SCANNING ELECTRON MICROSCOPY OF ANTENNAE OF *COCCINELLA SEPTEMPUNCTATA* (COCCINELLIDAE: COLEOPTERA)

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Abstract Scanning electron microscopy was done to describe the morphology of antenna of adult male and female ladybeetle, *Coccinella septempunctata*. Eleven-segmented, scaly antenna was 950 μm in male and 980 μm in female. Electron micrographs reveal the occurrence of eight types of sensillae, *viz*. chaetica (Ch), trichoidea (Tr), basiconica (Ba), campaniformia (Ca), ampucellaceous (Am), scolopalia (Sc), placoidea (Pl) and hook shaped sensilla on the antennae of male and female ladybeetles. Ch had long external-process, with base surrounded by membranous socket and the length was 60 and 70 μm in male and female ladybeetle, respectively. Sensillae Tr were distally curved and inserted into depression, 9.0 and 15 μm long in male and female ladybeetle, respectively. Sensillae Ba were cuticular peg-like and 0.57 and 0.70 μm long on the male and female antennae. Sensillae Ca were small dome-like and had diameter of 3.0μm in both the sexes. Sensillae Am were pit-like in appearance and the diameter of the pit was 1.2 and 1.5 in male and female ladybeetle, respectively. Sensillae Pl were elliptical sunken plates like in both the sexes. Sensillae Sc were broad at their apex and pointed at their tip, 2.5 μm. A single hook-shaped sensilla of 21.0 μm in length was present on male antenna only. Ch, Ca, Am, and Pl were almost similar in numbers in both the sexes. Sensillae Tr were more numerous on the male antenna and Ba were more numerous on the female antenna.

Key words Coccinellidae, ladybeetle, *Coccinella septempunctata*, scanning, antenna, sensillae.

Abbreviations: Scanning Electron Micrposcopy (SEM), Chaetica (Ch), Trichoidea (Tr), Basiconica (Ba), Campaniformia (Ca), Ampucellaceous (Am), Scolopalia (Sc), Placoidea (Pl)

1 INTRODUCTION

Antennae in insects are organs of taste, smell and stimulation (Wigglesworth 1972). They serve as sensory structures, as claspers in grasping prey and taxonomic placement in certain instances, and also help in sex differentiation, as in mosquitoes. The antennae also play kinetic roles and normally keep the nervous system in a state of tone in which it responds to stimuli of all kinds. Antennae of insects vary greatly in length, overall size, size of the individual segments, segmentation, setation and other aspects with the structures being closely related to their function.

Coccinellids (Coleoptera) are one of the most popular, attractive, and economically important biocontrol agents of a number of insect pests. *Coccinella septempunctata* Linnaeus has very wide geographical distribution and is a potent predator of aphids (Omkar and Srivastava 2001a). Despite its popularity and usefulness, there is very little information about its sense organs. Scanning electron microscopy (SEM) may reveal the desired information on the subject of investigation. Though, extensive SEM studies were made on the antennae of lepidopterans (Yponomeutidae) (Cuperus 1983) and some coleopterans (Cerambycidae, Curculionidae, Carabidae, Elateridae, Anobiidae) (Dyer and Sea-

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brook 1975, Isidoro and Solinas 1992, Kim and Yamasaki 1996, Merivee 1992; Okada *et al.*, 1992); there appear to be very little studies on the antennae of coccinellids. However, SEM studies of mouth-parts of the adult *C. septempunctata bruckii* in relation to feeding behaviour (Niijima *et al.* 1987), differences in antennal sensillae of male and female ladybeetle, *Semiadalia undecimnotata* (Jourdon *et al.* 1995), *Coccinella transversalis* (James 2001), *Propylea dissecta* (Pervez 2002) and *Pseudoscymnus tsugae* (Broeckling and Salom 2003) have been made. Antennae being one of the prime sensory organs, such studies may reveal differences in the antennal structure, types and number of sensillae, which may be indicative of their differential function. Hence, an attempt was made to conduct SEM investigations of the antennae of both sexes of *C. septempunctata* in order to mark sex specific differences in the antennal sensillae and to understand probable roles of the various sensillae of male and female ladybirds.

