Journal of the Egyptian Society of Parasitology, Vol.43, No.2, August 2013
J. Egypt. Soc. Parasitol., 43(2), 2013: 481 – 491

COMPARATIVE STUDY OF THE SENSILLA ON ANTENNA AND MAXILLARY PALPS OF FIVE CULICINE MOSQUITOES IN SOHAG GOVERNORATE

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Abstract

Mosquitoes are unquestionably the most medically important arthropod vectors of disease. The maintenance and transmission of the pathogens that cause malaria, lymphatic filariasis, and numerous viral infections are absolutely dependent on the availability of competent mosquito vectors. Although the medical community has known for over a century the role played by mosquitoes in the transmission of malaria and lymphatic filariasis, these diseases continue to have a devastating influence on less privileged populations throughout the tropical and subtropical regions of the world.

The present study was done to identify the different culicine mosquitoes in four different districts in Sohag Governorate and to describe and compare the various types of sensilla located on their antenna and maxillary palps using scanning electron microscopy.

Key words: Culicine mosquitoes, Antenna, maxillary palps sensilla, SEM.

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Introduction

The life of female mosquito is governed by its orientation responses to stimuli from different resources such as oviposition site, nectar, mating and by far the most important stimulus which is host seeking (*Seenivasagan et al,* 2009).

Mosquitoes sense host emanations, and olfactory cues, through the activation of sensory neurons are housed in hair-like sensilla. These olfactory sensilla are located on the antennae, the maxillary palps, and the proboscis. They constitute the mosquito's peripheral olfactory system (Hill *et al*, 2002). The sensilla and sensory mechanisms play a significant role in host seeking behavior of mosquitoes, which enable them to transmit various diseases to humans (Mclver and Charlton, 1982). The process of host finding is a directed flight toward the potential host with aid of visual and chemical orientation (Bowen, 1991).

The chemical identification of the host is based upon perception of attractants such as carbon dioxide, ammonia and lactic acid (Bosch *et al*. 2000). Other mosquito phagostimula-

tors are amino acids and adenine nucleotides. Alanine in blood plasma is known to be particularly attractive for *Cx. pipiens* (Alan *et al*, 2006).

Antennae are the major site for sensilla and the chemoreceptors located on the sensilla which detect and discriminate between air-borne stimuli and guide the mosquitoes to suitable human host. Other sensory structures present on other parts of the body, viz. maxillary palps, labellum, tarsi, ovipositors also play an important role in mosquito behavior (Seenivasagan *et al*. 2009).

The significant difference among various types of sensilla would possibly reveal their functions. The porous sensilla are olfactory and contact chemoreceptors while the aporous sensilla would play the role of mechanoreception (Seenivasagan *et al*, 2009).

The present study has been carried out to identify the identify the different culicine mosquitoes in four districts in Sohag Governorate and to describe and compare the various types of sensilla located on their antenna and maxillary palps using scanning electron microscopy.

Materials and Methods

The present study was done in Sohag Governorate, one of Upper Egypt Governorates located about 470 kilometer to the south of Cairo. Mosquitoes were collected from indoors and outdoors by hand collection, spray sheet collection and box traps from four districts (Tema, El-Maraga, Akhmeem and El-Monshah). The collected mosquitoes were directly examined by using dissecting and light microscope. Taxonomic identification of adult mosquitoes was carried out according to the keys (Paul *et al,* 1943; Natvig, 1948; Mikhail *et al*, 2009).

Electron microscopic examination of antenna and maxillary palps of the adult mosquitoes were processed according to (Hayat, 1981) as follow: Specimens were washed in saline to remove any stuck particles, fixed in 5% gluteraldehyde for 24-72 hours and washed in sodium tachodylate buffer of pH 7.3 for 4 times each 15 minutes. Fixation followed by adding 1% osmium tetroxide for 2 hours. Then the samples were washed again in the same buffer for 3 times, dehydrated in ascending concentration of ethanol 30%, 50%, 70% and 90% for 10 minutes each, double immersed in absolute ethanol for 24- 48 hours. After that the specimens were cleared in xylene for overnight and air-dried. The samples were incubated in 20 -25 ^{0}C stacked in double scotch tape carbon coated with gold. Examination was done and morphometric analysis was measured using the measure interactive function.

