VANKADARA SIVAPRASAD - VINEET KUMAR (*)

SCANNING ELECTRON MICROSCOPIC STUDIES ON THE HEAD APPENDAGES AND ITS SENSORY STRUCTURES OF AN IMMATURE STAGE OF *SPILARCTIA OBLIQUA* (WLK.) (LEPIDOPTERA ARCTIIDAE) A PEST OF MULBERRY (*MORUS* SP.)

(*) *Electron Microscopy Division, Central Sericultural Research and Training Institute, Manandavadi Road, Srirampura, Mysore -570 008, India*
(*) *Corresponding author;* email ID: vinkumar2006@rediffmail.com

Sivaprasad V., Kumar V. – Scanning electron microscopic studies on the head appendages and its sensory structures of an immature stage of *Spilarctia obliqua* (Wlk.) (Lepidoptera Arctiidae) – a pest of mulberry (*Morus* sp.).

The mouthparts and antennae of the second and third instar larvae of *Spilosoma obliqua* Wlk., were studied using scanning electron microscope. The three segmented antenna bears sensory structures which are olfactory in function. The labrum and mandibles are also parts of the mouthparts and have their own function in selection and intake of food. Eight apical sensilla known to be olfactory are observed on the distal end of the maxillary palpus. The gustatory structures *i.e.,* two sensory structures (ALP1 and ALP2), a fine sensory projection (STC), two sensory setae (SL1 and SL2) are observed on the maxillary palpi. A single pair of labial palpi, considered to be mechanoreceptors, is also observed. Further, the functional morphology of the sensory receptors on the head capsule of other lepidopterous larvae have also been discussed.

KEY WORDS: *Sensory receptors, head appendages, S. obliqua, mulberry, ultrastructure.*

INTRODUCTION

The mouthparts and its accompanying sensory structures in lepidopterous larvae have been a subject of study to understand the systematics, sensory physiology and host plant selection (TEWARI *et al.,* 1996). The head capsule possesses various types of sensory receptors *viz.*, basiconi, trichoid and styloconic, which perceive stimuli from the external environment (SCHOONHOVEN, 1972; REESE and CARLSON, 1974; DEVITT and SMITH, 1982; KUMAR and TEWARI, 1995). The function of several types of sensory receptors on mouthparts of lepidopterans have been confirmed by behavioural and electrophysiological studies which indicate that the apical sensilla on the maxillary palpus are olfactory (HANSON and DETHIER, 1973; DETHIER, 1977; SINGH and GOEL, 1990) while medial and lateral sensilla styloconica on the galea and sensory receptors on the epipharynx are gustatory (DETHIER and KUCH, 1971; BLOM, 1978). The sensory receptors on the maxilla of *Bombyx mori* and their probable function have been studied earlier (ISHIKAWA *et al*., 1963; ISHIKAWA and HIRAO, 1965; AKAI, 1976; TAZIMA, 1978). KUMAR and TEWARI (1995) have explained the role of head sensory receptors of *B. mori* in discrimination of food plant. Recently, NAIR *et al.,* (2013) carried out the scanning electron microscopic studies on the mouthparts of *B. mori* developed on artificial diet. Further, an investigation of microscopic structures of mouthparts sensillae in the larvae of eri silkworm, *Phylosamia ricini* has been undertaken by BARSAGADE *et al*., (2013).

Mulberry (*Morus* sp.), the only food source of silkworm, *Bombyx mori* Linn., is of great economic importance to the sericulture industry. The quality and quantity of leaf produced has a direct bearing on the cocoon production and in turn, the economy of the rural farmers (KUMAR *et al.,* 2009). The mulberry foliage is often hampered by the infestation of insect pests, parasites and pathogens (KUMAR *et. al.,* 2001). Among insects, Bihar hairy caterpillar, *Spilosoma obliqua* Wlk., is one of the major pests of mulberry and a total foliage crop loss in mulberry by the pest was estimated around 4.90% during 1996-97 (KUMAR, 2011). A survey of literature revealed that no ultrastructure study has been carried out, so far, on the head appendages and its sensory organs of the pest. Therefore, current study deals with head appendages and its sensory receptors in immature larvae of *Spilarctia obliqua* has been undertaken to understand the functional morphology at the ultrastructure level under a scanning electron microscope.

