Article

Ultrastructural studies on the sensory structures of the larval antennal and maxilla-labial palpi complex of the *Oryzaephilus surinamensis* (Coleoptera: Silvanidae)

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Abstract

Oryzaephilus surinamensis L. (Coleoptera: Silvanidae) is an important tropical stored grain pest that inflicts grain damage by acting as an external feeder. .The larva is more voracious feeder than the adults. The sensilla present in the insects antenna and mouth parts can perceive various senses from the environment and host plant. They can act as mechanoreceptors, thermoreceptors hygroreceptors, olfactory receptors and gustatory receptors. Therefore, in the current study, the morphology, structure, and distribution pattern of the sensilla found on the antennae, maxillary and labial palpi of the larvae of *O. surinamensis* were mainly investigated through scanning electron microscopy Seventeen different types of sensilla, including six types trichoidea, seven types of basiconica, two types of chaetica, one type of styloconica and coeloconica were identified in the antenna of the larvae. Similarly, 23 sub-types of sensilla have been found in the maxillo-labial complex of mouth parts: five types of trichoidea, eight types of basiconica, seven types of styloconica, two types of coeloconica, and one type of digitiformia. In both maxillary and labial palpi sensory complex, a unit of 13 sensilla was identified in which there is always a thick central sensilla encircled by other sensilla. The ultrastructural details of all the sensilla can underlie future electrophysiological and behavioral investigations.

Keywords *Oryzaephilus surinamensis*, Grain, sensilla, antenna, maxilla-labial sensory complex, scanning electron microscope

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1 Introduction

All insects remain covered with sensory structures called sensilla which are concerned with sensing the taste, smell, sound, touch, temperature and vision (Shields, 2011). Classification of insect sensilla is based on its morphological features, origin, surface texture and location on the body part (Zacharuk, 1985; Zacharuk and

Shields, 1991). It has been found elsewhere that in insect larvae the sensory organs are mainly congregated in and around the antenna and appendages of mouth parts surrounding maxillary and labial palpi (Benham and Ryan, 1978; Dubinskas et al., 1997).

These sensilla help insect to locate feeding resources and assess the quality of food before ingesting (Zacharuk and Shields, 1991; Liu et al., 2011; Song et al., 2014).In addition to that, different categories of sensilla give the most significant cues which help the insect to detect their exact microhabitat (Shorey, 1973) and communicate through chemical signals (Regnier and Law, 1968). The sensory structures present on antenna, mouthparts and ovipositors can detect any odor (Panda, 1995). The olfactory cues and feeding preferences of the herbivorous insects depend on the types of sensilla and therefore knowledge of these various kinds of sensilla can be very useful to develop novel management strategies of insect pest (El-Ghany et al., 2017; EL-Aziz et al., 2018).

Ultra-structural studies regarding the larval sensory structure of beetles have already been obtained for different families of beetles (Bloom and Zacharuk, 1982; Bloom et al., 1982; Zacharuk and Shields, 1991; Dubinskas et al., 1997) and amidst them Curculionidae and Carabidae are the most widely studied families where larval sensory structures of 22 and 15 species have already been documented (Bland, 1983; Speirs et al., 1986; Chan et al., 1988; Chaika and Tomkovich, 1997; Tomkovich and Chaika, 2001; Sinitsina and Chaika, 2003).

The description of adult sensilla present in the antenna and mouth parts are plentiful in stored grain pests (Hu et al.,2009; Seada et al., 2018). But depiction of larval stages of holometabolous insects is also very significant to decipher diverse processes, such as feeding specificity, detoxification of toxic substance, survival strategies, mortality and identification of taxonomically essential characters (Montserrat et al., 2023).

However, much less focus has been given on the larvae of *Oryzaephilus surinamensis* which has been considered as most widespread polyphagous stored grain pest nowadays (Nurul et al., 2019). Therefore, in the present paper the ultra structural studies of antenna and mouth part sensilla of Saw-toothed Grain Beetle, *Oryzaephilus surinamensis*, L. was carried out. This obnoxious pest can consume at least 31 suitable foods and now globally present in 104 countries. The percentage of infestation in the countries of Africa, Asia, Europe, North America and Oceania are 48%, 54%%, 43%, 48% and 50% respectively (Gourgouta et al., 2023). Infestation of this pest may occur at storage, manufacturing or retail level mainly based on various factors like food availability, temperature in different areas, food processing practices and interaction with other species (Nurul et al., 2019). Saw-toothed Grain Beetle is considered as "external feeder" because they feed on grain dust or debris but never enter into the grain and may cause devastating damage through contamination. Zulaikha et al., (2018) have reported in Malaysia that the omnivorous nature of Saw-toothed Grain Beetle poses a serious threat in upcoming days and as such it can also become a devastating pest in our country namely, India. In Indonesia Saw-toothed Grain Beetle has been considered as one of the major pests of rice (Kalshoven, 1981; Cogburn, 1991). Saw-toothed Grain Beetle causes weight loss of broken seed surface, leads to broken germ, broken endosperm and makes conducive microhabitat for fungal contamination also (Busvine, 1980; Robinson, 2005; Backel et al., 2007).

At present, there is very little published literature concerning the investigation of the sensilla located on the antennae and mouth parts of larval *O. surinamensis* which are concerned with detection of ecologically pertinent stimulus leading to finding suitable habitat, food-resource. However, Roppel et al. (1972) have demonstrated 2 basiconic and 3 trichoid sensilla in the adult *O. surinamensis* but the study was restricted to the antenna only.

Therefore, due to the lack of data regarding sensory structure on *O. surinamensis* larvae, the current study was undertaken to investigate the external morphology, distribution pattern and role of sensilla on antenna and mouth parts focusing on maxillary and labial palpi through scanning electron microscopy.

2 Materials and methods

2.1 Collection of sample or specimen

Adults were collected from cashew the nut of a local store house in Raiganj (2021), West Bengal, India. They were reared in 500 ml plastic jars containing 100 grams of cashew nut in the laboratory and maintained at 25 ± 1℃, 65 ±5% RH and with 24 hours darkness. Three Saw-toothed grain beetle (*Oryzaephilus surinamensis*) 3rd instars larvae were separately kept in different petri dishes without food for 24 hours before fixation to reduce contamination of the mouth and antenna with diet (White, 1986).Then specimens were etherized and collected in eppendorf tubes within 70% ethanol.