Results

Collected adult mosquitoes were microscopically examined and were identified as: 1- *Culex pipiens molestus* (Forskal 1775), 2- *Culex antennatus* (Beker 1903), 3- *Culex pusillus* (Macquart 1850), 4- *Aedes caspius* (Pallas 1771) and 5- *Culiseta longiareolata* (Macquart, 1902)

Table I: Numbers and percentages of adult mosquitoes collected in Sohag G.

Species	No. collected	Percentage
Cx. pipiens molestus	3600	72%
Cs. longiareolata	500	10%
Ae. Caspius	400	8%
Cx. pusillus	350	7%
Cx. antennatus	150	3%
Total	5000	100%

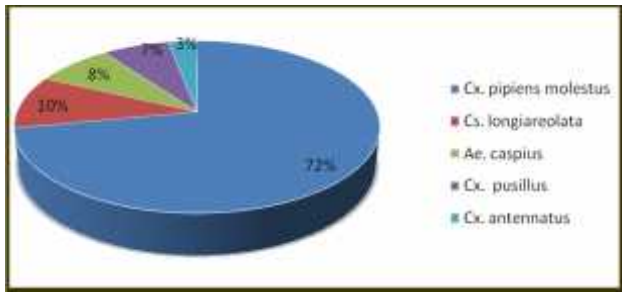

Chart I: Numbers and percentages of collected mosquitoes

Table II: Numbers and percentages of each species from the 4 districts.

Species Discrete / Tema district	*Cx. pipiens molestus*		*Cs. longi-areolata*		*Ae. Cas-pius*		*Cx. pusil-lus*		*Cx. anten-natus*		Total
	No.	%	No.	%	No.	%	No.	%	No.	%	
	843	74.8	100	8.9	85	7.5	80	7	20	1.8	1128
El Maraga district	970	69.7	143	10.3	116	8.3	102	7.4	60	4.3	1391
Akhmeem district	922	69.7	157	11.8	106	8	100	7.5	40	3	1325
El Monshah district.	865	74.8	100	8.7	93	8	68	5.9	30	2.6	1156
Total	3600		500		400		350		150		5000

Cx. pipiens molestus was the most predominant type in the four districts with a highly significant value(=< 0.001). Chart (I & II).

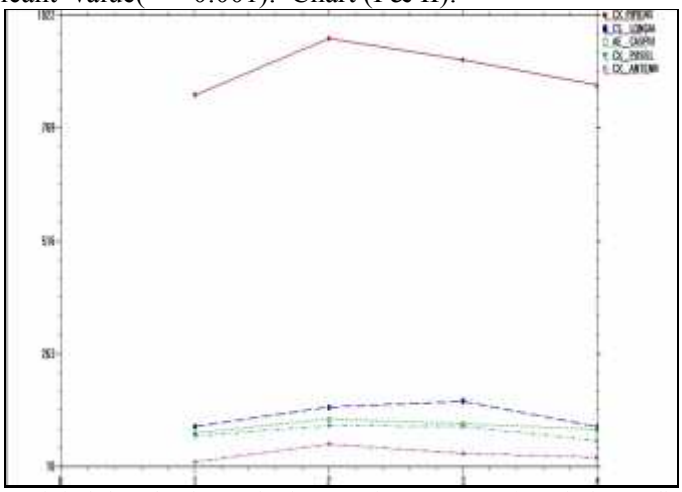

Chart II: Line plot statistical chart showing predominancce of *Cx. pipiens molestus* in four studied districts.

Antennae and maxillary palps of the collected mosquitoes were examined by SEM for the different types of sensilla.

Antenna: The overall morphological organization of the antenna appears to be conserved: Each antenna is divided into 13 flagella and the flagella are covered with sensilla which are sensory organs responsive to mainly the chemical stimuli and other types of stimuli such as mechanical, thermal, hygro stimuli. Electron microscopic study of the five collected species of mosquitoes revealed that the antennae are formed of three parts, namely scape, pedicel, and flagellum (fig. 1). The flagellum consists of 13 flagellomeres. Among the morphological features, length appeared as a prominent attribute to differentiate between the various types of sensilla. On the basis of size, shape and structural features, five types of sensilla have been identified and described.

