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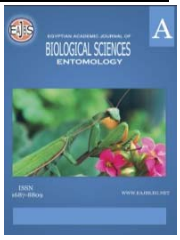

Scanning Electron Microscopic Studies of the Sting Apparatus of Honeybee, (*Apis mellifera*) (*Hymenoptera, Apidae*).

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ABSTRACT

The structure of different parts of the stinging apparatus of honeybee *Apis mellifera* was studied by scanning electron microscope in adult foraging workers for the first time. We reveal the association of the setaceous sheath and the alarm scent as it is due to the presence of the newly discovered Egypty gland.

INTRODUCTION

Insects as other animals and even some plants have their way in defending themselves against their enemies. The defense is either through biting by their jaws, through allergic substances secreted by special sensillae or by more effective substances delivered by stinging apparatus. Stinging apparatus of honeybee acts as an ovipositor and as a defensive organ in the queen but only as a defensive organ in workers (sterile females). Stinging apparatus had been studied by numerous investigators (Kraepelin, 1873; Cheshire, 1886; Zander, 1900; Betts, 1923; Leuenberger, 1929; Snodgrass, 1956; Dade, 1962; Hermann and Douglas, 1976 and Weiss, 1978) mainly on light microscopy level. The sting had been examined on scanning microscopy level only by Shing and Erickson (1982) and the main aim of their study was the structure and distribution of the sensillae on the sting. Many researchers were wondering about the main source of the scent produced by the sting of the workers alarming their sisters from a coming intruder. All agreed that the setaceous sheath is the main source but they are not completely confessed with this result as the sheath is not glandular in structure. In this work, we study the fine structure of the whole sting apparatus of honeybee workers on a scanning microscopic level in order to give a more detailed and clear description of the different parts of the sting and also to reveal the main source of the alarming odor.

MATERIALS AND METHODS

1- Experimental insects:

This study was to be fulfilled on adult workers honeybee (***Apis melllifera***) obtained from Honeybee Research apiary, Plant Protection Research Institute, Sakha station, Kafr El Sheikh Governorate, Egypt. Honeybee colonies were visited for collection of bee samples. Adult worker bees were collected at the entrance of the hive.

2- Preparation of Material for Scanning Electron Microscopy:

The bees were dissected using a stereo binocular microscope (WILD M3B Herbrugg Switzerland).The stings were carefully excised from the freshly collected worker bees with fine forceps and iris scissors. The collected material was then transferred into an Eppendorf tube and preserved in fixative solution (5% glutaraldehyde).

3-Procedure for Scanning Electron Microscope:

After fixation, the specimens were washed twice with sodium buffered saline. This was followed by post-fixation in 2% osmium tetroxide. After secondary fixation, specimens should be dehydrated in a series of ascending alcohols (30%, 50%, 70%, 90% (two washes), 100% (3 or 4 washes) each for 15 minutes), in order to eliminate the small amount of water remaining in the tissue. After dehydration, the specimens were dried in a Critical Point Drying Apparatus, mounted on cupper stubs with carbon conducting paint in the desired orientation. The stubs were placed inside the sputter coater and coated with a thin layer of gold in a Denton vacuum evaporator (SPi-module). The specimens were viewed in a scanning electron microscope (Jeol JSM 6510L) operated at an acceleration voltage of 20 kv. The results of scanning were preserved as photographs used in this presentation. The scanning electron microscopic procedure was carried out at the SEM unit, faculty of agriculture, El Mansoura University, Egypt.

RESULTS

The sting apparatus of honeybee worker is situated inside a cavity formed as a result of the union of the tergal and sternal plates of the 7th abdominal segment. This cavity is called the sting chamber and the whole of the sting apparatus is enclosed within it when not in use.

The sting apparatus was found to be consisting of three main parts (piercing, motor and venom parts) which have a considerable different function according to its structure and position (Fig.1).

