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SCANNING ELECTRON MICROSCOPIC INVESTIGATING DIFFERENT TYPE OF SENSORY SENSILLAE ON THE HEAD APPENDAGES OF ASIAN TIGER MOSQUITO, *AEDES ALBOPICTUS (SKUSE)*

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Abstract

In *Aedes albopictus* various types of sensillae were on the head and its appendages with distinct morphological features. The antennae and maxillary palps play a major role in host detection, and other sensory-mediated behaviors. Six long sensilla chaetica were arranged in circular shape and spread uniformly across the diameter of the antennal flagellomers. Maxillary palp is a relatively simple organ compared to antennae, consists of five segments, which densely covered dorsal side with flattened scales, but chemosensory sensilla on ventral side. Thus, a desirable model for exploring neuromolecular networks surrounds chemo- and mechanosensation. Proboscis analyses gustatory code via food intake and involved in identification. Labellum exhibits the most of sensory structures on the proboscis. Numerous sensillatrichoidea distribute on antennal flagellomers.

A significant difference in diameter of sensillacoeloconica that protruding from distal tip of antennal flagellomers. Sensillachaetica with greater morphological variation were distributed throughout *Ae. albopictus* head and appendages. Variation between different sensillae indicates their functions. Aporous sensilla play the mechanoreceptive role but, porous sensilla were contact chemoreceptors and olfactory ones. *Aedes aegypti'*sensory mechanisms were extensive studied, about Asian tiger mosquito, *Ae. albopictus* sensory systems is insufficient.

Key words: *Aedes albopictus*, SEM, Antenna, Maxillary palp, Proboscis, Sensilla

Introduction

The behavioral responses of female mosquitoes to find their host are a vital factor in their potential for disease transmission. A daytime biting Asian tiger mosquito, *Aedes albopictus* (Skuse) (Diptera: Culicidae) is viewed as a potential vector of several arthropod-borne viruses (arboviruses), including the most common human arboviral pathogen, the dengue virus (DENV). This caused significant concern among many of the scientists and public health officials about the possibility that this species' range expansion would raise the risk of arthropod-borne virus transmission Gratz (2004), Mitchell (1995) and Lambrechts *et al.* (2010) for *Aedes albopictus*. Olfaction is the primary sensory way used by mosquitoes search for long distance host / oviposition sites (Davis and Bowen, 1994). Antennae are the main olfactory sensors of mosquitoes and are therefore well-equipped with a wide range of sensilla. Odors were essential sensory signals which showed female mosquitoes to their favorite blood meal hosts (Takken and Knols, 1999; Zwiebel and Takken, 2004; Pitts and Zwiebel, 2006; Lu *et al,* 2007; Bohbot *et al,* 2014).

The sensory receivers, sensilla exist in numerous forms and are placed with special locations at maxillary palp, antennae, proboscis, etc. Each sensilla is specialized in receiving a well-defined stimulus like changes in temperature and humidity, mechanical effects and any kind of odors. The behavioral responses of female mosquitoes to find their host are a vital factor in their potential for disease transmission.

There are approximately 174 sense organs present in flagellum of *Culex* mosquitoantennae (Dhanalakshmi *et al,* 2018). The sensilla on mosquito antennae were studied by Ismail (1964) for culicine and anopheline females, Seenivasagan *et al.* (2009) for *Aedes albopictus* and Ibrahim *et al.* (2018) for *Culex pipiens*. SEM was effective for external structure and distribution survey (Slifer *et al,* 1959). Zacharuk (1984) examined insects' sensory structures and classified olfactory sensilla based on wall thickness and pore distribution. Mclver (1982) and Sutcliffe (1994) found that 90% of sensilla on mosquito antennae have an olfactory function, and Maekawa (2011) added that proboscis play an essential role during host-seeking.

Sensory organs of antennae are essential for their activities interaction (Ismail, 1962). Sensillatrichodea have receptor cells for oviposition (Bentley *et al,* 1982) and nectar-sources (Davis, 1977). Mosquito antennal sensillae were studied (Coluzzi, 1964; Hill *et al,* 2009). Bohbot *et al.* (2014) studied maxillary palp sensilla of *Ae. aegypti* and its potential involvement with sensory modalities. Dhanalakshmi *et al.* (2018) reported that female mosquito found its hosts by produced carbon dioxide.

