FINE STRUCTURAL STUDIES ON MAJOR LARVAL MOUTH PART SENSILLA OF ANTHERAEA ASSAMENSIS, AN ENDEMIC SILK MOTH SPECIES OF NORTH EAST INDIA IN REGARD TO SENSORY PHYSIOLOGY

Anita G Paul[1], Sudip Dey*[2] and Jatin Kalita[3]
[1]Department of Zoology, U.C.C., Umiam,
[2] Electron Microscopy Division, SAIF, North Eastern Hill University, Shillong-793022, India
[3]Department of Zoology, Gauhati University, Guwahati-14, India
*For correspondence. (sudipdey.dey1@gmail.com)

Abstract: The larval mouth part sensilla trichodea in *Antheraea assamensis* revealed differential distribution pattern of pores and lumen in different parts of the same sensilla. Pores were sparse in the tip as compared to the middle portion. Similarly lumen was well developed near the base but it was not observed in the tip. Sensilla cheatica, on the other hand were represented by porous as well as aporous types. Two types of sensilla basiconica (uniporous and multiporous), an aporous sensilla digitiform and placoid sensilla were detected. Most of the sensilla were found to be innervated by the dendrites of one or more sensory neurons. The fine structural details and distribution of dendrites, thickness of walls of sensilla, porosity of sensilla, presence ,absence and the nature of lumen of different sensilla have been studied, and their significance in sensory physiology are discussed in the light of available literature.

Keywords: *Antheraea assamensis*; sensilla; mouth parts; TEM

1. Introduction:

The muga silkmoth, *Antheraea assamensis* Helfer syn. *Antheraea assama* Westwood, belonging to the order Lepidoptera and family Saturniidae of the class Insecta, is endemic to the Brahmaputra valley of Assam, and the foothills of East Garo hills of Meghalaya in India.. It is an economically important sericigenous insect, producing an exquisite and lustrous golden yellow silk of high economic value. The larval forms of *Antheraea assamensis* thrives well on the aromatic leaves of the host plants *"Som"* (*Machilus bombycina* King ex Hook. F.) and *"Soalu"* (*Litsea polyantha* A. Juss). *Antheraea assamensis* being a semi domesticated insect exhibits a wild living and feeding habit in the larval stages, necessitating outdoor rearing of the larvae. As a result, the delicate worms are exposed to extremes of climatic conditions such as heavy rain, hailstorm, moisture, temperature stress etc as well as natural predators & parasitoids such as birds and insects.

In order to overcome the aforementioned problems associated with outdoor rearing, it appears that successful domestication or indoor rearing of the worms will be extremely important for sustenance and improvement of the muga silk industry as this would ensure controlled environmental conditions favorable for growth and development of the worm. Although numerous attempts have been made to rear the worms under indoor conditions, it had not been successful especially in large scale, due to a number of problems. It was therefore thought that for attaining success in indoor rearing, it is extremely important to gain a detailed knowledge about the feeding behavior of the insect, its sensitivity to host plant and specialization in structural features of its sensory system. It is amply highlighted in literature that adaptational diversity of an insect is best expressed by its specialized cuticular sensory structures called sensilla[1-3].Hence, it appears that the first step in understanding the complexity of behavior of an insect should involve detailed examination of its sensory structures, as mostof the insect behavior is known to be under nervous control[4].Further, sensory receptors play important roles in different behavioral responses such as host plant selection, food searching behavior, and in distinguishing host plants from the non-host plants. Host selection in phytophagous insects consists of a sequence of behavioral responses to an array of stimuli associated with host and non-host plants, and the insect sensory receptors enable them to perceive these stimuli[5].The role of insect mouthparts and host plant leaf