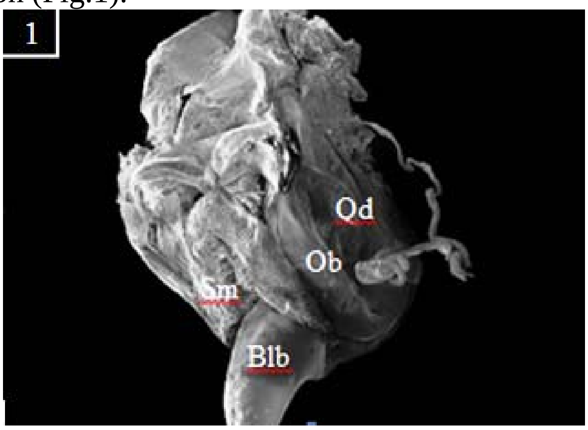

Fig. 1: SEM micrograph of a sting pulled out of honeybee worker: Blb- bulb, Sm-setaceous membrane, Ob-oblong plate and Qd-qudrate plate.

Piercing Part:

It’s the exposed part of the stinging apparatus and it consists mainly of three hollow structures which are the dorsal stylet and the two ventral lancets. They are in close contact enclosing a channel known as the venom canal.

The stylet is dorsal in position and has a smooth surface without any sculpture. It is pointed at its distal end while it increases gradually in width tell its proximal end which becomes enlarged to form the bulb, in which the venom sac opens. The two lateral ends of the bulb are diverged forming the 2nd right and left rami (Fig. 2).

Lancets are the main movable components of the sting shaft which formed of the two lancets and the stylet. They are two serrated retractable rods equipped laterally with saw like teeth pointed anteriorly. These teeth are known as the barbs (Fig. 3). These barbs differ in shape and size alongside the lancets. The most posterior barbs are the smallest with a wide base and short apex. The base of the barbs progressively extends and the apex height increases and becomes sharper (Fig. 4). The barbs are nearly arranged in a straight line on the external lateral side of each lancet. It was found that there are 10 barbs on each lancet (Fig. 5). The lancets are connected to the triangular plates with the first ramus of the sting (Fig. 2).

The stylet and the two lancets are enclosing a central canal extending throughout the bulb and the sting shaft. This canal is known the venom canal (Fig. 5) as the content of the venom sac discharged inside it. Movements of the lancets drive the venom out of the venom canal.

The width of the sting shaft increased progressively from posterior to anterior (Fig. 4) for one-third of its length, and then the width of lancets is fixed. The width of the stylet is increased gradually for some distance then there is a dramatic increase which forms the bulb. So there is an enlargement of the lateral sides of the stylet from the posterior to anterior (Fig. 6).

The bulb of the sting is surrounded by the setaceous membrane (Fig. 7) which protrudes as a hood over it and is directly connected to the sting sheath. The setaceous membrane has numerous setae and it appears to be provided with cuticular enfolding (Fig. 9, 10). The membrane has a basal undivided part from which the setae emerged. These Setae are either bi- or tri-furcated.

In the present study, we found a gland that was not mentioned before and we gave it the name of Egypt gland (Fig. 10). This gland consists of two lopes, one on each side of the sting bulb. The gland is composed of glandular units and has a convoluted surface.

2- The motor part which is responsible for movement of the sting. It is formed of three different pairs of cuticular plates (quadrate, oblong and triangular plates) attached with the sting chamber (Fig. 11)**.**

The quadrate plates are two large quadrilateral sclerites one on each side. As seen in Fig. 11 the quadrate plates articulate anteriorly with the dorsal posterior angle of the triangular plate. The dorsal margins of the quadrate plates protrude into the body cavity as a flat apodeme, which is attached to the muscles inside the body of the bee. The quadrate plate gland or the Koshewnikow gland is situated on the upper part of the quadrate plate and may be involved in the secretion of one or more of the alarm pheromones (Fig. 12).

The oblong plate is a paired horizontally elongated plate and its anterior end is continuous with the second ramus that connects the bulb of the stylet (Fig.11).

