

## Mouthparts and stylet penetration of the lac insect *Kerria lacca* (Kerr) (Hemiptera:Tachardiidae)

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### ABSTRACT

Hitherto less known aspects on mouthpart morphology and penetration mechanism of the lac insect *Kerria lacca* have been explored. Unique details of the mouthparts, i.e. morphology of labium and stylets and salivary sheath have been brought out. The gross morphology of the mouthparts though resembled other plant sucking homopterans; a two-segmented labium with symmetrically distributed six pairs of contact-chemoreceptors on its surface was distinct; the mandibular stylets had serrations on its extreme apical region, while the maxillary stylets had their external surface smooth with parallel longitudinal grooves on their inner surface. Formation of flanges, salivary sheath and penetration pathway observed along with probing and penetration of the stylets intracellularly up to the phloem cells, as illustrated herein, are the addition to the existing knowledge on the structural details of the mouthparts and the feeding behavior thereupon.

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### 1. Introduction

The phytophagous lac insect, *Kerria lacca* (Tachardiidae: Hemiptera) secretes true lac, the only resinous compound of animal origin having an immense economic importance. This insect had been reported from Oriental and Indo Malayan regions (Chamberlin, 1923; Varshney, 1976; Ben-Dov, 2006). It generally infests more than 400 plant species (Sharma et al., 2006), and feeds on the host tissues such as stem and sometimes petiole. Its mouthparts are specialized for piercing the plant tissue and sucking the sap like other phytophagous insects. Unlike many plant sucking homopterans, the lac insect inserts its mouthparts only once in its life-cycle, thereafter remains sedentary and continues feeding (Imms and Chatterjee, 1915). Within few hours of their emergence, the first instar nymphs find a suitable feeding site and successfully penetrate their stylets into the plant tissue to reach the vascular bundles.

In the lac insects too, the unique mouthparts have been acquired as a result of their diverse feeding mechanism during evolution.

Also, the phloem is their well known target similar to other piercing and sucking insects as these utilize the transported fluid for efficiently fulfilling their nutritional requirements (Kehr, 2006). Mouthparts of hemipterans play an important role in host location, feeding and in transmission of viral and bacterial pathogens (Pollard, 1968; Backus, 1988). Extensive studies are available on the stylet penetration and feeding mechanism by the sap sucking homopterans such as aphids and whiteflies (Evert et al., 1973; Walker, 1985; Rosell et al., 1995).

However, little is known about the finer aspects of mouthparts' structure and their role in locating the phloem tissue within the host plant (Pollard, 1973). The position of sensilla on the labial tip in hemipterans makes it more interesting as it is the preliminary contact point between the food source and the insect during the events of host location and feeding. Studies done on the mouthpart morphology of the lac insect described it to be a piercing and sucking type as usually found among the Coccidae (Imms and Chatterjee, 1915; Misra, 1931; Roonwal, 1962; Krishnaswami et al., 1964). Though few studies explain the labium and stylets of lac insects, information on the ultrastructural morphology and stylet penetration is not available.

Hence the present study examined the ultrastructural morphology of the clypeolabral shield, labium and stylet fascicles.