The present work aimed to investigate and describe sensilla types on head capsule and appendages; maxillary palp, proboscis and antenna of *Aedes albopictus* females by scanning electron microscope (SEM).

Materials and Methods

Specimens of *Aedes albopictus* adults were obtained from the laboratory colony maintained under controlled conditions of 25±1°C, 70-80% PH and a photoperiod of 12L: 12D for more than 15 generations in the Institute of Zoomorphology, Cell biology and Parasitology at Heinrich-Heine-University (Düsseldorf, Germany). For SEM investigation, ethyl acetate was used to kill the adults and fix them for 3 days within 2.5% glutaraldehyde in a solution of 0.1 cacodylate buffer (pH 7.4) to ensure hardening of tissues. Adults were dehydrated exposed to ascending acetone concentrations (50, 70, 80, 90, 96, & 100%) for 5min in each change. Then they were exposed to critical point drying and coated with gold using sputter-coating device for SEM (LEO1430VP) examination. Head capsule and its appendages; antennae, maxillary palp and proboscis were examined for various types of taxonomic sensory structures.

Statistical analysis: Length and width (M±SE) of each sensilla was compared using software SPSS (Version 14.0 for Windows evaluation version).

Results

Among the morphological characteristics, length has emerged as a significant attribute for differentiating the kinds of sensilla (Tab. 1). Various forms of sensilla were defined and mentioned based on shape, scale and structural features. Results of this work have been recorded by a basic survey of the various types of sensillae on the vertex (V), antenna (An), maxillary palp (Mp) and proboscis (Pr) that observed on *Ae. albopictus* such as sensillae coeloconica, sensillae chaetica, sensillae trichoidea, sensillae basiconia.

Head: Like all Pterygota, have annulated antennae. Head contained multiple sclerites fused together to form the head capsule. Compound eyes (Ce) covered a large head portion. The epicranial suture was quite uncharacteristic. Its median section the coronal suture (Cs) and two branches just above scape (S) formed frontal sutures. Head area beneath front sutures was the frons. A thin strip of vertex (V) or interocular space lied between compound eyes. Two sensillal types scattered over head vertex was pointed, thick-walled, articulated setae, and some of taxonomic value. Microscopic head dorsal side showed two different types of spine-like structures and many scales. i- Sensillachaetica (Ch) were thick-walled, long articulated and sturdy bristle externally grooved arising from a socket distributed close to compound eye as two types: large sensillae chaetica (Ch I) measured 194.08 ±0.09µm in length & 2.58-±0.45µm in width basally and small sensillae chaetica (Ch II) measured 89.73±0.03µm in length & 2.24± 0.25µm in width basally. ii- Grooved pegs (Gps) were deeply grooved, thick-walled and medium in size, few in number and distributed along compound eyes, measured

57.98±0.076µm in length & 2.84±0.082µm in width basally (Figs. 2a, b & c).

Antenna: *Ae. albopictus* antennae filamentous in shape, segmented and consisted of three major parts, scape (S), pedicel (P), and flagellum (FL), varying in shape and size with thirteen flagellomers (Fig. 1a & c).