Sensilla trichoidea: These are hair-like structures, numerously distributed on each segment of antennal flagella. These sensilla are of different sizes measuring 13-57µm in length. They are either pointed or blunt, arising from sockets (fig. 2), and are classified into the following types based on their morphology:

(a) Pointed *trichoidea* sensilla (fig. 3): hair-like, tapering at the end and further classified into two types based on the length; long and short pointed sensilla trichoidea.

(b) *Blunt trichoidea* (fig. 3): hair like with the tip of the sensillum slightly blunt and based on their length, further classified into long and short blunt-tipped sensilla.

Sensilla trichoidea are the most abundant sensilla observed in the five types of adult mosquitoes and were found with nearly equal numbers and distribution along the flagellomeres.

Sensilla chaetica: Sensilla chaetica are arranged in a whorl at the base of each the flagellomeres 2-13 and distributed evenly around the circumference of all flagellomeres in the antenna (fig. 4). They are the largest sensilla measuring 305-325µm in length. They are thick walled and externally grooved by sturdy bristles arising from a socket (fig. 5) with sharp-pointed tips. They occur in two distinct types; large and small types (fig. 4). There is no difference in distribution of this type of sensilla in the five collected types of the studied adult mosquitoes. They are from 6 to 8 in number at each flagellomere in the five species.

Grooved pegs: These are short, typical peg shaped, thick-walled and deeply grooved measuring 4.5-9µm in length, they are found all over the whole flagellomeres (fig. 6). This type of sensilla was observed only on *Cx. pipiens molestus* and *Cx. antennatus* but not found on any of the other studied mosquitoes.

Maxillary palps (fig. 7)

Capitate peg sensilla: These are pegs, club-shaped sensilla broadened at the tip and arising from a circular depression. Capitate pegs are 13.0±1µm long (fig. 8). This type of sensilla was found

in *Cx pipiens molestus*, *Cx. antennatus* and *Aedes caspius* only.

Sensilla campaniformia: Campaniform sensillum is dome shaped, located on the distal end of maxillary palp, having diameter of 5.7±1µm (fig. 9). This type was observed only on *Aedes caspius* mosquito.

Discussion

During the present study five species of Culicine mosquitoes were detected in Sohag Governorate, they were *Culex pipiens molestus*, *Cx. antennatus*, *Cx. pusillus*, *Aedes caspius*, *Culiseta longiareolata*. Among these five species *Cx. pipiens molestus* was the most predominant one presenting 72% and it was the predominant type in the four study districts presenting 74.8% of the collected sample in Tema district, 69.7% in El Maraga district, 69.7% in Akhmeem district and 74.8% in Al Monshah district. Khalil (1981) found that *Cx. pipiens* was the most predominant species in Upper Egypt. El-Nady *et al*. (2004) revealed that *Cx. pipiens molestus* (70.1%) was the most predominant larvae in Sohag Governorate.

Identification of mosquito species is important for ecological research studies interested in geographic distribution, abundance and behavior of the different vectors (Bass *et al*, 2007).

The present study described various types of sensilla on antenna and maxillary palps on the collected five mosquito species as a conserved part of the sensory system by using scanning electron microscopy in a trial to study the relationship between mosquito host seeking and vectors of parasitic disease in Sohag G.

On the basis of size, shape and structural features, five types of sensilla have been identified and described: Sensilla trichoidea, Sensilla chaetica, Grooved pegs, Capitate peg sensilla, Sensilla campaniformia.

Mosquitoes sense host emanations, and olfactory cues, through the activation of sensory neurons are housed in hair-like structures. These olfactory sensilla located on the antennae, the maxillary palps, and the proboscis and constitute the mosquito's peripheral olfactory system (Hill *et al*, 2002). Among the different types of sensilla present on culicine mosquito sensilla trichodea and grooved pegs are the olfactory types (Ismail, 1964; McIver and Charlton, 1970; McIver and Hutchinson, 1972; McIver, 1973, 1978, 1982; Boo and McIver, 1975 and Pitts and Zwiebel, 2006). Antennal trichoid and grooved peg sensilla of mosquitoes have been shown to house the olfactory receptor neurons that detect many of the odors involved in eliciting vector-related behaviors (Hill *et al,* 2009).

