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Deepak Barsagade
Department of Zoology, MJF Educational Campus, RTM Nagpur University, Nagpur MS India 440033

Arun Khurad
Department of Zoology, MJF Educational Campus, RTM Nagpur University, Nagpur MS India 440033

Mina Chamat
Department of Zoology, MJF Educational Campus, RTM Nagpur University, Nagpur MS India 440033

Hemraj Meshram
Department of Zoology, MJF Educational Campus, RTM Nagpur University, Nagpur MS India 440033

Manoj Thakre
Department of Zoology, MJF Educational Campus, RTM Nagpur University, Nagpur MS India 440033

Shruti Gharade
Department of Zoology, MJF Educational Campus, RTM Nagpur University, Nagpur MS India 440033

Ganesh Gathalkar
Department of Zoology, MJF Educational Campus, RTM Nagpur University, Nagpur MS India 440033

Rani Thakre
Department of Zoology, MJF Educational Campus, RTM Nagpur University, Nagpur MS India 440033

Correspondence:
Mina Chamat
Department of Zoology, MJF Educational Campus, RTM Nagpur University, Nagpur MS India 440033

Sex Specificity of Antennal Sensilla in Eri Silkworm *Philosamia ricini*

Deepak Barsagade, Arun Khurad, Mina Chamat, Hemraj Meshram, Manoj Thakre, Shruti Gharade, Ganesh Gathalkar and Rani Thakre

Abstract
The antennae of both sexes of eri silk moth *Philosamia ricini* were examined using scanning electron microscopy. The antennae are bipectinate type segmented consisting of scape, pedicel and flagellum. The flagellum consists of about 32 similar segments known as annuli. Different types of sensilla are present on the scape, pedicel and flagellum functioning as a sensory perception. Böhm bristle and sensilla trichoidea are found on scape and pedicel. The flagellum consists of sensilla chaetica (SCH), sensilla trichoidea (ST), sensilla trichoidea curvata (STC), sensilla basiconica (SB), sensilla squamiformia (SQU) and sensilla styloconica (SS). Sensilla trichoidea is further divided into sensilla trichoidea-I (ST-I), sensilla trichoidea-II (ST-II) and sensilla trichoidea- III (ST-III). The flagellum contains SCH, ST-II, ST-III, STC, SB, SQU and SS in female and SCH, ST-I, ST-II, ST-III, STC, SB, SQU and SS in male. ST-I sensilla is sex specific and present on male antenna.

Keywords: Eri silkworm, *Philosamia ricini*, antenna, sensilla, SEM.

1. Introduction

Antenna is one of the head appendages consisting of three parts; scape, pedicel and flagellum [1]. The adult antennal flagellum is divided into many small annuli conformed in Saturniid and Noctuid's moths [2, 3, 4] and annulations are an important step in the metamorphosis of adult antenna [5, 6, 7, 8, 9, 10, 11].

Light and scanning electron microscopic (SEM) studies on the antennae of silkworm reveal the presence of various types of sensilla showing ultrastructural variation in sexes [4]. The antennae are major sense organ that receives environmental information and specific communication through a variety of sensillum receptors [12]. The sensilla are cuticular special appendages for chemoreceptive, olfactory, hygrothermal-receptive and mechanoreceptive functions [13, 14].

Various types of sensilla including Böhm bristle, sensilla chaetica (SCH), sensilla trichoidea (ST), sensilla trichoidea curvata (STC) are known having specialized functions. The Saturniid moths often show species- specific sexual diversity in fine morphology of the antennal sensilla [3, 4, 5, 15, 16, 17]. The present SEM studies on the antennae of female and male moth of *Philosamia ricini* were undertaken to elucidate the surface micro- morphology of antennal sensilla.

2. Materials and Methods

The fresh eggs were brought from Eri silkworm Seed Production Centre, Central Silk Board, Mirza (Assam) during the year 2012-2013. The larvae were reared till cocoon formation in the Departmental insectaries. The emerged adult silk moth were sacrified. The antenna were separated from male and female silk moths under stereoscopic binocular and used for the study.

An antenna of adult eri silkworm, *P. ricini* was examined by both light and scanning microscopy. For scanning electron microscopy antenna were washed thoroughly with distilled water and fixed in 10% formalin for a period of 12 h, dehydrated in various grades of alcohol, cleared in acetone, dried and fixed on the metallic stub at different angles with the help of Fevicol and processed for gold coating and scanned under the Jeol (JSM 6380A) Scanning Electron Microscope (SEM) at Visvesvaraya National Institute of Technology (VNIT), Nagpur.