MATERIALS AND METHODS

The larvae of *S. obliqua* were reared on the fresh mulberry leaves in laboratory at 26±2°C temperature and 65±4% relative humidity.

PREPARATION FOR SEM STUDY

The head capsule of second and third instar larvae of *S. obliqua* bearing sensory receptors were separated and fixed in 2.5% glutaraldehyde prepared in 0.2 M cacodylate buffer (*p*H 7.2) for 2 hr, washed in cacodylate buffer followed by double distilled water dehydrated in ethanol series, critically dried in Critical Point Dryer (EMS-850), coated by gold (20 nm thickness) in Sputter Coater (EMS-550), mounted onto copper stubs. The

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coated samples were observed under a transmission electron microscope (JEOL 100 CX II, Tokyo Pvt. Ltd. Japan) attached with Scanning device (ASID 4 D) at 20 kV.

RESULTS AND DISCUSSION

The head capsule of *S. obliqua* is dark brown to black in colour, antero-posteriorly compressed and bears two large epicranials or parietals (PT) running antero-dorsally and are separated by an inverted "Y"- shaped epicranials sutures. The frons is a triangular sclerite located between the two parietals. Similar to the observations on other lepidopterous insects *viz., Lymantria marginata* (SINGH and GOEL 1990) and *Plusia orichalcea* (KUMAR and GOEL 1986), the ocellar area is characterized by the presence of six ocelli (OC) and dorsal to the antennal socket (Fig. I, 1). The sensory structures mainly a pair of antenna, maxillary lobe and maxillary palp on the maxilla are situated on both the lateral side of the head. The detailed morphology of each receptor is discussed in detail and compared to the sensory receptors of other lepidopterous insects larvae.

The labrum (LB) is a broad flat lobe of the head and hangs downwards over the mandibles (MB) and articulates distally with the clypeus for a dorso-ventral movement. Symmetrically arranged six setae in groups on each half of the labrum dorsally (Figs. I, 1-3 and II, 1-2). BARSAGADE *et al*., (2013) revealed that the labrum is horseshoe shape sclerotic structure and a deep groove found on the middle region of anterior side which differentiate the two arms like structure of the labrum. On the dorsal surface of groove about six sensilla tyrichoidia and short sensilla trichoidea are also seen in *P. ricini*. A pair of mandibles (MB) in *S. obliqua* are located in the oral cavity, and are strong biting and chewing jaws which are hinged to the head for a transverse movement (Figs. I, 1-4 and II, 1-2). Each mandible has two articulate surfaces *i.e.,* epicondyle and hypocondyle, epicondyle fits into the cavity on the cranium. Three crenulated sharp incisors (small arrows in Fig. II, 1) and the flat molar teeth are present on the distal end of each mandible (Fig. II, 1, 2) with the changed feeding nature of succeeding instars, an incisor becomes blunt and flat. BARSAGADE *et al*., (2013) reported that the mandibles of *P. ricini* are unsegmented strong sclerotized triangular shaped structure with five teeth like denticles found on the anterior margin of the mandibles. The similarity of the labrum and mandibles in lepidopterous caterpillars were described by DOWNEY and ALLYN (1979); KUMAR (1983); KUMAR and GOEL (1986) and SINGH and GOEL (1990) in their respective studies except the naming of structures.