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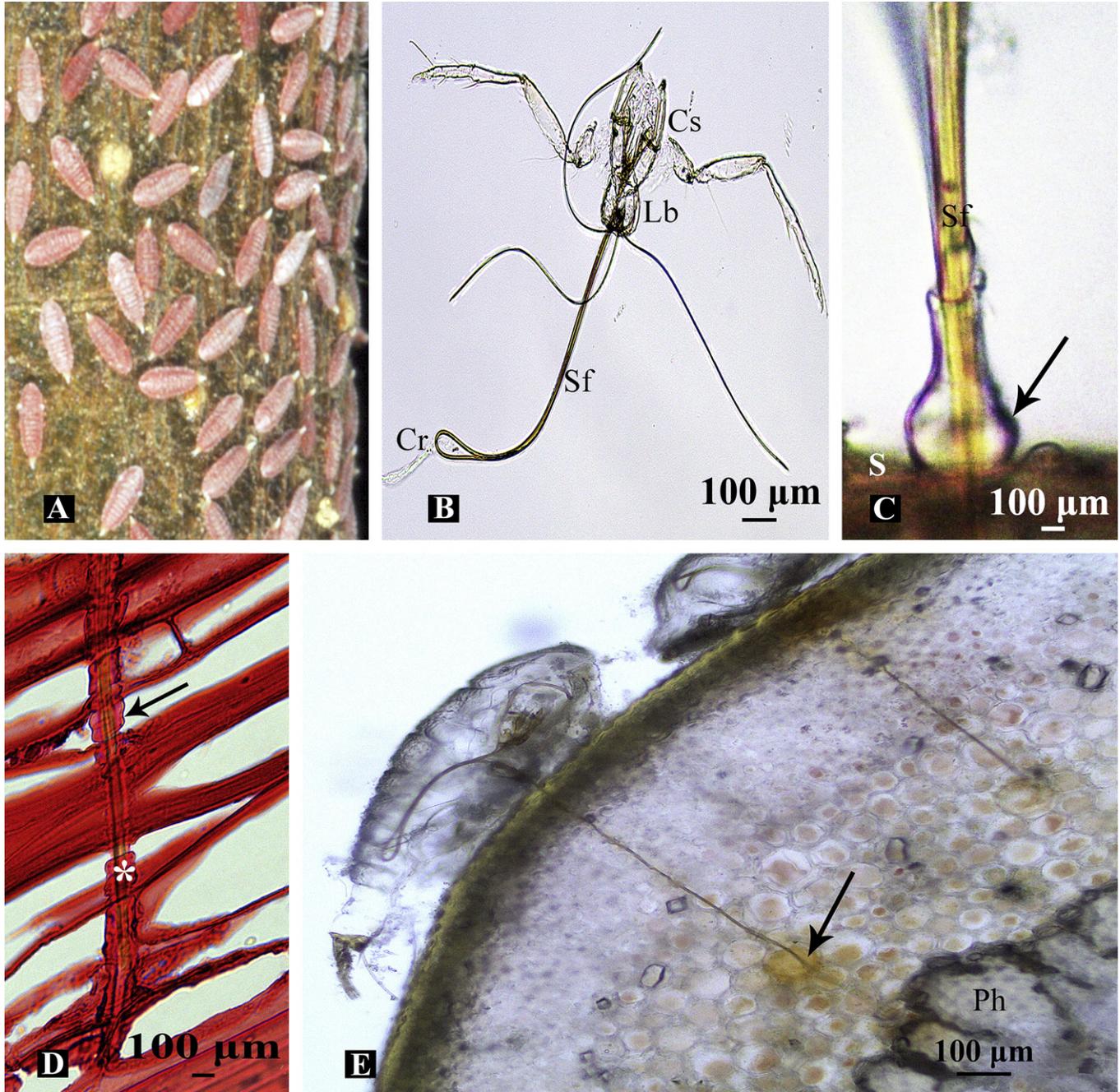
Also, the penetration pathway of the stylets and salivary sheath within the host tissue was explored. Light microscopy and scanning electron microscopy have been used for observations. The observations thus obtained could be useful in unraveling the details behind the feeding behavior and stylet penetration by the lac insect.

## 2. Materials and methods

The stock culture of brood lac with about to hatch gravid females was collected from Botanical Garden, University of Delhi, India, and was inoculated on *Ficus religiosa*. The first instar nymphs were

allowed to feed on stem and petioles and collected on alternate days for a week. For elucidating the stylet penetration pathway infested petiole and stem with approximately 120 first instar nymphs settled per 2.5 cm<sup>2</sup> known to be a good settlement were selected (Figs. 1A and 3A). Emerging first instar nymphs from the brood lac collected in parafilm coated petri plates were fixed for further investigations. Both fresh and fixed material was used for the study of mouthparts.

