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Evaluation of the Internal Morphology of Antennal Sensilla in *Scirpophaga Incertulas* for Male and Female by Scanning Electron Microscopy

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The Scirpophaga incertulas (Walker), trivial name Yellow Stem Borer (YSB) is a serious pest contributing to significant yield loss to rice. YSB detects host/mate cues through olfactory receptors (sensilla) on antennae, which is critical for its survival, adaptability, and perpetuation. Though YSB is a destructive pest, its mechanism of olfaction is poorly understood. Using a scanning electron microscope, it is attempted to detect distinct sensilla, decode their characteristics, and compare the antennal sensilla of both males and females. Sensilla trichodea (S.T), Sensilla chaetica (S.Ch), Sensilla styloconica (S.St), Sensilla squamiformia (S.Sq), Sensilla coeloconica (S.Co), Sensilla cavity, and Bohm bristles (BB) are the seven distinct kinds and subtypes of sensilla are recognized. Sexual dimorphism was observed in the arrangement of scales over the antenna in males and females. Interestingly, among the subtypes, the S.T-I was observed only in males. Furthermore, Sensilla coeloconica without a fence (type II) noticed in our studies was also reported earlier in other monophagous lepidopteran pests. The statistical analysis of the number and size of sensilla clearly indicates their involvement in sexual differentiation. This study may help in better understanding the processes of communication, identification of plant volatiles, oviposit site, and mate in YSB.

Keywords: Antennal Sensilla; Chemosensory; Olfaction receptors; SEM; Yellow Stem Borer (Scirpophaga incertulas).

Rice is the widely distributed dietary staple food in the world and is cultivated mostly in Asia. India is the second-largest producer of rice globally. According to the reports of Food and Agriculture Organization (FAO), November 2022[1] the rice production is forecasted at a record of 173 million tonnes in India. Rice, being a major crop plant, is affected by the innumerable number of pests that hamper its crop production. Especially in India, approximately 100 insect species infest rice, and 20 are considered major pests[2]. Among these, *Scirpopagha incertulas* Walker is the dominant and most destructive pest that leaves most of the rice plants at a vulnerable stage[3]. The *Scirpophaga incertulas* Walker, appellative Yellow Stem Borer (YSB) belongs to the order Lepidoptera of the family Crambidae is monophagous and attacks rice plants. The larvae of *S. incertulas* can be recognised by frail tillers and hills, Dead Heart (centre leaf drying) in the vegetative stage, and White Ear Head (grain-less panicle) at the reproductive stage. The larvae are detrimental to plants at various stages[4]. The *S. incertulas* alone reduces about 10-90% yield throughout the Indian subcontinent during the last two decades[5].

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Effective measures have been developed in the recent past to control *S. incertulas* using biopesticides[6-7], pheromone lures[8], transgenic rice plants[9], the effect of temperature[10-11], and the knockdown of genes through RNAi[12]. Antennae of insects have a predominant role in the olfaction mechanism that help insects in locating host for its food, identifying mate and better oviposition sites[13]. *S. incertulas* remains one of the most understudied groups of rice pests and scarce knowledge exists till date on the general morphology of antennae, distribution, location and function of its different types of sensilla. The antenna consists of different types of sensilla which play a prominent role as sensory receptors such as chemo-receptors, hygro-receptors, thermo-receptors, mechano-receptors, sensory neuron receptors, olfactory receptors and proprioceptive. The response of male *S. incertulas* for pheromone has been investigated, but the behavioral response of both adult male and female *S. incertulas* for its host-derived semiochemicals has not been studied yet. In the present study, scanning electron microscopy (SEM) is undertaken to evaluate the internal morphology of antennal sensilla of both the sexes of *S. incertulas*. Antennae of both male and female *S. incertulas* are compared for the distribution, location, number, and different types of antennal sensilla. This report is the first of its kind on *S. incertulas* antennal sensory organs that provides the morphological feature for future behavioral, IPM and electrophysiological experiments.

MATERIALS AND METHODS

Insects assortment

Instars of *S. incertulas* larvae were collected from the infested paddy fields at Dichpally, Nizamabad, Telangana, India, and were released onto the rice plants (TN 1 variety) grown under the laboratory conditions in the pots. The samples were kept on a 16:8h photoperiod, at the temperature of 27±2ºC and relative humidity 60±5% till pupation as per the rearing technique of *S. incertulas*[14]. The *S. incertulas*adults emerged from pupae were kept separately in the test tube provided with a small piece of paddy leaf and wet cotton ball until it is shifted to the RUSKA Lab's, College of Veterinary Science, P. V.Narasimha Rao Telangana Veterinary University, Rajendra Nagar, Hyderabad, India, for Scanning Electron Microscopic examination.