The triangular plate is a relatively small plate (triangular in shape) situated just above the anterior end of the oblong plate and in front of the quadrate plate. It articulates by the dorsal angles of its base with the anterior angle of the quadrate plate and by its lower angle with the upper edge of the oblong plate. The apex (anterior angle) of the triangular plate is continuous with the first ramus as seen in (Fig.11).

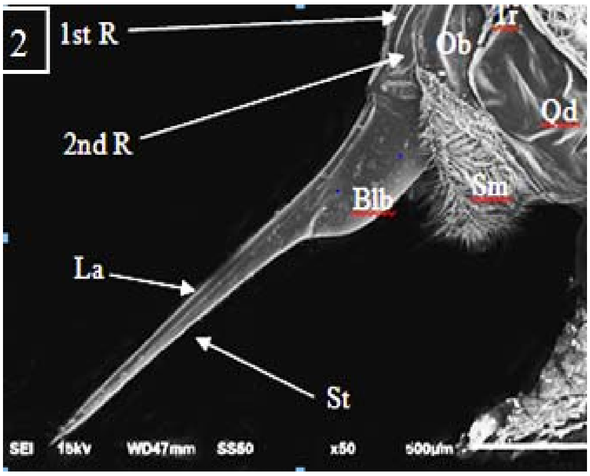

Fig. 2: SEM micrograph showing lateral view of a sting dissected out of a worker honeybee**:** St-stylet, La-lancet, Blb-bulb, Sm-setaceous membrane, Qd-qudrate plate, Tr-triangular plate, Ob-oblong plate, 1st R-first ramus and 2nd R- second ramus.

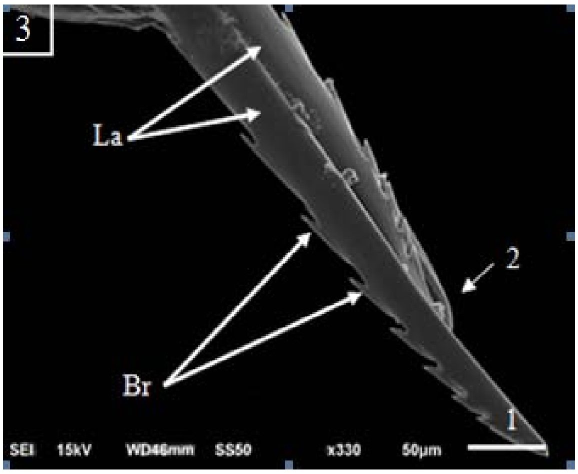

Fig. 3: SEM micrograph of the distal part of the lancets in alternating position: La-lancet, Br-barbs, 1-the pointed end of the lancet and 2-the most distal part of the stylet

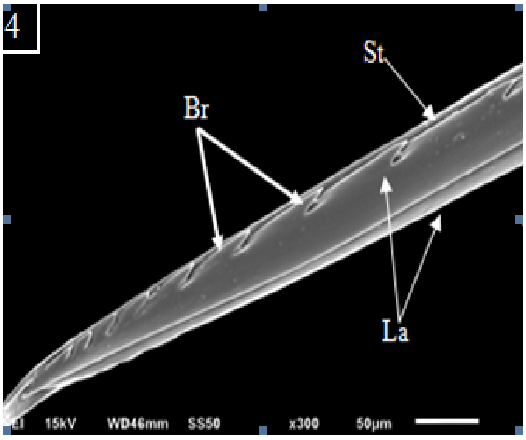

Fig. 4: SEM micrograph illustrating the variation in size and sharpness of barbs alongside the lancet. The distance between the barbs increased progressively from posterior to anterior:Br-barbs, St-stylet and La-lancets.