In this study, Sensilla trichoidea was the most abundant type observed in each of the five collected mosquitoe species with nearly the same distribution along the flagellomeres, and of different sizes measuring 13-57µm in length arising from sockets with four subtypes; long pointed, short pointed, long blunt and short blunt. The morphological classes of sensilla trichodea appear to be conserved among the culicine mosquitoes. This apparent morphological conservation may indicate an evolutionary constraint on the pe-

ripheral olfactory system in culicine mosquitoes which may, in turn, translate into a conservation of function as well (Molaei *et al,* 2007). According to Ismail (1964) both culicine and anopheline mosquitoes have this sensillum type. The majority of antennal sensilla are of this type and are thus considered to be the principal ‘‘drivers’’ of various behaviors (Hill *et al*, 2002).

The grooved peg sensilla were found to be short, typical peg shaped, thick-walled and deeply grooved, only noticed on *Cx. pipiens molestus* and *Cx. antennatus* antennae; but was not found on any of the other three types. This finding coincides with the fact that *Cx. pipiens molestus* remains to be the main vector of *Wuchereria bancrofti* in Egypt and *Cx. antennatus* was claimed to be a suggestive vector for this parasite (Gad *et al,* 1995).

Grooved peg sensilla were proved to be of potential importance in host seeking behavior. They had been proved by studies in various mosquito species to show sensitivity to human sweat components, including ammonia (Geier *et al*, 1999; Meijerink *et al*, 2001) and lactic acid (Davis *et al*, 1976). Moreover, behavioral studies also implicate these odors, especially ammonia, in host attractiveness (Braks *et al*, 2001), while lactic acid seems to have a synergistic effect in combination with other odors (Dekker *et al*, 2002; Bernier *et al*, 2003; Smallegange *et al,* 2005).

Gubler (2004) reported that grooved pegs are with high sensitivity to lactic acid in *Aedes aegypti* the primary vector of dengue, yellow fever and other arboviruses in the world emphasizing the importance of this type of sensilla to the host seeking and disease transmission processes. *Anopheles gambiae s.s.* the main vectors of human malaria were proved to have grooved pegs closely resembling those of *Ae. aegypti* and other culicines (Pitts and Zwiebel, 2006).

Trichoid sensilla with the conserved morphology among culicines and grooved peg sensilla were said to respond to overlapping sets of host odors and that these sensilla may therefore be part of a generalistic host sensing mechanism with host specific information being derived from the combined information of these inputs (van den Broek and den Otter, 2000).

Sensilla chaetica were found in the present study to be long, stiff, ridged hair–like structures that were set into a socket at the base and radially distributed in whorls at the flagellar margins. This type of sensilla was present in all five mosquito species collected with no differences in morphology or distribution along the antennae. They were reported to be mechanoreceptive sensilla (Hill *et al,* 2002).

On the maxillary palp in addition to sensilla chaetica, other sensory structures namely capitate pegs, sensilla campaniformia and some non-innervated structures such as microtrichia were observed. The capitate pegs that were classified among the aporous non olfactory sensilla were proven to respond to trace breath gases; amylacetate and acetone in *Ae. aegypti* (Kellogg, 1970).

Campaniform sensilla which consist

of a domed cap that is hinged surrounding ring of raised cuticle were reported. McIver and Siemicki (1978) reported this type of maxillary palp sensilla in *An. stephensi* the leading vector of malaria in India, parts of Asia and the Middle East (Corby-Harris *et al*, 2010). McIver and Siemicki (1978) also stated that this type of sensilla was deficient in *Aedes* and *Culex* mosquitoes. In more recent study Seenivasagan *et al*. (2009) reported campaniform sensilla on the maxillary palps of *Aedes albopictus* the emerging major vector of Chikungunya virus in the epidemics in most parts of southern India (Das *et al*, 2012) and now in Arabia (CDC, 2012).