2 MATERIALS AND METHODS

Laboratory emerged, 15-day-old male and female *C. septempunctata* were used in the study. The antennae of freshly anaesthetized male and female were dissected out, dehydrated and fixed in glutraldehyde (2.5%) (v/v) with phosphate buffer (pH 7.4) for 12 hours at 40C. The fixed antennae were washed with phosphate buffer (pH 7.4) thrice by dipping for 20 minutes each. The antennae were re-washed 3-4 times with distilled water and then dehydrated by passing through a series of ethanol, *viz.* 30, 50, 70 and 90, (10 minutes in each). They were further dehydrated in 100% ethanol by giving two changes for 20 minutes each. After dehydration, the materials were cleared by treating in pure acetone (20 minutes each). The specimens were then allowed to air-dry and mounted on bronze stubs with the help of carbon adhesive tape with desired face of antennae upward. The mounted antennae were allowed to dry for three hours. Gold coating of antennae was done by ion sputter. Discharge at 1.2 KV and 7 mA was preferred for five minutes as it gives a finer and more uniform film. Antennae of both the sexes were photographed under Scanning Electron Microscope for the surface ultrastructure of the samples at accelerating voltage of 5 KV with 15 WD at different magnifications. Six specimens of both the sexes were observed.

The morphological feature and length of antennae in both sexes were recorded from scanning electron micrographs. The sensory structures found were named following the terminology of Faucheus(1985) and Jourdon *et al.*(1995). Number of each type of sensilla was counted along the length of the antennae. Average length and basal diameter of each sensilla were obtained from micrographs.

3 OBSERVATION AND DISCUSSION

3.1 General sensory apparatus of antennae

The antennae of male and female ladybeetles were located in front of the eyes at the dorso-lateral corners of the frons and their gross morphology were similar. They consist of eleven annuli; the scape, the pedicel and a flagellum of nine annuli in both the sexes. The annuli were cylindrical in shape and generally the length decreased near distal end of antennae (Fig. 1a, b). The length of female antenna (9720.00 ± 9.57 μm) was slightly higher than that of male (950.00 ± 8.16 μm).

The larger size of female antenna may be attributed to relatively bigger body size of female than of male. The surface of antennae was not smooth but scaly and the scales were arranged linearly.

At apical ends of antennae, when magnification was less, sensillae of three different sizes were observed in both the sexes: (i) long sized, (ii) medium sized, and (iii) small sized (Fig.1c, d).

3.2 Ultrastructure of antennal sensillae in two sexes of ladybeetle

At higher magnifications, it is possible to distinguish several types of sensillae as well as a few less common forms according to their gross structure. Higher sensillar density was noticed on two proximal annuli in both sexes.

