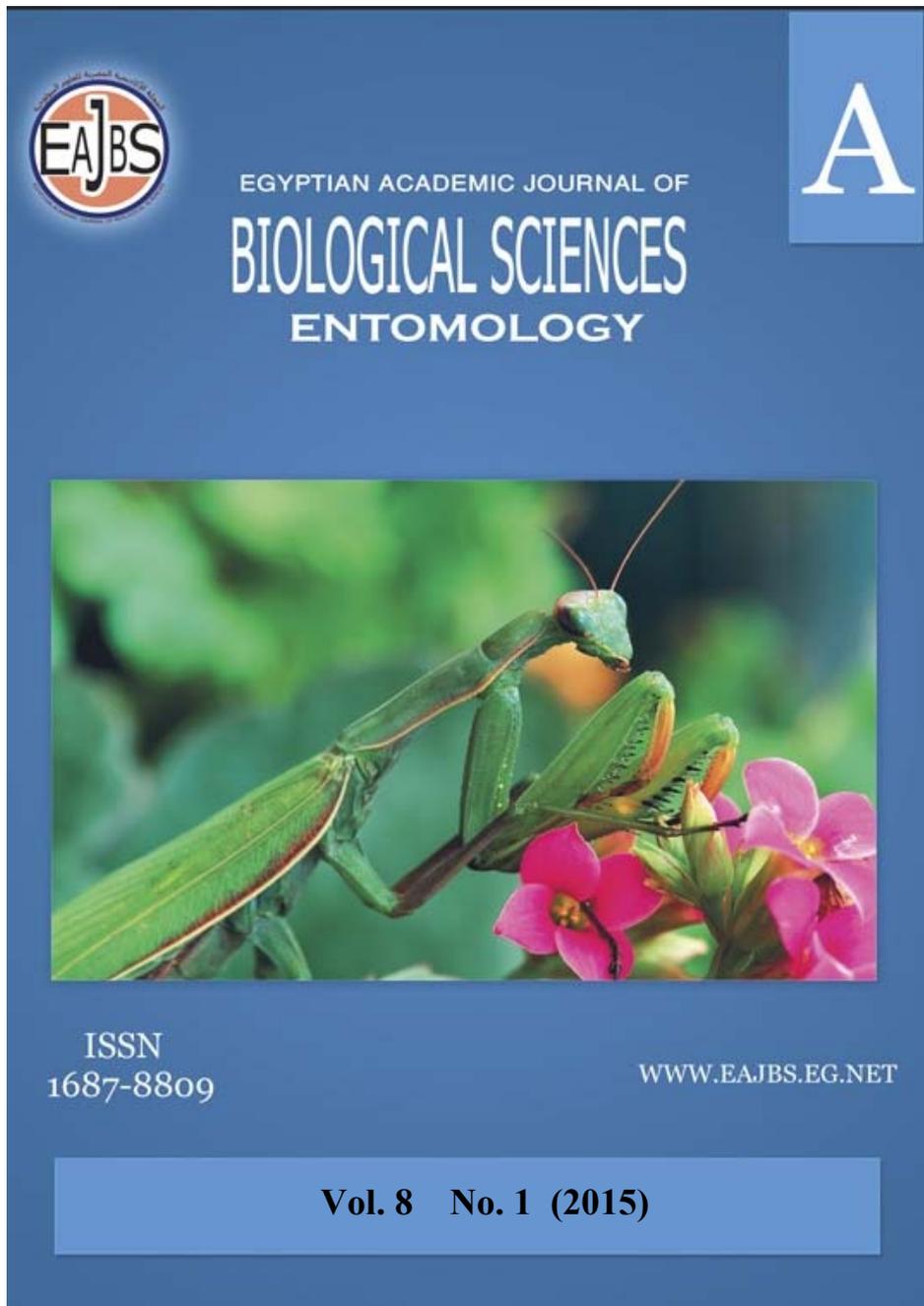


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Electron microscope study on workers antennae and sting lancets of three subspecies of honey bee *Apis mellifera* L. (Hymenoptera: Apidae) and its bearing on their phylogeny

