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Full Length Research Paper

Ultrastructure of antennal sensillae of the samsum ant, *Pachycondyla sennaarensis* (*Hymenoptera: Formicidae*)

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Black ant (Samsum), *Pachycodyla sennarrensis*, stings and injects venom and inflicts allergy (a rare clinical problem) due to its local and systemic reaction, which is considered as a health hazard amongst Saudi society. Thus, black ant is a source of serious concern for the government and experts as well. Ultramorphological variations, distribution, differential sensillae counts (DSC) and total sensillae counts (TSC), were identified and estimated as a complementary part of the peripheral nervous system on the antennae of worker samsum ant, *P. sennarrensis* in order to understand its behavioral ecology. Based on scanning electron micrographs, four types of sensillae constituted with three trichoid types, which is an abundant form with a high distribution density at the apex, but a low density at subsequent proximal flagellomere of the antenna and a placoid type of sensillae (a rare form mostly found in the middle of the flagellum, that is, on the 4th, 5th and 6th flagellomere) were categorised. It is documented that nonporous trichoid type of sensillae are mechanoreceptors and thermoreceptors, whereas, the placoid types are olfactory receptors. Present findings in an indigenous species in Saudi Arabia may help in understanding the ecological behaviour of the ant, which subsequently may form the basis in producing its effective control measure in future.

Key words: Samsum ants, *Pachycondyla sennarrensis*, ultrastructure, antenna, sensillae,

INTRODUCTION

Medically important ant, *Pachycondyla sennaarensis,* is known to cause anaphylactic shock upon stinging allergic persons that may deteriorate into serious health conditions. Related and relevant studies provided evidences of their hazardous nature with varying distribution density depending on seasons in almost all localities in different regions of Saudi Arabia (Al-Shahwan et al., 2006; Al-Khalifa et al., 2009). Immense odor-evoked responses are exhibited amongst insects cues from diversified sources of plants and pheromone from other insects, owing to olfactory sensory neurons (OSN) associated with the sensillae, are expressed in seven diverse family of trans-membrane odorant receptors (Ors) (Laissue and Vosshal, 2008).

However, insect sensillae have been the subject of exhaustive reviews (Schneider, 1964; Altner and Prillinger, 1980; Keil and Steinbrecht, 1984; McIver, 1985; Zacharuk, 1985; Altner and Loftus, 1985; Zacharuk and Shields, 1991). A detailed scanning electron microscopy (SEM) survey of the sensillae on the antennae of many ant species was previously reported by Hashimoto (1990). The antennae of adult insects have various types of sensillae with different functions and play an important role in various behaviors during adult life. Antennal sensillae are important sensory receptors involved in host location and discriminatory behaviors (Schneider, 1964; Ochieng et al., 2000). Sensillae are the antennal

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Abbreviations: DSC, Differential sensillae counts; **TSC,** total sensillae counts; **ST1,** sensillae trichoidea 1; **ST2,** sensillae trichoidea 2; **ST3,** sensillae trichoidea 3; **SPL,** placoid sensillae; **SEM,** scanning electron microscopy; **OSN,** olfactory sensory neurons.

derivatives that are distributed all over its surface to perform sensory functions. Density, diversity and ultrastructure of sensillae are explicitly and explicably linked with the behavioral ecology of ants (Faucheux et al., 2006). Polymorphic shapes of sensillae constitute different types, which have been assigned different functions. Basiconic type acts as food and CO_2 receptor, trichoids as pheromone receptors and coeloconic sensillae as water and ammonia odor receptors (Laissue and Vosshall, 2008).

In the current study, scanning electron microscopy (SEM) was used to study the ultrastructure of the different types of antennal sensillae of the samsum ant worker, *P. sennaarensis*. Simultaneously, the differential sensillae counts (DSC) together with the total sensillae counts (TSC) in a unit area, mm^2, in the different segments of the antenna flagellum were counted to understand and interpret their possible functions.

MATERIALS AND METHODS

Experimental ants

Studied colonies were collected from different localities of Riyadh region, Saudi Arabia Kingdom, and were housed in a plastic nest bottle within a large plastic box (45 x 30 x 18 cm) that was used as a foraging area with Fluon-coated walls to prevent ant escape. The insectaries were maintained at 28 ± 1°C, relative humidity of approximately 30% and a 12:12 h light: dark regime. Colonies were provided fresh water in glass tubes sealed with cotton wool and fed daily on dead insects, grinded rice and wheat seeds or breads, and weekly with apple pieces.