All flagellum segments were more or less similar, but basal one varied in a few details. Its third length inside the pedicel's funnel was pigmented and surrounded by cuticular microse-tae; basal segment, scape (S) of an irregular chitins' shell, collar shaped and concealed behind an extended, spherical second segment or pedicel. Pedicel (P) is a mechano-sound receptor (Johnston organ), with a few scales (Sc) and thick-walled short articulated sensillae trichoidea (Tr). Antennal sensillae performed most olfaction-driven behaviors such as host-seeking, oviposition and nectar-feeding location. Other thirteen segments and flagellum carried several important sensory organs for behavior and receipted many external stimuli (Figs.1b & c). Microscopic antenna showed several kinds of spine-like, hole-like, hairy sensory structures and few scales at flagellum first segment measured 62.07±0.65µm in length & 22.98±0.05µm in width basally. Sensory structures were of differed lengths, short or very long, small pit-like ones on flagellamers: i- Sensillae chaetica (Ch): Long and thick-walled articulated bristles raised from a socket with delicate serrations around \bottom of grooves with strong-pointed. Basally, within each flagellomer 2-13, six long sensillae chaetica arranged in circular shape and spread uniformly along antennal diameter of flagellomers. SEM showed sensillae chaetica surface longitudinal grooves composed of two distinct subtypes, large sensillae chaetica (Ch I) measured 278.87±0.034µm in length & 2.17±0.046µm in width basally and small one (Ch II) measured 187.45-±0.078µm in length & 1.83±0.092µm in width basally, with fewer numbers of small sensillae chaetica on the first two antennal segments (Figs.1b & c). ii- Sensillae trichoidea (Tr): Hair-like not articulated slightly pigmented setae raised from sockets, tipped, smooth and numerously distributed on antennal segments. Thickness was evidently more regular than straight trichoidea, hair-like olfactory organs, and most numerous on flagellomeres 2-13. Two pointed subtypes of sensillae trichodea, one long (Tr I) measured 48.34 ±0.14µm in length & 0.98±0.076µm in width basally and one short (Tr II) measured 37.67±0.87µm in length & 0.67±0.34µm in width basally. Number of long sensillae trichodea reduced from distal ends to proximal ones with a smooth surface more frequent than short sensillae trichodea (Figs.1b & 2e). iii- Grooved pegs (GPs): Medium in size, thick-walled, peg shaped, deeply grooved, and measured 68.56±-0.04µm in length & 2.78± 0.316µm in width basally (Figs.1c, d & e; 2d). iv- Sensillae basiconica: Saddle-shaped, thin-walled structures with curved tips (sensory pegs), irregularly distributed sharp, tipped trichoidea of antennal segments chemo-sensilla. Two subtypes, long sensillae basiconica (Ba I) more concentrated in flagellum first basal segment, measured 29.89±0.078 µm in length & 0.74±0.065µm in width basally and short one (Ba II) on the all antennal segments, measured 19.9±0.96µm in length & 0.68±0.087µm in width basally (Fig.1b). v- Sensillae coeloconica (Co): Medium sized, thick walled, peg organs sunk into antennal wall depressions. On cuticle surface, pegs as circular openings, protruded from distal flagellum tip, with narrow cuticular hole on bottom of a pit, as round openings with a diameter of 4.89±0.083µm in cuticule of one pin inside pits (Fig. 1b)

Maxillary palp: Relatively simple compared to antennae, with five segments dorsally heavily covered with flattened scales. The five main sensilla types, with fourth segment exhibited the most of sensory structures included microtrichia, scales, capitate pegs, grooved pegs, sensillae basiconica, sensillae chaetica and sensillae campaniformia. i- Capitate pegs (CP): Chemosensory sensillae distributed on ventral side of maxillary palp segments number 2, 3 & 4, spatula/spoon formed at distal end and arise from circular depression measured 13.23±0.09µm in length & 0.68± 0.087µm in width basally (Figs. 3e & f). ii- Grooved pegs (GPs): Four robust, long, thick walled, longitudinally grooved, found on 1, 2 & 3 maxillary palp segments measured 98.917±0.35µm in length & 2.69± 0.075µm in width basally & 4 long grooved pegs basally (Figs. 3b, c, d & f). iii- Sensillae basiconica (Ba): Smooth surfaced, widened basally and tapered distally on segments number 1, 3 & 4, surrounding sensillae campaniformia, measured 23.54±0.076µm in length & 0.84±0.047µm in width basally (Fig. 3d). iv- Sensillae campaniformia (Ca): Dome-like on ventral side of maxillary palp segments number 2, 3 & 4, measured 12.97± 0.074µm in diameter (Figs.3b, c, d & f).

v- Sensillae chaetica (Ch): Long, thick-walled articulated bristles and externally grooved sturdy bristle raised from a socket with gentle serrations around base of grooves with strong-pointed. Two types; large sensillae chaetica (Ch I) measured 78.499±0.098 µm in length & 1.94±0.98µm in width basally and small sensillae chaetica (Ch II) measured 45.62±0.214µm in length & 1.23±0.67 µm in width basally (Figs.3b, c, d & f).

