proved that sensilla trichoidea and sensilla chaetica work as mechanoreceptors (Schneider, 1964; Agren, 1977; Chapman, 1998).

In conformity to the present observation Backus (1988) had documented presence of a few sensilla in antenna similar to other Sternorhyncha. Present study is supported by the observation of Koteja (1980) and Le Rü et al. (1995A) respectively. Both of them had noted different types of sensilla in mealy bugs of Pseudococcidae. The diversity of sensilla in mango mealy bug is mainly documented in the three terminal segments. One pair basiconic sensilla possessing grooves with 'pegs' was found in the most apical antennomere and these sensilla act as receptors for sensing smell. The present finding is in consonance with Le Rü et al. (1995A) who had also recorded that 'peg sensilla' and 'multiporous sensilla' with pores and fissures can perform the role of olfactory receptors in mealy bugs. However, observation of absence of olfactory plate in the current observation is contradicted by Bromley and Anderson (1982) who had confirmed the presence of olfactory plate organ in some other sternorrhyncha. One pair coeloconic sensilla emerging from an stiff socket were also situated in the ventral division of the terminal flagellomere and are supposed to have either thermo and/or hygroreceptorrole. In consonance to the present obsrvation Le Rü et al. (1995 A) has made similar reports related to the presence of coeloconic sensilla in flagellomere of the cassava mealy bug, P. manihoti.

The *D. mangiferae* labium comprised of three segments and the terminal part was found to be thinnest. Short trichoid hairs with mechano-receptive role was found to be present symmetrically at the two sides of median groove and were 10 pairs in number. Such trichoid hairs sensilla serve mango mealy bug to assess the labium insertion pattern into the host-plant tissue. This current finding resembles closely to that of Le Rü et al. (1995B) and Alliaume et al. (2018). They had noted trichoid sensilla with mechano-receptive functions in *P. aceris* and *P. mannihoti.* Backus (1988) has recorded low number of mechanoreceptors in heimpteran labial tip. Five pairs of long sensilla chaetica was also encountered in labium of *D. mangiferae*. The presence of such sensilla chaetica in labium performing mechanoreceptive functions is similar to the other Sternorrhyncha like Aphididae (Wensler, 1977; Tjallingii, 1978) and in Aleyrodidae (Walker and Gordh, 1989).This kind sensilla chaetica was situated at labial tip on two sides of the central labial groove and keep a direct contact with that groove. All these factors i.e. position, contact of these sensilla is indicating that they may be involved to scan the physical characteristics of plant surface and thus manipulates stylet for penetration.

A chemosensilla possessing a grooved surface that is supposed to help in olfaction is found in the present study. Such observation corroborates to the study of Le Rü et al. (1995B) who have also recorded grooved uniporous and multiorous peg sensilla with olfactory function in *P. mannihoti*. The specific feeding behavior might have induced such low incidence antennary and labial sensilla (Chapman, 1982). The presence of a spine like stylet emerging from the labial tip with a clearly visible central food groove and fused with maxillary and mandibular stylet were also seen in the current observation. Such observation corresponds to that of Alliaume et al. (2018) who had noted somewhat similar structure in *P. aceris*.

Mealy bug leg was found to be composed of coxa, trochanter, femur, long tibia, tarsus ending with a curved tarsal claw. The claw have a pair of claw digitules that are longer than claw. Sirisena et al. (2015) had reported mostly similar segments in the legs of other mealy bugs. Trichoid hairs of the legs function as a mechanoreceptor and can sense any kind of stretch of the cuticle due to touch or flexion. The single short and blunt basiconic sensilla in the ventral portion of coxa is supposed to function as gustatory or olfactory receptor. Probably such receptor helps to select proper host-plant surface for sucking of sap.

Wax released from mealy bug wax pores never allows wetting and is also responsible for making skin impervious. Two categories of wax pores, trilocular pores and quinquelocular pores were encountered in the present study. The distribution of trilocular pores was even on the two sides of dorsum and venter. Sirisena et al. (2015) have recorded similar trilocular pores while studying other types of mealy bugs. Quinquelocular pores with pentagonal appearance were present densely in venter adjacent to vulva of *D. mangiferae* which is in agreement with the findings of Srisena et al. (2015) who have also observed similar organization and distribution of quinquelocular pores adjacent to vulva in, *Coccidohystrix insolita* (Green, 1908).

One of the characteristic features of mealy bug of Pseudococcidae is the presence of cerarii and ostiole at edge of the body (Sirisena et al., 2015) but it was found lacking in mango mealy bug. However, in *D. mangiferae* that belongs to Margarodidae family of Coccidea these three features were absent.

4 Conclusion

The current study of ultra and sensory structures of *D. mangiferae* present in various body parts like antenna, mouth parts, legs and wax pores will be very much beneficial to comprehend food perceiving mechanism. This current study is worthwhile as informations gathered during this study will be advantageous to learn mealy bug taxonomy. The role of wax and wax pores in mango mealy bug biology can also be realized.

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