The first instar nymphs were placed in 10% KOH for few hours to clear the internal body contents, then cleared in distilled water and the mouthparts dissected under Leica EZ4 stereozoom microscope at 35x. The samples were then dehydrated in a graded series of ethyl



**Fig. 1.** Light Micrographs of first instar on *Ficus religiosa* stem. (A) Settlement on stem; (B) Mouth parts showing clypeolabral shield (Cs); labium (Lb); a stylet fascicle (Sf) and crumena (Cr); (C) Salivary flange (arrow) and S indicating stem surface; (D) Stylet fascicle (\*) and salivary sheath (arrow) in a section; (E) Petiole section showing stylet fascicle (arrow) and Ph indicates phloem.

alcohol 50%, 70%, 90% and 100%, followed by clearing in xylene and mounting in DPX mountant.

The plant tissue samples were prepared through hand sectioning and clearing in lactic acid and phenol, then stained in 1% aqueous solution of safranin, dehydrated in graded series of ethyl alcohol, xylene and mounted in DPX. Similar procedure was followed for the unstained sections. These samples were then examined and photographed using Leica DM500 research microscope attached with DFC290 digital camera.

For scanning electron microscopy, dissected mouthparts and plant tissues obtained through hand sectioning were fixed in 2.5% glutaraldehyde for 12 h at 4 °C, rinsed in phosphate buffer saline (PBS-0.1M, pH 7.2) for 10 min and cleaned in ultrasonic mini cleaner for 30 s, followed by dehydration in graded series of 70, 80, 90 and 100% ethyl alcohol, and then dried with chemical dryer. The samples were then mounted on aluminum stubs with carbon tape and sputtered with gold: palladium alloy (18 nm) in a SC7620 mini sputter coater with a pressure of  $10^{-1}$  mbar and examined under Zeiss EVO MA10 SEM at 15.00–20.00 kV and magnifications ranging from 237x to 19.14 kx. For imaging in environmental mode (EP), freshly cut sections of infested stems were directly observed in cold stage with a BS (Back Scattered) Detector. All the measurements are based on a sample size of  $n = 10$ .

### 3. Results

#### 3.1. Mouthparts

The mouthparts of *K. lacca* are hypognathous, with salient parts being the clypeolabral shield (Cs), labium (Lb) and the stylet fascicle (Sf) (Fig. 1B). The labium is membranous and two segmented with a longitudinal groove on its anterior surface, and with sensilla in symmetrical configurations on either side of the groove. The stylet fascicle remains coiled inside a membranous pouch ‘crumena’ within the body comprising of two inner maxillary stylets and partially surrounded by the slightly shorter, outer mandibular stylets.

#### 3.2. Clypeolabral shield (Cs)

The clypeus and the labrum are fused forming a ‘clypeolabral shield’ on the ventral aspect beneath the head. The surface of this shield is smooth,  $141 \pm 0.001 \mu\text{m}$  long,  $91.5 \pm 0.001 \mu\text{m}$  wide and flattened, giving a slightly convex appearance, and covering the maxillary and mandibular bristles (Fig. 2A).

#### 3.3. Labium (Lb)

The labium is membranous, conical, pouch like and composed of two segments, the first segment being approximately twice as the second in width (Fig. 2A1), its anterior surface has a deep groove extending the entire length enclosing the stylet fascicle. Investigations reveal that the stylet fascicle was positioned off center in the labial groove. The labium is  $55 \pm 0.001 \mu\text{m}$  long and  $68.5 \pm 0.001 \mu\text{m}$  wide.

Labium is covered with symmetrically arranged sensilla on each side of the labial groove. The first labial segment consists of a single pair of sensilla. There are five pairs of sensilla on the second segment and the sculpturing is smooth. All sensilla are hair like, with or without pores and are of the chemo- as well as mechano-sensory type. The sensilla are slender, slightly curved and directed toward the distal end of the labium, and range from  $5.31 \pm 0.001 \mu\text{m}$  to  $16.29 \pm 0.012 \mu\text{m}$  (Fig. 2B).