2.2 Sample preparation for scanning electron microscopy

The collected 3rd instar larvae were washed two times in 70% ethyl alcohol with the help of paintbrush in a watch glass (separately with high care) and then the samples were dehydrated using an ascending series of ethanol 10 min each in 70%, 80%, 90% and then twice in 100% for 20 min to dehydrate the specimen properly (Li, 2021) and the samples were ready for further processing. Metal coating and specimen observation was done as per methodology of Nandi and Chakraborty (2021). The test samples were then kept on stack of tissue papers for air drying. Afterwards best three 3rd instar larvae were mounted in two separate aluminum stubs. Thereafter sample loaded stubs were inserted within a Q150T ES sputter platinum coater in 10^{-3} millibar pressure for 2 minutes, in 25 mA current to get 10 nanometer coating layers of platinum on samples. Coating was done four times and each cycle was run for 30 seconds. After coating, samples were kept inside a highly vacuumed Scanning Electron Microscope (SEM) chamber. All samples were examined using Carl Zeiss EVO 18 Scanning Electron Microscope which was completely controlled by PC loaded with software - SMART SEM, Carl Zeiss. The tungsten warmed fiber was used to give electron beam at 15 kV from various edges for every platinum covered specimen. Thereafter micrographs were collected from different locations on the antennae, labial pulps and maxillary pulps to observe different sensilla structures of 3rd instar larvae.

The segmental length of each antenna segment was measured. The types of sensilla found on samples were named according to the classification system of Schneider (1964).

2.3 Measurement of sensilla length and width

During SEM micrograph study length, width and distribution of different types of sensilla found on antennal region and mouth parts of 3rd instar larvae were noted. The mean length and diameter of each type of sensilla and the standard deviations were calculated from either two or three sensilla of each type based on their distribution (Li, 2021).

3 Results

3.1 Antennal sensilla

The antenna of *O. surinamensis* 3rd instar larva consists of three segments, apical or terminal, followed by sub-apical and basal Fig1. a & b. The total length of larval antenna is 178.7±0.8 µM. The apical segment is the shortest part having a length of 9.49±1.16. The longest segment is the sub-apical segment with a length of 118.7±1.27. The most proximal segment is called basal segment and which is 51.85±0.70 µM long. A total of 17 types of sensilla is found in larval antenna of *O. surinamensis* (Table 1). The various types of sensilla of antenna are trichoidea, chaetica, basiconica, stylloconica and coeloconica (Table 1). The detailed accounts of these sensilla are given below.

Table 1 Types and features of antennary sensilla (ST: Sensiila trichoidea, SB: Sensilla basiconica, SDG: Sensilla digitiformia, SS: Sensilla styloconica, SCo: Sensilla coeloconica)

Types of sensilla	Length (μM) (Mean±SD)	Diameter (μM) (Mean±SD)	Shape	Tip	Wall
ST1	14.65±0.10	1.46±0.02	Straight	Pointed	Smooth
ST2	12.64±0.10	1.56±0.01	Straight	Pointed	Grooved
ST3	19.63±0.10	3.15±0.05	Straight	Pointed	Grooved
ST4	24.54±0.02	3.54±0.01	Straight	Blunt	Grooved
ST5	11.516±0.10	8.12±0.00	Leaf-like, larger	Pointed	Rough
ST6	4.68±0.001	6.63±0.15	Small leaf like projection	Blunt	Smooth
Sch1	37.29±0.03	3.11±0.01	Spine like	Sharp	Smooth
Sch2	42.43±0.12	3.10±0.01	Slightly curved	Pointed	Smooth
SB1	7.48±0.10	1.52±0.01	Slightly curved	Pointed	Smooth
SB2	5.22±0.10	1.63±0.05	Arrow head	Pointed	Smooth
SB3	6.56±0.06	1.47±0.05	Curved	Pointed	Smooth
SB4	5.61±0.005	1.42±0.01	Short &straight	Blunt	Ribbed
SB5	5.42±0.10	1.62±0.01	Short & slightly curved	Blunt	Rough
SB6	5.21±0.10	1.74±0.005	Tapered at the middle and wide at the base and tip	Blunt	Grooved
SB7	4.17±0.34	2.55±0.03	Peg like	Pointed	Smooth
SS	2.74±0.70	2.12±0.02	Small peg like	Blunt and porous	Rough
SCo1	2.20±0.005	2.61±0.001	Sensilla in pit and inside a raised cuticle	Blunt and porous	Grooved
ST1	14.65±0.10	1.46±0.02	Straight	Pointed	Smooth

3.1.1 Sensilla trichoidea

Six sub-types of sensilla trichoidea are identified in the antenna (Fig. 1-4). Sensilla trichoidea type-1 (ST1) is straight and pointed sensilla with smooth wall. The shaft is 14.65±0.10 μM long. The base is widest and the diameter is 1.46±0.02 μM and it gradually tapers from the base towards apex. It is found in almost all the segments of antenna. It arises from an un-socketed cuticle (Fig.1, Fig.2 & Fig.3). Sensilla trichoidea type-2 (ST2) are slightly shorter than type-1. It has a length of 12.64±0.10μM and base diameter of 1.56±0.01μM. It is straight pointed and grooved sensilla which arise from socket-less area. They are found in basal and sub-apical segment (Fig. 1 & Fig. 2). Sensilla trichoidea type-3 (ST3) is longer than both ST1 and ST2 and has a shaft measuring 19.63±0.10 μM. Just like the other two types it has also widest base with a diameter of 3.15±0.05 and then tapers towards apex. It is grooved sensilla and arises from flexible socket and only found in the terminal segment of antenna (Fig. 3). Sensilla trichoidea type-4 (ST4) is straight sensilla with a blunt tip. It is the longest type among all the sensilla trichoidea and characterized by a length of 24.54±0.02 μM and basal diameter of 3.54±0.01μM with grooved wall. It is also located only in the apical segment of the larval antenna and arises from raised cuticular area (Fig. 3). Sensilla trichoidea type-5 (ST5) have a characteristic leaf-like shape. These sensilla are 11.516±0.10 μM long and 8.12±0.005 μM broad at the base. In fact it is the widest among all the sensilla encountered in the antenna of the larvae. They have rough surface and sharply pointed tip. They can only be found in the terminal segment of antenna (Fig. 3 & Fig. 4). Sensilla trichoidea type-6 (ST6) is flat, leaf-like and stout structure. They have smooth surface and round blunt tip. The shaft is non-existent. The length and basal width of these sensilla were found to be 4.68±0.001 μM and 6.63±0.15 μM. The base is wider than the length of the shaft (Fig. 3 & Fig. 4).