The antennae and maxillae are the major sensory receptors located on the head capsule of *S. obliqua* larvae and each receptor has its own specific function in discrimination and induction of food as also confirmed in *B. mori* larvae by KUMAR and TEWARI (1995) and TEWARI *et al.,* (1996). NAIR *et al.,* (2013) reported that the antennae and maxilla of *B. mori* larvae are the most important entities in mouthparts with regards to feed selection and the same are considered to be organs of chemoreceptin (taste and olfaction) involved in feeding behaviour. The antenna of *S. obliqua,* is the first sensory appendicular organ of the head present laterally on either side just above the bases of the mandibles. The base of the antenna is set into a pit of retractile membranous anticaria, an observation also described by KUMAR (1983) and SINGH and GOEL (1990) in lepidopterous insects. The scape, pedicel and flagellum are the main parts of the antenna. The proximal scape by which antenna is articulated to the head, the distal pedicel is longer and cylindrical in shape, whereas, the two segmented flagellum present beyond the pedicel. The proximal segment (FL I) of the antenna bears two cone like sensory basiconicae (FSB1 & FSB2) and a sensory projection (FSP) whereas, FL II is delicate and distal part of the flagellum. Three sensory basiconicae (PSB1, PSB2 & PSB3) and a sensory projection (PSP) are other sensory structures of the antenna lie situated on the distal end of the pedicel (Figs. I, 5 and 6). Similar description was also made in past in several lepidopterous larvae (KUMAR, 1983; KUMAR and GOEL, 1986; and SINGH and GOEL, 1990) except an additional sensory basiconicae (PSB3) in the present study on *S. obliqua*. The distal membranous part of the pedicel also bears two setae AN1 and AN2, where AN1 is exceptionally long seta.

The maxillolabial-hypophrayngeal complex is a united large composite structure of the maxillae, labrum and hypopharynx. The complex contributes to the underneath of the gnathal preoral cavity and basally supported by triangular hypostomal lobe (Figs. I, 2-3 and II, 3).

The maxilla consists of maxillary lobe and maxillary palp. The maxillary lobe has two cone like sensory structures (ALP1 and ALP2) each ending into a knobbed papilla and a fine sensory projection of a step cone (STC) (Figs. II, 4-6). On the distal end of maxillary lobe and laterad to ALP1 and APL2, two blunt setae SL1 and SL2 are also present. An elongated fine seta (AL1) is further attached near the anterior base of the maxillary lobe (Fig. II, 6). SINGH and GOEL (1990) also explained similar sensory structures, projection and setae in *L. marginata* under light and electron microscopic study.

The terminal end of maxillary palpi (MP) bears eight small sensory pegs arranged irregularly in *S. obliqua* (Figs. II, 5 and 7). KUMAR and TEWARI (1995) and TEWARI *et al.,* (1996) reported nine apical sensilla in *Bombyx mori*, which are responsible for olfaction. However, eight apical sensilla have been reported earlier in *B. mori* by AKAI (1976). Further, the presence of eight apical sensilla in different lepidopterous larvae *viz., Heliothis zea* and *H. virescens* (BAKER *et al*., (1986); *Agrotis ipsilon* (REESE and CARLSON (1974); *Euxoa missoria* (DEVITT and SMITH, 1982) and *Lymantria marginata* (SINGH and GOEL, 1990) have been confirmed in past. Moreover, in few other lepidopterous larvae the morphology of eight small apical sensilla have been studied where five have tapering cones with a single pore at the apex, similar to gustatory receptor, and remaining three have blunt cones with many minutes pores on exaggerated irregular surface which are similar to be olfactory sensilla. Thus the maxillary palpi are probably both gustatory and olfactory in function (DETHIER and KUCH, 1971). Since, we did not observe considerable difference in morphological characteristics on eight apical sensilla, thus it is felt that all the sensilla may have the only function of olfaction. KUMAR and TEWARI (1995) conducted an experiment by ampulated the antenna and maxillary lobe from their respective positions from both the side of *B. mori* larvae and found that the larvae lost its power of taste and smell thus started feeding on the non-host plant which indicates that maxilla and antenna together play an important role in gustation and olfaction. BARSAGADE *et al*., (2013) reported that the maxilla of eri silkworm, *P. ricini* is a fused structure composed of maxillary palp, stipe and cardo. They further mentioned that the sensilla trichoidia are present on the maxillary palp with association of prominent sensilla basiconica,