Proboscis: An essential head appendage for host identification. Recognition of pseudo-thermo targets not only antennae and maxillary palps, but also proboscises. Labellum exhibited the most of sensory proboscis' structures on. Three major types: i- Sensillae chaetica (Ch): Located on labellal outer edge (Lb) as two distinct types surrounded by a numerous micro-trichia; long sensillae chaetica (Ch I) measured 69.718±0.076µm in length & 1.87±0.063µm in width basally and short sensillae chaetica (Ch II) measured 58.23±0.045µm in length & 1.76±0.076µm in width basally (Figs. 4b, c & d). ii- Grooved pegs (GPs): Six grooved pegs around ventral side of labium and dorsal side of labellum, robust, long, thick walled and longitudinally grooved measured 61.56±0.087µm in length & 2.67±0.076µm in width basally (Figs. 4a, c & e). iii- Sensillae basiconica (Ba): Thin-walled structure irregularly distributed as chemosensilla. Two subtypes on proboscis; long (BaI) measured 22.65±0.023 µm in length & 0.84±0.12µm in width basally and short (Ba II) measured 17.90±0.34µm in length & 0.59.3-±0.43µm in width basally (Figs. 4c, d & e).

Table 1: Measurements (µm) of sensilla types on various head appendages of Asian tiger mosquito, *Ae. albopictus*.

Sensilla type	Attribute	Vertex		Antenna		Maxillary palp		Proboscis	
		Length	Width	Length	Width	Length	Width	Length	Width
SensillaChaetica	Ch I	194.08 ± 0.09	2.58 ± 0.45	278.87 ± 0.034	2.17 ± 0.046	78.499 ± 0.098	1.94 ± 0.98	69.718 ± 0.076	1.87 ± 0.063
	Ch II	89.73 ± 0.03	2.24 ± 0.25	167.45 ± 0.078	1.83 ± 0.092	45.62 ± 0.214	1.23 ± 0.67	58.23 ± 0.045	1.76 ± 0.076
Sensillatrichoidea	Tr I	-	-	48.34 ± 0.14	0.98 ± 0.076	-	-	-	-
	Tr II	-	-	37.67 ± 0.87	0.67 ± 0.34	-	-	-	-
Grooved pegs	Gps	57.98 ± 0.076	2.84 ± 0.082	68.56 ± 0.04	2.78 ± 0.316	98.917 ± 0.35	2.69 ± 0.075	61.56 ± 0.087	2.67 ± 0.076
Sensillabasiconica	Ba I	-	-	29.89 ± 0.078	0.74 ± 0.065	23.54 ± 0.076	0.84 ± 0.047	22.65 ± 0.023	0.84 ± 0.12
	Ba II	-	-	19.9 ± 0.96	0.68 ± 0.087			17.90 ± 0.34	0.59.3 ± 0.43
Sensillacoeloconica	Co	-		4.89 ± 0.083 *		-	-	-	-
Sensillacampaniformia	Ca	-	-	-	-	12.97 ± 0.074 *		-	-
capitate peg	Cp	-	-	-	-	13.23 ± 0.09	1.87 ± 0.087	-	-
Scales	Sc	64.87 ± 0.35	22.28± 0.046	62.07 ± 0.65	22.98 ± 0.05	58.46 ± 0.076	23.45 ± 0.016	57.93 ± 0.087	24.23 ± 0.034

Discussion

In the present study, different kinds of sensillae were detected on head and appendages of *Ae. albopictus'* females with distinct morphological features. Mosquitoes use five kinds of stimuli to locate hosts, CO_2, heat, body odor, namely visual indications and water vapors. The compound eyes, sensillae coeloconica, capitate pegs, grooved pegs and sensillae trichoidea were respective sensilla responded to these stimuli. As soon as a mosquito has landed it start to taste its host, perceiving first by tactile setae and chemo-sensilla contact present on tarsi and labellum sensillae, McIver (1978) for *Ae. aegypti* female. In *Aedes* and *Anopheles*, two subtypes of sensillae chaetica were identified and second type sensillae chaetica found dorsally close to flagellomers edge (Bowen, 1995). In *Ae. aegypti,* grooved pegs sense components of sweat including ammonia and lactic acid (Davis and Sokolove, 1976). Bhattacharya *et al.* (2016) reported that *C. quinquefasciatus* played an essential role for initiating and facilitating disease transmission by establishment of an effective vector-host transmission cycle for various pathogens in different environments.