surface in insect-plant relationship has been reviewed by Chapman (1977)[6]. It is also well known that sensilla present on the mouthparts of insects play significant roles in feeding activities [7,8].Thus, mouthparts and the associated sensory structures appear to be directly related to the foraging and feeding behavior of phytophagous insects and their larval forms. Although many detailed studies have been carried out on the sensory structures of different lepidopteran species [9-11].very few reports on sensory system of *"Muga"* silkworm, *Antheraea assamensis* are available in existing literature [3,12-17]. As far as the mouthpart sensilla of *"Muga"* silk worm is concerned, only some scattered information exists in literature [12, 13, 17, 18-20]. The aforementioned studies on mouth part sensilla, however were restricted mainly to surface micro structural features and gross morphology investigated through scanning electron microscopy. Reports on studies concerning fine structural aspects of mouth part sensilla of *A.assamensis* involving Transmission electron microscopy are lacking as revealed from the existing literature. It is needless to mention that studies correlating morphological features of sensilla with the fine structural aspects such as dendritic organization, nature of the dendritic sheath, details of lumen of sensilla shaft, characteristics of the wall of the sensilla at the base & apex etc. are important in understanding the structural and functional physiology of the mouth part sensilla. Keeping these in view, Tansmission electron microscopy of major mouth part sensilla of *A.asamensis* have been undertaken to acquire an in-depth knowledge on the physiology of the mouth part sensilla.

2. Materials and methods:

2.1. Sample collection:

The first larval stage of the *"muga"* silk moth, *Antheraea assamensis* (Figs. 1.) were collected from the three sites, The Central Muga and Eri Research and Training Institute, Ladoigarh, Assam, India; The Regional Muga Research Institute, Boko, Assam, India and the Field station of Central Silk Board, Nongpoh, Meghalaya, India during the period 2010-2013.Samples were collected during the four seasons, pre-monsoon, monsoon, post-monsoon and winter.

Figure 1: Photograph of the First larval stage of the silk moth, *Antheraea assamensis*

For the purpose of Transmission Electron Microscopic (TEM) study, excised heads of larvae were fixed in modified Karnovsky's fixative [21].

2.2. Sample preparation:

2.2.1. Transmission electron microscopy:

For TEM studies, standard methods for TEM sample preparation was followed [22]. The head were excised from the larvae, and, the mouthparts were separated very carefully, by viewing under a dissection microscope. The different components of the mouth parts were further trimmed and cut into smaller pieces of approximately 1mm x 1mm size and fixed in modified Karnovsky's fixative [21] having the composition of 20g of para-formaldehyde dissolved in 250ml of 0.2 M sodium cacodylate buffer at 60°C. The volume was increased to 480ml by adding double distilled water. To this, 20ml of 25% glutaraldehyde and 12.5g of anhydrous calcium chloride were added. After 4 hours of fixation in the above primary fixative, the samples were washed thoroughly in 0.1 M sodium cacodylate buffer. Post fixation was carried out in 1% osmium tetroxide prepared in

the same buffer for 1 hour at 4°C. Fixed samples were dehydrated in ascending grades of acetone with two changes of 15 minutes each. From dehydrated samples, the residual acetone was removed by keeping the samples in propylene oxide for 30 minutes. This was followed by infiltration carried out gradually in different proportions of propylene oxide mixed with embedding medium {Araldite CY212- 10ml, DDSA (dodecenyl succinic anhydride- 10ml, DMP-30 [Tri- (di-methyl-amino-methyl) phenol] - 0.4ml, and di-butyl phthalate- 1ml}.

Embedding of the samples was carried out in the araldite embedding medium using beem-capsules. The embedded blocks were kept at 50°C in an embedding oven for 24hours. The temperature was then raised to 60°C and the embedded samples were kept for 48 hours to complete polymerization.

After complete polymerization of the embedded samples, ultra-thin sections (600A°-800A°) were cut in an RMC ultra-microtome, MT-X, with a diamond knife. The sections were collected on copper grids and stained with saturated alcoholic solution of uranyl acetate for 10 minutes at room temperature in the dark, followed by lead citrate for 5 minutes.[23] The stained sections were examined in a Jeol JEM 2100 transmission electron microscope at an accelerating voltage of 80 kV.

