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Surface ultrastructure of mouthparts in predatory bugs *Andrallus Spinidens* (F.) and *Amyotea Malabarica* (F.) (Pentatomidae: Heteroptera)

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Abstract
To better understand feeding adaptations, the fine structure of the mouthparts in *Andrallus spinidens* (F) and *Amyotea malabarica* (F) (Pentatomidae: Heteroptera) were studied using scanning electron microscopy. Above studied species from subfamily Asopinae attack not only on soft body insect pests but other adult insect species also and controlling pest population in crop fields naturally. Piercing and sucking type of mouthparts in studied bugs, consists of labrum, labium, a pair of mandibles and a pair of maxillae. There are nine types of sensilla present on the mouthparts of the predatory bug. These are sensilla basiconica (SB I, SB II, SB III, SB IV), sensilla trichodea (ST I, ST II, ST III), sensilla trichodea curvata (STC), sensilla styloconica (SST). All the sensilla present on the mouthparts play an important role in predatory success. Each mandibular stylet tip has three long pointed hooks and five irregular teeth help in tear tissue and maxillary stylet has food canal and salivary canal.

Keywords: Andrallus spinidens, Amyotea malabarica, mouthparts, sensilla, scanning electron microscopy

Introduction

Heteroptera is one of the most abundant groups of order Hemiptera. The Heteroptera includes a diverse assemblage of insects that have become adapted to a broad range of habitats - terrestrial, aquatic and semi-aquatic. Heteropteran bugs are serious crop pest and valuable predators (Carver *et al*., 1991; Schaefer and Panizzi, 2000 and Triplehorn and Johnson, 2005) [7, 20, 24]. Pentatomidae is one of the most important family in the Heteroptera distributed worldwide. These bugs are phytophagous feed on plant tissue and some species especially from subfamily Asopinae are natural enemies, feed on agriculture pest mainly slow-moving soft bodies insect like larvae of Lepidoptera and Coleoptera so, these species used as biocontrol agent against agriculture pest (De Clercq, 2000) [10]. Asopinae predatory bugs distributed all over the world with 357 species belonging 63 genera (Thomas, 1992, 1994; Grazia *et al*., 2015) [13, 22, 23]. These insects have elongated, piercing-sucking mouthparts which arise from the ventral (hypognathous) or anterior (prognathous) part of the head capsule (Richards and Davies, 1979) [18]. The mandibles and maxillae are long and thread-like, interlocking with one another to form a flexible feeding tube (proboscis) (Cohen, 1990) [9]. The bugs inject digestive enzymes through the mouthparts into the prey's body, and then suck up the digested and liquefied tissue. The mouthparts of hemipterans contain two channels, one through which is pumped salivary fluid into the organism and the other through which the fluids are sucked back into the insect. Both nymphs and adults are predatory in nature; they are voracious feeders and eat until their stomachs are full, and when shortage of food, they engage in cannibalism. (Schaefer, 1996; De Clercq, 2000) [10, 19]. Several prior studies reported on morphology of the mouthparts of Pentatomidae and specially focused on the general structure of mouthparts but only seven predatory Asopinae bugs were studied, *Canthecona furcellata* (Wolff) by Barsagade and Gathalkar (2016) [2], *Eocanthecona furcellata* (Wolff) by Rani (2009) [17] and Praveen *et al*, (2015), *Perillus bioculatus* (Fabricius) by Cobben (1978) [8] and Praveen *et al.*, (2015); *Podisus maculiventris* (Say) by Cohen (1990) [9]. *Picromerus bidens* (Linnaeus), *Picromerus lewisi* (Scott) *Cazira bhoutanica* (Shouteden) by Wang *et al.*, (2020) [25]. Prior research on *Andrallus spinidens* and *Amyotea malabarica* focus only on taxonomy and life cycle. There were no detail reports on mouthparts. Aim of this study to provide the first detailed fine morphological characterization of the mouthparts of two Asopinae bugs *A. spinidens* and *A. malabarica* using Scanning electron microscopy (SEM). To know role of the mouthparts structure play in their feeding process.