Scanning electron microscopy

Workers Samsum ant, *P. sennaarensis*, were first anaesthetized in a freezer (4°C) for 2 min and their heads were removed. Antennae were carefully excised from the antennal sockets with a fine forceps (Fisher Scientific, Germany) at 10x under a dissecting stereomicroscope (Olympus SZX12, Japan), and immediately were fixed overnight in 4% glutaraldehyde at 4°C. They were rinsed in a phosphate buffer three times for 15 min and each was subjected to post fixation in 1% osmium for 2 h. Also, they were bathed three times for 15 min in water and then dehydrated in a graded alcohol series (30, 50, 70, 80, 90 and 100%) in each case for one hour. Each individual antenna was mounted on aluminum stubs with double-sided sticky tapes. Immediately before microscopy, antennae were coated with gold in a high resolution sputter coater (Hitachi E-1010). Antennae were then examined under SEM (JEOL – JSM 636, Japan).

The criteria adopted by Schneider (1964), Zacharuk (1985) and Laissue and Vosshall (2008) constituted the basis for the present distinction amongst the categories of sensillae. Sensillae were identified for their different types and were counted and measured on the screen through micrographs. Mapping and differential counts were taken into 20 antennae for comparison, and 10 loci in each antennae to include their mean was calculated with standard deviation using least significant test (LST).

Total sensilla counts (TSC)

Numbers of sensillae were counted on scanning electron microscope in 10 preview of the area 38.8 x 29.1 µm and were then calculated in per square millimeter. As a result, the mean and standard deviation were used to evaluate the relative distribution density of sensillae in different flagellomeres, starting from the apex to proximal ends (Table 3).

Differential sensillae counts (DSC)

Under each view of the area 1129.08 μm^2, different categories of sensillae were counted and their relative percentage on each of the 10 observations were used to calculate the mean and standard deviation used in evaluating the specific role of each type. However, no datum is reported in this aspect and as a result, the present finding would signify the specific role related to each type.

Statistical analysis

Sensillae on different segments (flagellomere, 1 - 11) were measured, identified and counted amongst worker individuals. Measurements were taken from individuals and the means were calculated with standard deviations. However, data on total and differential numbers in different flagellomeres were counted and calculated through the scanning electron micrographs and the means were separated by the least significant difference (LSD) test. Consequently, significant values were obtained ($P < 0.05$) using version 12.0. software package of SPSS.

RESULTS

General description of the antennae of P. sennaarensis

The geniculate type of antennae (2.3 ± 0.4 mm in length, n = 20) comprised three components; the scape (0.1 ± 0.09 µm; n = 20) at its proximity, the pedicel (0.9 ± 0.2 µm; n = 20) in the centre and eleven segments (the flagellomeres) in its flagellum (1.2 ± 0.2 µm). The apex or the first segment of the flagellum is longer and larger than the other segments, while the cuticle of the antennae is black with smooth and finely finished surface.

Types of sensillae

Myriad sensillae are concentrated on the first flagellomere declining subsequently in the second to eleventh segment. Pedicel is exhibited scarcely in located sensillae. Sensillae are composed of a central piece of cuticle in the shape of a thin and long filament with short pedal inserted into a socket bound by membranous ring. The central shaft can freely move in the socket in all direction to maximize its effective functioning. Based on the morphology, size and function, four distinct categories of the sensillae were identified, out of which, three belonged to the trichoidea group and one to placoid.

Sensillae trichoidea 1 (ST1) (*Bohm bristles* sensillae)

The ST1 sensillae are peg-shaped triangular structures

Table 1. Morphometery of the antenna of *P. sennarrrensis* with sizes of the flagellomeres (F_1 - F_{11}) and their segments, mean and total area.