3. Results:

3.1. Fine structure of major larval mouthpart sensilla:

3.1.1. Sensilla trichoidea:

3.1.1.1. Sensilla trichodea typeI (STrI):

A transverse section of sensillum trichoidea showed that the sensilla are innervated by three dendrites (Fig.2).
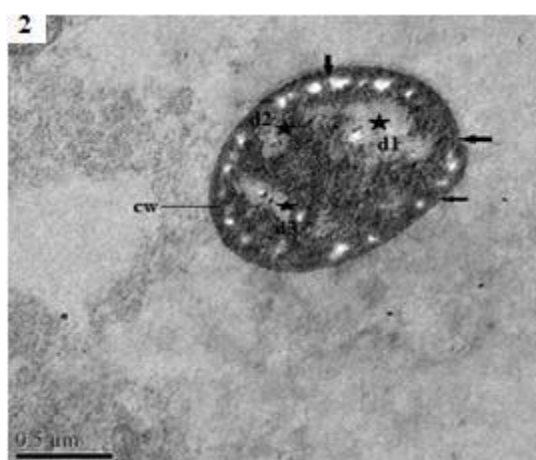

Figure 2: TEM image of the transverse section of the apical region of the hair shaft of sensillum trichoideum type I showing three dendrites (d-asterix), a cuticular wall (cw) with pores (arrows)

The dendritic sheath is not clearly visible as it may be present in close apposition with the inner layer of the cuticular wall. The cuticlar wall is found to be thick and few pores are observed (Fig.2.). The lumen of the sensilla is found to be filled with electron dense material.

3.1.1.2. Sensilla trichoidea typeII (STrII):

Cross section of sensillum trichoidea typeII reveals the presence of a thick electron dense cuticular wall. Few pores are also observed on the cuticular wall. The sensillum was found to be innervated by four dendrites (Fig.3).
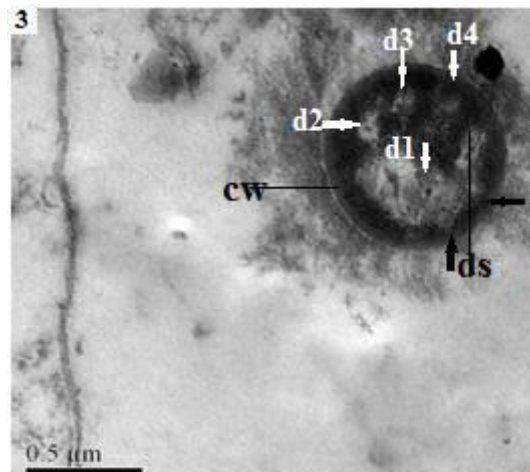
Figure 3: TEM image of the distal region of sensillum trichoideum type II showing the innervations of four dendrites (d1-d4)(white arrows). The electron dense dendritic sheath (ds) partially compartmentalizes the dendrites. Neurotubules are observed in the dendritic regions (d1). Cuticular wall (cw) is thick with few pores (black arrows).

The electron dense dendritic sheath lying in close apposition to the inner surface of the cuticular wall was found to be invaginated into the lumen, partially compartmentalizing the dendritic segments. In one of the dendritic regions a few neuro-tubules are also observed (Fig.3).

3.1.2. Sensilla chaetica:

3.1.2.1 Sensilla chaetica typeI (SChI):

The transverse section of the distal region of the hair shaft of sensilla chaetica type I (located at the labrum) shows two dendritic segments within the sensilla sinus, encased by the dendritic sheath in close proximity to the inner layer of the cuticular wall.
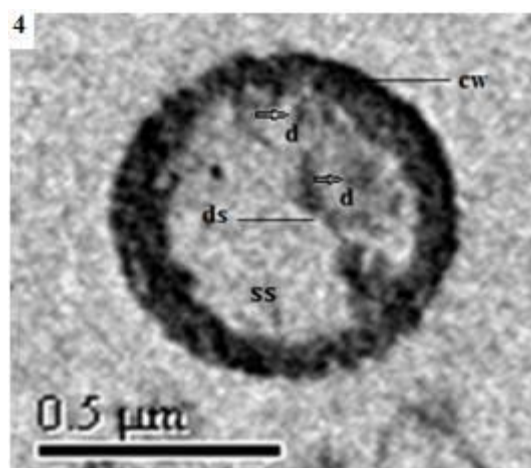

Figure 4: TEM image of a cross section of sensillum chaeticum, showing two dendrites (d) (arrows) enclosed within a dendritic sheath (ds), the sensillar sinus (ss) and a thick cuticular wall (cw)

The cuticular wall is found to be thick and aporous (Fig.4).