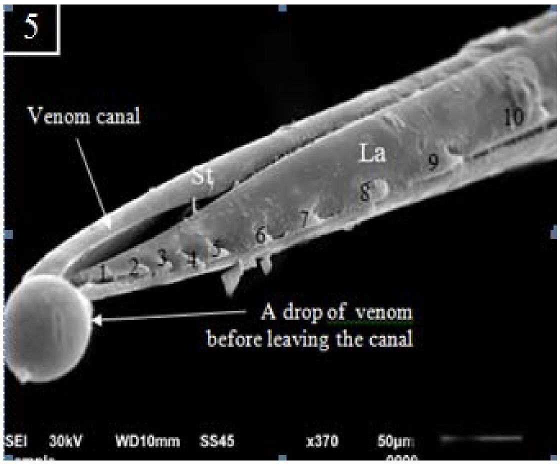

Fig. 5: SEM micrograph of worker honeybee sting illustrating the venom canal and a drop of venom released from the canal: St-stylet and La-lancets.

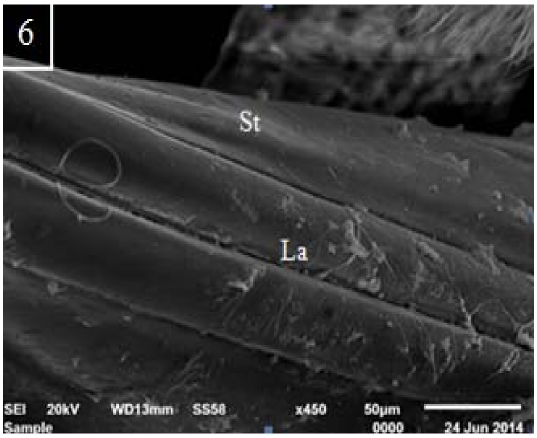

Fig. 6: SEM micrograph of the middle part of the sting shaft of worker honeybee: St-stylet and La-lancets.

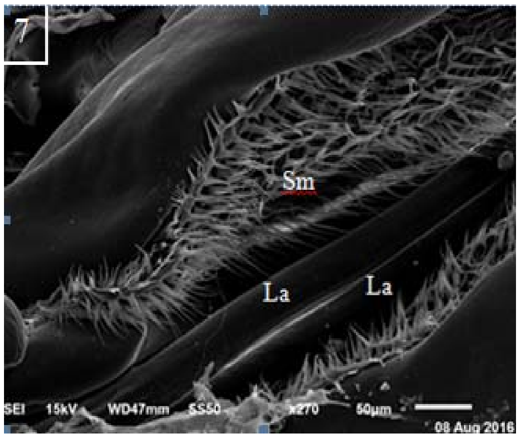

Fig. 7: SEM micrograph showing a ventral view of the basal part of the sting shaft of worker honeybee covered by the setaceous membrane: Sm-setaceous membrane and La-lancet

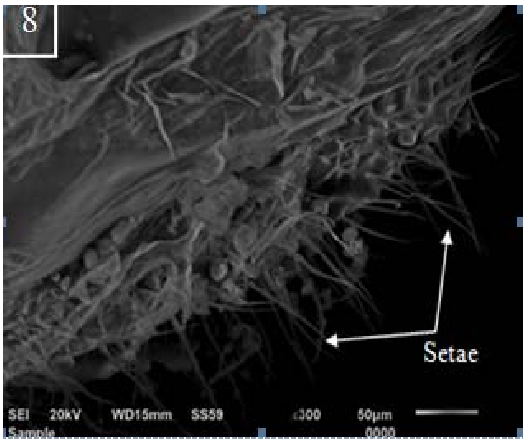

Fig. 8: SEM micrograph showing a magnified view of the basal part of the setaceous membrane in which the setae appear to be embedded in a sticky, glandular secretion that looks like fine ice particles