3.1.2 Sensilla chaetica (Sch)

These are the longest hair-like spikes found in the antenna and depending on the length and shaft curvature there are two sub-types of it (Fig. 3). Sensilla Chaetica type-1 has straight shaft, pointed tip and smooth wall. It has a length of 37.29±0.03 μM and basal diameter of 3.11±0.01 μM. The distribution of these sensilla is restricted to the apical segment only. Sensilla Chaetica type-2 has curved shaft, pointed tip and smooth wall. It is slightly longer than type-1 measuring 42.43±0.12 μM in length of 3.11±0.01 μM in base diameter. The distribution of these sensilla is restricted to the apical segment only.

3.1.3 Sensilla basiconica (SB)

These are thicker, shorter, straight or slightly curved sensilla arising from shallow socket. It has seven sub-types from SB1-SB7 (Fig. 1-4 & Table 1). Sensilla basiconica type-1 is short slightly curved antennary sensilla having a length of 7.48±0.10 μM and a base diameter of 1.52±0.01 μM. These sensilla end in pointed tip and smooth surface. Only basal segment has this kind of sensilla. These sensilla arise from an inflexible socket (Fig. 1d). Sensilla basiconica type-2 has a shape of arrowhead with broadest base and very tapering apex. The shaft has a length of 5.22±0.10 μM and a base diameter of 1.63±0.05 μM. These sensilla have pointed tip and smooth surface. These sensilla are inserted in the inflexible socket. It is also restricted to basal segment only (Fig. 1d). Sensilla basiconica type-3 is 6.56±0.06 μM long and 1.47±0.05 μM wide at the base. They have a slightly curved shaft and smooth surface which are inserted in round flexible socket. These are found in the sub-apical and apical segments and remain attached to a flexible, round socket (Fig. 2). Sensilla basiconica type-4 are straight, ribbed and short sensilla with a blunt tip and with a length of 5.61±0.005 and basal diameter of 1.42±0.01. They are only restricted to sub-apical segment. These sensilla arise from flexible socket (Fig. 2). Sensilla basiconica type-5 bear short and slightly curved shaft with rough surface, flexible socket and distinctly blunt tip. These are 5.42±0.10 μM long 1.62±0.01 μM wide at the base. These are only found in the sub-apical segment (Fig. 2). Sensilla basiconica type-6 is one the most unique sensilla basiconica with tapered middle and wider base and apex. These are aporous, grooved surfaced sensilla with a blunt tip. The short shaft of these sensilla has a length of 5.21±0.10 μM and basal width of 1.74±0.005 μM. The distribution of these sensilla is also restricted to sub-apical segment (Fig. 2). Sensilla basiconica type-7 is characterized by smooth peg-like short shaft with a pointed tip arising from an inflexible socket. These are 4.17±0.34 μM long and widest at the base amongst all sensilla basiconica with a basal diameter of 2.55±0.03 μM. These can only be found at the tip of antenna in the last segment (Fig. 3 & Fig. 4).

3.1.4 Sensilla stylloconica (SS)

These are very small peg like sensilla which come out from round socket. The base and apex are slightly wider than the middle of the shaft. These sensilla have a small length of 2.74±0.70 μM and basal diameter of 2.12±0.02 μM. Sensilla stylloconica can only be located at the tip of the terminal antennary segment (Fig. 4).

3.1.5 Sensilla coeloconica

These are small round projection type sensilla on the pit. Only one such sensillum can be located at the tip of the antenna. The length is 2.20±0.005 μM and basal width is 2.61±0.001 μM. They are characterized by grooved surface, blunt and porous tip (Fig. 4).