3.1.2.2 Sensilla chaetica typeII (SChII):

The transverse section of the apical region of the hair shaft of sensilla chaetica typeII (located at the mandible) reveals that the cuticular wall of the sensillum is thick with some pores on it, which connect the surface to the sensillar lumen. In the central region of the lumen of the sensilla, two dendrites are observed (Fig.5).
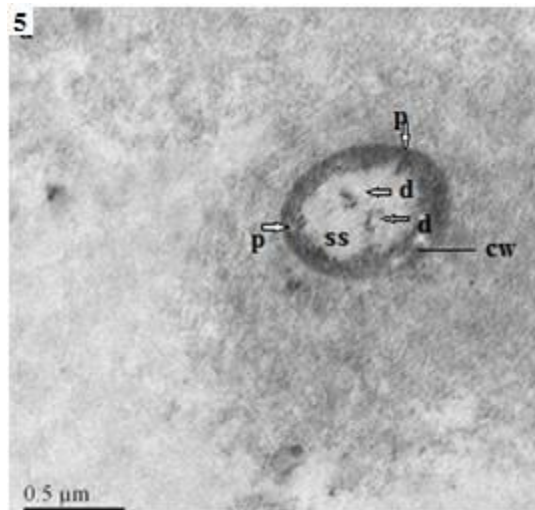

Figure 5: TEM image of a cross section at the apical region of the hair shaft of sensilla chaetica type II showing the innervations of two dendrites (d- arrows) with a thick cuticular wall (cw) and pores (p).

The dendritic sheath, however, is not distinct.

3.1.3. Sensilla styloconica:

3.1.3.1 Sensilla styloconica type I (SStyI):

The transverse section of sensillum styloconicum type I reveals the presence of four distal dendritic segments on the periphery of the lumen, close to the inner layer of the cuticular wall lying within the dendritic channels. In one of the distal dendritic segments (Fig.6), a few prominent round neurotubules are present. The cuticular wall is found to be relatively thick.
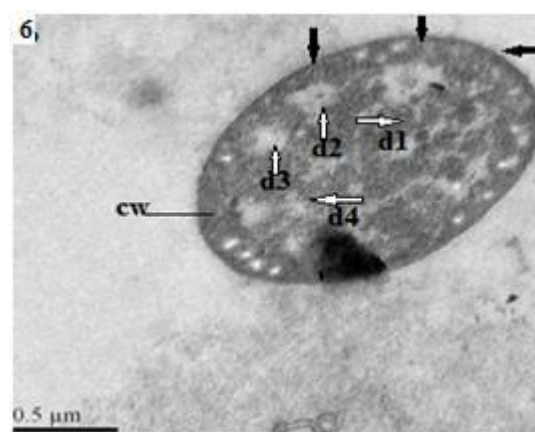

Figure 6: Cross section of the distal region of sensillum styloconicum innervated by four dendrites (d1-d4) (white arrows) showing neurotubules, particularly in d1and d4, and cuticular wall (cw) showing pores (black arrows).

Few pores are observed on the cuticular wall.

3.1.3.2. Sensilla styloconica type II (SStyII):

A cross section of sensillum styloconicum shows the presence of four distal dendrites surrounded by the dendritic sheath. The dendritic sheath is found to be elaborate, surrounding individual dendrites and presumably providing support for them (Fig.7).
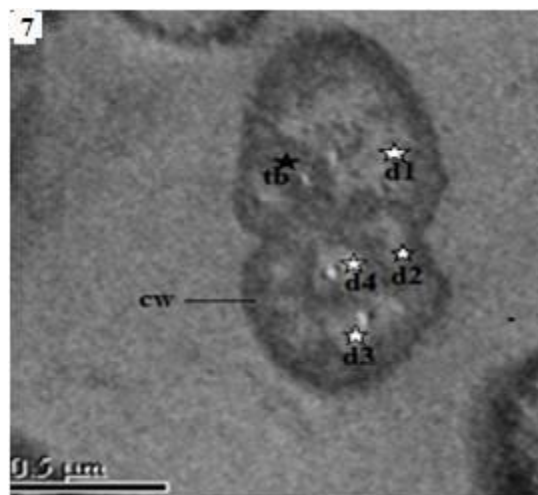