Three types of sensilla trichodea, as uniporous sensilla trichodea type I and II and non-porous sensilla trichodea were identified. The

first segment is covered with a pair of non-porous sensilla trichodea inserted in a flexible socket. The second segment bears four pairs of sensilla trichodea type II and pair of sensilla trichodea type I on the anterior and lateral surface, and symmetrically positioned besides the labial groove. Sensilla trichodea type I are long, uniporous (terminal pores) and are more curved at their apex in comparison to sensilla trichodea type II. The sensilla trichodea type II is also uniporous within a flexible socket and varies in length, ranging from  $5.31 \pm 0.001 \mu\text{m}$  to  $9.35 \pm 0.001 \mu\text{m}$ . A pair of short and robust sensilla trichodea is present on either side of the labial groove, where stylet fascicle exits the labium and found to be in contact with the stylets (Fig. 2C).

#### 3.4. Stylet fascicle (Sf)

The stylet fascicle is needle like, composed of two each of maxillary and mandibular stylets. The length and diameter of the stylet fascicle is  $1110 \pm 0.016 \mu\text{m}$  and  $5 \mu\text{m}$ , respectively, and approximately twice (2.02) the body size ( $549 \pm 0.006 \mu\text{m}$ ). The average length of the stylet is  $1343.2 \pm 0.001 \mu\text{m}$  and  $1326.4 \pm 0.015 \mu\text{m}$  for the maxillary and mandibular stylets, respectively.

The mandibular stylets (Md) are located on lateral aspects of the maxillary stylets and are only slightly shorter (0.98). Their most distinguishing feature are the fine serrations on a smaller region of the apex, i.e. serrated ridges on the external surface tapering toward the end, and the extreme tip with a cusp on the median surface. These cusps are most prominent at the apex and thereafter gradually disappear as these reach the tip. Nearly half of these cusps bear notch like projections on their surface which are parallel to the adjacent serrations, and their inner surface is smooth (Fig. 2D–F).

The paired interlocked maxillary stylets appear slightly thicker and are partially enclosed by the mandibular stylets. These stylets at their extreme tip are asymmetrical and form an opening (Fig. 2G), with a smooth external surface and grooved inner surface when viewed individually (Fig. 2H). These stylets are held together by parallel annular rings which form a series of nodes in a zip-like fashion for appositioning (Fig. 2I).