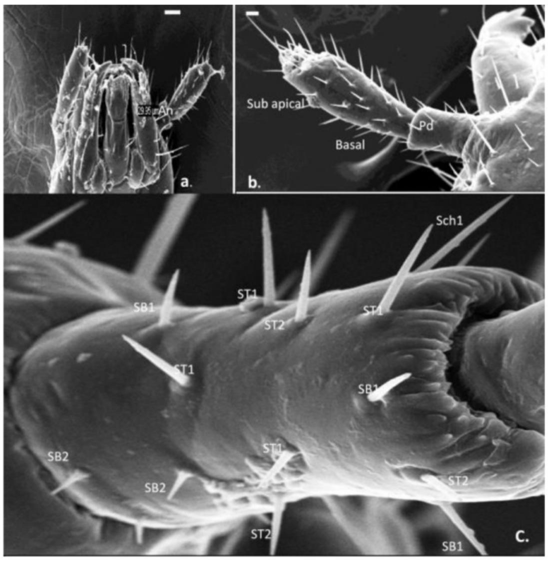

Fig. 1

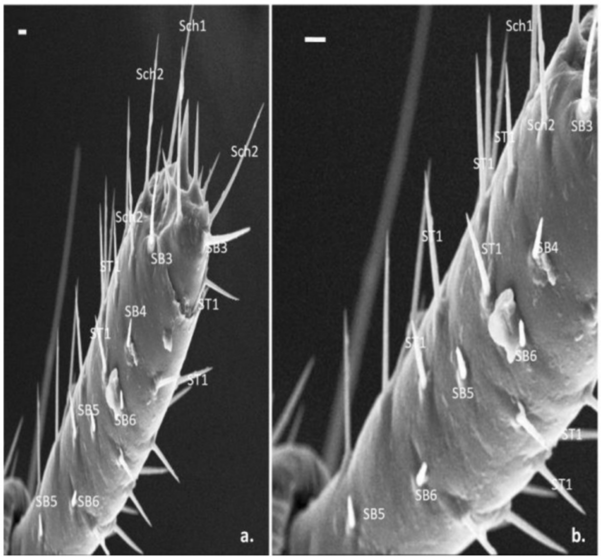

Fig. 2

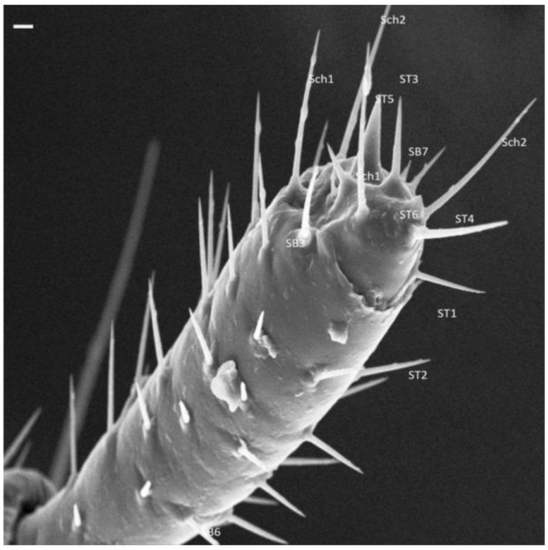

Fig. 3

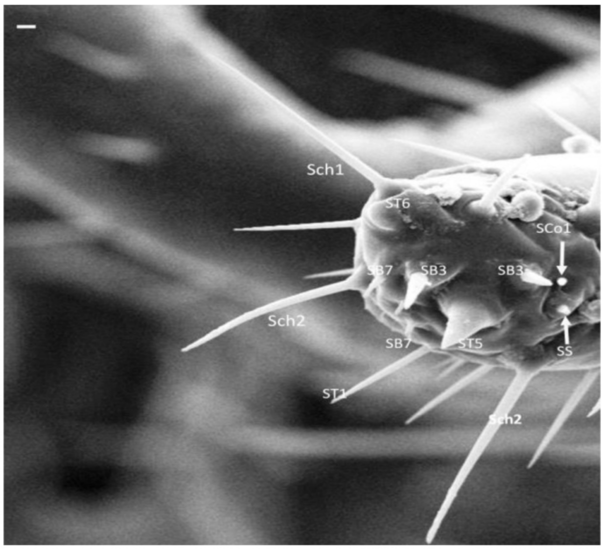

Fig. 4

Fig. 1: Antenna and head complex of larva and adult *Oryzaephilus surinamensis* L. **a.** Head complex containing mouthparts and antenna of 3rd instar larvae of *O.surinamensis* L **b.** Close up of antennae showing three different parts(apical, sub-apical and basal). c. Magnified image of basal segment of larval antenna. Sc:Scape, Pd: pedicel, Fla: Flagellomeres. ST1-ST2:Sensilla trichoidea type-1 and 2, SB1-SB2: Sensilla basiconica type1and type2, Sch1: Sensilla chaetica type-1. Scale bars are 10 µM, 100 µM and 2 µM. **Fig. 2:** a. Sensilla of apical and sub-apical complex of 3rd instar larvae of *Oryzaephilus surinamensis* L. b. Close up of sub-apical segment antenna. ST1-ST2: Sensilla trichoidea type-1 and 2, SB1-SB6: Sensilla basiconica type-1 to type-6, Sch1 and Sch2: Sensilla chaetica type-1 and type 2. Scale bars: 4µM and 4µM. **Fig. 3:** Close up of Sensilla of apical segment of 3rd instar larvae of *Oryzaephilus surinamensis* L. top view. ST1-ST6: Sensilla trichoidea type-1-type-6. SB7-SB8: Sensilla basiconica type-7 and type-8, Sch1 and Sch2: Sensilla chaetica type-1 and type 2. Scale bar-4 µM. **Fig. 4:** Close up of side view of Sensilla of apical segment of 3rd instar larvae of *Oryzaephilus surinamensis* L.ST1: Sensilla trichoidea type-1, ST-5 and ST-6: Sensilla trichoidea type-5 and type-6. SB7-SB8 and SB9 : Sensilla basiconica type-7 and type-8 and type 9. SS1: Sensilla stylloconica, SCo1: Sensilla coeloconica type-1 Scale bar, 4 µM.

3.2 Sensilla of maxillolabial-complex of mouth part

The larval mouth parts of *O. surinamensis* are strictly tailored for chewing and consist of an upper roof-like labrum, one pair of unattached beak-like mandibles, symmetrically arranged two maxillae with four segmented maxillary palpi and one bifurcated galea and one labium with labial palpi containing five segments (Fig. 5). The sensory field of various types of sensilla can locate concentrically at the tip of the most distal segment both maxillary and labial palpi (Fig. 6b). These sensory fields can be called as maxillary and labial sensory complex. The main features of the various types of sensilla present in the maxilla-labial complex are depicted in the Table 2.

Table 2 Types and features of Sensilla of maxillo-labial complex of 3rd Instar larva (ST: Sensiila trichoidea, SB: Sensilla basiconica, SDG: Sensilla digitiformia, SS: Sensilla styloconica, SCo: Sensilla coeloconica).

Types of Sensilla	Length(μM)	Diameter(μM)	Shape	Tip	Wall
ST1	17.12±0.12	1.70±0.007	Straight	Pointed	Smooth
ST2	19.41±0.40	1.55±0.005	Straight	Pointed	Smooth
ST3	21.99±0.381	1.94±0.071	Straight	Blunt	Grooved
ST4	13.54±0.10	2.16±0.043	Slightly curved	Blunt	Smooth
ST5	2.54±0.05	2.1±0.01	Leaf-like, broad at the base	Sharply pointed	Smooth
SB1	4.75±0.051	2.28±0.068	Spine	Pointed	Smooth
SB2	21.29±0.117	1.83±0.085	Curved and long	Slightly blunt	smooth
SB3	3.12±0.005	1.52±0.005	Peg like	Round, flat, porous	Grooved
SB4	2.52±0.01	1.72±0.02	Finger like	Blunt	Smooth
SB5	1.43±0.02	1.31±0.005	Small peg	Round & porous	Grooved
SB6	4.39±0.115	1.22±0.011	Elongated finger like	Blunt	Grooved
SB7	3.92±0.01	1.43±0.002	Finger like	Blunt & round	Grooved
SB8	3.80±0.05	1.02±0.064	Chili shaped	Slightly Pointed & porous	Grooved
SDG	18.34±0.01	1.94±0.02	Elongated rope-shaped	Blunt	Grooved
SS1	5.17±0.049	2.33±0.01	Broadest, finger-like	Blunt &round	Grooved
SS2	0.52±0.10	1.57±0.0.01	Flat & round	Flat &porous	Smooth
SS3	1.73±0.037	1.12±0.02	Small pointed peg	Slightly pointed	Grooved
SS4	1.52±0.01	1.44±0.02	Peg like	Bifurcated	Smooth
SS5	2.44±0.01	2.08±0.05	Broad spike like	Round	Grooved
SS6	1.4±0.01	1.00±0.01	Broadest at the base	Slightly pointed	Smooth
SS7	1.47 ±0.04	0.75±0.025	Peg like	Blunt and round	Smooth
SCo1	0.91±0.02	0.75±0.03	Small projection like sensilla in pit	Nipple like	Smooth
SCo2	1.92±0.05	1.63±0.011	Flat and round	Nipple like	Smooth

3.2.1 Sensilla on the maxilla and maxillary palp

The first three segments of maxillary palp are cylindrical and last segment conical ending in a flat round maxillary sensory complex (Fig.6b).Total number of sensilla present in these segments is very few in first three segments (Fig.6a) and very difficult to examine. The detailed account of sensilla is given below.