Materials and Methods

Scanning electron microscopy (SEM) studies were carried out in Sophisticated Analytical Instrument Facility (SAIF) of Indian Institution of Technology (IIT) Mumbai, India.
The mouthparts of *A. spinidens* and *A. malabarica* were dissected out and washed thoroughly with distilled water. Boil in 10 % aqueous KOH solution for 10 minutes. After boiling, the tissue was washed several times in distilled water to remove the traces of KOH. The materials were dehydrated in ascending order alcoholic grade, cleared in xylene and dried at room temperature. Then materials were mounted on carbon-coated metallic stubs and were coated with platinum coating in the JOEL coating unit. After coating materials were observed in a JOEL JSM- 7600 F.

Result and Observation

Mouthparts of *A. spinidens* and *A. malabarica* are piercing and sucking type like other heteropteran bugs that located on anteroventral side to clypeus of head. Mouthparts consist of triangular cone-shaped labrum, four segmented labium, a pair of mandible and a pair of maxilla. The two opposing maxilla and mandible that fit together to create a feeding canal and salivary canal. The mandible covered the maxilla on both lateral sides and forming a stylet that was enclosed inside the labial groove. (Fig.1 A).

Labrum

The labrum was elongated, triangular in shape. Anteriorly it was attached with clypeus. Anterior half was wide and smooth and distal half narrow and wrinkle. It partially inserted into the labial groove and it passed over the first segment of labium in both pentatomid bugs (Fig.1 B and 2B).

In *A. spinidens* and *A. malabarica* proximal end of the labrum was broad and distal end was narrow with rough surface. The rough surface consists of semicircle ridges with cuticular pores (Fig.1 C). The length of labrum was measured about 1.77 ± 0.025 mm and 0.22 ± 0.06 mm in width in *A. spinidens*. The length of *A. malabarica* was measured about 1.64 ± 0.05 mm and 0.18 ± 0.04 mm in width (Table 1). Labrum of both bugs *A. spinidens* and *A. malabarica* contains sensilla basiconica (SB).

Labium

The labium is long, arising from a head capsule and it was divided longitudinally up to the tip, A groove in the labium is used to house the maxillary and mandibular stylets. The labium is located in between the first and second pair of legs' coxae when the animal is at rest or non-feeding. The labium divides into first, second, third and fourth segments. Morphologically all these segments differ in size and shape. The first labial segment was short, uniform and broadest than other labial segment. Labial groove was very wide open enough to accommodate the labrum. Length and width of first segment of labium were measured about 1.08 ± 0.001 mm and 0.53 ± 0.04 mm respectively in *A. spinidens* while, in *A. malabarica* it measured approximately 1.01 ± 0.04 mm lengths and 0.44 ± 0.01 mm width (Table 1). In both studied species sensilla basiconica I present on this segment (SB I).

The second segment was the longest than remaining three segments, widened from base and then straight with smooth surface and base looked like a band between 1st and 2nd segments.

In *A. spinidens*, the second segment of labium was measured about 1.54 ± 0.005 mm in length and 0.40 ± 0.07 mm in width. In *A. malabarica*, it measured about 1.75 ± 0.015mm in length and 0.31 ± 0.013 mm in width. Four types of sensilla present on this segment in both species, these were sensilla basiconica SB I, SB II, SB III and SB IV. Three pairs of sensilla basiconica IV (SB-IV) present at the proximal end of the second segment in row (Fig. 1 E and 2 C).

In *A. spinidens*, third segment of labium is uniform and measured about 0.97 ± 0.006 mm in length and 0.45 ± 0.02 mm in width while, in *A. malabarica* it was measured about 0.833 ± 0.01 mm in length and 0.29 ± 0.02 mm in width (Table 1). In both species three type of sensilla present on this segment, sensilla basiconica SB I, SB II and SB III.

The fourth segment was conical in structure and shorter than the other three segments. Enormous numbers of sensilla were present on the entire segment and maximum number of sensilla found especially near the tip. A pair of sensilla basiconica present on the joint of the third and fourth segments. In *A. spinidens*, fourth segment of labium was measured about 0.97 ± 0.25 mm in length and 0.31 ± 0.05 mm in width while, in *A. malabarica* it was measured about 0.42 ± 0.08 mm in length and 0.17 ± 0.015 mm in width (Table 1). In both species seven types of sensilla were noted on these segments. These were sensilla trichodea ST I, ST II, ST III, sensilla trichodea curvata (STC), sensilla basiconica SB I, SBIII and sensilla styloconica (SST) (Fig. 1F and 2F).