Of eight types of sensillae found on antennae

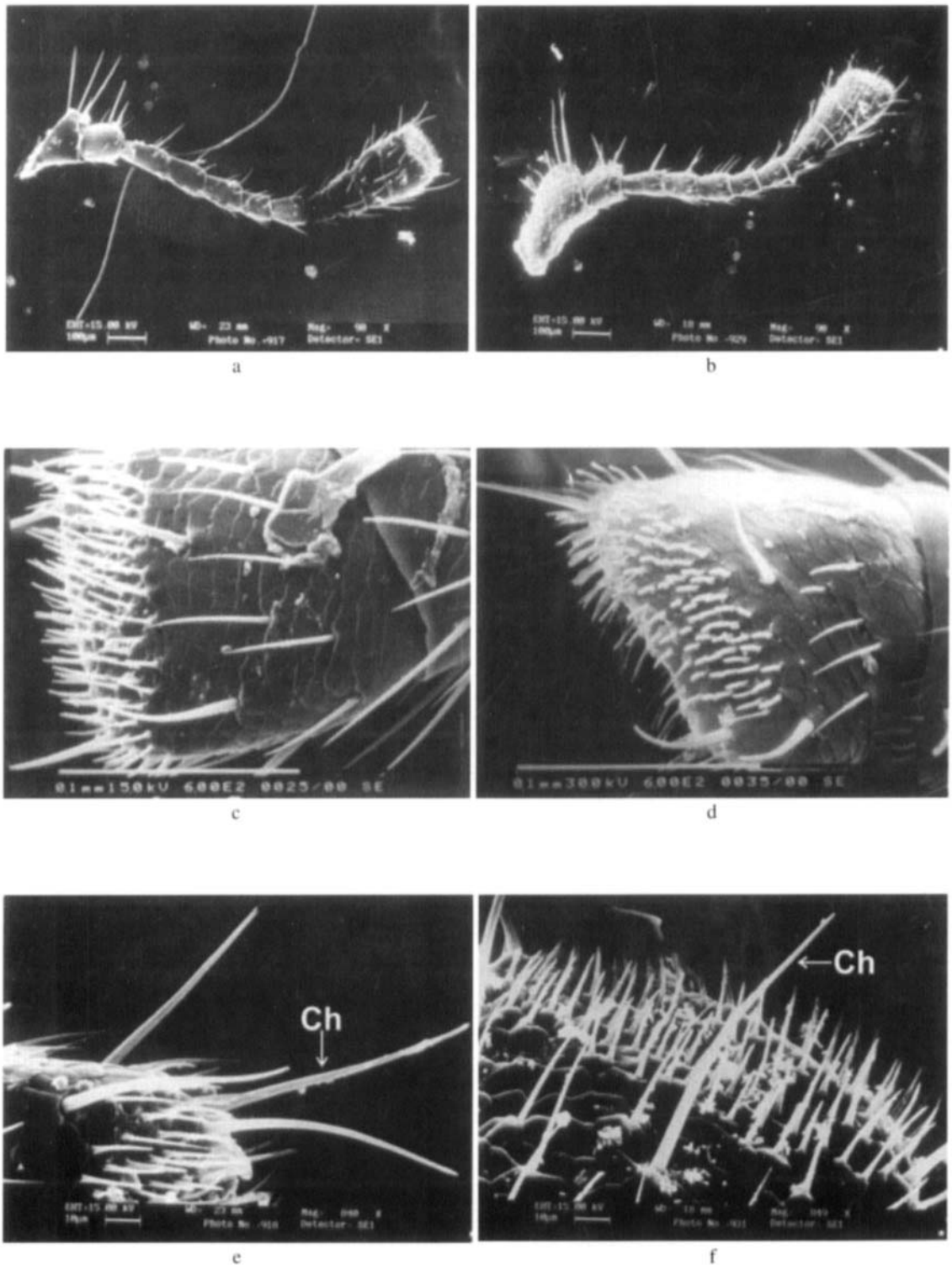

Fig. 1 Scanning Electron Micrographs showing (a) the entire antenna of male, (b) the entire antenna of female, (c) the apical segment of male antenna, (d) the apical segment of female antenna, (e) Sensilla Chaetica (Ch) in male, and (f) Sensilla Chaetica in female *Coccinella septempunctata*.

of the two sexes, chaetica, trichoidea, basiconica, campaniformia, ampucellaceous and placoidea were common. Sensillae, scolopalia and hook shaped sensilla, were found only on the antenna of male. Sensillae Ch, Tr and Ba were the most common sensillae found on the antennae of *C. septempunctata*. Various functions have been ascribed to these sensillae in insects and present observations are discussed in the light of the probable functions of the different sensillae as reported in other insects.

3.3 Sensilla Chaetica (Ch)

Sensillae Ch were long hair like structures and emerge from between the scales. Their base was surrounded by a membranous socket, and extended from the antennal surface. Their long axis forms a right angle with antenna. Distally, it tapers uniformly into simple extremities with closed tips. They were present in large numbers and distributed over the entire antennae. Higher magnification showed that cuticle of the sensilla was thick, poreless and marked by longitudinally arranged furrows. Length of Ch in female (72.50 ± 5.00 μm) was more than that of male (62.50 ± 2.89 μm). The base of sensilla had average diameter of 4.0 μm in both the sexes (Fig. 1e, f).

Sensilla Ch has been reported from the antennae of insects belonging to different families of Coleoptera *viz*. Chrysomelidae (Ritcey and McIver 1990), Carabidae (Daly and Royan 1979), Curculionidae (Isidoro and Solinas 1992), Coccinellidae (Jourdon *et al*. 1995; James 2001 Pervez 2002, Broeckling and Salom 2003). They are ascribed to mechano- and chemoreception in coccinellids, *Semiadalia undecimnotata*, *Coccinella transverguttata* and *P. tsugae* (Jourdon *et al*. 1995, Wipperfurth *et al*. 1987, Storch 1976, Broeckling and Salom 2003).