Figure 7: The cross section of sensillum styloconicum type II, showing the innervations of four dendrites (d1-d4)(white asterix), with a tubular body (tb)(black asterix) like structure.

In the same section, a tubular structure closely apposed to the cuticular wall is also observed (Fig.7).   Since it has been amply highlighted in existing literature that the uniporous galeal styloconic sensilla are innervated by five bipolar neurons[24].it is possible that the fifth dendrite terminated at the base of the sensillum by forming a tubular body.

In an enlarged view of a section of the sensilla (Fig.8), four dendrites are found to be enclosed by the dendritic sheath and a tubular body with electron dense material is separated from the others by a septum within the dendritic sheath.
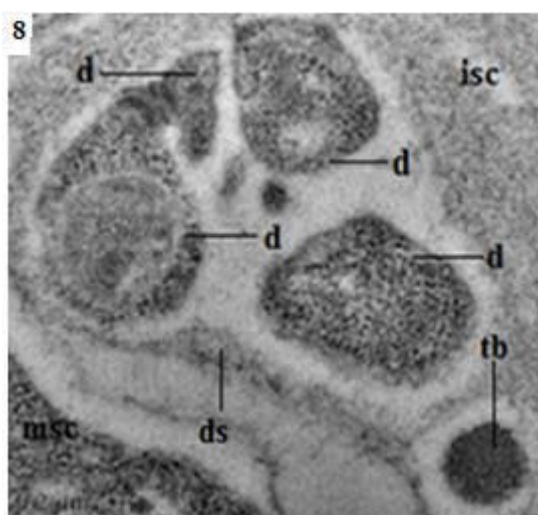

Figure 8: Enlarged view of the sensillum showing the four dendrites (d) enclosed in a dendritic sheath (ds), and a tubular body (tb) like structure with electron dense material separated from the others by a septum within the dendritic sheath.

The dendritic sheath envelopes the tubular body, possibly the fifth dendrite all around the circumference. The entire structure is found to be enclosed by the inner sheath cell (isc) and the intermediate sheath cell (msc). The dendritic segments are filled with neurotubules (Fig.8). In the section around the ciliary region, the dendritic

sheath could not be detected, and the dendritic segments are located within the ciliary sinus wrapped by the inner sheath cell.
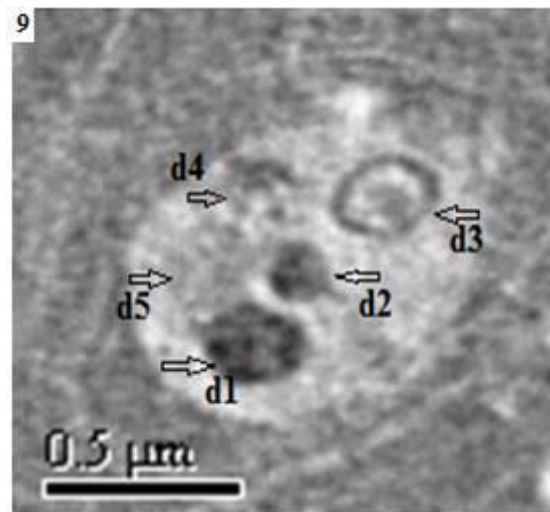

Figure 9: The cross section of the ciliary region of the sensillum. Section showing absence of dendritic sheath of the dendritic segments located within the ciliary sinus (cs) surrounded by the inner sheath cell. Some dendrites (d1 and d3) showing the microtubular arrangement along the periphery.

Neurotubules are observed along the periphery of some of the dendrites (Fig.9).