Sensillae on proboscis may respond to signals in blood through penetration. The most abundant sensilla detected was sensillae trichoidea with other kinds of grooved peg sensilla, sensillae chaetica and sensillae basiconica on antennae. Similar sensillae were in *Anopheles stephensi* (Boo, 1980), Culicidae (Mclver, 1982), *Ae. aegypti*, *Ae. atropalpus*, *Ae. epactius*, and *Cx. pipiens* (Bowen, 1995), *Aedes* and *Anopheles* spp. (Amer and Mehlhorn, 2006), *Ae. albopictus* (Seenivasagan *et al,* 2009), *Culex* spp. (Dhanalakshmi *et al,* 2018) and *Cx. pipiens* (Ibrahim *et al,* 2018).

In the present study, sensillae coeloconica protruding from the distal tip of flagellum in thick peg organs sunk into antennal wall depressions, but in *Anopheles* and *Ae. Aegypti*, they were detected at the flagellum tip without protrusion. Ismail (1964) and Mclver (1982) reported that beside hygro- and thermo-reception, sensillae coeloconica played the role of short-range olfaction. Seenivasagan *et al.* (2009) detected two sensillae coeloconica protruding in a tubular structure from terminal tip of flagella in *Ae. albopictus*. Sensory structures; such as sensillae tric-hoidea, sensillae ampullacea, sensillae coeloconica, sensillae basiconica, and sensillae cheatica on female antennae (Steward and Atwood, 1963; Dhanalakshmi *et al,* 2018) for *Culex*. Antennae and maxillary palps detected host and other sensory-mediated behaviors (Maekawa *et al,* 2011).

In studies of McIver (1970; 1972) for culicine mosquitoes and Bowen (1995) for *Culex* and *Aedes* species, reported that the grooved peg sensillae were two subtypes; short and long grooved peg sensillae. However, Seenivasagan *et al.* (2009) reported a significant variation in length and tip structure, which grooved peg sensillae suggested a potential difference in the odor molecules sensing system. Bowen (1995) found that short grooved pegs contained sensory cells which stimulated by lactic-acid, meanwhile, in *Ae. atropalpus* (Coquillett), *Ae. aegypti*, *Cx. pipiens* and *Ae. epactius* the lactic acid exciting cells were absent.

The maxillary palps comprised five segments, and contained other sensory structures such as, grooved pegs, sensillae campaniformia, capitate peg, Sensillae basiconica and certain non-innervated structures like cu\ticular projections, scales and microtrichia. This agreed with Mclver and Hudson (1972), Mclver and Siemicki (1975) and Mclver (1982) for other mosquito species and Seenivasagan *et al.* (2009) for *Ae. albopictus*. Kellogg (1970) reported that capitate pegs respond to acetone, amyl-acetate and n-heptane. In the present work, the sensillae campaniform was detected on ventral side of maxillary palp segments number 2, 3 & 4, sensillae chaetica with delicate serrations around grooves base and classified into two kinds; large Sensillae (Ch I) and small Sensillae (Ch II). Similar structure was reported in *An. stephensi* (Mclver and Siemicki, 1975) and *Ae. albopictus*by (Seenivasagan *et al,* 2009).

In this study, the maxillary palp was a relatively simple organ and desirable for exploring neuromolecular networks surrounding chemo- and mechano-sensation. Maxillary palp of *Ae. albopictus* female consists of five segments, densely covered dorsally with flattened scales. Capitate pegs, chemosensory sensillae were distributed on ventral side of maxillary palp segments number 2, 3 & 4. The fourth segment was the most of sensory one including microtrichia, scales, capitate sensillae basiconica and sensillae campaniformia. This agreed with Mclver and Hudson (1972), Mclver and Siemicki (1975), Mclver (1982) and Angelini and Kaufman (2005) for mosquito species and Seenivasagan *et al.* (2009) for *Ae. albopictus*. Lu *et al.* (2007) and Bohbot *et al.* (2014) found three innervated sensory organs on maxillary palp, capitate sensillae basiconica, which were porous hairs with 3 neurons of chemosensory sensitive to CO_2, octenol and human skin odorants, and sensillae campaniformia and chaetica were mechanoreceptors holding one sensory neuron.