Mandibles

The pair of mandibles are modified into long stylet and wrapped into the labium tube in both species. In *A. spinidens* and *A. malabarica* the mandibles are long and laterally show curvature with rows of transverse ridges (TR) on the outer surface. Tip of the mandible consists of three long, irregular, backwardly pointed sharp hooks (HO), with five irregular teeth (TO) in dorso-lateral position. Among them first and second teeth present at the anterior side, while third teeth present at mid-dorsally and fourth and fifth teeth present on dorsolateral side (Fig. 1I and 2H). Inner surface of the mandible has three longitudinal grooves. The first and third inner grooves of the mandible are smooth and middle groove with square shape texture (Fig. 2H). In *A. spinidens*, length of mandible was 4.7 ± 0.06 mm and width 0.036 ± 0.001mm. In *A. malabarica*, length of mandible was approximately 4.2 ± 0.12 mm and width 0.023 ± 0.04 mm (Table 1).

Maxillae

In both species *A. spinidens* and *A. malabarica* interlocked pair of maxilla was long, slender and pointed toward apex. Externally the maxillary surface was smooth while, internally a series of ridges and grooves were present. The right and left maxillary style were asymmetrical. These two opposing maxillae held together by grooves, they form salivary canal and food canal (Fig. 1J and 2I). In *A. spinidens* length and width of maxillary stylet were measured about 4.8 ± 0.15 mm and 0.046 ± 0.001 mm respectively. In *A. malabarica* length and width of mandible were measured about 4.4 ± 0.05 mm 0.034 ± 0.002 mm respectively (Table 1).

Types of sensilla present on mouthparts

Sensilla basiconica (SB)

Sensilla basiconica (SB) were the largest and widely spread all over labial segments of mouthparts, Four different types of sensilla basiconica were observed in both studied species SB-I, SB-II, SB-III and SB IV. SB-I was straight with blunt tip, longitudinal grooves on the surface, this sensilla inserted in a flexible, cylindrical socket. (Fig. 1G). SB-II was shorter than sensilla I and roughly oval in shape with blunt tip. SB-III was shorter than SB-II. It was oval in shape with a smooth surface; inserted in a flexible, cylindrical socket (Fig.1D and 2D) SB-IV was present in three pairs in the junction of first and second labial segments and one pair present in the junction of third and fourth labial segment. SB- IV shorter than SB II, smooth surface with flexible socket and pointed tip (Fig. 1E and 2C)

Sensilla trichodea (ST)

Sensilla trichodea (ST) were present on the fourth segment of labium. Four different types of Sensilla trichodea were present in both studied species ST- I, ST-II, ST-III and STC. ST-I was long, hair-like sensilla, inserted into a flexible socket. Walls of the sensilla were smooth with a pointed apex. ST-II was slender and similar in appearance to sensilla trichodea -I, but shorter than STI with a sharp tip and occurred almost all over the surface of the labial tip. Sensilla trichodea III was similar in appearance to sensilla trichodea II, but shorter than STII. Sensilla trichodea curvata (STC) was long, pointed and present at the periphery of the labium on both the dorsal and ventral regions. These are slightly bent at the tip region (Fig.2 F and G).

Sensilla Styloconica (SST).
Sensilla styloconica generally had a broad base and long stem with smooth cuticular walls and blunt tip. These sensilla found near the tip of the proboscis. (Fig. 1H and 2F). Length and width of all sensilla present on mouthparts of both studied species are given in Table 2.

Table 1: Length and width of mouthparts of A. spinidens and A. malabarica

Sr. No.	Mouthpart	Length (mm)		Width (mm)	
		A. spinidens	A. malabarica	A. spinidens	A. malabarica
1.	Labrum	1.77 ± 0.025	1.64 ± 0.05	0.22 ± 0.06	0.18 ± 0.04
2.	Labium	4.5 ± 1.83	4.0 ± 1.77	-	-
	Seg I	1.08 ± 0.001	1.01 ± 0.04	0.53 ± 0.04	0.44 ± 0.01
	Seg II	1.54 ± 0.005	1.75 ± 0.015	0.40 ± 0.07	0.31 ± 0.013
	Seg III	0.97 ± 0.006	0.833 ± 0.01	0.45 ± 0.02	0.29 ± 0.02
	Seg IV	0.97 ± 0.25	0.42 ± 0.18	0.31 ± 0.05	0.17 ± 0.015
3.	Mandible	4.7 ± 0.06	4.2 ± 0.12	0.036 ± 0.01	0.023 ± 0.01
4.	Maxilla	4.8 ± 0.15	4.4 ± 0.05	0.046 ± 0.001	0.034 ± 0.002

