COMPARATIVE FINE STRUCTURE OF THE FEEDING MOUTH BRUSHES AND SIPHON OF FIVE CULICINE MOSQUITO SPECIES (DIPTERA: CULICIDAE)

ABSTRACT:
Comparative morphological characters of feeding mouth brushes and siphon of fourth larval instars of five culicine mosquito species were studied using scanning electron microscope in order to investigate their structural differences. The filament of the lateral palatal brushes (LPB) of Aedes (Ae.) caspius shows a distal rake-like structure with pointed teeth. The filament of LPB of Culex (Cx.) pipiens resembles that of Cx. antennatus and Cx. pusillus in that the teeth of the filament are less pointed than those of Ae. caspius. In Cx. perexiguus, the teeth of LPB are almost absent. The siphon of Ae. caspius bears prominent spines compared to the fleshy siphon of Cx. pipiens, Cx. antennatus, Cx pusillus and Cx. perexiguus. The structures described in this study will be helpful in the identification of the five culicine mosquitoes.

INTRODUCTION:
Mosquitoes are the single most important taxon of arthropods affecting human health globally (Parmanand et al., 2008; Farajollahi and Price, 2013). Despite centuries of control efforts, mosquito-borne diseases are flourishing worldwide (Tolle, 2009). Accurate identification of vectors is crucial to initiate aggressive control measures (Farajollahi and Price, 2013). Classical studies used to identify mosquitoes using standard morphological and pictorial keys.

Scanning electron microscopy (SEM) has not been used extensively in describing the external morphology and fine structure of mosquito larvae, although it is frequently employed in the observation of the eggs (Alencar et al., 2003; Junkum et al., 2004) or adult mosquitoes (Seenivasagan et al., 2009; Kong and Wu, 2010). The structures of taxonomical interest that are used in taxonomic keys include the siphon, anal segment and head capsule. Comparable studies have been underwent on mouthparts of muscoid (Kovács, 1988), phlebotomines (Brinson et al., 1993), Culicoides (McKeever et al., 1994), and other insect species.

Therefore, the aim of this study was to describe and compare the structure of the feeding mouth brushes and siphon of the fourth larval instar of Culex (Cx.) pipiens, Cx. antennatus, Cx. perexiguus, Cx. pusillus and Aedes (Ae.) aegypti using the scanning electron microscope.

MATERIAL AND METHODS:
Immature stages of mosquito were collected from a drainage canal in El Berka, a district located on the eastern border of Cairo. Collection was carried out by means of a long handed net. Immatures were poured into plastic bags (Nasco Whirl-pack 40 g filline, USA) and transported to the central laboratory in picnic-ice boxes containing cold water (12-20°C) over which floated the bags. Fourth-instar larvae were identified according to Kirkpatrick (1925) and Harbach (1985 & 1988). Immatures of
mosquito were maintained in walk-in insectaries under controlled conditions.

Five fourth instar larvae of each species were fixed in 2% glutaraldehyde, dehydrated, dried to the critical point, sputtered with gold ions, and examined using a SEM (Hitachi S-570), SEM unit, Faculty of Agriculture, Ain Shams University. Observations were made mainly on the siphon and feeding mouth brushes.

**RESULTS AND DISCUSSION:**

Most keys for identifying mosquito larvae are based exclusively on the characteristics of the 4th instar (Clark-Gil and Darsie, 1983). However, previous studies revealed that the structures were found to be similar in all larval instars, showing equivalent distributions and relative sizes, including the terminal structures of the siphon and setae of the head capsule (Schaper and Hernández-Chavarria, 2006). The main structural differences were observed in the lateral palatal brushes, comb scales, and pectin. Consequently, observations of such structures in the fourth larval stage were found to be reasonable representative of the studied species.

The lateral palatal filament is among the structures involved in the production of water jet, and consequently the magnitude of the processing rate (Lacoursière et al., 1999). The lateral palatal filament of *Ae. caspius* larvae showed a distal rake-like structure with pointed teeth (Fig. 1 a), whereas filament teeth of *Cx. pipiens* (Fig. 1 b), *Cx. antennatus* (Fig. 1 c), and *Cx. pusillus* (Fig. 1 d) were less pointed. Lateral palatal filament of *Cx. perexiguus* larvae was devoid of teeth (Fig. 1 e). Structural differences are interpreted as expressions of the different feeding strategies the larvae use to optimize food intake in relation to available space (Widahl, 1988). The main role of the filaments of the lateral palatal brushes (LPB) was to generate and maintain water and particle flow around the larval head (Rashed and Mulla, 1990). Suspension feeders must sustain large flow pattern around the larva to ensure sufficient particle entrapment (Lacoursière et al., 1999). In *Ae. aegypti*, most of the LPB filaments were provided with stout pointed teeth at the tips which were useful in the brushing of food material, while the filaments of the anteromedian palatal brush (APBr) in *Culex* species were branched (Rashed and Mulla, 1990). The authors added that in *Ae. aegypti*, the APBr contained long blade-like filaments and short teeth like elements. They concluded that the main function of mandibular and maxillary brushes is to direct and concentrate particle flow.
In addition to the chemical factors associated with the food substances, the physiological and environmental conditions of the larvae play an important role in regulating the ingestion rate of suspended particles by mosquito larvae (Rashed and Mulla, 1989). Among Cx. tarsalis, Ae. aegypti, and An. albimanus, the authors found that Ae. aegypti larvae were the most rapid feeders.