3.4 Sensilla Trichoidea (Tr)

Sensillae Tr were long hair like structure, distally tapered, smooth walled and placed in a depression. They were often slightly curved just above their bases and projected outwards at 50-70° angles from the base towards antennal shaft (Fig. 2a, b). The sensillae lacked cuticular collar at the base. Tr was longer in female (14.87 ± 2.25 μm) than that in male (10.75 ± 1.50 μm), with an average diameter of 1.2 μm in both sexes. 7-8 sensillae were arranged in a circular manner on each annulus but they were more numerous at the apical end of male antenna.

Sensillae Tr were also recorded on antennae of male and female of Cerambycidae (Dyer and Seabrook 1975), Carabidae (Daly and Ryan 1979, Kim and Yamasaki 1996), Elateridae (Merivee 1992, Merivee *et al*. 1997) and Coccinellidae (James 2001, Pervez 2002). They have been ascribed several olfactory roles, *viz*. selection of habitat, oviposition site, prey and intersexual communication in coccinellids (Jourdon *et. al*. 1995). Tr and a specialized organ composed of chordotonal receptors, Johnstons organ, are known to perceive sound waves in a number of insects (Romoser 1981).

3.5 Sensilla Basiconica (Ba)

Sensillae Ba were blunt tipped, smooth walled, relatively stout pegged, inserted into a small dome often straight and sometimes slightly curved towards the antennal shaft. It was considerably shorter than Ch and Tr and was more in number at apical end of female antenna than that of male. It was 0.75 ± 0.06 μm long in female and 0.57 ± 0.02 μm in male ladybeetle. The surface of sensilla was smooth, thick and poreless (Fig. 2c, d) and presumed pore lacked its tip. It was abundant at the apex of eleventh annuli.

Sensillae Ca have also earlier reported from the antennae of other coleopterans *viz*. *Conotrachelus nenuphar* (Curculionidae) (Alm and Hall 1986), *Carabus fiduciarius saishutoicus* (Carabidae) (Kim and Yamasaki 1996), and *Agriotes obscurus* (Elateridae) (Merivee 1992). They were modified forms of Ch and Tr and serve as compression and stretch receptors (McIver 1985, Zacharuk 1985). Sensillae Ba are largely responsible for olfaction and hygroreception in insects, (Isidoro and Solinas 1992, Okada *et al*. 1993, Kellogg 1970). In lepidopterans sensillae Ba were specifically generalists and