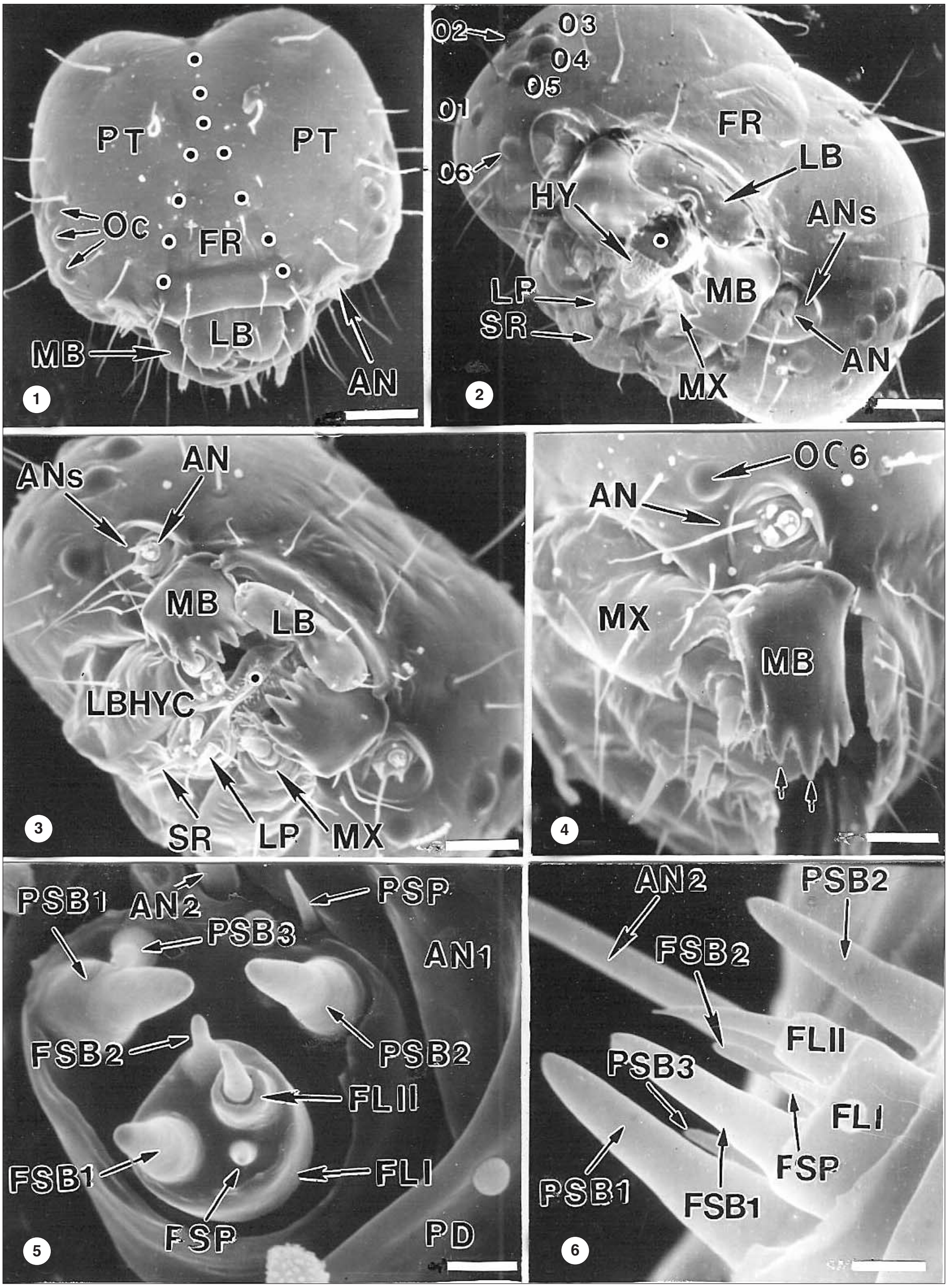

Fig. I – Scanning electron microscopic figures of head capsule and its appendages of second instar larvae of *Spilarctia obliqua.* 1. Anterior view of a complete head capsule showing antennal (AN) and labrum (LB) articulation, parietals (PT), ocelli (OC), mandibles (MB) and inverted Y shaped epicranial sutures (ʘ). (Scale Bar = 15 µm). 2. Frontal view showing antennal (AN) mandibular (MB) articulation with the epicranium; frontals (FR), Labrum (LB), Ocelli (O1-O6), Hypopharynx (HY), Antennal socket (ANs), Labial palpi (LP), Maxilla (MX) and Spinneret (SP) and ʘ indicate the preoral cavity (Scale Bar = 30 µm). 3. Enlarged view of head reveals the antenna (AN) located in the antennal socket (ANs), Labrum (LB), Mandibles (MB), Labiohypopharyngeal complex (LBHYC), maxilla (MX), Labial palpi (LP) and Spinneret (SP) (Scale Bar = 10 µm). 4. A part of antero-ventral view of capsule showing ocelli (OC6), antenna (AN) and mandibles (MB). Small arrows reveal the pointed incisors (Scale Bar = 10 µm). 5. Three segmented antenna showing the second segment pedicle (PD) with three sensilla basiconica (PSB1, PSB2 and PSB3), sensory projection (PSP, two setae (AN1 and AN2), third segment of the antenna divides into flagellums I and II (FL1 and FL II), further two sensory basiconica (FSB1 and FSB2) and a sensory projection (FSP) located on FL I (Scale Bar = 1.0 µm). 6. Enlarged view of antero-lateral view of antenna reveals the three sensilla basiconica (PSB1, PSB2 and PSB3) on pedicle, two sensory basiconica (FSB1 and FSB2) and a sensory projection (FSP) located on flagellum I (FL I) and flagellum II (FL II) (Scale Bar = 1.0 µm).