Scanning Electron Microscopy (SEM)

After being decollated, 15 adult individuals of both sexes of *S. incertulas* were collected in different Eppendorf tubes. These samples were first postfixed for 4 hours in 2% aqueous osmium tetroxide after being fixed for 24 hours at 4°c in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2). Additionally, after being dehydrated in a series of graded alcohols, samples were dried to the critical point using a CPD machine. The processed samples were positioned over the stubs and then coated with gold layer using an automated sputter coater for three minutes. The samples were scanned using an SEM at the laboratory with necessary magnifications[15].

Terminology and Statistical Analysis

Identification of sensilla, categorization, and nomenclature were modified from terms employed in earlier investigations[16-17]. The given values of different types of sensilla in this study are mean of triplicates. The mean value, their respective standard errors and the data were analyzed by oneway ANOVA (Holm Sidak method) using statistical software Sigma Plot (version 12.0). Antennae of both the sexes of *S. incertulas* were compared which were set to *P* d" 0.05 and this was contemplated significant.

RESULTS AND DISCUSSION

No information exists about the morphology, distribution and types of sensilla of *S. incertulas*till date. This is the first report to illustrate about the seven different types of sensilla identified in *S. incertulas*,Sensilla trichodea (S. T) subtypes S. T-I, S. T-II and S. T-III, Sensilla chaetica (S. Ch), Sensilla styloconica (S. St), Sensilla squamiformia (S. Sq), Sensilla coeloconica (S. Co), Sensilla cavity and Bõhm bristles (BB). Based on the SEM observations sensilla identified in *S. incertulas*are identical to those described earlier in most of the lepidopterans, *O. nubilalis*, *Z. dixolophella*,*T. batesi*,*C. medinalis*, *C. remissa,*and *P. interpuctella*[18-23].

Antennal Morphology

The morphology of the antenna was filamentous in both the sexes of YSB. The antennae were located between the compound eyes (Fig. 1A) and were similar in a structure consisting of scape, pedicle, and flagellum (Fig. 1B). The antennae of both the sexes were covered with scales arranged in rows and overlapping immediately next segment on the dorsal side. The scales were tightly packed or aggregated on the male antenna (Fig. 1C) whereas in females they were loosely arranged (Fig. 1D). The ventral surface of the antenna has a honeycomb mesh like appearance. The ventral and dorso-ventral surfaces of the antennae showed no significant differences in organization and pattern of sensilla distribution between males and females. Sexual dimorphism has been observed differentiating female and male (female antennal length was more than that of the male). The scape was the largest segment of antenna which

Table 1. Mean ± SE length of the scape, pedicel, and antennal flagella *S. incertulas*.

Antennal Segments	Male		Female	
	Length (µm)	Width (µm)	Length (µm)	Width (µm)
Scape	285.6 ± 2.9[a]	242.6 ± 3.3[b]	214.6 ± 0.8[b]	152.4 ± 0.9[b]
Pedicel	73.5 ± 0.7[a]	143.2 ± 0.9[a]	51.7 ± 0.9[b]	123.3 ± 1.4[b]
Flagellomeres	2702.9± 22.2[a]	2379.0 ± 22.9[b]	3594.2 ± 43.1[a]	2446.6 ± 26.7[b]

Values comparing sexes within the same row followed by the same letter are not significantly different.

Table 2. Mean of number of different types of sensilla in male and female antennae of *S. incertulas*

Types of Sensilla	Number of Antennal Sensilla	
	Male	Female
Sensilla Trichodea- I	287.3 ± 1.45[a]	0 [b*]
Sensilla Trichodea- II	309.0 ± 3.05[b]	352.0 ± 4.04[a]
Sensilla Trichodea- III	205.7 ± 2.18[b]	220.7 ± 2.18[a]
Sensilla Chaetica	102.0 ± 1.52[b]	147.0 ± 1.53[a]
Sensilla Styloconica	25.3 ± 0.33[a]	27.6 ± 0.33[a]
Sensilla Squamiformia	42.3 ± 1.45[a]	31.0 ± 2.08[b]
Sensilla Coeloconica	61.0 ± 1.52[a]	67.6 ± 1.45[a]
Böhm Bristles	24.0 ± 2.64[a]	22.6 ± 0.88[a]

Mean ± SE,($p \leq 0.05$), values sharing the same alphabet within the same row are not significantly different according to the one way ANOVA (Holm Sidak method). *Sensilla Trichodea I are absent in females.