3. Results

The bipectinate antennae were present on the head region of the moth. The length of antenna was slightly longer in male than female (Table 1). Each antenna was divided into three parts; scape, pedicel and flagellum (Fig. 2a). The flagellum consists of many similar segments called annuli. There were 32 annuli in female and 29 annuli in male antenna. Each annulus contains two pairs of barbs from 1-26 annuli in female and on 1-23 annuli in male. The 27 annuli of female contain 3 barbs 1 outer and 2 inner, while from 28-32 annuli a pair of barbs (one outer and one inner) was present. In male annuli 24 had 2 barbs on the inner side while 25-29 annuli had only one barb on the inner side. At the end of each barb spike like sensillae were noticed (Table 2). Morphology under SEM showed varied differences in both sexes of adult of *P. ricini.*

Table 1: Morphological observations of antenna of *P. ricini* moth.

Sr. No.	Morphometry of antennae	Female (mm)	Male (mm)
1.	Total length of antenna	10.796 ± 0.79	10.833 ± 0.43
2.	Length of scape	0.660 ± 0.41	0.699 ± 0.56
3.	Length of pedicel	0.341 ± 0.20	0.359 ± 0.15
4.	Length of flagellum	9.795 ± 0.49	9.775 ± 2.41

Table 2: Distribution of number of spikes on the various annuli of male and female.

Barbs present on the annulus		No. of annuli containing spikes on the barbs					
		Male annuli			Female annuli		
		Spike I	Spike II	Spike III	Spike I	Spike II	Spike III
Outer side of annuli	Barb I	1-23	0	0	1,3-6,8,13,14,18-20,22,24,25,27-29,31,32	7,9-12,15,16,23	2,17,21
	Barb II	0	0	0	5,7-10,12,15	0	0
Inner side of annuli	Barb I	1-29	0	0	3-8,10,11,13-15,17,21,22,26-32	1,2,9,12,16, 18-22,23-25	0
	Barb II	0	0	0	2,15,16	0	0

SEM observations show the presence of various types of sensilla like Böhm bristle (BB), sensilla chaetica (SCH), sensilla trichoidea (ST), sensilla trichoidea curvata (STC), sensilla basiconica (SB), sensilla squamiformia (SQU) and sensilla styloconica (SS) on different parts of antenna of both the sexes of *P. ricini* moth. Further, sensilla trichoidea on the basis of their length was divided into sensilla trichoidea-I (ST-I), sensilla trichoidea-II (ST-II) and sensilla trichoidea-III (ST-III). Sensilla trichoidea-I was observed only in the male moth (Table 3).

Table 3: Morphometry of different types of sensilla present on various parts of antenna.

Sensillae present		Male		Female	
		Length (µm)	Width (µm)	Length (µm)	Width (µm)
Scape	Böhm bristle	215.5±3.61	2.5± 0.01	215.5± 1.52	2.5± 0.28
	Sensilla Trichoidea-I	95.32± 2.55	3.7± 0.03	-	-
	Sensilla Trichoidea-II	-	-	46.66±3.66	4±0.173
	Sensilla Trichoidea-III		-	28±2.18	3.3± 0.03
Pedicel	Böhm bristle	215.5± 3.73	2.5± 0.02	215.5± 2.05	2.5± 0.27
	Sensilla Trichoidea-II	-	-	46.66± 3.93	4± 0.18
	Sensilla Trichoidea-III	-	-	28± 2.23	3.3± 0.04
Flagellum	Sensilla Chaetica-I(SCH-I)	-	-	275.4±1.35	0.182±0.03
	Sensilla Chaetica-II(SCH-II)	70± 3.45	7.5± 0.32	77±1.978	8± 0.21
	Sensilla Trichoidea-I(ST-I)	95.32±2.63	3.7± 0.53	-	-
	Sensilla Trichoidea-II(ST-II)	43.1±4.1	3.4± 0.01	46.66±2.45	4± 0.01
	Sensilla Trichoidea-III(ST-III)	26.5±3.01	3± 0.71	28± 3.10	3.3± 0.04
	Sensilla Trichoidea Curvata (STC)	31.2± 4.38	3.8±0.12	33.07± 2.51	3.8± 0.23
	Sensilla Basiconica(SB)	11.3± 2.03	5.3± 0.23	11.3± 1.01	5.3± 0.42
	Sensilla Styloconica(SS)	15±1.48	6.8± 0.08	16.58±1.83	6.8± 0.65
	Sensilla squamifornia(SQU)	16.2±1.72	-	16.2±1.72	-

3.1 Böhm bristles (BB)

These were leaf like structures found on the scape and pedicel of both the sexes. They were long sharply tapered and had sclerotise ring at the base. They were similar in male and female moth (Fig. 2b, d).