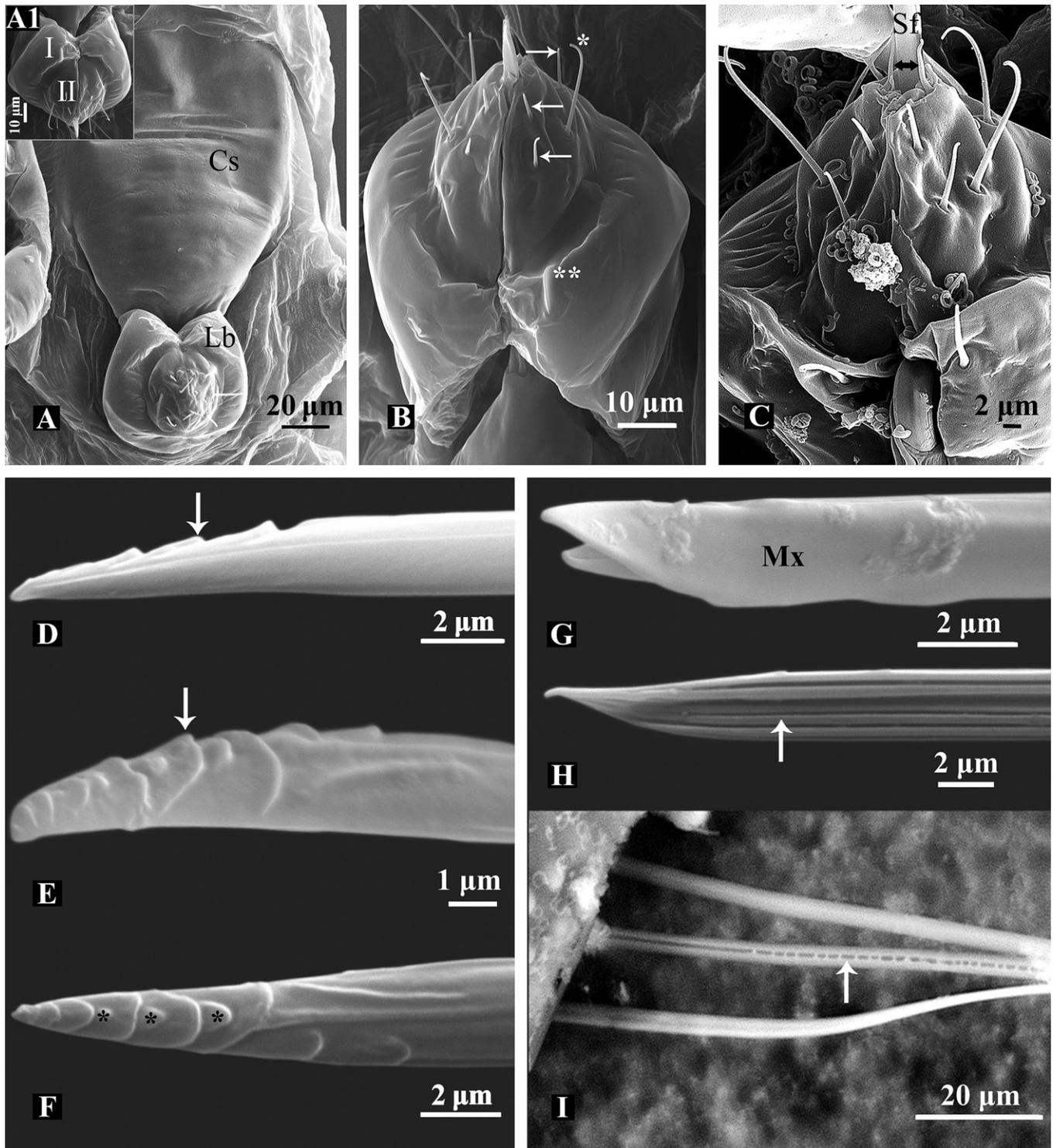
#### 3.5. Stylet penetration

##### 3.5.1. Probing

With emergence the nymphs can crawl up to a distance of approximately 10 m to find a suitable infestation site. During probing the insect taps its antennae vigorously on the surface of the plant and it is presumed that sensilla present on the antennae may respond to the physical stature and the volatile compounds of the plant. The tapping on the plant surface and subsequent insertion of the stylet to the epidermis may take 20–60 s.

##### 3.5.2. Stylet penetration

Feeding sites with maximum infestation were excised for observing the stylet pathway. The penetration on the surface is marked with the deposition of salivary sheath material termed as flanges, which are somewhat pear-shaped (Figs. 1C and 3B). These flanges were found to be in continuation with the internal portion of the sheath. The stylets were capable of penetrating the plant tissues through the epidermal cells (Fig. 3C), and no penetration was observed in the natural openings, viz. stomata and lenticels. The stylets were found to be perpendicular to the surface, penetrating both the ridges as well as furrows but maximal feeding sites were observed on the latter (1:10). Stylet penetration beyond the epidermis may take few minutes in order to reach the phloem tissue, traversing the parenchyma and cortical cells of the host plant. Penetration and simultaneous secretion of saliva was enabled