Table 3. Mean ± SE length of sensilla in male and female antennae of *S. incertulas*

Types of Sensilla	Length (µm) of Antennal Sensilla	
	Male	Female
Sensilla Trichodea- I	56.8 ± 0.65 µm	-*
Sensilla Trichodea- II	38.0 ± 0.41 µm	42.4 ± 0.23 µm
Sensilla Trichodea- III	26.3 ± 1.06 µm	22.4 ± 0.98 µm
Sensilla Chaetica	37.2 ± 0.60 µm	41.2 ± 0.62 µm
Sensilla Styloconica	17.8 ± 0.12 µm	22.4 ± 0.38 µm
Sensilla Squamiformia	25.4 ± 1.89 µm	23.2 ± 1.26 µm
Böhm Bristles	11.8 ± 0.26 µm	10.8 ± 0.23 µm

*Sensilla Trichodea-I are absent in female antennae of *S. incertulas.*

was longer and wider in males when compared to females with the significant difference of t = 27.62 and P <0.001. The pedicle was the smallest segment of the antenna. The flagellomeres of the male antenna were longer and wider which was significantly different (t = 8.48 and $P<0.001$) from that of the female. The flagellum was cylindrical in shape consisting of flagellomeres with no significant difference between males and females (ranging from 32-33 in females and 30 -31 in males) t = 0.768, P = 0.949 (Table1).

Morphology of sensilla

Sensilla are the microscopic hair-like olfactory chemosensory receptors present on the insect's antennae. Seven different types of sensilla on the ventral and dorsoventral surfaces of *S. incertulas* flagellomeres, namely, (i) Sensilla trichodea (S. T), (ii) Sensilla chaetica (S. Ch),