3.2 Sensilla chaetica (SCH)

These were the blunt and most striking spike- like present on

the antenna. They were found at the middle region of each segment, and also distributed on terminal end of the barbs. On the basis of length SCH was differentiated into long sensilla chaetica (SCH-I) and short sensilla chaetica (SCH-II). The surface of SCH covered with radial ridges (Fig. 3a, c, e, f, 4c).

3.3 Sensilla trichoidea (ST)

These were the most numerous sensilla present on the antenna and were commonly known as sensory hairs. The sensilla trichoidea had been characterized on the basis of their length as long, medium and short (ST-I, ST-II and ST-III) trichoid sensilla. ST-I was present only in male antennae (Fig. 2d, 3a, b, 4a, c).

3.4 Sensilla squamiformia (SQU)

SQU were short, scale like abundantly distributed on the flagellum of both sexes of adult moths. The sensilla were broad at the base and pointed towards the tip (Fig. 3c, d).

3.5 Sensilla basiconica (SB)

SB present on dorso- ventral surface of terminal annulus and barbs of flagellomere of both sexes. These were commonly called as pit pegs. The pit was shallow with inward slanting spines. The sensory pegs were arising from the base of pit. The number of sensilla basiconica was more in female as compared to the male moth (Fig. 3d, 4b).

3.6 Sensilla styloconica (SS)

Sensilla styloconica were present on 27 to 32 annuli in female and 24-29 annuli in male flagellum. They were short, cone shaped, sensory structure mounted at the apices of stout cuticular pegs. The peg arises from the distal edge of the segment in the sensory area. Each peg was provided with one cone at its tip. The number of sensilla styloconica was more in female moth as compared to male moth (Fig. 3e, f).

3.7 Sensilla trichoidea curvata (STC)

These were present on the flagellum of antennae of both sexes of moth. The shaft of the sensilla was long, pointed and slightly curved towards the apex (Fig. 3b, 4a).