3.1.4. Sensilla basiconica:

3.1.4.1 Sensilla basiconica typeI (SBaI):

In the transverse section of the multiporous basiconic sensillum, a rough and thin cuticular wall, serrated externally can be observed. A number of pores are also observed on the wall. The inner surface of the cuticular wall is found to be lined with a thick electron dense layer. The centre of the sensillar lumen is occupied by a number of distal dendritic branches (Fig.10).
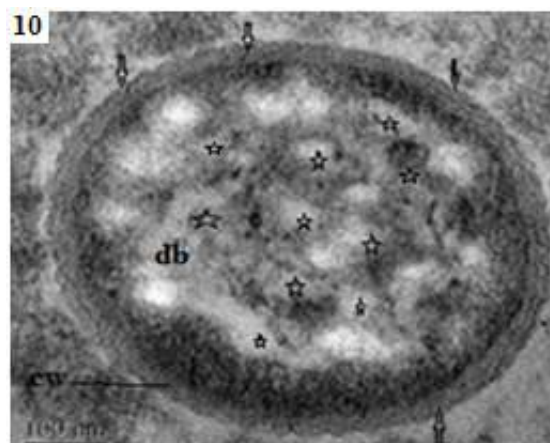

Figure 10: TEM image of cross section of multiporous sensillum basiconicum type I showing thin cuticular porous wall and dendritic branches (db) within the sensillum also showing micritubular structure in some.

In the cross section of the uniporous basiconic sensillum type I, the cuticular wall is found to be thick. The lumen of the sensillum is innervated by five dendrites enclosed by a dentritic sheath (Fig.11). Dendritic sheath is elaborate, completely compartmentalizing some of the dentrites.
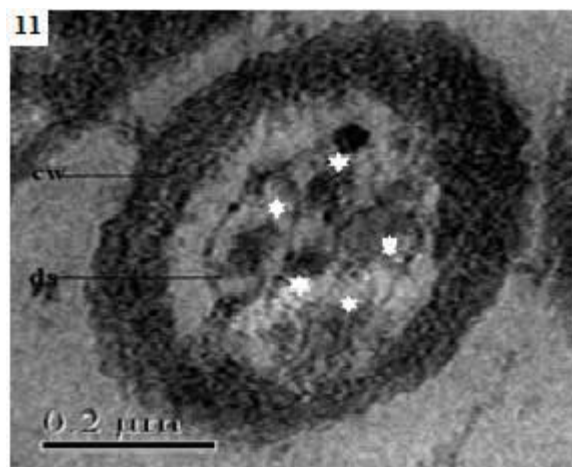
Figure 11: Cross section of uniporous sensillum basiconicum typeI, showing pore on the cuticular wall and innervations of four dendrites (asterix).

Neurotubules are also present (Fig.11).

3.1.4.2. Sensilla basiconica typeII (SBaII):

In the transverse section of the sensillum basiconicum type II, two distal dendritic segments are observed in the sensillar sinus (Fig.12).
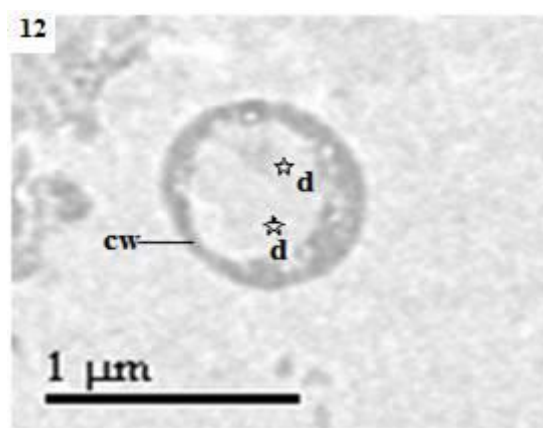

Figure 12: TEM image of cross section of sensillum basiconicum type II, showing two dendritic segments with microtubules in the sensillar sinus

The dendritic sheath is found to be closely apposed to the cuticular wall and no pore is observed on the wall. The cuticular wall is found to be relatively thin with electron dense material in some parts of its inner surface.