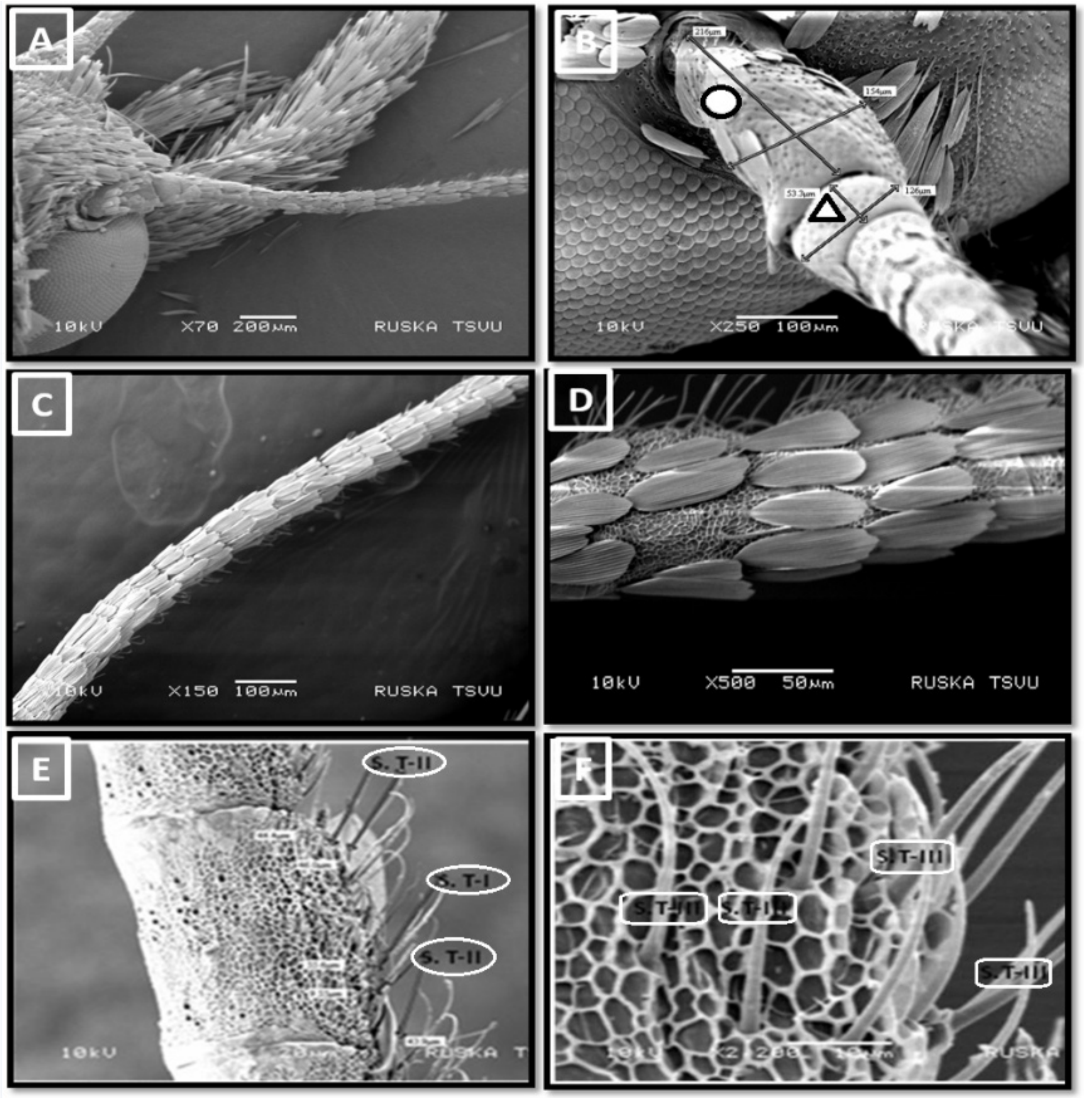

Fig. 1. Represents the different regions and different types of sensillae on the antenna of *S. incertulas*(A) Photo-micrographs of female *S.incertulas* antenna situated between the compound eyes; (B) Scape is marked with a circle, pedicel with a triangle; (C) Dorsal view of male antenna tightly covered with the scales (D) Scales on the female antenna are arranged loosely; (E) A single segment of male antenna showing different types of sensilla trichodea (S. T-I and S.T-II) differentiated based on their size and (F) Closer view of shorter sensilla trichodea(S.T-III) on female antenna.

(iii) Sensilla styloconica (S. St), (iv) Sensilla squamiformia (S. Sq), (v) Sensilla coeloconica (S. Co), (vi) Sensilla cavity and (vii) Bõhm bristles (BB) have been identified.

Sensilla trichodea (S. T)

Sensilla tricodea is abundantly present on theantennae of both the sexes. Three different subtypes of S.T have been identified namely, S. T-I, S. T-II, and S. T-III respectively. S. T-I is only observed in males, they are longest, straight and slightly curved at the apex. S. T-II is hook-shaped which is curved toward the top with a conical tip (Fig. 1E). The number of S. T-II on male and female antennae shows higher significance with $t = 16.50$ and $P < 0.001$ (Table 2). The female S. T-II antenna is longer than the male ones. S. T-III is the smallest of the three and is bent close to the antenna surface (Figure 1F). S. T-III was more common in females than in males and there was a significant difference between both the sexes($t = 5.75$ and $P < 0.001$) (Table 2). The mean length of S. T-III was higher in males than in females (Table 3). The sensilla trichodea of *S. incertulas*was classified into three types according to their size and shape in the antenna and arranged side by side. Sexual dimorphism in the distribution of Sensilla trichodea (S.T) was observed in both sexes. S.T-I were abundant on the males' antennae and absent on the females. A similar type of distribution of S. T-I was found in other Lepidoptera *Z.dixolophella*[19], *P.interpunctella*[23] and has also been observed in other lepidopteran species viz., *H. armigera*[24], *C. remissa*[20]; *C. pomonella* and *C. succedana*[25]. Contrary to the present findings, S.T-I was also present in the female antenna of *M. separata*[26], and in addition, S.T in *C. medinalis* was classified into four types[21]. These sensilla play a key role as receptors for the detection of plant volatiles and mate cues[18, 21, 27]. Particularly in females, these types of sensilla are known to identify their own