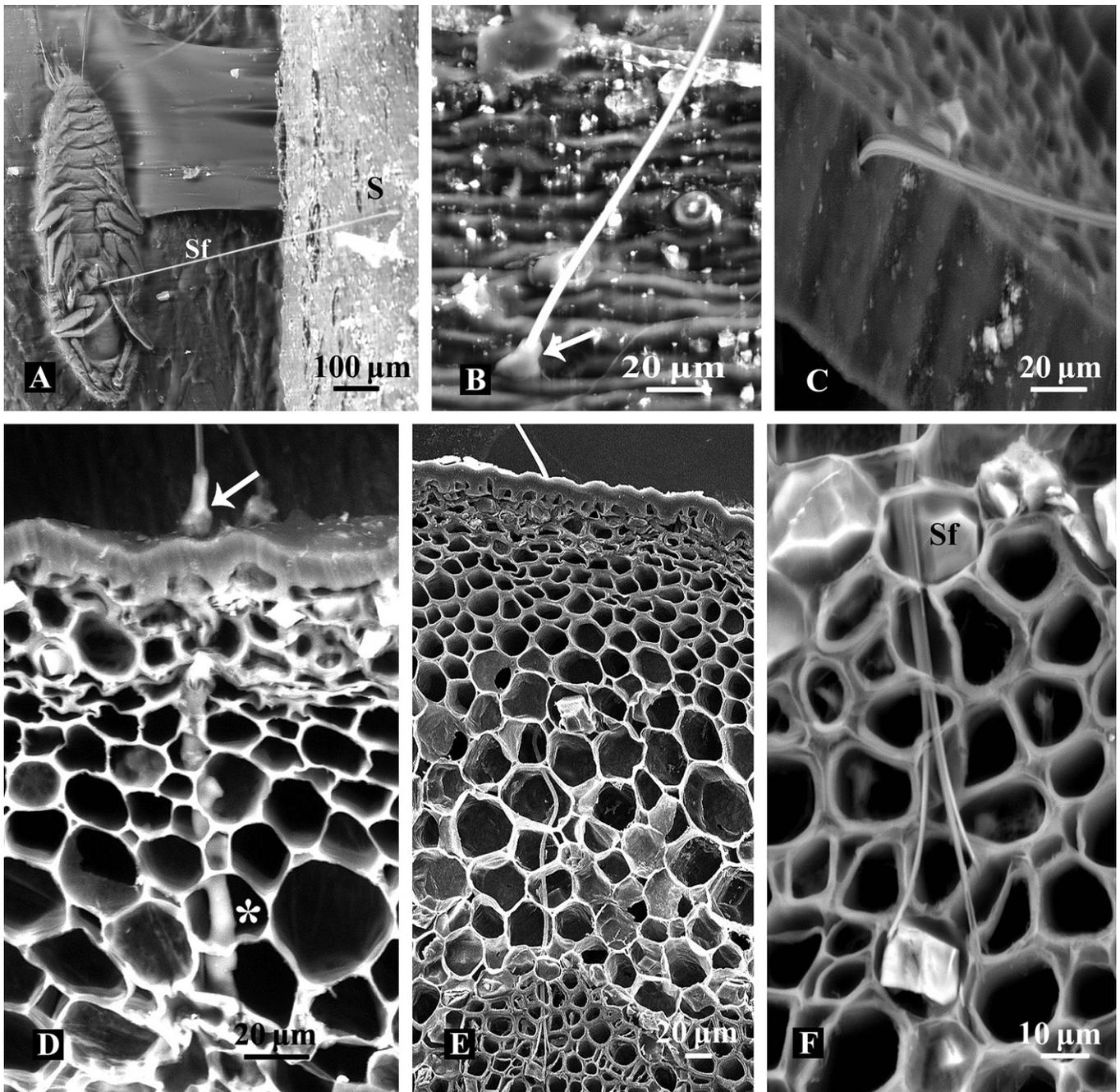


**Fig. 2.** Scanning electron micrographs of stilet components. (A) Clypeolabral shield (Cs); (A1) Two segmented labium; (B) Labium with symmetrically arranged sensilla - uniporous trichodea sensillum type I (\*); type II (arrow) and non-porous trichodea sensillum (\*\*); (C) Second labial segment- (↔) showing the short sensilla touching the stylet fascicle (Sf); (D-F) Lateral; dorso-lateral and dorsal views of mandibular stilet showing serrated edges (arrows) and cusps (\*); (G) Maxillary stylets (Mx) showing the opening and inward curving of lower stilet; (H) Inner surface of maxillary stilet with longitudinal grooves (arrow); (I) Interlocking of maxillary stylets indicated by (arrow).