Table 2: Length and width of sensilla present on mouthparts of A. spinidens and A. malabarica

Sr. No.	Part of Mouthparts	Type of sensilla	Length (mm)		Width (µm)	
			A. spinidens	*A. malabarica*	*A. spinidens*	*A. malabarica*
1.	**Labrum**	SB	26.6 ± 0.05	25.38 ± 2.64	3.66 ± 0.03	4.80 ± 0.07
2.	**Labium**					
	Seg I	SB I	40.0 ± 2.02	19.64 ± 3.51	3.77 ± 0.45	4.46 ± 2.53
		SB II	15. 5 ± 3.25	10.7 ± 4.21	1.75 ± 0.032	4.20 ± 0.09
		SB III	8.80 ± 0.04	6.25 ± 0.21	1.5 ± 0.08	2.14 ± 0.012
	Seg II	SB I	52.3 ± 0.14	29.4 ± 2.57	4.40 ± 0.03	4.5 ± 0.05
		SB II	35.7 ± 0.02	19.64 ± 1.08	3.24 ± 0.05	3.9 ± 0.5
		SB III	12.9 ± 1.52	8.9 ± 1.20	2.38 ± 0.16	1.57 ± 0.016
		SB IV	11.9 ± 1.05	13.1 ± 0.05	4.76 ± 0.02	4.60 ± 0.065
3.	Seg III	SB I	38.0 ± 3.45	25.2 ± 2.55	3.77 ± 0.025	2.67 ± 0.055
		SB II	16.5 ± 1.85	17.8 ± 4.28	1.75 ± 0.42	2.83 ± 0.008
		SB III	7.34 ± 0.002	9.43 ± 0.023	1.27 ± 0.14	1.47 ± 0.002
4.	Seg IV	ST I	73.5 ± 7.45	73.9 ± 6.75	2.94 ± 0.02	3.12 ± 0.16
		ST II	43.1 ± 4.24	49.8 ± 3.22	2.6 ± 0.05	2.81 ± 0.27
		ST III	16.7 ± 0.04	25.0 ± 2.20	2.45 ± 0.23	2.60 ± 0.78
		STC	39.2 ± 0.15	58.3 ± 2.80	3.20 ±1.52	4.16 ± 0.52
		SB I	14.7 ± 4.53	34.3 ± 4.2	4.27 ± 1.55	3.12 ± 0.05
		SB III	11.7 ± 3.11	9.37 ± 0.02	2.15 ± 0.22	2.08 ± 0.08
		Styloconica	19.14 ± 0.06	17.5 ± 0.08	8.51 ± 0.23	7.4 ± 0.02
SB –Sensilla basiconica, ST- Sensilla trichodea and STC- Sensilla trichodea curvata.						