The larval siphon showed modifications among the five mosquito species. The siphon of Ae. caspius larvae bore prominent spines (Fig. 2 a). The siphon of Cx. pipiens (Fig. 2 b), Cx. antennatus (Fig. 2 c), Cx. pusillus (Fig. 2 d), and Cx. perexiguus (Fig. 2 e) were similar in that they were fleshy compared to that of Ae. caspius.

Fig. 1. Scanning electron micrograph of lateral palatal filament of fourth instar larvae of Aedes caspius (a) showing that the filament has a distal rake-like structure with pointed teeth indicated by arrow head. Filament teeth are less pointed in Culex pipiens (b), Culex antennatus (c) and Culex pusillus (d). Teeth are almost absent in Culex perexiguus (e).

Feeding behavior of mosquito larvae consists of foraging along the bottom of the container, and stationary filter feeding while suspended from the surface film (Kinney et al., 2013). The lateral brushes serve to filter and collect suspended food particles from water medium. Merritt and Craig (1987) found that the ability of mosquito larvae to capture fine particles may be enhanced by mucosubstances secreted onto the palatal brushes. In contrast, Widahl (1988) reported that culicid larvae do not exhibit filter feeding; he concluded that transport and entrainment of particles in a laminar flow toward the mouth by means of the movement of mouth brushes play a more significant role in particle retention that does direct adhesion onto the lateral palatal brush filaments.

Mouth brush function varied among the species studied. The mouth brushes of Ae. aegypti appeared in the 3rd and 4th instars as a tangle of long structures, obstructing the view of individual mouth parts. Furthermore, the filaments of these brushes have a terminal rake-like structure, composed of four to five big teeth, located at an angle of approximately 90° with respect to the rest of the smaller teeth in the filament. This development may be important in the feeding of larvae since it could provide for better grasping of food particles, including bacterial films and protozoa (Rashed and Mulla, 1990). The development of these filaments might be correlated with a change in diet during larval maturation (Rashed and Mulla, 1990). It was assumed that 3rd and 4th instars ingest large organic particles and can be seen feeding on surface films in culture containers. The big filaments on the lateral palatal brush could enable the larvae to capture and feed on proportionally larger food items (Dahl et al., 1993).
Fig. 2. Scanning electron micrograph of comb and pecten of fourth instar larva of *Aedes caspius* (a) showing that the siphon bears prominent spines indicating by arrow head. Siphon is fleshy in *Culex pipiens* (b), *Culex antennatus* (c), *Culex pusillus* (d) and *Culex perexiguus* (e).

Morphometric comparisons of the different structures were not analyzed because those dimensions may have been altered during processing, particularly during the critical point drying, as has been previously described by various authors (Schaper and Hernández-Chavarría, 2006).

The structures described in this paper will be helpful for more accurate identification of mosquito larvae, a significant task when retrospective analysis of preserved water samples containing mosquito immature is needed.

REFERENCES:


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Tewfick et al., Comparative Fine Structure of the Feeding Mouth Brushes and Siphon of Five Culicine Mosquito Species

دراسة مقارنة للتركيب الطاهري الدقيق لأجزاء الفم والأنبوب التنفسي في بعض أنواع البعوض الكيولسيني (ناتية الأجبة: كيولسيني)

مها كمال توفيق، نهلا محمد وسيد، بلال أحمد سليمان

قسم علم الحيوان، كلية العلوم، جامعة السويس، السويس، مصر

تم دراسة التركيب المجهري الدقيق باستخدام تقنية الميكروسكوب الإلكتروني الماسح للأشكال الخارجي لأجزاء الفم والأنبوب التنفسي في بعض أنواع البعوض الكيولسيني بهدف الإطلاع على الاختلافات بينهم والتي يمكن استنادها إلى الأنواع أو الطرازات، وتشير هذه الدراسة إلى احتمالية استخدام تقنية الميكروسكوب الإلكتروني الماسح للأشكال الخارجي لأجزاء الفم والأنبوب التنفسي كدليل مائي للتفاوت بين الأنواع...

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