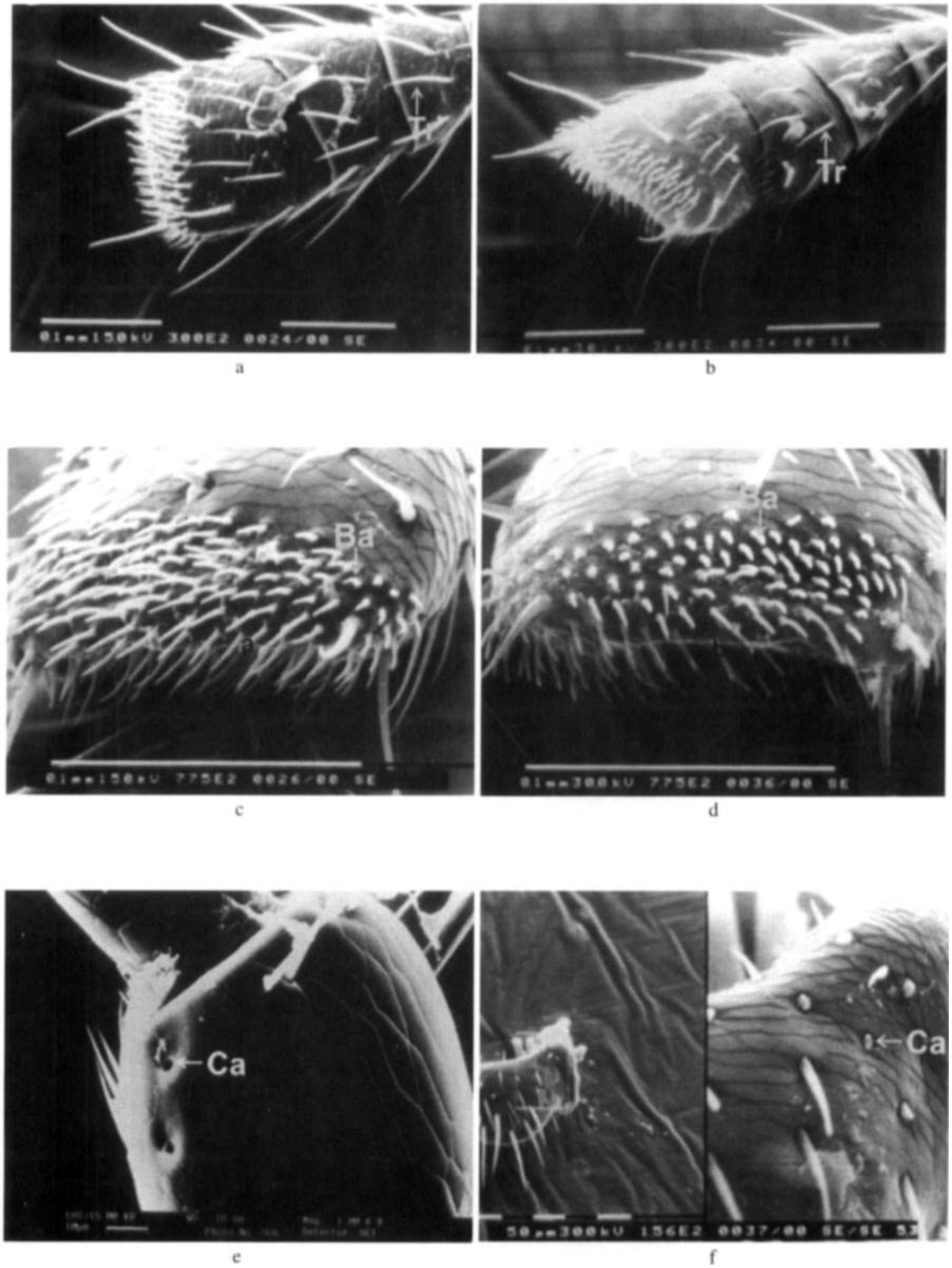

Fig.2 Scanning Electron Micrographs showing (a) arrangement of Sensilla Trichoidea in three apical segments of male antenna, (b) arrangement of Sensilla Trichoidea in three apical segments of female antenna, (c) Sensilla Basiconica (Ba) on the apical segment of male, (d) Sensilla basiconica on the apical segment of female, (e) Sensilla Campaniformia (Ca) in male, (f) Sensilla Campaniformia in female *Coccinella septempunctata*.