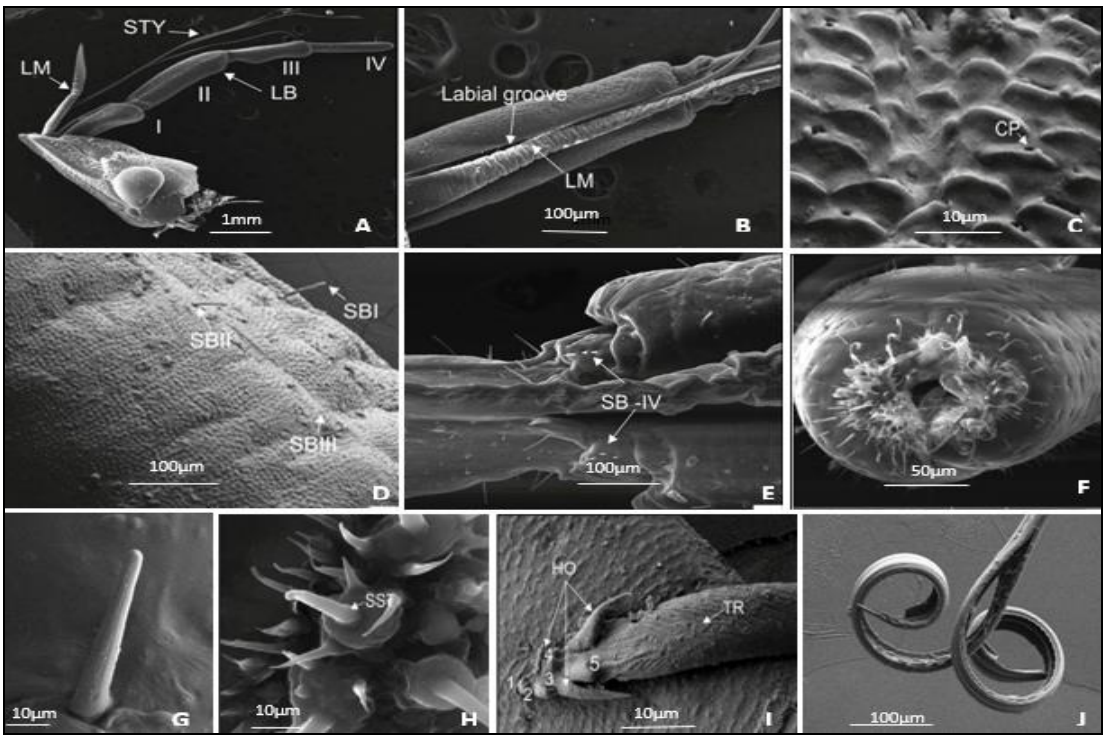

Fig 1: Scanning electron microscopic (SEM) structure of mouthparts of *Andrallus spinidens.*

(A) Ventrolateral view of mouthparts showing labrum, four segmented labium and stylet,(B) Ventral view of mouthpart showing Labrum and labial groove, (C) Labrum showing cuticular pore, (D) First labial segment showing sensilla basiconica (SBI, SBII, SBIII), (E) Three pair of sensilla basiconica IV present on the junction of labial segment 1st and 2nd, (F) Tip of fourth labial segment (G) Sensilla basiconica I, (H) Sensilla styloconica (I) Mandibular stylet showing hooks, teeth, and transverse ridges (J) Maxillary stylet. LM-Labrum, LB- Labium, STY- Stylet, SB- Sensilla basiconica, CP-Cuticular pore, SST- Sensilla styloconica, HO- Hooks, TR- Transverse ridges.