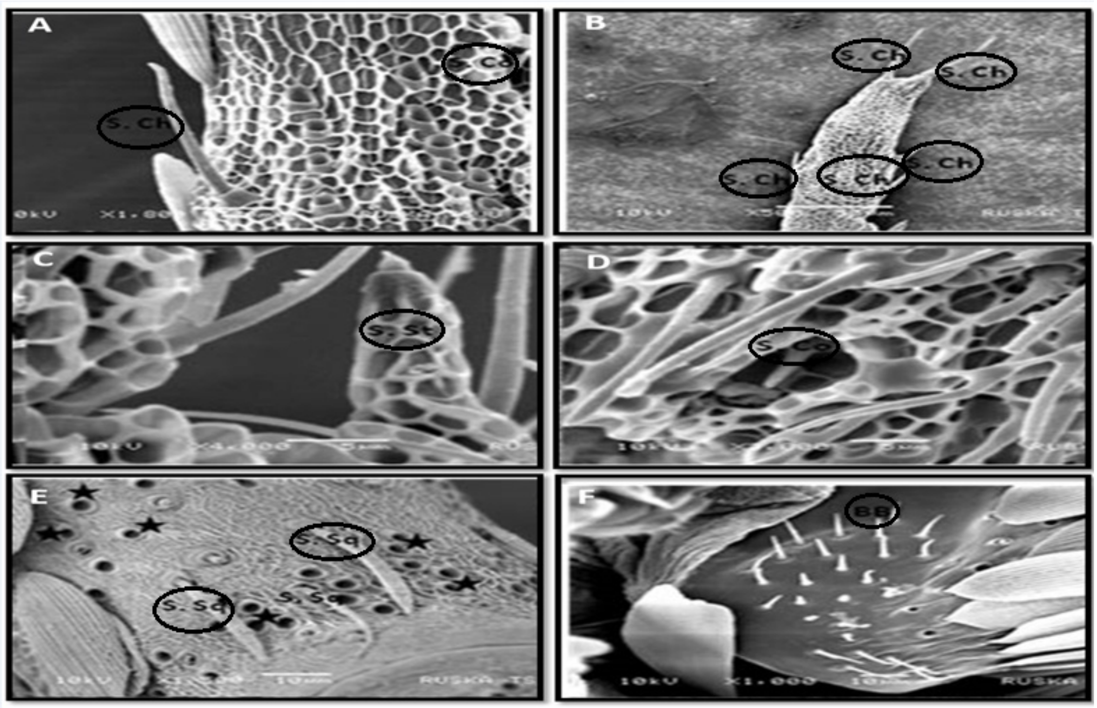

Fig. 2. Scanning electron micrographs of different types of sensillas of *S. incertulas* antenna (male and female). (A) SEM micrographs showingSensilla Chaetica (S. Ch) on male antenna segment, sensilla coeloconica (S. Co) and honey comb mesh like surface, (B) shows the presence of more number of Sensilla Chaetica (S. Ch) on the tip portion of female antenna,(C) Cylindrical shaped Sensilla styloconica (S. St) with pointed tip on the distal region of flagellum, (D)Small peg like structure protruding out from depressed region, Sensilla coeloconica (S. Co) without fence, (E)Sensilla squamiformia (S. Sq) and sensilla cavities (S. Ca) marked as star and (F)Böhm bristles (BB) present on the scape region

sex pheromones; either by attracting oviposite or by repelling it [28-29]. S. T-III is reported to serve as chemosensor and contact mechanoceptor[30].

Sensilla Chaetica (S. Ch)

These are the second copious sensilla wherein the S.Ch are emerging out straight from the round bulged socket, but slightly curved at its apex (Fig. 2A). They are distributed evenly along the ventral surface and few are noted at the central region. At the tip region 4-6 S. Ch (Fig. 2B) are observed and these are longer than the S.Ch found on the ventral and central region of flagellomeres. The S.Ch in females are more in number and are longer compared to males with higher significant values of t = 17.26 and $P < 0.001$ (Tables 2 & 3). The distribution pattern of sensilla chaetica on each antennal segment of *S. incertulas* was even, but a higher number of S. Chare observed at the tip portion. Our current findings with respect to type of distribution along the antennal segment are in agreement with the previous reports in *H. armigera*[24],*C. remissa*[22], *M. separata*[26], and *S. littolaris*[31]. The rigid structure of S. Ch arising from a socket may suggest the role of protection[26] and according to several studies in some lepidopteran families, these may also function as chemotactile and mechanotactile receptors[18,31, 32]and proprioceptors[16].