by the mandibular and maxillary stylets, respectively. This was found to inhibit the phloem sealing mechanism of the host, enabling continuous sap sucking. The stylets along with the salivary sheath traverse intracellularly through different planes in a zig-zag manner to reach the phloem cells. The sheath traverses in a single, beaded tubular structure encasing the stylets (Fig. 1D); these are slightly broader at the point where the stylet fascicle has just

penetrated (Fig. 3D). Stilet penetration into the cortical cells was marked with slight distortion of cells and formation of dark patches around their terminating regions where as the epidermal cells were found to be intact (Fig. 3E). The fascicle segregates into three at the tip after reaching the phloem cells (Fig. 3F).

Stilet penetration upto the phloem and pre-settlement were observed on host as well as non-host plant species, as major part of



**Fig. 3.** Scanning electron micrographs showing stylet penetration. (A) First instar nymph inserting stylets (Sf) into the stem (S); (B) Salivary flange (arrow); (C) Stylet penetration on the outer surface of petiole; (D) Salivary flange (arrow) and intracellular presence of salivary sheath (\*); (E) Stylet fascicle traversing through different planes; (F) An enlarged view of stylet fascicle (Sf) mandibular and maxillary stylets reaching the phloem tissue.

the stylet fascicle remains in a coiled state while the insect was feeding indicating that it can overcome the varying phloem distance in different hosts (Fig. 1E). Formation of waxy filaments over the anal aperture and brachial plates indicates continuous feeding and final settlement not observed in non-hosts of *K. lacca*.

#### 4. Discussion

##### 4.1. Mouthparts and sensilla

The mouthpart morphology of *K. lacca* nymphs is similar to that of other piercing and sucking homopterans (Backus and McLean, 1982; Backus, 1988; Leopold et al., 2003; Zhao et al., 2010).

Earlier work done indicated a conical labium and a rostralis consisting of four filamentous chitinous structures arranged within a tube, with the mandibles on the outer and maxillae on the inner side (Krishnaswami et al., 1964). The present details on the ultrastructural morphology show the labium to be segmented and bearing hair-like sensilla over its surface resembling contact chemoreceptors. This is similar to the ones detected and described in the subfamily Peiratinae of Reduviidae (Brozek and Chlond, 2010). Similar to the mealybug, *Phenacoccus manihoti* (Calatayud and Le Ru, 2006) and whitefly, *Bemisia tabaci* (Rosell et al., 1995), chemo- as well as mechanoreceptors were found in the nymphs of *K. lacca*. The sensilla on the surface of the labium help in the detection of the plant surface but the specific role of these sensilla

need investigations. In several hemipterans their putative functions like discrimination between host and non-host tissues, and chemo-localization of salivary flanges are known (Backus, 1988). In fact these are evolved to detect external and internal plant cues for orientation, host acceptance, to control feeding tissue location (Backus and McLean, 1982; Walker and Gordh, 1989), and responding to gustatory and olfactory stimuli produced by the host plant (Rani and Madhavendra, 1995). The two short, robust sensilla trichodea (type II) on the apex of labial groove opening are likely to have a role in directing the stylet fascicle and acting as a clamp (Leopold et al., 2003). The mechanoreceptors on the first labial segment play a role in transmission of stimuli for the positioning and movement of stylets from the labial tip as seen in *Brevicoryne brassicae* (Tjallingii, 1978). This presumably detects the degree of labial telescoping or bending during probing.