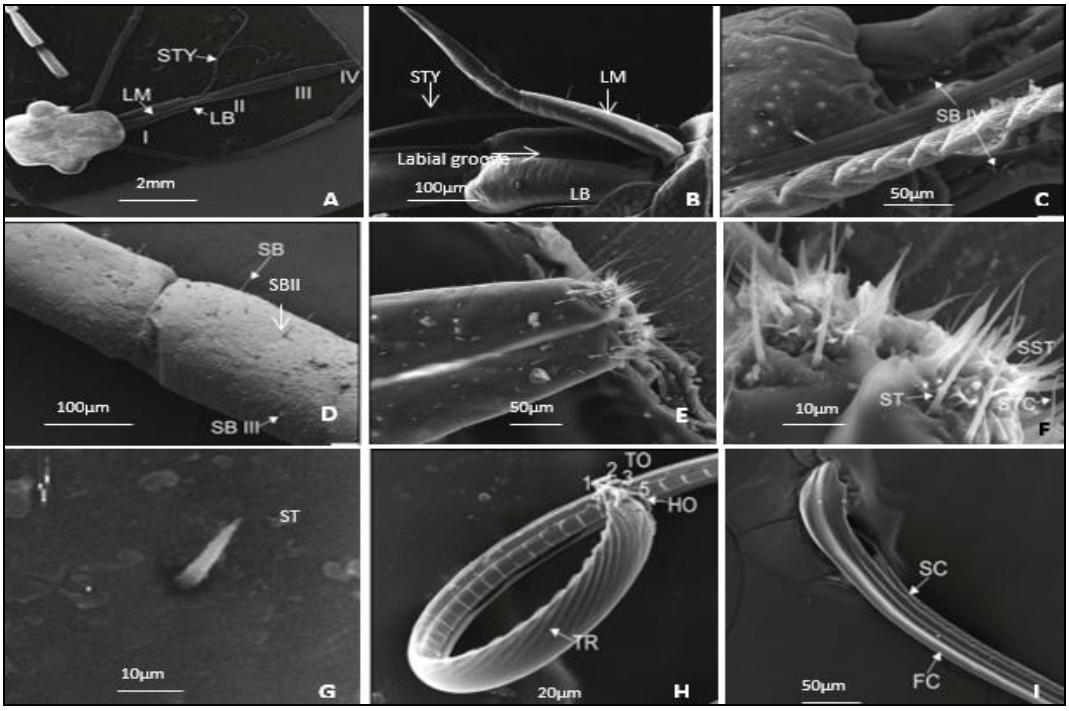

Fig 2: Scanning electron microscopic (SEM) structure of mouthparts of *Amyotea malabarica.*

(A) Ventral view of mouthparts show labrum, four segmented labium and stylet,(B) Latral view of mouthparts showing Labrum and labial groove, (C) Three pair of sensilla basiconica IV present on the junction of labial segment 1st and 2nd, (D) Second and third labial segments showing sensilla basiconica (SBI, SBII, SBIII), (E) Fourth labial segment showing different type of sensillae (H) Tip of fourth labial segment showing Sensilla styloconica, Sensilla trichodea and sensilla trichodea curvata (I) Mandibular stylet showing hooks, teeth, and transverse ridges (J) Maxillary stylet. LM-Labrum, LB- Labium, STY- Stylet, ST- Sensilla trichodea, STC- Sensilla trichodea curvata, SB- Sensilla basiconica, CP-Cuticular pore, SST- Sensilla styloconica, HO- Hooks, TR- Transverse ridges, SC- Salivary canal, FC- Food Canal.

Discussion

The mouthparts of *A. spinidens* and *A. malabarica* were piercing and sucking types present on the antero-ventral side of the head. The labium (lower lip) was a long-modified rostrum of a Heteroptera consisting of a four-segmented and enclosing two pairs of the long maxillary and mandibular stylets as found in other true bugs (Schuh and Slater, 1995) [21]. Similarly, the maxillary stylet was on the inner side and mandibular stylet at the outer side. The maxilla and mandible modified into long needle like slender stylet that fit together and forming a food canal for taking liquid food and salivary canal for deliver saliva, this structure is also called as proboscis (Cobben, 1978; Boyd *et al*., 2002; Boyd, 2003) [5, 6, 8]. The narrow, triangular labrum (upper lip) present at the base of labium anteriorly attached with clypeus and posterior end was narrow partially inserted into the labial groove and pass over first segment of labium in both pentatomid bugs found similar with previous study on predatory bugs *Canthecona furcellata* (Wolff) (Barsagade and Gathalkar, 2016) [2]. The long labrum found in *A. spinidens* and *A. malabarica* may be helpful in these predatory bugs to grip the proximal portion of the stylets together during feeding as noticed by Wang *et al.,* (2020) [25] [25]. The labrum surface of *A. spinidens* and *A. malabarica* rough consist of semicircular ridges with cuticular pores similar to study in seed bugs (Wang *et al.,* 2017) [26].

The labium of *A. spinidens* and *A. malabarica* were four segmented as like other heteropteran bugs (Barsagade and Gathalkar 2016; Wang *et al.,* 2017) [2, 26]. The length of the first segment was shorter than the second segment and width of this segment thickened than other segments in both species. This enables the proboscis to swing forward and make it easier for predation as noticed by De Clercq (2000) [10].

Similarly, the second segment was longest and widened from base and then straight with smooth surface and looked like a band between first and second segments. Similar type of result reported in previous studies of three predatory stink bugs of family Asopinae (Wang *et al*., 2020) [25]. Tip of the labium is shorter than the other segment and contains a number of sensilla, in *A. spinidens* and *A. malabarica.*

During present study, it has been observed that tip of mandible of *A. spinidens* and *A. malabarica* consist of five irregular teeth (TO) where first and second teeth present on anterior side, third teeth present on mid-dorsal side while, fourth and fifth teeth present on dorsolateral side with three longs, irregular, sharp pointed hooks (HO) similar to earlier worker (Wang *et al*., 2020) [25]. According to Faucheux