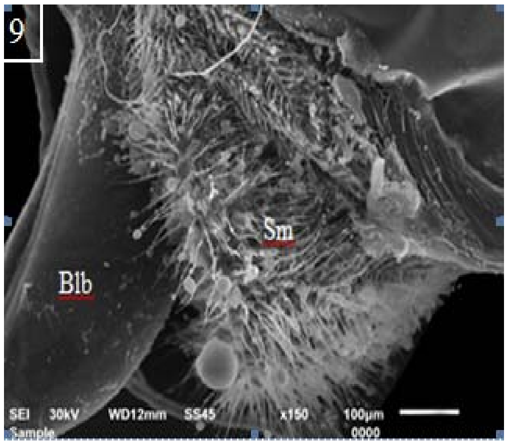

Fig. 9: SEM micrograph of the of worker honeybee sting showing the setaceous membrane appears as a hood over the bulb: Blb-bulb and Sm-setaceous membrane.

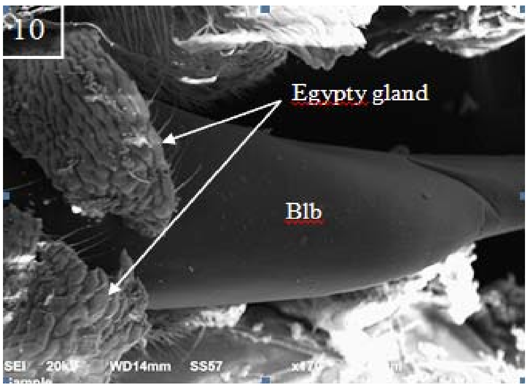

Fig. 10: SEM micrograph of worker honeybee sting showing the Egypty gland.

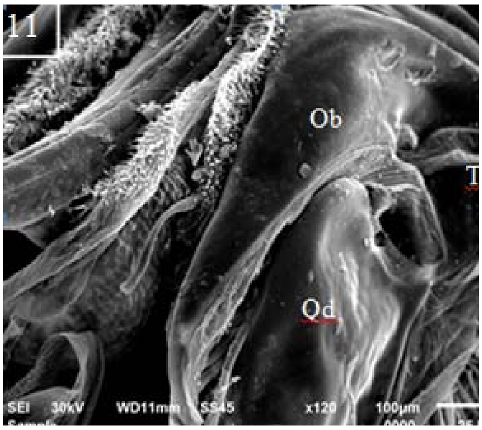

Fig. 11: SEM micrograph of a worker honeybee sting showing the motor part: Ob-oblong plate, Qd-quadrate plate, Tr-triangular plate**.**

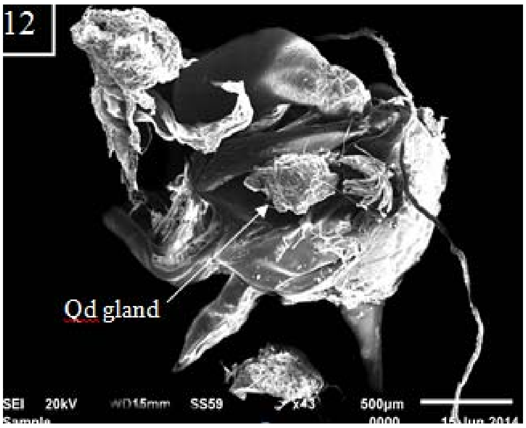

Fig. 12: SEM micrograph of a worker honeybee sting showing the quadrate plate gland (Qd gland).

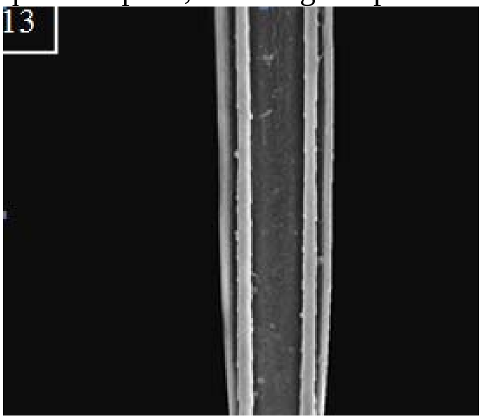

Fig. 13: SEM micrograph of worker honeybee sting showing the rail like structure of the stylet.