4. Discussions:

Related studies[25] suggest that sensilla trichoidea which are without a flexible socket, have multiple pores on a thick wall as observed in the aforementioned sensilla located in the labial palps of the muga silkworm in the current study. Further, the sensilla are innervated by one to three neurons and this type of sensilla is reported to have an olfactory function. The three dendrites and the pores observed in the sensilla trichoidea type I in the labial palps of the muga silkworm confirm the innervations necessary for a chemosensory, possibly olfactory function.

Transmission Electron Microscopy (TEM) of the cross section of the type II trichoid sensillum showing the presence of four dendrites with cuticular pores indicate that they may function as chemo-receptors . It has been reported that the number of olfactory receptor neurons in most olfactory sensilla ranges between one to five[26]. In this context, it is to be noted that trichoid sensilla is also reported to be equipped with one to five neurons.[27] Structures of olfactory sensilla are generally believed to be well conserved, consisting of cuticular pores through which odor is perceived, despite their considerable variability in the gross morphology[28].Thus, the presence of sensilla trichoidea in the mouth parts of *A. assamensis* suggests the importance of olfactory receptors in detecting odors emanating from the host plant.

Sensilla chaetica are reported to function as both mechano- and chemo-receptors[29,30],and such dual function is generally ascribed to the presence of a dendrite not attached to the hair shaft but forming a tubular body at the base[31,32]. Hence, the presence of two dendrites observed in the labral sensilla chaetica in *A. assamensis* suggest that, in the muga silkworm it may play a significant role in chemoreception, probably as a contact chemoreceptor[30]. and help the larvae to assess the leaf texture and quality[33]. Chemicals from the leaf surfaces are believed to stimulate larval contact chemoreceptors and this is the normal way by which these receptors operate when the larva first encounters a leaf surface[34-36]. According to Bland et al., (1998)[37], contact chemoreceptive sensilla on the mouthpart are primarily used to taste food and determine the chemical characteristics of the substrate.

Sensilla chaetica observed on the paired mandibles of *A. assamensis* has also been reported in other lepidopterans [38]. However, in other lepidopteran larvae, they are reported to be aporous [39], while they are multiporous in *A.assamensis*. The multiporous nature of these sensilla indicates their chemosensory function, probably in olfaction. Also, the presence of two dendritic segments without the dendritic sheath and the presence of pores on the cuticular wall of the sensilla suggest that they may function as chemoreceptors, since chemo receptors are usually innervated by two to four neurons with the dendritic sheath usually ending at the hair base and the dendrites emerging from it [33]. These sensilla are believed to respond specifically to volatiles emitted by the host and non-host plants, and assist larvae in the selection of host plant[40]. Thus, sensilla chaetica on the mandibles of the muga silkworm may help the larvae in host plant recognition. It is also highlighted in literature that sensilla located on the mandibles help to monitor the hardness of food and modulate the power of the adductor muscles [41]. The mandibular sensilla chaetica may function in a similar manner in *A.assamensis*.

The sensillum styloconica present on the labial palps of *A.assamensis* has been reported in other lepidopteran larvae [39, 42]. The presence of numerous pores on the surface and the occurrence of four dendritic segments in the cross section of the sensillum suggest their chemoreceptive function. They probably play a role in olfaction and help the larvae to perceive odors emitted by the host plant and thereby assist the larvae to differentiate host from non-host plants.

The two uniporous sensilla styloconica, present medially and laterally, in the mesal lobe of the paired maxillary galea of the muga silkworm has been reported in different lepidopteran larvae [43,44]. which may play an important role in host plant recognition, assisting the larvae to distinguish the host plant by assessing the plant volatiles and are likely to function as the main larval gustatory organs.