(1975) [11], in heteropteran bugs, the number of mandibular teeth ranges from 4 to 40 but in phytophagous bugs contains significantly lower and less variable number of teeth. Cobben (1978) [8], reported that the predatory pentatomid bug *Perillus bioculatus* (Fabricius) uses their irregular teeth and sharp recurved hooks as a piercing, scratching or filing device that helps in the mechanical disruption of tissues. The mandibular structures of predatory stink bug differ from seed bug *Pyrrhocoris sibiricus* in the position and number of teeth (Wang *et al*., 2017) [26]. In the present study of predatory pentatomid bugs shows presence of pointed and sharp recurved hooks on the mandible. Similarly, in *A. spinidens* and *A. malabarica* interlocked pair of maxillae observed long, slender and pointed toward apex. These two opposing maxillae are held together by grooves and form salivary canal and food canal. Interestingly the tip of the right and left maxillary stylet was asymmetrical in both predatory bugs which may be helping in the feeding mechanism as observed earlier (Cobben, 1978; Cohhen, 1990) [8].

Different nine types of sensilla found on four segments of the labium in *A. spinidens* and *A. malabarica.* The basic shapes of sensilla present on labium were morphologically similar to sensilla observed in other heteropteran bugs such as *Canthecona furcellata* (Barsagade and Gathalkar, 2016) [2], in *Eocanthecona furcellata* (Rani *et al.,* 1994) [16] and in *Perillus bioculatus* and *Eocanthecona furcellata* (Parveen *et al*., 2015) [15]. It may be performing chemosensory, mechanosensory and thermo-hygrosensitive functions like other heteropteran bugs (Cobben, 1978; Brozek, 2013; Rani *et al*, 1994) [3, 8, 16].

Labrums of *A. spinidens* and *A. malabarica* consist of sensilla basiconica (SB) with flexible socket and blunt tip similar to sensilla observed in three predatory bugs (Wang *et al*. 2020) [25]. Sensilla basiconica was present on labrum as well as four segmented labia in the form of four sub-types, SB I, SB II, SB III and SB IV. These sensilla widely spread all over labial segments. Morphologically it contains a flexible, cylindrical socket and blunt tip. The presence of flexible socket is characteristic of mechanosensilla (McIver, 1975; Altner and Prillinger, 1980; Frazier, 1985) [1, 12, 14], Three pairs of sensilla basiconica (SB IV) were present in row on the junction between the first and second labial segment and one pair of it between the third and fourth labial segment, Similar type of sensilla was present in other heteropterans such as in *Pyrrhocoris sibiricus* (Wang *et al*., 2017) [26] and in three predatory stink bugs from Asopinae (Wang *et al*., 2020) [25].

In *A. spinidens* and *A. malabarica* the sensilla trichodea I was smooth in surface and inserted in a flexible socket observed on the tip of the labium. The sensilla trichodea II was morphologically similar to ST I but shorter in length as similar to other heteropterans bugs (Rani *et al.,* 1994; Barsagade and Gathalkar, 2016) [2, 16]. According to Parveen *et al*., (2015) [15], sensilla trichodea were most abundant near the labial tip in such a way that they make first contact with the substratum during feeding and are mechanosensilla nature also noted in other bugs (Altner and Prillinger, 1980; Frazier *et al.,* 1985) [1, 12]. The sensilla trichodea present on the tip of the labium in *A. spinidens* and *A. malabarica* may work as mechanosensilla to sense the host body during feeding.

In present study, both predatory bug species shown sensilla styloconica (SST) on the tip of labium, similar type of sensilla reported previous in *Perillus bioculatus* and *Eocanthecona furcellata* on the labium tip (Parveen *et al*., 2015) [15]. This sensilla was described as sensilla basiconica type A by Rani *et al.,* (1995) [16], and sensilla basiconica (SB I) in *Canthecona furcellata* by Barsagade and Gathalkar, (2016) [2]. According to Parveen *et al*., (2015) [15], predatory pentatomid bugs having two types of chemoreceptive sensilla styloconica and peg on the tip of the labium may have enhanced ability to sense prey diversity than phytophagous bugs. During the present study, sensilla styloconica, abundant on the labial tip of *A. spinidens* and *A. malabarica* that may be showing chemoreceptive function.

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