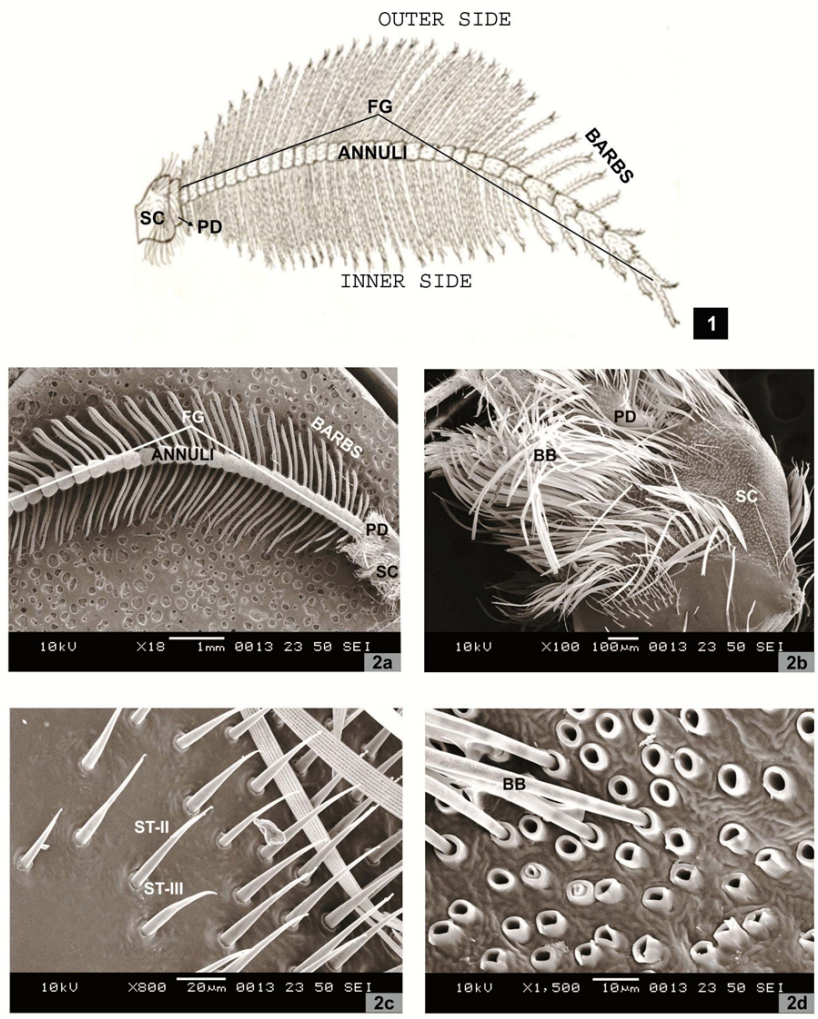

Fig 1: Female antenna (diagrammatic).

Fig. 2(a-d): Scanning electron microscopic (SEM) photomicrograph of antennae of *Philosamia ricini* adults.

2a- SEM structure of female antenna showing scape (SC), pedicel (PD), and flagellum (FG).

2b- SEM of female antenna showing Böhm bristle (BB) on scape (SC), pedicel (PD).

2c- Magnified view of scape showing sensilla trichoidea ST-II and S-III.

2d- Magnified view of scape showing base of Böhm bristle (BB).