In this context, it is to be noted that food plant recognition is predominantly governed by the presence of eight taste neurons present in the two styloconic sensilla of the maxillary galea [43]. These two sensilla have been reported to be the main taste receptors as confirmed by behavioral and electrophysiological studies [43]. The ultra structure of these sensilla are reported to be conserved across lepidopteran species [43], each sensillum being innervated by five bipolar neuron i.e. four chemosensory and one mechano-sensory [44]. This may also be true for *A. assamensis*, as the transverse section of the sensillum revealed the presence of four dendrites which appear to be the chemosensory cells and the fifth dendrite is the tubular body of a mechano sensory cell. Un-branched dendrites lack wall pores, and, the presence of a tubular body suggests a combined mechano-sensory and gustatory function [44]. The tubular body at the base of the sensillum is a typical mechano-sensitive structure [29]. Thus, the presence of both types of cells in the uniporous styloconic sensilla in *A.assamensis* as revealed from the current study is indicative of a bimodal, chemo-mechano-sensory function of the aforementioned sensilla. In this context, it is to be noted that mechano-receptive sensilla are used by many insects to obtain information on the surface texture of the leaf through continuous contact with the leaf [45]. Out of the eight cones observed in the sensilla basiconica type I, three with multiple pores on the surface are believed to be olfactory, while five with a single apical pore are believed to be gustatory [46]. Moreover, the

dendritic branches and the serrated appearance of the thin cuticular wall with pores on the basiconic sensilla of *A.assamensis* is typical of olfactory structure and hence, an olfactory function of the aforementioned sensilla is indicated [47]. It is therefore logical that the basiconic sensilla in the mouth part assist the larvae to detect and perceive the plant volatiles.

The five dendritic segments observed in basiconic cones on the maxillary palp suggest a gustatory function [48]. Thus, the two morphological types of basiconic sensilla on the maxillary palps of *A.assamensis* indicate a dual olfactory and gustatory function. These sensilla help the larvae to perceive the leaf odor and also to assess the chemical nature of the leaves. Moreover, according to Wasserman and Itagaki (2003) [49], maxillary palpi are believed to be in direct contact with a feeding substrate and are able to perceive odors directly. As a result, the larvae are able to distinguish between the fresh aromatic leaves and the dry leaves with changed or reduced aroma. Further, the tubular structure observed on the inner wall of the cuticle at the basal region of the basiconic peg on the maxillary palp suggests the presence of a mechanosensory neuron which is terminated at the base of the peg to form a tubular body.

The aporous sensillum basiconicum with a blunt tip detected on the lobarium of the maxillary galea has been reported in other lepidopteran larvae [48, 50]. According to Faucheux, (1995) [51] the sensillum may be mechanoreceptive, responding to food particles before they enter the hypo pharyngeal region. As reported in *Mamestra configurata* [48], it may also function as a thermo-hygrosensitive sensillum. According to Steinbretch and Muller (1978) [52], hygroreceptors are modified mechanoreceptors and are mediated by sensillar structure consisting of both dry and wet receptor neurons as well as a temperature neuron [53]. The essential features of thermo-hygroreceptors are the absence of pores, an inflexible socket directly arising from the cuticle with one to two outer dendritic segments [48]. These features being present in the type II basiconic sensillum of the muga silkworm is indicative of its function as a thermo-hygrosensitive sensillum.

5. Conclusion:

Transmission electron microscopy of major larval mouth part sensilla such as different types of sensilla trichodea, sensilla chaetica, sensilla basiconica and sensilla styloconica could reveal their fine structural details relevant to the sensory physiology of the silk moth larvae. The distribution pattern and number of the dendrites; porous or non porous nature of the wall of the sensilla, arrangement of dentritic sheath, localization of neurotubules, presence or absence of lumen in different parts of the sensilla etc. could be resolved precisely with the help of Transmission electron microscopy. The study suggests that along with the morphological characterization of different mouth part sensilla with the help of scanning electron microscopy, the fine structural details through transmission electron microscopy are extremely important in understanding the mechano- sensory, chemosensory and olfactory functions of the mouth part sensilla in the silk moth, *A.assamensis* endemic to the North East India. The detail knowledge on different types of mouth part sensilla in *A.assamensis*, as revealed from the present study will be important in developing appropriate strategies for indoor rearing of the silk moth larvae since the feeding behavior of the larval stages in insect are governed by the body sensilla in general and mouth part sensilla in particular.

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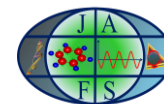
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