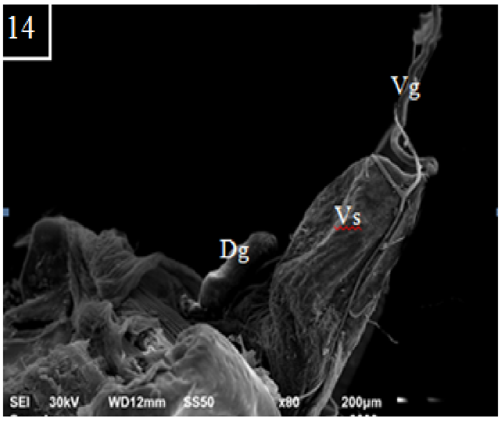

Fig. 14: SEM micrograph of a worker honeybee sting showing the venom apparatus: Vs-venom sac, Vg-venom gland and Dg-Dufour gland.

Movement of the Sting:

When the lancets are removed from the shaft as in (Fig. 13) a rail-like structure appears on the stylet. On both the lancets there are two grooves that are fitted well with the rail of the stylet and thus they are able to slide freely forward and backward on the rail of the underside of the stylet.

3- The Venom Apparatus:

It appears to be consisting of two glands (an exocrine gland called venom or acid gland and an endocrine gland called alkaline or Dufour’s gland), and the venom sac (fig. 14)**.**

The venom gland is a long, thin and distally bifurcated gland. It consists of a secretory filamentous region, connected to a reservoir at its proximal portion, in which the venom is stored (Fig. 14).The venom sac with its tapering posterior end discharged venom into the cavity of the bulb of the sting. The venom sac is pouch-like structure and stores the venom secreted from the venom gland. The folds on the venom sac were arranged concentrically. The venom sac did not rupture when the sting was removed from sting chamber.

Dufour’s gland lies to the left of the venom sac. It is much smaller and shorter than the venom gland. It is slightly convoluted tube with a relatively thick wall (Fig. 14).

DISCUSSION

As a rule of nature, all living organisms have developed one or more strategies to defend against their enemies (Okada 1984, Matsuura and Sakagami, 1973 and Matsuura, 1988). The sting apparatus of some Hymenoptera is directly derived from the ovipositor and acts as an effective defense weapon for the colony (Hermann, 1984; Britto and Caetano, 2005). Honeybee species (only the workers and the queen) are known for their defensive behavior which is elicited by visual stimuli, such as moving a dark object, and is enhanced by alarm pheromone secreted from special glands of the attacked nest mates. 'Stinging response' is the ending stage of the bee defensive behavior, which involves the penetration of the sting and the release of venom from the venom sac (Boch *et al.,* 1962).

The general structure of the bee's sting differs but little from that of the ovipositor of other Hymenoptera, and it is only in certain details that the sting is specialized for its specific function of ejecting the poison liquid from the reservoir of the poison gland (Snodgrass, 1933). The present study demonstrates the composition of the honeybee (*Apis mellifera*) stinging apparatus by using the scanning electron microscopy. Our observations are similar to those of Snodgrass (1910, 1925, and 1933) who provided an overview of the anatomy of the ovipositor apparatus of honeybee but his studies were based only on light microscope. Shing and Erickson (1982) studied the ultrastructure of the sting but they focused only on Sensory pegs, hair plates, and campaniform sensilla associated with the sting of the honeybee. They had shown that the length of the sting was proportionate to the body size of the worker bees (*Apis mellifera*) measuring approximately 2.3 mm in length.