No doubt, studying of the sensilla on antenna and maxillary palps of five culicine mosquitoes was not only of great importance in Sohag Governorate, but also allover Egypt. This is critical with the re-emerging of *Ae. aegypti* the main vector of dengue fever (El-Bahnasawy *et al*, 2011a) and yellow fever in Aswan Governorate (Heikal *et al*, 2011) and Toshka project Shoukry *et al*, 2012). Besides, El-Bahnasawy *et al*, 2011b) recorded *An. multicolor, An. sergentii,* and *An. algeriensis* in Toshka reclaimed area on the Egyptian-Sudanese border, and that *A. sergentii* is a malaria-vector and *A. multicolor* is a suspected vector and that chloroquine resistant *P. falciparum* could be introduce to Egypt from Sudan.

Conclusion

The present study identified five different culicine mosquitoes and estimated their prevalence in four different districts in Sohag Governorate. Moreover, five types of host-seeking sensilla were differentiated by SEM illustrating their ultrastructural characters as well as their distribution on the antenna and maxillary palps. As these sensilla play a significant role in host seeking which enables them to transmit various parasitic and viral diseases, we hope this work will be of benefit in future planning of mosquitoes control.

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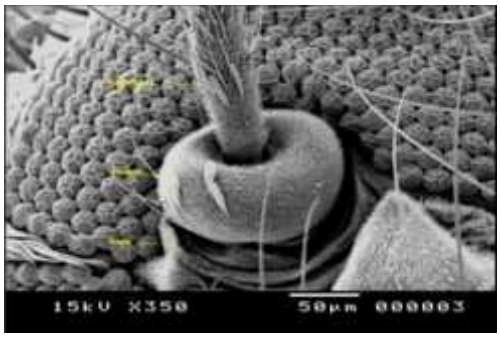