Flagellomere	Mean width/mm with standard deviation	Mean length/mm with standard deviation	Mean area/mm^2 with standard deviation	Total area/mm^2 with standard deviation
F_1	0.138±0.03	0.353±0.09	0.048±0.01	0.096±0.009
F_2	0.123±0.01	0.123±0.08	0.015±0.009	0.03±0.009
F_3	0.123±0.02	0.107±0.09	0.013±0.009	0.026±0.01
F_4	0.107±0.03	0.092±0.01	0.0098±0.009	0.0196±0.009
F_5	0.092±0.003	0.107±0.02	0.0098±0.008	0.0196±0.008
F_6	0.092±0.003	0.107±0.05	0.0098±0.008	0.0196±0.009
F_7	0.092±0.002	0.107±0.05	0.0098±0.008	0.0196±0.008
F_8	0.076±0.01	0.107±0.06	0.0081±0.009	0.0162±0.009
F_9	0.076±0.01	0.107±0.07	0.0081±0.009	0.0162±0.01
F_{10}	0.076±0.01	0.107±0.07	0.0081±0.007	0.0162±0.01
F_{11}	0.061±0.01	0.384±0.07	0.023±0.006	0.046±0.12
Pedicle	0.078±0.02	0.964 ± 0.24	0.07±0.02	0.14±0.35
Scape	0.046 ±0.01	0.057± 0.03	0.026±0.032	0.052±0.023

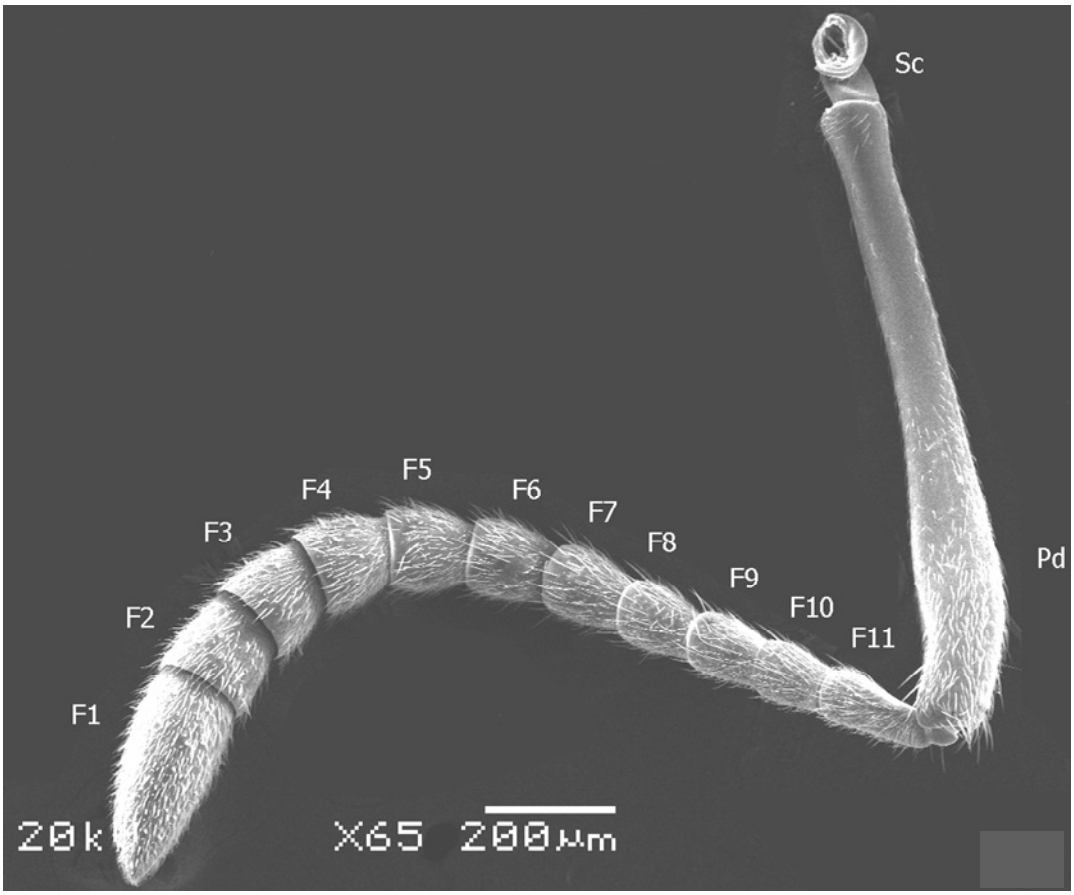

Figure 1. Scanning electron micrographs of the excised antenna of worker *P. sennarrensis* with 11 flagellomeres, (F1 – F11) and a pedicel with scape.