Sensilla trichoidea

These are mainly spine-like pointed sensilla coming out from an inflexible-socket. There were five sub-types of these sensilla identified. Sensilla trichoidea type-1 (ST1) is present on the first three basal segments and comes out of an inflexible socket. These are straight pointed spine like sensilla with smooth surface and measure 17.12±0.12 µM in length and 1.70±0.007 µM in basal width (Fig. 6b).Sensilla trichoidea type-2 (ST2) is also pointed straight spine like sensilla with smooth surface and inserted in inflexible socket. They can be located in the second segment of maxillary palpi. But these are slightly elongated with a length of 19.41±0.40 µM and basal width of 1.55±0.005 µM (Fig. 6b). Sensilla trichoidea type-3 (ST3) is long (21.99±0.381 µM) and wide base sensilla with grooved surface and blunt tip. These can be found singly at the base of double-segmented galea (Fig. 6b). Sensilla trichoidea type-4 (ST4) is slightly curved and contains pointed tip. These are strictly found at the galea and remain attached to an inflexible socket. The length and base width are 13.54±0.10 µM and 2.16±0.043 µM respectively of this kind of sensilla trichoidea. Sensilla trichoidea type-5 (ST-5) is very rare and unique structure and found to be present at the ventral portion of the junctional point of third and fourth maxillary palpi segment. These are always present in series of 3 or 4. They are short and wide sensilla and look like leaves. The width was measured to be 2.1±0.01 µM and length was 2.54±0.05 µM. Sensilla of this kind suddenly taper at the apex and bears sharply pointed tip and smooth surface. These are always attached to some inflexible socket (Fig. 7c).

Sensilla basiconica (SB)

These are shorter, blunter and stouter than sensilla trichoidea and inserted in inflexible or flexible socket. Mostly they possess blunt rounded tip. Eight different kinds of sensilla basiconica have been encountered in the larvae of this insect. Sensilla basiconica type-1 (SB1) are short and pointed sensilla bearing smooth surface and present at the junction of terminal two segments of maxillary palpi. The length and basal width are subsequently 4.75±0.051 µM and 2.28±0.068 µM. They arise from inflexible socket (Fig. 6a). Sensilla basiconica type-2 (SB2): The main characteristic feature of these types of sensilla is the elongated shaft and presence in a group. These are strictly restricted in the double-segmented galea and inserted in an inflexible socket. They are the longest among all types of sensilla found to be present in the maxilla or labium with a shaft length of approximately 21.29±0.117 µM and base width of 1.83±0.085µM. The surface of this kind of sensilla is grooved and ends in blunt tip (Fig. 6b & c).Sensilla basiconica type-3 (SB3) is a short peg-like flat-round containing sensilla and bearing grooved surface. These sensilla contain pores at the tip and are strictly located at tip of maxilla in the maxillary sensory complex. The length and basal width are subsequently 3.12±0.005 and 1.52±0.005 µM. They also come out from an inflexible socket(Fig. 6b). Sensilla basiconica type-4 (SB4) is characterized by finger like appearance having same width from base to apex and getting tapered gradually towards the tip. These are also located only at the maxillary sensory complex. They possess blunt tip and smooth surface. The length and base width were found to be 2.52±0.01 µM and 1.72±0.02 µM (Fig. 7a & b). Sensilla basiconica type-5 (SB5) is the smallest among all types of sensilla basiconica and has length of 1.43±0.02 µM and base of 1.31±0.005 µM. These are peg-like projections and contains unique blunt and porous tips. These are inserted in a flexible socket and like SB2 and SB3 located only at the periphery of maxillary sensory complex (Fig. 7a & b). Sensilla basiconica type-6 (SB6): These are elongated finger-like sensilla with grooved surface ending in blunt tip. The straight shaft bears length of 4.39±0.115 µM. The basal width was recorded to be 1.22±0.01 µM. They also have flexible socket like SB4 and restricted to the maxillary sensory complex (Fig. 7a & b). Sensilla basiconica type-7 (SB7) is spine-like sensilla with grooved surface and blunt round tip. The length and width of the base are 3.92±0.01 μM and 1.43±0.002 μM respectively. These sensilla are also found at the periphery of the maxillary sensory complex and arise from an inflexible socket (Fig. 7a & b). Sensilla basiconica type-8 (SB8) have unique chili-like smooth surfaced structure originating from a flexible socket and slightly curved at the apex bearing blunt and porous tip. These are present singly at the ventral surface of the second segment of maxillary palpi. The measured length and basal width have been found to be 3.80±0.05 μM and 1.02±0.064 μM (Fig. 7c).

Sensilla digitiformia (SDG)

These are present singly in the ventero-lateral surface at the junction of third and fourth maxillary segments. These are very elongated rope-like structure arising from one un-socketed area. The length and basal width are 18.34±0.01 μM and 1.94±0.02 μM (Fig. 7c).

Sensilla styloconica (SS)

These are short, flat structures and arise from a round base. Four sub-types of sensilla stylloconica have been found in maxillary-sensory complex (Fig.6b&c).Sensilla styloconica type-1 (SS1) is the longest and widest sensilla lying at the middle of maxillary sensory complex surrounding which all other sensilla are present at the tip. It comes out from a round an elevated base. The length and base width were found to be 5.17±0.049 μM and 2.33±0.01 μM. They have grooved surfaces and round blunt aporous tips (Fig.7a & b). Sensilla styloconica type-2 (SS2) also comes out of a very flat round base. The length is almost non-existent, only base exists. The measured basal width was found to be 1.57±0.0.01μM and length was calculated as 0.52±0.10μM. The tip of these sensilla is blunt and porous (Fig. 7a & b).Sensilla styloconica type-3 (SS3) has round flexible-base and present in a pair side by side at the periphery of maxillary sensory complex. The base is wider and gradually tapers down to the apex. The surface of this kind of sensilla is grooved. These are 1.73±0.037 μM long and 1.12±0.02 μM wide at the base (Fig.7a&b).Sensilla styloconica type-4 (SS4) is smaller than SS3 and present singly in the maxillary sensory complex. These sensilla have almost the same diameter from base to tip and end in unique bifurcated porous tip. These are 1.52±0.01 μM long and 1.44±0.02 μM wide at the base (Fig.7a & b).