Like other dipterans, the proboscis is an essential head appendage of the mosquito in host identification, which analyses gustatory code throughout food intake, especially good in view of the fact that blood-sucking mosquitoes frequently reach vessels below the host skin using this probe, using it to quantify CO_2 -activated thermo-sensing behavior. Protruding mosquito proboscis contributes unforeseen to distance host identification. Recognition of pseudo-thermo targets needed antennae, maxillary palps, and proboscises. Also, the proboscis can be separated from CO_2 identification needed to elicit activation of the mosquito. Maekawa *et al.* (2011) reported the potential role of female proboscis was essential organ to search for the host and the labellum exhibits the most of sensory structureswith three major kinds of sensillae; sensillae chaetica (Ch) surrounded by a numerous microtrichia and divided into two distinct kinds; long (ChI) and short (Ch II). Six grooved pegs (GPs) detected ventrally at the base, robust, long, thick walled and grooved longitudinally. Sensillae basiconica were thin-walled structure irregularly distributed chemo-sensilla. They were divided into two subtypes; long (BaI) and short (BaII). This agreed with Amer and Mehlhorn (2006) for *Aedes* and *Anopheles*, Hill and Smith (1999) for *Ae. aegypti* and Seenivasagan *et al.* (2009) for *Ae. albopictus.*

Two kinds of grooved peg sensillae, short and long were in some *Aedes* and *Culex* species but, the highly significant kinds of grooved difference in length of these two grooved peg sensilla and in tip structure indicated a possible variation in odor molecules perception mechanism. As olfactory receptors, sensillae trichoidea detected its host skin carboxylic acid, and the produced different chemicals, but as compared to *Culex,* in *Aedes* spp. the commonest ones was sensillae trichodea (Seenivasagan *et al,* 2009)

Conclusion

The odor detection and chemoreception of these sensillae may theoretically aid in evaluating female mosquito vector capacity. The outcome results on the sensory structures on head and appendages of *Ae. albopictus* by SEM clarified their sensory structures and their potential role in mosquito behavior.

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Explanation of figures

Fig. 1: SEM of *Ae. albopictus* female. a, c) anterior view and lateral view of head, showed proboscis (Pr), maxillary palp (Mp), compound eyes(Ce), vertex (V), coronal suture (Cs), antennae (An), pedicle (P), scape (S), Flagellomeres (FL) with various sensilla, grooved peg sensilla (Gps), large sensilla chaetica (Ch I), small sensilla chaetica (Ch II). b) Antennal flagellomeres with short pointed sensilla trichodea (Tr II), long pointed trichodea sensilla (Tr I), large sensilla chaetica (Ch I), small sensilla chaetica (Ch II), long sensilla basiconica (Ba I), short sensilla basiconica (Ba II) and sensilla coeloconica (Co). d, e) lateral view of grooved peg sensilla (Gps).

Fig. 2: SEM of *Ae. albopictus* female, a) lateral view of head showed compound eyes (Ce), vertex (V) with few sensilla, grooved peg sensilla (Gps) placed into a pit with two rings of cuticle, large sensilla chaetica (Ch I), small sensilla chaetica (Ch II) and many scales (Sc); b) dorsal view showed vertex, coronal suture (Cs), part of pedicle (P); c) large sensilla chaetica (Ch I); d) lateral view of antenna with grooved sensilla (Gps) and few scales (Sc); e) lateral view of pedicle (p) with few sensilla trichodea (Tr) and scales (Sc).

Fig. 3: SEM of maxillary palp (Mp) in female *Ae. albopictus* a) lateral view of head showed maxillary palp, compound eyes (Ce), Pedicle (P) densely covered with scales (Sc) and proboscis (Pr); b, d) lateral view) showed various types of sensilla, capitate pegs (Cp), sensilla campaniformia (Ca) at the distal end, grooved peg sensilla (Gps), large and small sensilla chaetica (Ch I, Ch II), respectively, Sensilla basiconica (Ba) and covered heavily with scales (Sc) on dorsal side; c, e, f) ventral view showed capitate pegs (Cp), grooved sensilla (Gps), large and small sensilla chaetica (Ch I, Ch II), respectively.

Fig. 4: SEM of proboscis (Pr) in *Ae. albopictus* female, a) ventral view showed six grooved peg sensilla (Gps) at base and compound eyes (Ce); b, c) dorsal view (Pr) covered heavily with scales (Sc), large sensilla chaetica (Ch I) and labellum (Lb); d, e) dorsal view showed grooved peg sensilla (Gps), long and short sensilla basiconica (Ba I, Ba II), respectively.