Sensilla Styloconica (S. St)

Sensilla styloconica are detected on the complete flagellum and a single sensillum was discovered at the end of each flagellomere. These sensilla are bulged at the base, a solid cylindrical edifice protruding out from it and having a stinger like structure at its tip (Fig. 2C). The average number of these sensilla present on males is less compared to the females (Table 2). No important distinctionis observed within the length of S. St. between males and females (Table 3). The contour of the sensilla styloconica and distribution on the antennal segment resembles *Z. dixolophella*[19]and *S. littoralis*[31], but is slightly different and unique (telson at its tip). Earlier this type of sensillum was also reported in several other insects like *A. segetum*[33], *M. sexta*[34], and *O. nubilalis*[18]. However, the function of S. St is unknown, as electrophysiological studies performed in several previous investigations suggest that these are thermo-hygrosensitive receptors[35-37].

Sensilla Coeloconica (S. Co)

These sensilla are smooth peg like structures projecting out from the shallow grooves on the antennal surface (Fig. 2D). S. Co are present 0-4 per flagellomere and were found more in females than male antennal segments which were not significant (t = 0.77 and P = 0.949) (Table 2). Sensilla coeloconicais are classified into two types i.e., S.Co I and S.Co II, depending on the presence or absence of circular fence[38]. However, in our study, we have identified only subtype II, without the circular fence around the peg like structures extruding out from the depressed regions. The presence of only S. Co II is one of the rare conditions observed in monophagous pests, *E. semipurpurella*[39]and *P. flammans*[40]. This type of sensillum was also reported in few other lepidopteran insects, *M. separata* [26], neopseustid moths[27], *C. punctiferalis*, *G. molesta* and *S. albicana*[41]. These sensilla are suggested to be responsible for olfactory stimuli; volatile odor of plants and identification of oviposition sites [22,42-43]. Though S. coeloconica has thermo-, chemo- and hygro receptor functions; these are also sensitive to humidity, CO_2 and water[44-46].

Sensilla Squamiformia (S. Sq)

Sensilla squamiformia are present among the scales of the antennae. These were scattered 2-4 per flagellomere on the dorsal surface (Fig. 2E) and few were observed on the head, but not taken into account. S. Sq appears like scales, but are narrow and tapered towards the tip (Fig.2B). S. Sq were considerably ample in males than in females (t = 4.34 and P = 0.003) (Table 2). S. Sq. noticed on males were longer compared to females (Table 3). In general, the characteristics of sensilla squamiformia are similar to scales, but are modified scales with tapered ends and are narrow [37]. Faucheux, 1999 has reported that this type of sensillumis frequently found on many other lepidopteran insects. Similar morphological structures and distribution were also observed on *Z. dixolophella*[19-20], *C. remissa*[22] ,*S. littoralis*[31], and*O. sacchari*[47]. These sensilla are surmised to have mechanoreceptive function [16, 37,48] or as wind velocity receptors in Coleoptera, *M. notatus*[49].

Sensilla cavity

Sensilla cavities are located on the basal segment and all over the surface of the *S.*

incertulas antenna. These are numerous pores of different sizes present irregularly on the antenna (Fig. 2E). Few sensilla cavities were also noticed underneath the scales; they were not reckoned and left unevaluated. Sensilla cavities are present on the overall antenna at different locations and size. These were suggested to function as contact chemoreceptors. Regarding the function of sensilla cavityit has been suggested that these may help insects in prevention of desiccation[30] and might play role in the perception of humidity and temperature[50]. In *C. medinalis* it was clearly illustrated that these cavities provide information to insects about the suitability of the plant and thus act as contact chemoreceptors[21].

Böhm bristles

Bõhm bristles are found on the scape that look like spines with smooth ends arising from small pits and these are found standing perpendicular to the antennal surface (Fig. 2F). The Bõhm bristles are very few in number and have no significant difference between males and females. Structurally, these sensilla are identical in both sexes (Table 2). The Böhm bristles are pointed setae located on the basal segments of the antenna of *S. incertulas*. Böhm bristles may function as mechanoreceptors[16]and these are responsible for sensing the position and movement of the antennae[51].

CONCLUSION

Finally, itmay be concluded that this is the first report on *S. incertulas* male and female antennal structure. In this SEM study, we have compared the antennae of *S. incertulas* (both males and females) and identified different types of sensilla, their distribution, location and size, which are not only sex-specific but also are unique when compared with other closely related species of lepidopterans. Further electrophysiological and TEM studies may confirm the function of these sensilla in identifying their host and mate cues, their communication processes and behavior. Therefore, future studies may also shed light on deploying of olfaction mechanism of *S. incertulas* and the development of IPM strategies.

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Conflict of interest

No conflict of interest

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