Fig. 1: Structure of antenna; scape, pedicel, and flagellum

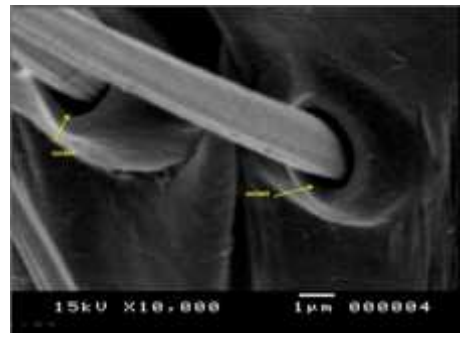

Fig. 2: Sensilla trichoidea arising from them

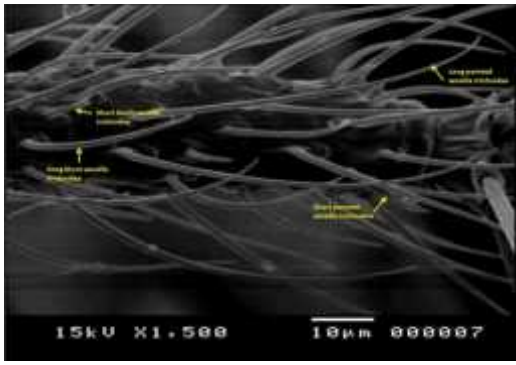

Fig. 3: Pionted and blunt sesilla trichoidea socket

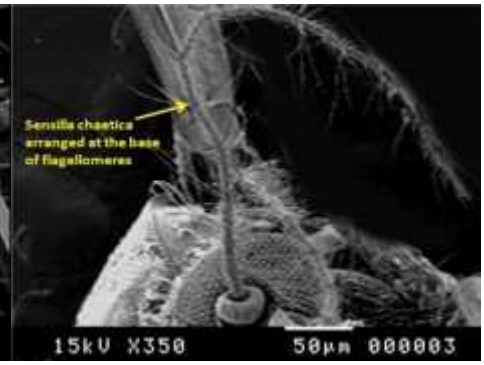

Fig. 4: Arrangement of sensillae chaetica

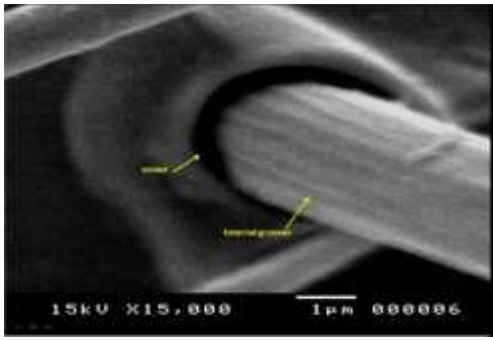

Fig. 5: Externally grooved sensilla chaetica

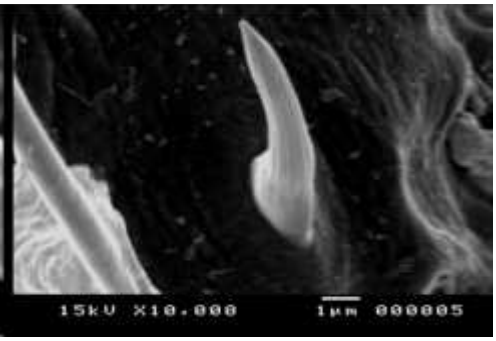

Fig. 6: Grooved pegs sensilla socket

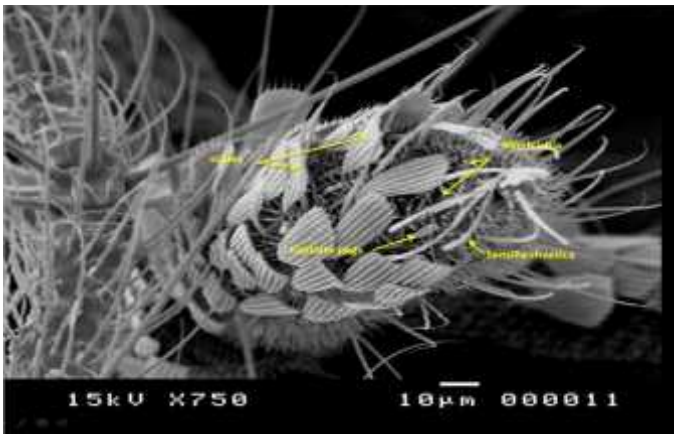

Fig. 7: Last maxillary palp segment of *Cx.pipiens molestus*

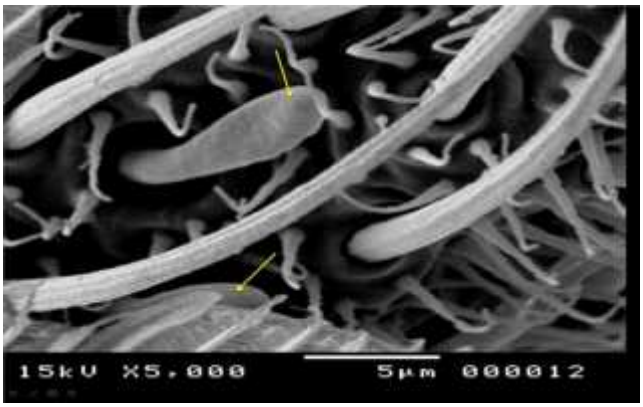

Figure 8 s: Capitate peg ensilla (arrows)

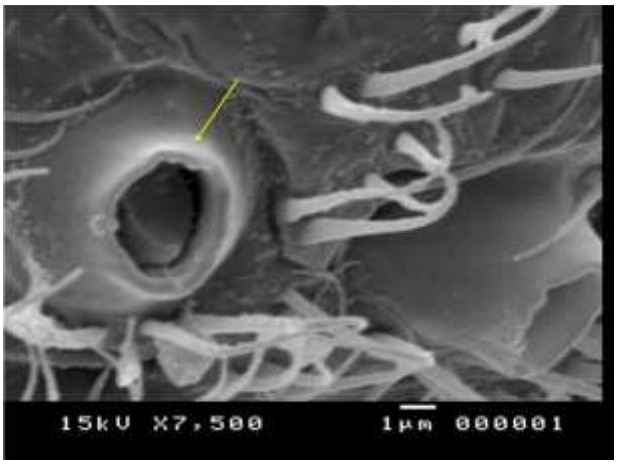

Figure 9: Sensilla campaniformia (arrow)