Sensilla coeloconica-1 (SCo1)

These are very small nipple like projection coming out from one pit. They are also present along the periphery of maxillary sensory complex. From base to the apex of the sensilla has same diameter, smooth surface and finishes in porous tip. It measures 0.91±0.02μM in length and 0.75±0.03μM in basal width (Fig.7a&b).

3.2.2 Sensilla of labium and labial-palpi sensory complex

The numbers of sensilla are very few in number in labium. One sensilla trichoidea and one sensilla chaetica are found attached to the basal segments; all other sensilla are found at the tip of labial palpi known as labial palpi sensory complex. The sensory complex of labial palpi is also present as twin structure just like maxillary palpi sensory complex (Fig. 8a).Various kinds of basiconic sensilla, sensilla styloconica and sensilla coeloconica are present in labial palpi sensory complex. The types of basiconic sensilla that are coinciding in the maxillary palpi sensory complex and labial palpi sensory complex are SB2, SB3, SB4 and SB5. All these four kinds of sensilla are found singly.

Four unique kinds of sensilla present at the tip of the labial palpi and those were identified as Sensilla styloconica type-5 (SS5), Sensilla styloconica type-6 (SS6), Sensilla styloconica type-7 (SS7) and one sensilla coeloconica type-2 (SCo2).

Sensilla styloconica type-5 (SS5)

Sensilla styloconica type-5 is a robust sensilla in the middle of the sensory complex. The base is wide and arises from round elevated base ending in blunt round tip. These sensilla bear grooved surface and measures

2.44±0.01 µM in length and 2.08±0.05 µM in width (Fig. 8b).

Sensilla styloconica type-6 (SS6)

It is a nipple-like sensilla with very wide base and at the tip there is a small protuberance which is a distinguishably nipple-like structure arising from a round socket. These are 1.4±0.01 µM long and 1.00±0.01 µM wide at the base with smooth surface and pointed tip (Fig. 8b).

Sensilla styloconica type-7 (SS7)

These are finger-like smooth surface containing sensilla thattapers to a round blunt tip. They are inserted in a flexible round socket and present singly at the periphery of labial sensory complex. The length and basal width of these kind of sensilla have been found to be 1.47 ±0.04 µM and 0.75±0.025 µM (Fig. 8b).

Sensilla styloconica type-2 (SCo2)

These sensilla are inserted in a pit formed by raised cuticle and distributed in a pair at the periphery of labial-sensory complex. These are very short and thick measuring 1.92±0.05 µM in length and 1.63±0.011 µM in basal width. The wall of these sensilla is very smooth but ends with a porous tip (Fig. 8b).