#### 4.2. Stylet fascicles (mandible and maxillae)

The stylet fascicle enables penetration and feeding by the insect. The maxillary stylets of *K. lacca* are slightly longer than the mandibulars seen in Pentatomidae, Reduviidae and Lygaeidae (Pollard, 1973). Serrations on the apex of mandibular stylets are similar to those observed in the adults of *Bemisia tabaci*, and are known to cut and hold the host tissues (Rosell et al., 1995) and immobilize the body during moulting as in *Psammotettix striatus* (Zhao et al., 2010). The serrations on the mandibular stylets of predacious heteropterans (e.g., Reduviidae) are more numerous than those of the phytophagous heteropterans (e.g., Lygaeidae) (Boyd et al., 2002). These serrations and projections are flexible, might help in the directional movement of maxillary stylets, and as cleaning devices for removing salivary depositions from the maxillary stylets as reported in *Eupteryx melissae*, where these help to clear the mouthparts (Pollard, 1972). Maxillary stylets of *K. lacca* have a smooth outer surface, and are interlocked by ridges and grooves as in the salivary sheath producing phytophagous *Rhopalosiphum maidis* (Evert et al., 1973) and *Bemisia tabaci* (Rosell et al., 1995) which feed on the plant vascular fluids. The longitudinal grooves present on the inner surface of maxillary stylets might form the food canal and the salivary canal as reported on the basis of their transverse sections (Rani and Madhavendra, 1995).

#### 4.3. Salivary flanges, salivary sheath and stylet penetration

Maximal feeding sites were observed on the furrows which may help the insect to grip the substratum more tightly in order to facilitate insertion of stylets. Like in aphids the stylet penetration acts as a reflex following tarsal contact with the plant surface. During the initial penetration stages, the insect secretes saliva on the plant surface which solidifies forming a flange which acts as a fulcrum for further stylet movement. These flanges are pear-shaped as observed for *Rhopalosiphum maidis* and *Homalodisca coagulata* which seem to seal the labium, and are continuous with the internal portion of the salivary sheath supporting the “protection against leakage hypothesis” (Leopold et al., 2003).

The irregularly beaded salivary sheath along with the stylets traverses intracellularly through the plant tissue originating from the epidermis up to the phloem. In contrast in *Rhopalosiphum maidis* intercellular penetration was observed only in the epidermal and mesophyll tissues, and afterwards penetration up to the vascular bundle was intracellular (Evert et al., 1973). Salivary sheath was found to be unbranched in *K. lacca*, while a branched sheath is known from *Bemisia tabaci* (Chu et al., 2007) and *Homalodisca coagulata* (Leopold et al., 2003). Incidentally various functions are attributed to the salivary sheath during feeding, such as lubrication, sealing and preventing air contact with the injured tissues and as

an aid to directional control (Miles, 1964, 1968, 1972; Pollard, 1973; Crews et al., 1998)

Coiled stylet fascicle observed inside the body of *K. lacca* is similar to that of the first instars of *Bemisia argentifolii* (Freeman et al., 2001). It can be presumed that feeding in *K. lacca* occurs by tapping the antennae and labium on the plant surface, bending of the labium, secretion, and formation of salivary flanges on the plant surface, penetration of the epidermis by serrated mandibular stylets and simultaneous secretion of saliva through the maxillary stylets. Further penetration of the plant tissue is followed by protraction of the maxillary stylets and ultimately termination of the stylets in the phloem tissues occurs.

## 5. Conclusion

The present observations support the classification of *K. lacca* as salivary sheath feeder like aphids, whiteflies, leafhoppers and cicadas. This study has brought to the fore less known but interesting aspects related to the mouthparts of *K. lacca*. Like the modified clypeolabral shield which is not used for piercing and sucking, and segmented labium, ultrastructure of mandibular and maxillary stylets, and presence of sensilla which may have a role in feeding site selection. Presence of salivary flanges, salivary sheath and penetration pathway are reported in detail which could provide a comparison with other salivary sheath producing homopterans. Further transmission electron microscopic studies and cross sections of stylet fascicle may be useful in unraveling the role of labial sensilla based on the innervations of dendrites, structure of the sensilla, food and salivary canal, respectively. Also, biochemical and histochemical investigations are required to elucidate the nature of salivary sheath material and plant responses.

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