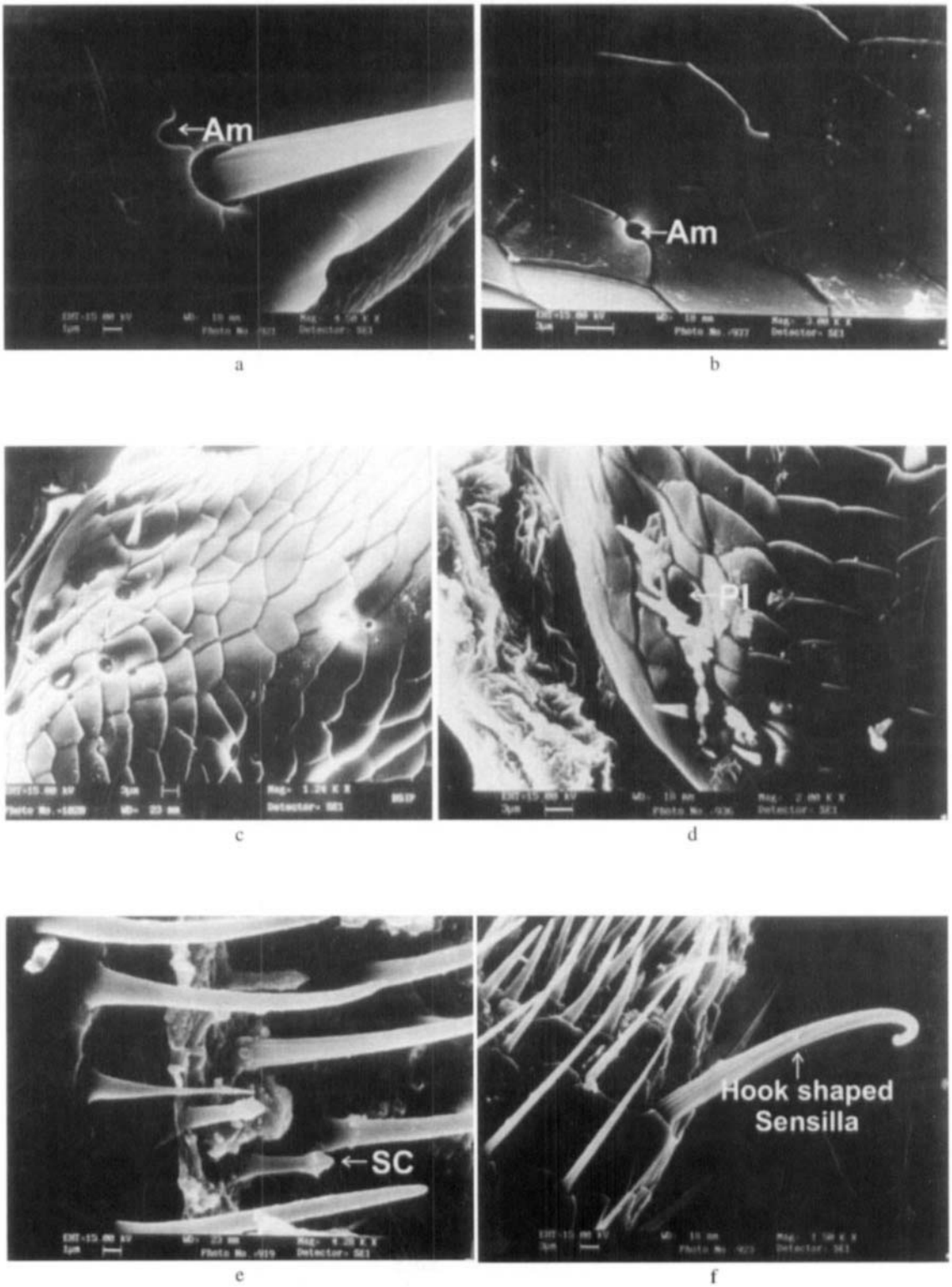

Fig.3 Scanning Electron Micrographs showing (a) Sensilla Ampucellaceous (Am) in male (b) Sensilla Ampucellaceous (Am) in female, (c) Sensilla Placoidea (Pl) at the terminal end of male, (d) Sensilla Placoidea at the terminal end of female, (e) Sensilla Scolopalia (Sc) in male and (f) hook shaped sensilla in male *Coccinella septempunctata*.

responded to odours of food and oviposition sites (Schneider *et al*. 1964, Cuperus 1983) and in coccinellid, *P*. *tsugae*, these were supposed to have contact chemoreceptive role (Broeckling and Salom 2003). Higher number of Ba on the antenna of female, *C*. *septempunctata* suggests their probable role in selection of oviposition sites and moisture reception, as it prefers to oviposit on moist and shady places, *e*.*g*. leaf surface (Omkar and Srivastava 2001b).

3.6 Sensilla Campaniformia (Ca)

Sensillae Ca were small dome shaped cuticular projections, situated at the centre of a slightly sunken portion of antenna. Though they were also found on mid annular segments but were more numerous on the pedicel of antennae of both the sexes (Fig. 2e, f). Their diameter was 3.0 μm in both the sexes.

In *C*. *septempunctata*, they probably help in the proper orientation of body parts.

3.7 Sensilla Ampucellaceous (Am)

Sensillae Am were pit-like in appearance and were evenly distributed on entire antennae of both the sexes of ladybeetle. The pits were deeply sunk and connected with the surface by a more or less elongate tube. The diameter of pits was 1.2 μm on male and 1.5 μm on female antenna (Fig. 3a, b).