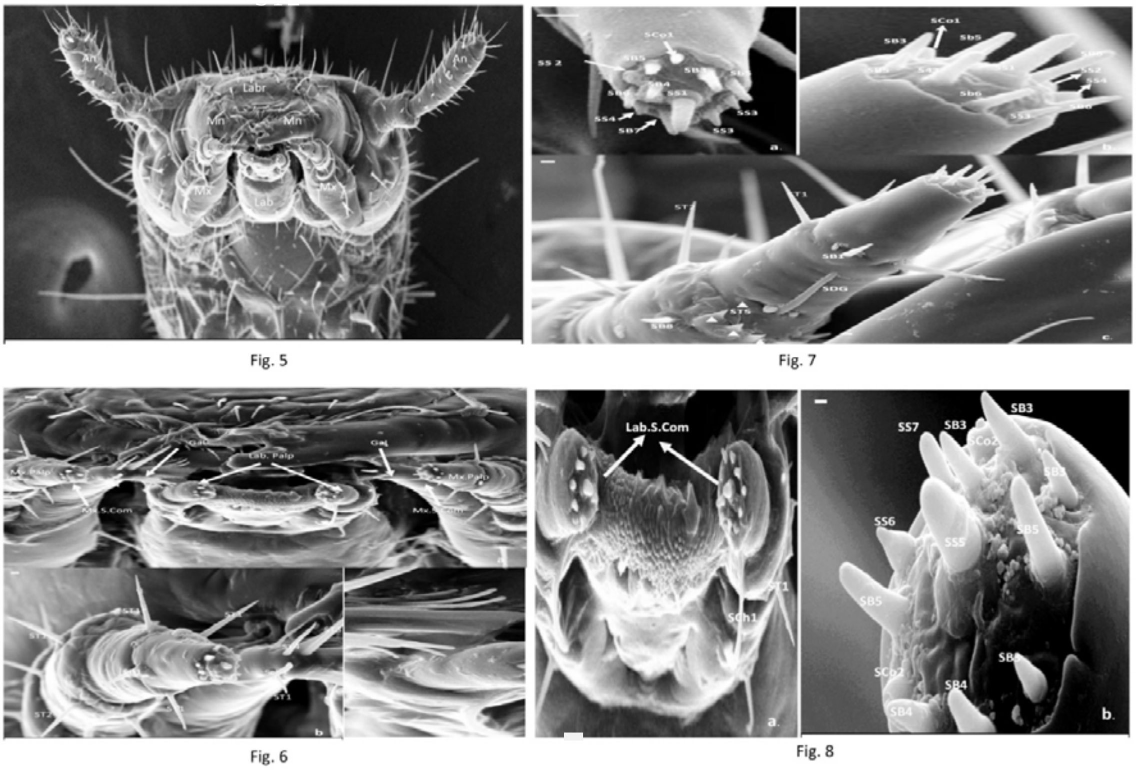

Fig. 5: Head complex of larval *O. Surinamensis* showing different subparts of chewing mouth parts and antennae. a. Labr-labrum, Max-Maxilla, Mn-Mandible, Lab-Labium, an-Antenna. **Fig. 6:** Mouth part detailed structure. a. Maxillolabial complex sub-parts . Mx.palp: Maxillary palp, Lab.palp:Labial palp, Mx.S.Complex: Maxillary sensory complex, Lab.S.Complex: Labial sensory complex, Gal: Galea of Maxilla. b. Close up of maxilla, ST1-ST4: Sensilla trichidea type1 to type-4. SB1: Sensilla basiconica type-1. SB2: Sensilla basiconica type-2. Scale bars: 2µM, 2µM and 2µM. **Fig. 7:** Maxillary sensory complex and different segments of maxillary palp.a. Maxillary palp sensory complex Top view, b. Maxillary palp sensory complex side view, c. Sensila located in the ventral parts parts of maxilla. SB2-SB8: Sensilla basiconica type-2 to type-8. SS1-SS4: Sensilla stylloconica type-1 to type-4, SDG: Sensilla digitiformia, Sensilla Coeloconica-1, Scale bars: 3 µM and 2 µM. **Fig. 8:** Labial sensory complex and associated sensilla, a. labial palp sensory complex at the tip of labium, b. Close up of labial palpi sensory complex showing the sensilla at higher magnification. Lab.S.Complex: Labial sensory complex, Sch1- Sensilla chaeticatype-1, SB2-SB7: Sensilla basiconica type -2 to type-7, SS5-SS6: Sensilla stylloconica type -5 t and type-6. SCo2: Sensilla coeloconica type-2. Scale bar; 400 NM OR 0.4 µM.

4 Discussion

In this study, morphology, structure as well as the distribution pattern of the sensilla found on antennae, maxillary and labial palpi of the larval *O. surinamensis* was mainly focused. Coleopteran larvae have been found to inhabit different trophic niche yet they have uniform topography and sensilla distribution (Alekseev et al., 2005). Most stored grain pest depend on chemical communication for detection, infestation and ingestion of food (Oehlschlager et al., 1988).

In this current study, seventeen different types of sensilla,including six types trichoidea, seven types of basiconica, two types of chaetica, one type of styloconica and coeloconica were identified in the antenna of the larvae by scanning electron microscope. The sensilla of the antenna are in direct contact with the environment and they are considered to be very important for survival of any insect (Nakanishi et al., 2009). The antennae of larval saw toothed grain beetle are found to be shortened by the reduction of the terminal segment, which is very similar to the finding of Alekseev et al. (2005) in the larvae of some beetle families. The main group of antennal sensilla found in the current study are sensilla trichoidea, sensilla basiconica and coeloconica is very much in close magnitude with the report of other Coleopteran larvae (Alekseev et al., 2005). The beetle larva always have uniform topography of sensory structures although beetle inhabit different trophic niche (Alekseev et al., 2005). The apical segment contains sensilla trichoidea, sensilla basiconica and sensilla chaetica as the most abundant types in addition to one coeloconic and one stylloconic sensilla and this observation in our current study is very much similar to the findings of Roppel et al. (1972) where combination of basiconic and trichoid sensilla was found to be present at the terminal segment and most abundant also .If all the segments of antenna is considered simultaneously, The most abundant type of sensilla was found to be sensilla trichoidea in which first two sub-types i.e. ST1 and ST-2 were located on all the segments and last two sub-types ST-3 and ST-4 are located on the terminal segment only. The external texture, sharp tips and aporous nature indicate that the first two types of Sensilla trichoidea act as mechanoreceptors in these insect (Schneider and Steinbrecht, 1968; Keil, 1997). The location of the last two types of sensilla trichoidea only in the apical segment suggests their probable role in sensing olfactory cues (Yang et al., 2009). Hamilton et al.(1999) have reported that a lady bird beetle, *Hippodamia convergens* showed no response to the olfactory cues due to the removal of sensilla trichoidea which conclusively proved their role in olfactory reception. The leaf-shaped ST5 and ST6 are presented on the tips of the distal segment of antenna and may have role in controlling the curvature and sense movement (Dai et al., 1990).

Second most abundant and most diverse kind of sensilla that were found in the antenna is the basiconic sensilla. Seven different kinds of basiconic sensilla have been noticed in the antenna. These are blunter and much shorter than the trichoid sensilla which are very in line with the study of Shields (2004). Basiconic sensilla containg all shapes and surface features have been found and those are cone shaped, peg-shaped, straight, curved, smooth walled or rough walled. Apical and sub-apical segments contain maximum number and type of Basiconic sensilla. Morphologically sensilla found over here are very similar to the sensilla basiconica encountered in beetles elsewhere (Ren et al. 2012, Yi et al. 2016, Lili et al., 2015). Basiconic sensilla mainly act as contact chemoreceptor or as odor receptor (Dethier, 1955). *Phoracantha semipunctata* (Coleoptera: Cerambycidae) were found to be very much adjusted to the volatile substances of the host plant only by the active participation of basiconic sensilla (Lopes et al., 2002). According to Ali et al. (2016), the basiconic sensilla of the last three segments of *Tribolium castaneum* take major part in locating food and removal of those segments leads to impaired food choice. Therefore, according to the current study it can be concluded that sensilla trichoidea and sensilla basiconica represent principal repertoire of olfactory sensilla. The two types of sensilla chaetica which are hair like long shafts also restricted to the terminal segments in the larval of *O.surinamensis*. Sensilla chaetica are significantly longer compared to other types of sensilla present on the lateral part of antennal segments. This kind of longer sensilla chaetica has also been reported to be present in the antenna of pollen beetle *Meligethes* (Odonthogethes) (Li et al., 2021).The location of these sensilla to the tip of the antenna indicates that they may have contact-chemoreceptive function (Ryan, 2002). Isidoro (1998) have demonstrated similarly that in *Psylliodes chrysocephala* sensilla chaetica act as contact chemoreceptor and responded to the plant chemical waxes.

Only one sensilla styloconica is present in the terminal segment of antenna which comes out from a round flexible base. The rough surface indicates its probable role in chemoreception. The chemosensory and gustatory role played by sensilla styloconica was demonstrated clearly by Schoonhoven and Dethier (1966) when it was noticed that these sensilla were responding to water,salt, glucose and sucrose concentration. These may also function as thermo-hygroreceptors (Schoonhoven, 1967).

Single coeloconic sensillum in the cavity was identified at the terminal segment only. These receptors are embedded in a cavity in which odorescent molecules are trapped and hence they perform the act of olfactory receptor,CO_2 receptor. Similar type of coeloconic sensilla with a cavity was also registered in another stored grain beetle larva, *Tribolium castaneum* (Coleoptera: Tenebrionidae) (Ryan and Behan, 1973).