The main part of the sting is the sting shaft (Betts, 1923) or sometimes called the sting sheath (Cheshire, 1886, Snodgrass 1910, 1925) which consists of the stylet and two barbed lancets. In the present work, the lancets were found to be serrated or barbed while the outer surface of the stylet appears to be smooth with no barbs or sensillae. According to Snodgrass (1925; 1956) this shaft may act in part as a sense organ that continuously registers movement, weight or other mechanical forces for the sting, while Hermann and Douglass, (1976) have reported that there are Pressure sensitive receptors (probably campaniform sensillae) that are present on the sting lancets and stylets of worker bees. Shing and Erickson (1982) have determined that such sensillum is associated with each of the barbs except those distal. Dade (1994) and Wu *et al* (2014) stated that the main role of these barbs is to provide one-way traction of the sting that makes it penetrate deeper into the flesh. This might help the bee to continue pumping the venom into the victim for a relatively long time after separation of the sting (Dade, 1994).

Our results showed that there are 10 acute barbs on each lancet. Our observations were similar to those of Wu *et al* (2014) who mentioned that there are two rows of barbs on the stinger of *Apis mellifera ligustica* each of which comprises about 10 acute barbs. However this number may vary according to the species. The number of barbs on the lancet were found to be 11 in *A. dorsata* and 10 in *A. cerana* and *A. florea* according to a documented observations made by Poore (1974). Ahmed *et al* (2015) observed that there are 7, 9, 10 barbs on the lancet of *A. m. ligustica, A. m. carnica* and *A. m. lamarckii,* respectively. These differences in the number of lancet barbs may play a role in calmness or violence of different honeybee species (Ahmed *et al,* 2015). In the present study it was also noticed that the distance between the successive barbs gradually increased anteriorly. Ramya and Rajagopal

(2008) assumed that this increase is to assist in the firm penetration of each acute barb into the victim's body.

The sting shaft extends anteriorly to form the bulb which is enfolded by the setaceous membrane. In the present study, it appears obvious that the setaceous membrane is provided with numerous biforked and triforked setae which were considered as the main source of the characteristic scent of the sting produced by the worker bees (Ghent and Gary, 1962). Maschwitz (1964) also mentioned that the setaceous membrane is a simple chitinous membrane, with a highly reduced epidermis and no secretory cells, but he did not definitely prove that this tissue constitutes the glandular source of these characteristic chemical signals of honey bee workers. Lensky *et al*. (1995) studied the fine structure of the setaceous membrane to evaluate its contribution in the defensive behavior of the worker in a bee colony. They observed the attraction, alarm and stinging reactions of guards to airborne volatiles from the various components of the sting in field conditions. Their results showed that the guards at the hive entrances displayed the highest attraction to volatiles of the setaceous membrane, and that of all the sting parts tested, setaceous membrane was the most attractive (60&70%) to the guards. However they did not find any characteristics of an exocrine gland, so they assumed that the setaceous membrane acts only as a platform to release pheromones which are secreted elsewhere. Such characteristics clearly appear in the basal part of the sting sheaths which are connected to the setaceous membrane (Cassier *et al.,* 1994). In the present work, a first record was done for a glandular structure at the base of the sting sheath which we gave it the name of Egypty gland. We think that this gland is responsible for the secretion of the pheromones that are accumulated on the setaceous membrane that in turn act as evaporation area for these pheromonal substances.

The present work, also demonstrates the presence of another gland called the quadrate plate gland on the sting of the worker bee. The quadrate plate gland was described previously in honeybee (*Apis mellifera*) only by Snodgrass (1956) who suggested that this gland produces secretions to the outside of the quadrate plate. These secretions might act as a lubricant for the shaft of the sting when venom is ejected. This gland was also observed in some hymenoptera species. For example Jessen and Maschwitz (1983) have provided a detailed overview of abdominal glands in a ponerine ant (*Pachycondyla tridentate*). In that study, an account was given of the glands associated with the sting apparatus itself (triangular plate gland, qudrate plate gland, spiracular plate gland and sting sheath gland). However only light microscopical data are available. Schoeters and Billen (2001) studied the ultrastructure of the whole sting apparatus of bumblebees and some other hymenoptera and mentioned that the quadrate plate gland is found on the sting of bumblebees and the other studied species, however, honeybee (*Apis mellifera*) was not included in their study.

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