that have a broad diameter of 0.56 ± 0.32 µm; n = 10 at the base with a sharp pointed tip and a short length of 0.67 ± 0.45 µm; n = 10. The total area of the first structure was measured as 0.96 ± 0.009; n = 10 (Table 1 and Figure 1). These sensillae are located at the proximal end or pedals of flagellomeres, and their number are

Table 2. Changes (percentages) in the mean values of the differential sensilla counts (DSC) on the different flagellomeres (F_1-F_{11}) and segments in the antenna of *P. sinnarensis.*

Flagellomere /segment	Percentages of different type of sensillae			
	Sensilla trichoidea (ST1)	Sensilla trichoidea (ST2)	Sensilla trichoidea (ST3)	Sensilla placoidea (PLS)
F_1	65.67±3.62	25.71±3.16	7.77±0.75	2.01±0.48
F_2	61.24±2.95	21.75±3.74	10.5±0.49	6.25±±0.25
F_3	64.24±2.95	18.67±4.65	9.56±1.1	7.56±1.01
F_4	30.14±2.45	46.56±3.56	17.08±1.78	5.07±0.94
F_5	25.67±1.98	51.67±4.5	15.67±2.2	7.08±.86
F_6	20.45±2.67*	55.56±3.8	16.68±1.9	7.09±0.96
F_7	30.13±1.96	56.68±	7.01±0.69	5.01±0.45
F_8	40.23±2.54	47.15±2.45	6.10±0.97	5.15±.89
F_9	50.14±1.89	37.26±3.54	8.19±1.54	3.16±0.91
F_{10}	68.64±2.13	19.37±2.94	7.10±1.30	4.17±1.31
F_{11}	72.57±4.6*	19.07±2.59	5.44±0.85	2.91±1.5
Pedicel	0	91.07±10.6	3.24±1.5	4.98±2.1
Scape	None	100	None	None

Table 3. Total sensilla counts (TSC) in different flagellomers (F_1 - F_{11})* and segments of the antenna.

Flagellomer	Number of sensillae in 38.8 X 29.1 µm	Number of sensillae/mm²
F_1	25±1	22222±501
F_2	22±2	19552±478
F_3	18±1	15997.1±333
F_4	16±1	14219±232
F_5	13±2	11553.5±158
F_6	10±1	8887.3±232
F_7	9±1	7998.5±311
F_8	9±1	7998.5±358
F_9	8±1	7109±212
F_{10}	7±2	6221.1±189
F_{11}	6±1	5332.3±203
Pedicle	4±1	3554.9±149
Scape	None	None

* Antennal segments.

estimated as 8887 ± 801/mm^2; n = 10 (Table 2).

Sensillae trichoidea 2 (ST2) (*Microtracheal sensillae*)

The ST2 sensillae are straight or slightly curved with a length of 20.8 ± 2.5 µm; n = 10, while fine sensillae are abundantly distributed all over the periphery in parallel rows on the flagellomeres. However, their number, 56888 ± 201/mm^2; n = 10 (Table 2), followed a declining trend towards the pedicel in the subsequent posterior segments. These are inserted into sockets that have membranous surroundings with a free space at the base (Figure 2).

Sensillae trichoidea 3 (ST3) (*Microtracheal sensillae*)

The ST3 are a relatively thicker type of trichoidea and shorter than chaetal sensillae in length 16 ± 2.7 µm; n = 10. They are broader at the base with a blunt tip and their number was calculated as 7108 ± 291/mm^2; n = 10 in the first falgellomere (Table 2 and Figure 3).

Placoid Sensillae (SPL) (*Coeloconic Sensillae*)

The SPL are peg-shaped or round cavities without hair shaft and located as grooves on the ventral side of flagellomeres, and these were observed only on the 3rd, 4th, 5th, 8th and 11th flagellomeres (Table 2 and Figure 4).

DISCUSSION

Distinct functions performed by sensillae constitute the basic criterion of morphological differences, size variations and the number of neurons housed in each of the sensilla. Multiporous sensillae (the trichoidea) were previously identified with different names like trichoidea with wall pores (Wibel et al., 1984; Ryan, 2002; Bleeker et al., 2004; Onagbola et al., 2008).