Sensilla on maxillary and labial palps are directly involved in the detection and in the evaluation of the quality of the feeding resources, through both tactile (Keil, 1997; Chapman, 1998) and chemical receptors (Steinbrecht, 1996; Liu et al., 2011). Indeed, the variety of sensilla, especially chemoreceptors, that has often been observed on the palpi of larvae and adult stages of insects enable them to perceive several types of chemicals from both plants and feeding sources (Ma, 1972; Nagnan-LeMeillour et al., 2000; Tang et al., 2014).

The successive larval instars of *O. surinamensis* do not differ too much other than the size. Therefore, fourth larval instar sensilla of maxilla-labial complex are demonstrated in the present study. The different varieties and number of sensilla are mainly located in the maxillary and labial palpi. Similar type of sensilla distribution pattern have been portrayed in so many larvae of beetles belonging to the families of Silvanidae (Roppel et al., 1972; Mitchell et al., 1979), Chrysomelidae (Mitchell et al., 1979; Bartlet et al., 1999), Elateridae (Zacharuk, 1971; Corbière-Tichanè 1973), and Tenebrionidae (Ryan and Behan, 1973).The sensory organs found in the maxillary palpi and labial palpi of this beetle larvae are clustered at the tip of the apical segment which similar to the sensilla found in Dytiscidae and Dermestidae of Coleoptera (Alekseevet al., 2005).

In the current study a total of 23sub-types of sensilla have been encountered in the maxillo-labial complex of mouth parts and those are five types of sensilla trichoidea, eight types of sensilla basiconica, seven types of sensilla styloconica, two types sensilla coeloconica, one type of sensilla digitiformia. Excepting a few types of sensilla trichoidea, basiconica and digitiformia most of the sensilla are found in sensory complexes of the maxilla and labium. The location of the sensilla trichoidea in basal few segments of maxilla and labium, their socket structure and surface texture might indicate mechano-sensory function in the mouth parts. Seo and Youn (2000) have clearly demonstrated that sensilla trichoidea play the role of mechano-receptors in *Hippodamia variegate* (Coleoptera: Coccinellidae). The leaf-like sensilla trichoidea (ST-5) found at the junction of third and fourth maxillary palpi segments may act as sensor of movement (Dai et al., 1990). Only SB1 and SB-2 are located in the basal or other segments. The pointed tip and socket nature of SB1 dedicate their mechano-receptive function. The galea of maxilla have dense tuft of long,curve sensilla basiconica type-2 containing smooth wall suggesting their probable role in gustatory and olfactory reception. Exactly similar type of basiconic sensilla have been reported to be present in the galae of maxilla in another stored grain beetle, *Rhyzopertha dominica* F. (Coleoptera: Bostrichidae) (Seada and Hamza, 2023). A single long rope like sensilla digitiformia was noted at the ventero-lateral surface of maxillary-palpi. It was found to be attached in the groove of the cuticle (Fig. 7c). Honomichl and Guse (1981) have described it as an aporous hair–like structure inserted in a canal of cuticle being attached to a superficial groove on the maxillary palpi. The functions of sensilla digitiformia still not clear as different roles were recommended for them by various authors. The assigned roles performed by sensilla digitiformia as per a range of reports are thermoreceptors, hygroreceptors (Honomichl and Guse, 1981), CO_2 receptors (White et al., 1974) and mechanoreceptor (Zacharuk et al., 1977).

In both the maxillary and labial palpi sensory complex, a unit 13 sensilla were identified in which always there are thick central sensilla encircled by other sensilla. This wheel-like structure of maxillary and labial palpi sensory complex is matching exactly with that of other Coleopteran beetle larval maxillo-labial sensory complex sensilla structure (Alekseev et al., 2005). As per several earlier reports, the apical sensilla present at the terminal ends of maxillary and apical palpi perform the roles of gustatory and olfactory receptors which help them to find host and mate (Zacharuk, 1985; Cui et al., 2023).

The sensilla basiconica (SB3-SB7) present in the sensory complexes of maxillary and labial palpi seem to perform the role of contact chemoreceptor due to their round, flat, blunt and porous nature (Lopes et al., 2002; Zacharuk 1985).Similarly, seven types sensilla styloconica were noticed in the maxillary and labial palpi. These sensilla have distinctive flexible round socket, short shaft, blunt or flat tip, cylinder, globe, finger shaped and grooved surface and as such comparable to sensilla basiconica which designates their role in olfactory reception. Similar finger-like, globe-like or cylindrical sensilla styloconica have also been observed in other stored grain pest larva like *Tribolium confusum* and *Tribolium castaneum* (Ryan and Behan, 1973). The contact chemoreceptor and gustatory function of sensilla styloconica has been demonstrated conclusively in several earlier publications (Zacharuk, 1985; Steinbrecht, 1996; Lopes et al., 2002).

Sensilla coeloconica appear like small nipple like structure with an aperture at the tip of them. These are peg like projections which remain completely embedded in cuticular structure. Similar types of sensilla coeloconica were also noted in the *Tenebrio molitor* larvae and Hygrobiidae and Dytiscidae larva of beetle (Ruschioni et al., 2019; Jager et al., 2024). As per the earlier reports sensilla coeloconica perform the task of thermo-and hygroreceptors. (Altner et al., 1981; Ruchty et al., 2009).The sensory complexes at the tip of both maxilla and labium consist of mainly Sensilla basiconica and styloconica and one or two sensilla coeloconica.

The sensory complex present on the maxillary palps bears five types of sensilla basiconica (SB3-SB7), four types of sensilla styloconica (SS1-SS4) and one type sensilla coeloconica. Whereas in labial palpi only three types of sensilla basiconica is found (SB3-SB5), SB6 and SB7 are lacking in them. Regarding sensilla styloconica from type-1 (SS1) to type-4(SS4) all these type are absent in labial palpi. On the contrary, three additional types of sensilla styloconica from type-5 to type-7 are found and instead of sensilla coeloconica type-1, type-2 is found to be present at the labial palpi. Both sensilla basiconica and styloconica comprising the sensory complexes perform the task together as contact chemoreceptors and this can be concluded for the pattern of their distribution and shape (Farazmand and Chaika, 2008; Alekseev et al., 2005; Giglio et al., 2013).The differences of the number and type of sensilla that are found among the different insects are due to their different food habit and habitat (Giglio et al., 2012).

This study which is the first report of antennary and mouth part sensilla of the larval *O. surinamensis* L. has shown the ultra-structural details of all the sensilla that can pave the way for the future electrophysiological and behavioral investigations in this very important stored grain pest found in the tropical countries.

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