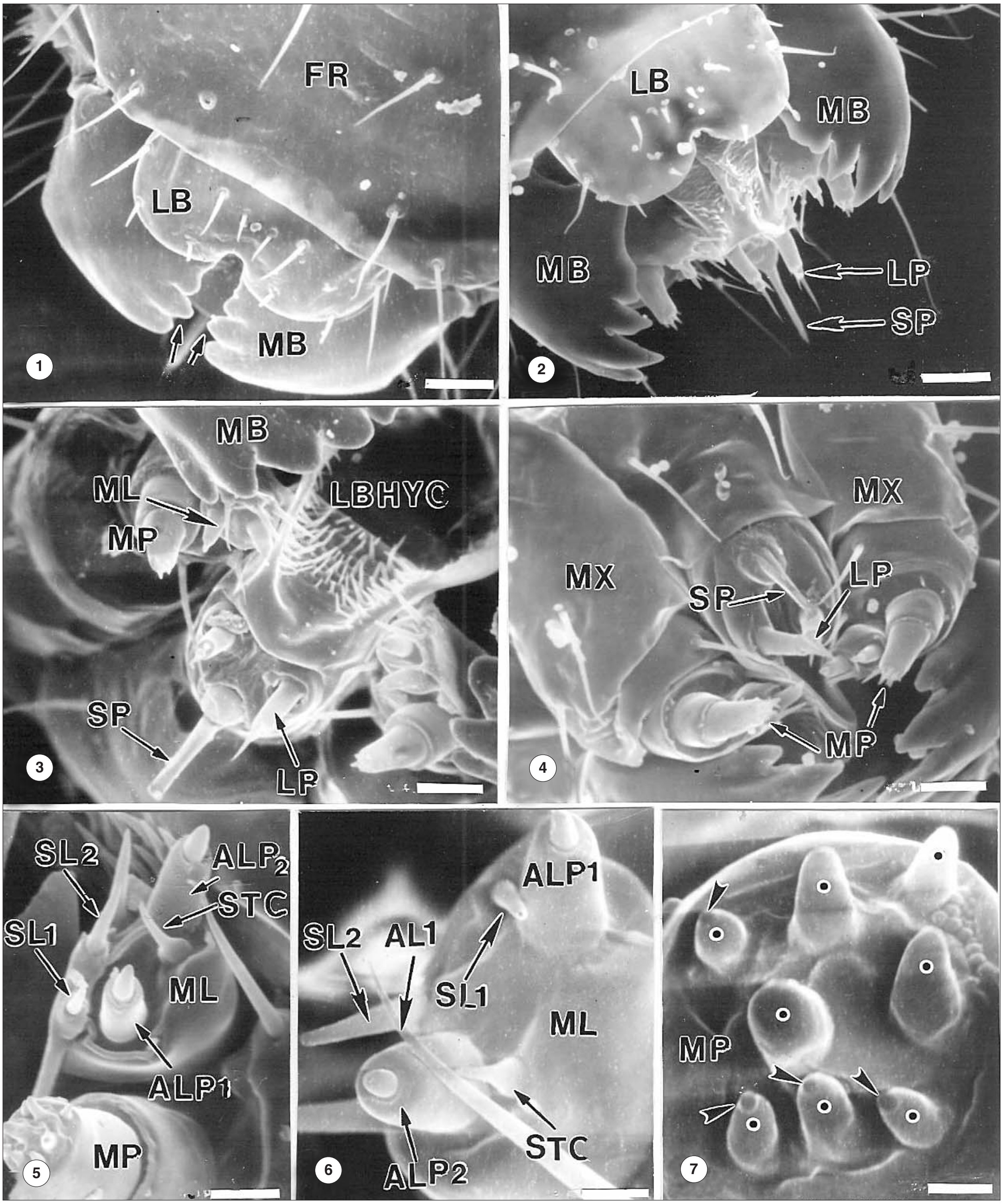

Fig. II – Scanning electron microscopic figures of head capsule and its appendages of second instar larvae of *Spilarctia obliqua.* 1. Antero-dorsal view of capsule reveals the labrum (LB), mandibles (MB) and frontals (FR). Small arrows indicate the incisors of mandibles (Scale Bar = 15 µm). 2. Anterior view of head showing labrum (LB), Mandibles (MB), Labial papli (LP) and Spinneret (SP). (Scale Bar = 6 µm). 3. Antero-ventral view of head capsule showing Labiohypopharyngeal complex (LBHYC), Maxillary lobe (LB), Maxillary palp (MP), Labial palpi (LP), Spinneret (SP) and Mandibles (MB) (Scale Bar = 1.0 µm). 4. Anterior view of head reveals two sides Maxilla (MX) anteriorly possessing Maxillary palpi (MP), Labial palpi (LP) and Spinneret (SP). (Scale Bar = 6 µm). 5. Enlarges view of terminated end of Maxilla divided into maxillary palpi (MP) and Maxillary lobe (ML). (Scale Bar = 1.5 µm). 6. Magnified view of anterior end of Maxillary lobe (ML) with various sensory structures. (Scale Bar = 2 µm). 7. Magnified view of distal end of Maxillary palp (MP) terminated with eight knobbed papillae (ʘ). (Scale Bar = 0.5 µm).

whereas the sensilla styloconica is located at the tip of each palp. In addition of these sensory structures a large group of short and pointed microtrichia occupied the ventral side of maxillary palp. According to NAIR *et al.,* (2013) the feeding behaviour in *B. mori* consists of three steps *i.e.,* attracting, biting and swallowing, performed in coordination of the structural components and the chemical stimuli available in the feed. The attracting behaviour is controlled by the sensilla basiconica in the antennae and the biting behaviour by those in the maxillae.

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