The sensillae trichodea curvata are proven to respond to a wide range of organic compounds including various pheromones (Dumpert, 1972; Martini and Schmidt, 1984). Electrophysiological studies confirmed pheromone receptor functions to trichoidea sensillae of *Neodiprion*

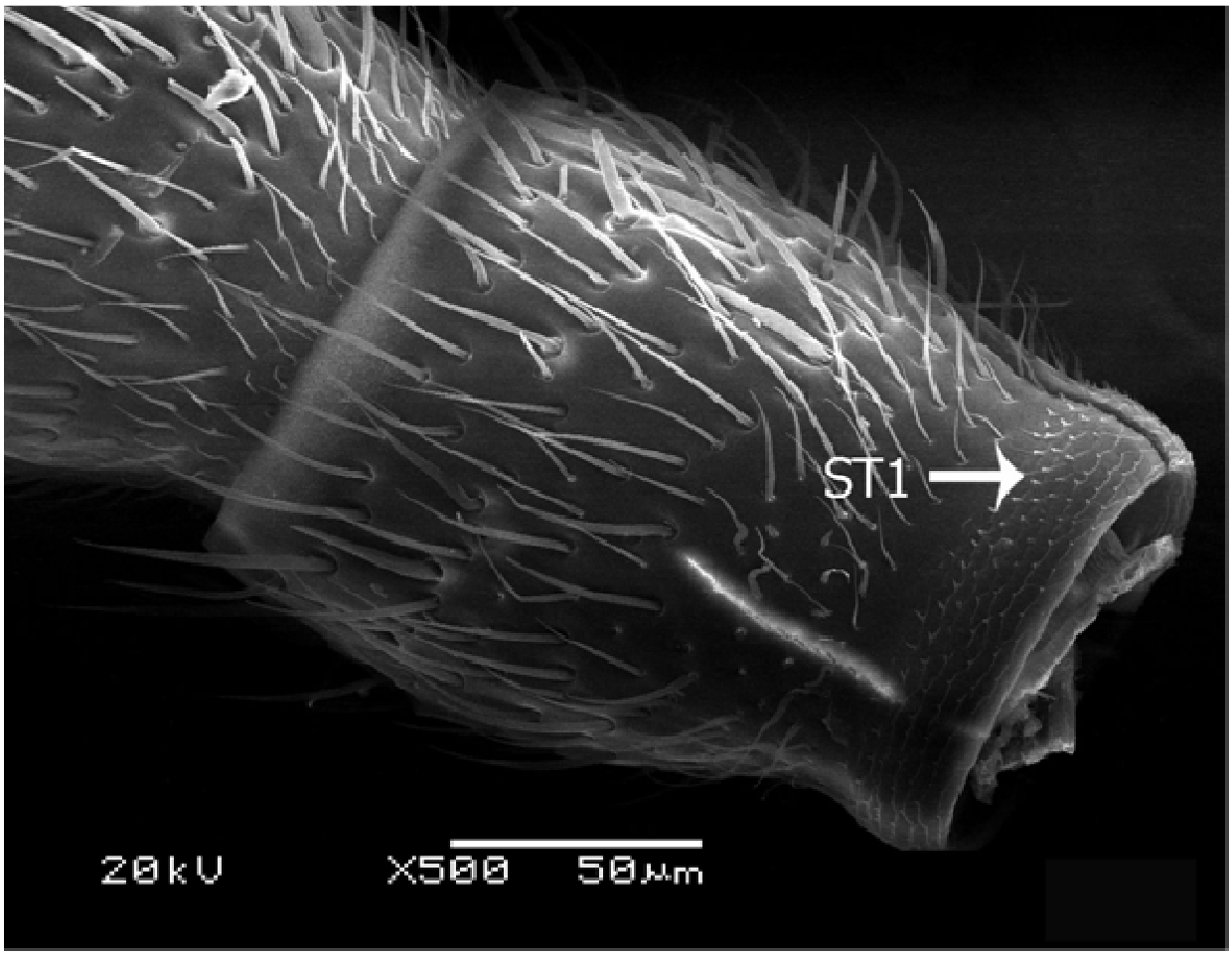

Figure 2. Scanning electron micrographs of a flagellomere with a pointer indicating sensilla trichoidea 1 (ST1).

sertifer Geoffory (Hymenoptera: Diprionidae) (Hansson et al., 1991). In *Rhopalicus tutela*, Pettersson et al. (2001) described pheromone receptor as a function to sensillae trichoidea. In *P. sennarrensis,* the sensillae are non-porous structures, and so, only the olfactory receptor function may be attributed to these sensillae due to their minimum (zero) role in sexual activities.