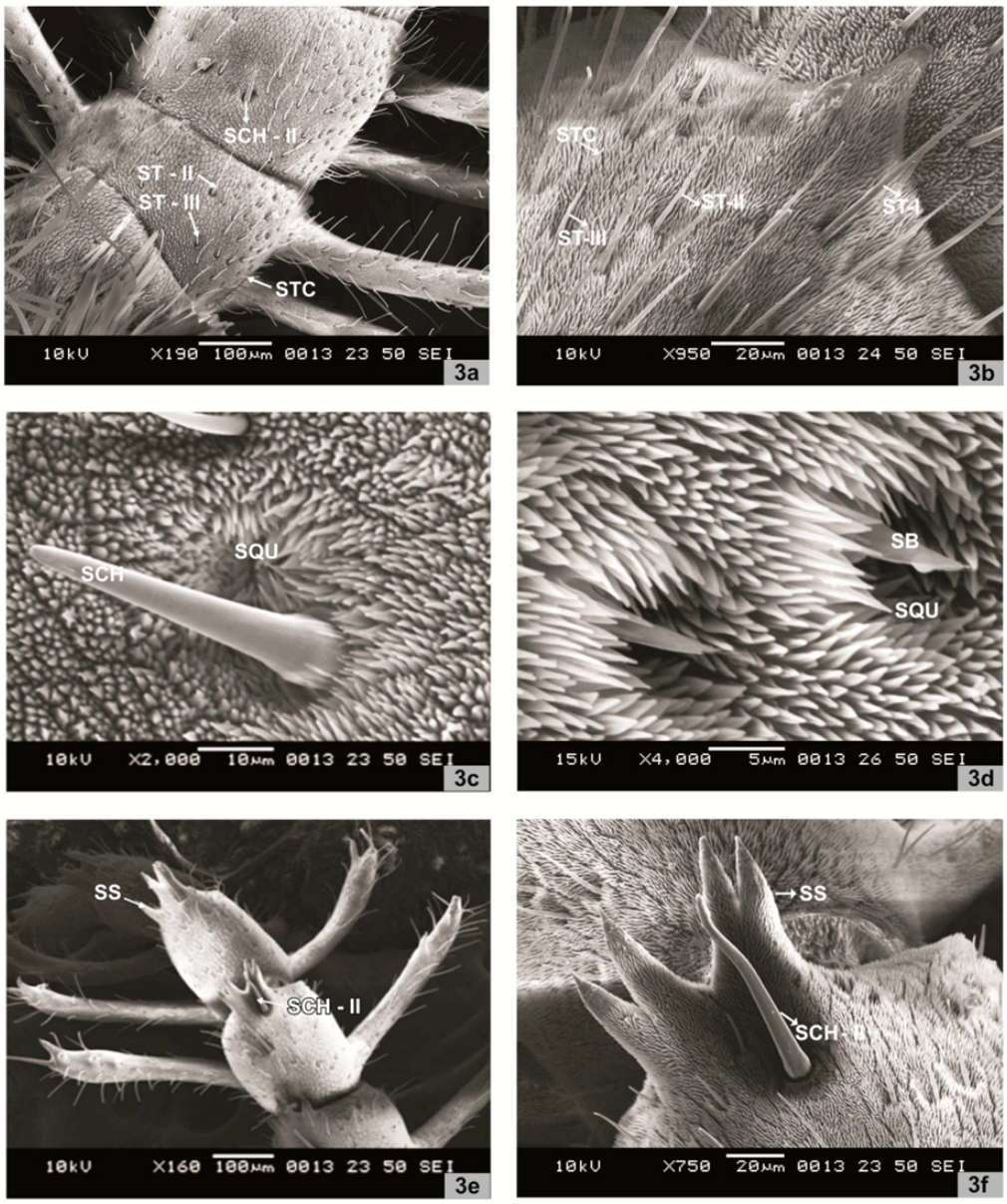

Fig 3(a-f): Scanning electron microscopic (SEM) photomicrograph of antennae in male and female adult of *Philosamia ricini.*
3a: Early annuli (flagellomere) of flagellum showing barbs and various type of sensillae in female antenna.
3b: Mid annuli of flagellum showing various type of sensillae ST-I, ST-II, ST-III and STC in male antenna.
3c: Magnified view of annuli showing SCH and SQU in male antenna.
3d: Magnified view of annuli showing SB and SQU in female antenna.
3e: Photomicrograph of terminal 29th annuli showing pair of barbs with various type sensillae SS, SCH-II, SS, SCH and STC in female antenna.
3f- Magnified view of fig:e showing SS and SCH.
ST-sensilla trichoidea, SCH-sensilla chateaca, STC-sensilla curvata, SB-sensilla basiconica, SS-sensilla styloconica, SQU-sensilla squmiformia