The sensillae are seemingly chemo-and thermo-receptors, as they perceive water vapours, carbon dioxide and thermal changes in orthropterans and dipterans (Waldow 1970, Davis and Sokolove 1975). The antennae of blood sucking bug, *Rhodnius prolixus* (Hemiptera, Reduviidae) were extremely sensitive to differences in temperature (Romoser 1981).

3.8 Sensilla Placoidea (Pl)

Sensillae Pl were elliptical sunken plates with a thin cuticle forming the outer lining of cells, surrounded by a narrow membranous ring. They were found on the scape, pedicel and the terminal annulus of both the sexes (Fig.3c, d).

These sensillae have also been reported on the antennae of ladybeetle, *P*. *tsugae* and were olfactory in nature (Broeckling and Salom 2003).

3.9 Male specific sensillae

Two types of sensillae were found on the antenna of male ladybeetle and constitute the principal element of antennal sexual dimorphism.

3.10 Sensilla Scolopalia (Sc)

Sensillae Sc was found on the terminal annulus of male antennae in between sensilla Ch and sensilla Tr. It gradually tapered from their base upto a certain length, followed by cuticular thickening, which is pointed towards apex. The cuticle was smooth (Fig. 3e). The length of the sensilla was 2.5 μm and the diameter at base was 0.6 μm. Functions of Sc are uncertain, but their presence on male antenna suggests their probable role in courtship and mate search.

3.11 Hook shaped sensilla

A single characteristic hook shaped sensilla was recorded for the first time on the lateral side of the apical segment of antenna of male (Fig.3 f). Its length was 21 μm. This was not found on female antenna. Thus, it reveals the sexual dimorphism in the antennae of male and female *C*. *septempunctata*. Its surface was smooth, poreless with longitudinal furrows and closed tip. Its function is yet not known; but possibly it facilitates the selection of mate. Though hook-shaped sensillae were also found in *A*. *tsugae* but critical examinations of the micrographs revealed that they were not similar to the ones found in this study. Hence terminology used by Broeckling and Salom(2003)was not used.

4 CONCLUSION

The present study reveals that:(i) antennae of *C*. *septempunctata* possess sensillae Ch, Tr, Ba, Ca, Am, Pl, Sc, and hook shaped sensilla, (ii) surface of the antenna was scaly, (iii) Ch, Ca, Am, and Pl were almost similar in number on antennae of both the sexes, (iv) sensillae Tr were more in number on male antenna, while Ba were more numerous on female antenna and the difference in the number of these sensillae suggested that they were probably associated with the specific

functions of male and female ladybeetles, (v) Sc and hook shaped sensillae were found only on the antennae of male ladybeetle.

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七星瓢虫(鞘翅目:瓢虫科)触角的扫描电镜观察

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用扫描电子显微镜对两性七星瓢虫的触角进行了观察,结果显示,鳞状触角分七节,分别为雄性 950.00 ± 81.16 μm 而雌性为 972.00 ± 9.57 μm 长。电镜下可观察到七星瓢虫触角有 8 种类型的感觉器(sensillae),即: chaetica(Ch),trichoidea(Tr),basiconica(Ba),campaniformia(Ca),ampucellaceous(Am), scolopalia(Sc),placodea(Pl)和钩状感觉器。Ch 在雄性长为 62.50 ± 2.89 而在雌性为 72.50 ± 5.00 μm。Tr 在雄性长为 10.75 ± 1.50 μm 而在雌性为 14.98 ± 2.25 μm 长。Ba 在雄雌两性中分别为 57 ± 0.02 μm 和 0.75 ± 0.06 μm 长。Ca 为半球状,其直径在两性中均在 3.0 μm。Am 的直径在雄雌中分别为 1.2 和 1.5 μm。Ca 为椭圆内陷盘状。Sc 在其顶部较阔而在末端尖锐,2.5 μm。在雄性触角上观察到一钩状感觉器,长 21.0 μm,它表明七星瓢虫在触角上的性别两型性。在雄雌中其感觉器 Ch,Ca,Am,Pl 的数量上接近。Tr 在雄性触角上数量较大而 Ba 则在雌性触角上数量较多。

关键词 七星瓢虫 扫描电镜 触角 感觉器