Placoid sensillae, on the other hand, are believed to be associated with chemoreceptive activities and that helps in food finding sensitivity through their secretory role similar to glandular openings. An example of that is the single sensillum of *Micropitis croceipes* that responded, in a dose dependent manner**,** to plant volatiles (Ochieng et al., 2000). Electrophysiological studies on the placoid sensillum in *P. sennarrensis* would further shed light of confirmation on this aspect. Bombristle type (Sensillae Tricohoidea 1, ST1) and Microtrichial sensillae (Sensillae Trichoidea 2, ST2) in *P. sennaarensis* are more likely to act as mechanoreceptors similar to that in *Bemisia tabaci* (Lin et al., 2007). Non-porous microtrichial sensillae described here are comparable to the non-sensory hairs on the antennae of some dipteran species (Shanbhag et al., 1999; Sukontason et al., 2007; Chen and Fadamiro, 2008). However, in some homopteran species, these are considered to be non-sensitive hairs (Mellor and Anderson, 1995; Lin et al., 2007). In *P. sennaarensis*, these microtrichial sensillae are termed as sensillae trichoidea, in which ST1 are quite long and non-porous. In other species, these types are also termed as non-innervated spinules, spines or trichomes (Shanbhag et al., 1999; Stocker, 2001; Fernandes et al., 2002; Lin et al., 2007). However, the basiconic sensillae that have been described in *Drosophila melanogaster* (Shanbhag et al., 1999), phorid fly, *Pseudacteon tricuspis* (Chen and Fadamiro, 2008) and *Phoracantha semipunctata* (Lopes et al., 2002) have not been observed in *P. sennaarensis*. Heavy density of non-porous sensillae trichoidea conforms with the mechanoreceptor or thermoreceptor's role in samsum ants.

This study herein, supposedly stand to be the first attempt to describe the antennal sensillae in *P. senna-arensis* workers using SEM, and might be considered as the first step towards a future investigation of the odorant receptors in this medically hazardous ant which, hope-