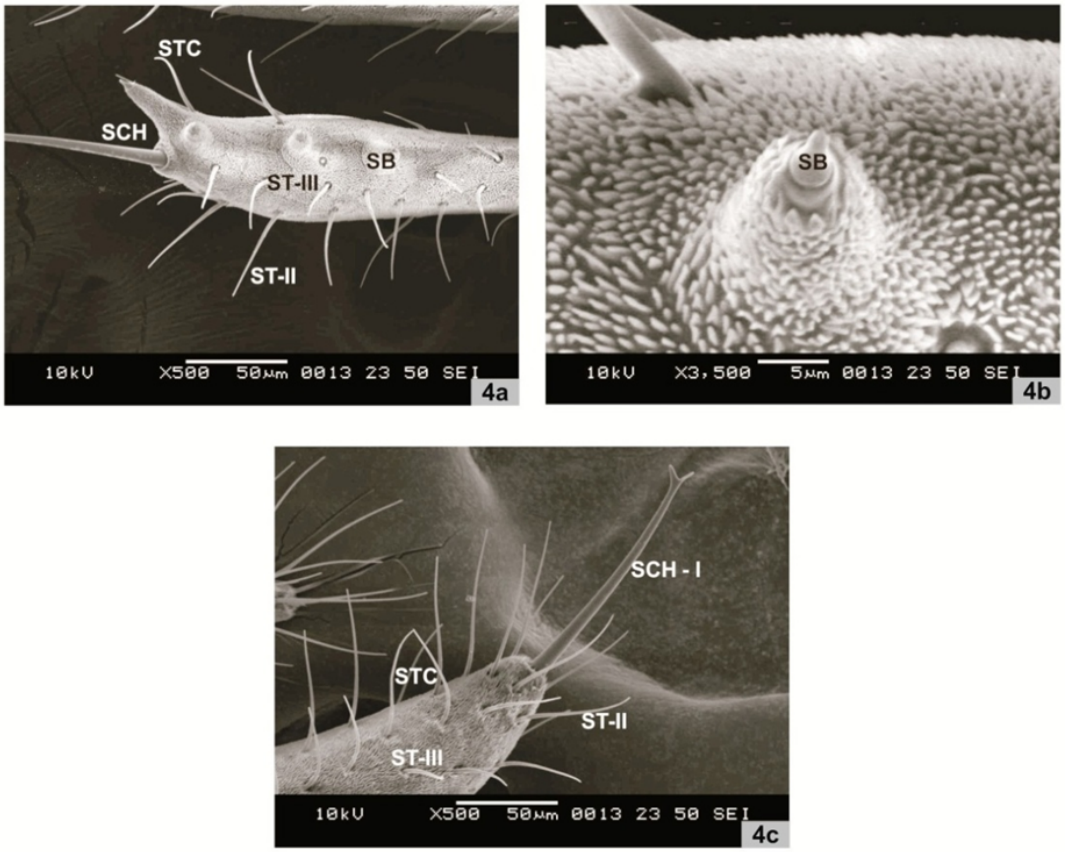

Fig 4(a-c): Scanning electron microscopic (SEM) photomicrograph of bars on antennae of *Philosamia ricini* adults.
4a: Magnified view of terminal end of barbs showing sensilla SB, ST-II, ST-III, STC and SCH in female antenna.
4b: Showing SB present on the barbs in female antenna.
4c: Magnified view of barbs showing sensilla SCH, ST-II, ST-III and STC in female antenna.
ST- sensilla trichoidea, SCH- sensilla chateaca, STC-sensilla curvata, SB-sensilla basiconica.

4. Discussion

The Böhm bristles are spine like sensilla [4] and are present on the antenna of *Trichoplusia ni*, *Heliothis zea*, *Prodenia ornithogalli* and *Spodoptera exigna* [15]. Callahan *et al*. [18] observed that, the Böhm bristles are dielectric wave guide aerials in the visible region and may also have mechanoreceptive function in Saturniid species. Schneider [19] confirmed the mechanoreceptive properties of the Böhm bristles through electrophysiological studies in *Apis mellifera*. The present SEM observations confirmed, presence of leaf like Böhm bristles on the scape and pedicel of antenna of *P. ricini* with morphological differentiation in both sexes might be performing the function of mechanoreceptors and chemoreceptors.

The sensilla chaetica (SCH) are blunt striking spines found on the middle annuli as well as barbs end. Similar arrangement of the sensilla chaetica has been confirmed earlier [15, 19]. According to Albert and Seabrook [17] the sensilla chaetica usually function as mechanoreceptors and contact chemoreceptors [4]. In *P. ricini* short sensilla chaetica (SCH-II) present on annuli of both sexes might be functional as contact chemoreceptors while, long sensilla chaetica (SCH-I) present on female annuli, is female sex specific character.

The trichoid sensilla-I (ST-I) is longest and characteristically observed only in the male. It is relatively straight but hooked at the tip. Structurally, the body of ST-I was found to possess spiral ridges as observed by earlier workers on the male antenna of Saturniids moths [15, 3, 4]. Bland [20] observed the trichoid sensilla on the antenna of adult *Hyperapostica* with blunt tip. In *P. ricini*, the trichoid sensilla II (ST-II) are shorter and more curved than the long ST-I while, ST-III is the shortest sensilla with blunt tip. The ST-I present only on the male antenna while, ST-II and ST-III has more population on the female antenna. In *A. polyphemus*, the long sensilla trichoidea (ST) are found only in male [21] and in *B. mori* ST-I is sensitive to sex pheromone emitted by the female [22]. The presence of ST-I in *P. ricini* male confirmed its sex specificity which might be female sex- pheromone sensitive during mating behavior. On the flagellum some scales appear to be sensory. Schneider and Kaissling [23] observed some sensory scales on the antenna of *B. mori* and called them sensilla squamiformia functioning as receptors. On the antenna of both sexes of *P. ricini*, numerous scales like sensilla squamiformia are found which might be the sensory receptors.

The sensilla basiconica responded to chemical responses [4]. In *P. ricini*, it has been found that the sensilla basiconica is present on antenna of both sexes and might be chemoreceptors in nature. Lin and Chow [16] observed that sensilla styloconica is innervated by 3 to 4 neurons. In our investigation, sensilla styloconica of *P. ricini* was found to possess cone and peg, of which cone was situated at the tip of peg and peg extended outwards for reception and increasing the area covered by the nerve ending of sensilla styloconica for olfaction, thus supporting earlier observations

[24, 15, 4]. Albert and Seabrook [17] observed similar structure of styloconica sensillum in the spruce bud-worm *Choristoneura fumiferana* functioning as olfactory receptors. Sensilla trichoidea curvata (STC) has been confirmed on *P. ricini* antennae as mechanoreceptors and contact chemoreceptors as suggested by [17, 4].

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