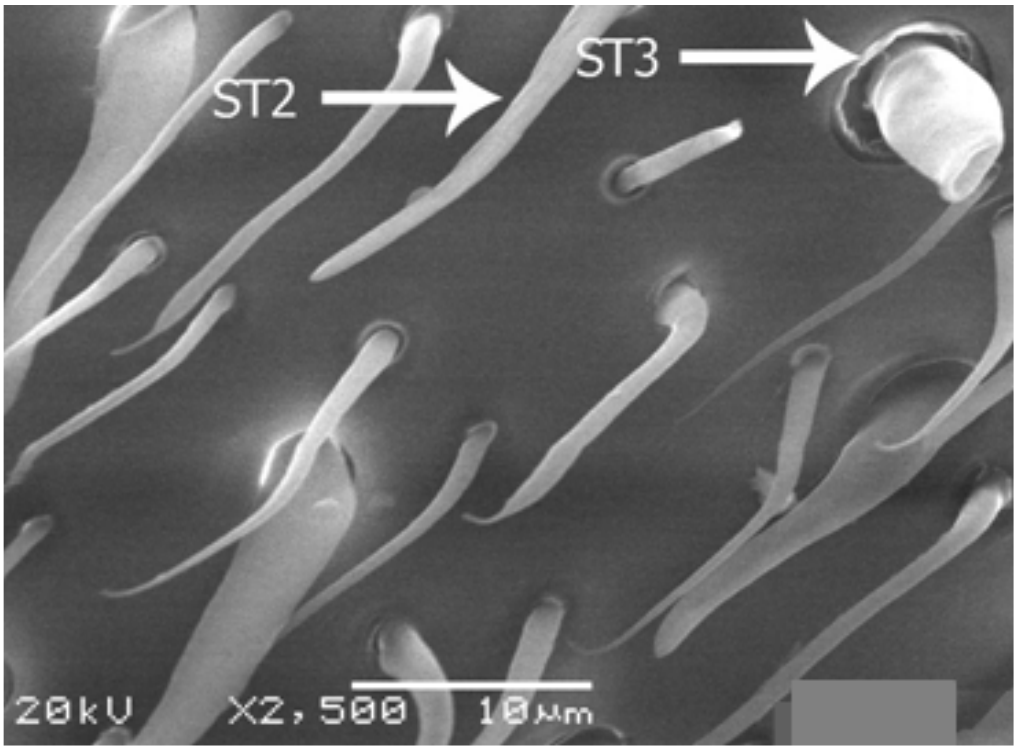

Figure 3. Scanning electron micrographs of a flagellomere with a pointer indicating sensilla trichoidea 2 (ST2) and sensilla trichoidea 3 (ST3).

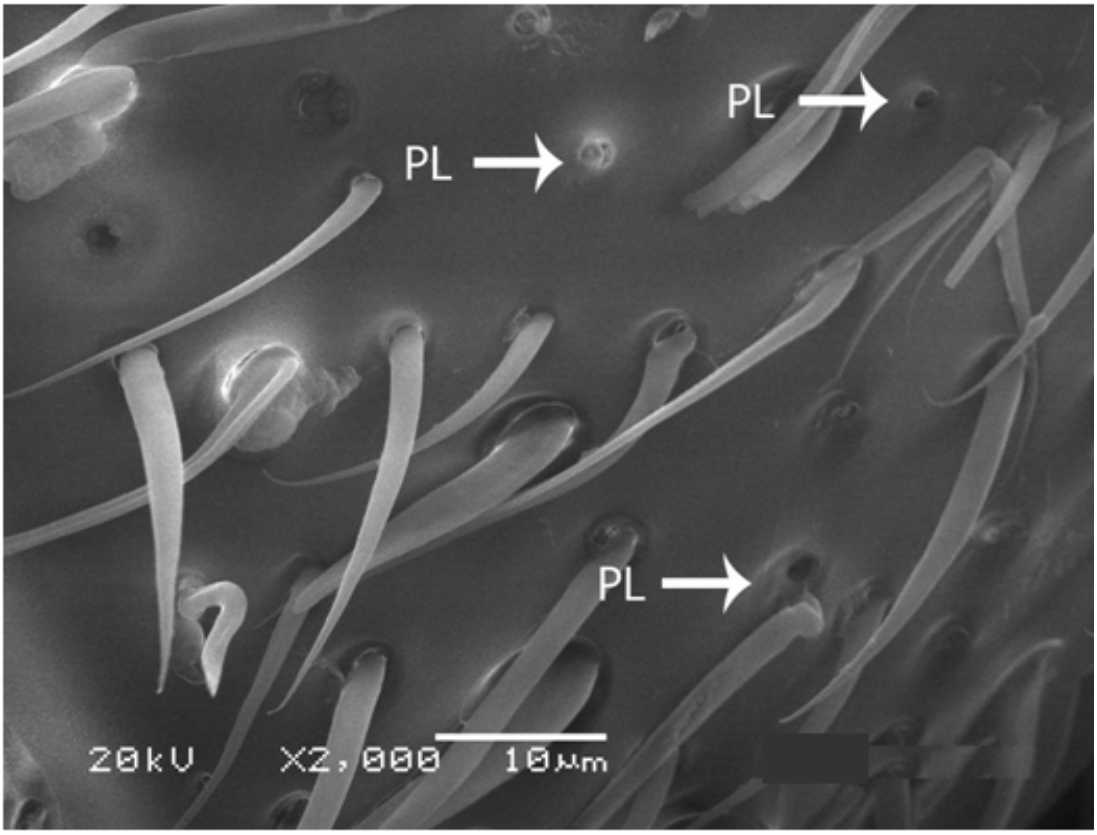

Figure 4. Scanning electron micrographs of a sensilla placoidea in *P. sennarensis*.

fully, may help in establishing an effective control strategy in the endemic regions of Saudi Arabia.

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