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ABSTRACT

Ultrastructure of antennae and sting of the honey bee workers in three subspecies of *Apis mellifera*; Egyptian *A. m. lamarckii*, Carniolan *A. m. carnica* and Italian *A. m. ligustica* was investigated. Twelve types of sensilla were recorded on the worker antennae; placodea, basiconica, trichodea types (A, B, C and D), ampullaceous, coeloconica, coelocapitular, non-innervated hair seta, campaniformia and sensilla chaetica. The latter is recorded for the first time on antennae of *A. mellifera*. The number and distribution of the various types of sensilla are recorded. Sensilla trichodea were found most frequently and were present on all the antennal segments. Sensilla placodea were present on the distal eight flagellomeres. Sensilla ampullaceous and sensilla coeloconica were present on the distal five while sensilla campaniformia were confined to the distal seven flagellomeres. The barbs carried on the lancets of sting differ in numbers among the workers of the three subspecies; 10, 9 and 7 in *A. m. lamarckii*, *A. m. carnica* and *A. m. ligustica*, respectively. Depending on morphometrical analysis, a dendrogram separated the Egyptian subspecies from the other two subspecies with a highly genetic distance of 0.25 between them. The Italian and the Carniolan subspecies were grouped into one main cluster with a genetic distance of 0.01 between them.

INTRODUCTION

Apis mellifera L. is originally distributed in Africa, Europe and the Near East. Up to 29 subspecies have been distinguished on the basis of morphological traits (Ruttner, 1988; Hall & Smith, 1991; Garnery *et al.*, 1992; Arias & Sheppard, 1996; Franck *et al.*, 2000 & 2001 and Sheppard & Meixner, 2003). Based on various phylogenetic parameters, *A. mellifera* has been estimated to have occurred between 0.7 to 1.3 million years ago (Ruttner, 1988; Cornuet & Garnery, 1991 and Arias & Sheppard, 1996). The taxonomy of honey bee has been in chaos for decades. Morphometric studies have grouped the available subspecies into five lineages (Ruttner, 1988 & 1992; Sheppard *et al.*, 1997 and Franck *et al.*, 2001).

These five main lineages: 'C' (the Carnica group that includes *A. m. carnica* and *A. m. ligustica*); 'W' (the north and western European honey bees that include *A. m. mellifera*, *A. m. iberica*, and *A. m. intermissa*); 'A' (the African group that includes *A. m. scutellata*, *A. m. capensis*, *A. m. lamarckii*, *A. m. litorea*, *A. m. adansonii*, and *A. m. unicolor*); 'Y' (The Ethiopian subspecies, Franck *et al.*, 2001); and the 'O' group (the Oriental or Middle Eastern group which includes *A. m. anatolica*, *A. m. caucasica*, *A. m. syriaca*, *A. m. pomonella*, and *A. m. cypria*) (Ruttner, 1992).

The common honeybee subspecies in Egypt was *A. m. lamarckii*, but now this subspecies confined to small districts in Upper Egypt, where it is kept in modern and mud tube hives. Two other subspecies; *A. m. carnica* and *A. m. ligustica* were also recorded in Egypt, probably due to importation. As a result, the present honeybee in Egypt is a hybrid between different subspecies which has its own characteristics so it needs more investigation. In Egypt, most beekeepers prefer Carniolan or Italian honeybee.

It was reported that insect antennae are the sensory organ carriers in insects (Wigglesworth, 1981). Amputation of antennae in *Plodia interpunctella* Hubner caused 81% failure of tested couples to mate (Ahmed & El-Sayed, 1995).

Few scanning electron microscopic studies of worker honey bees, *A. mellifera*, have been carried out earlier (Slifer, 1970 and Dietz & Humphreys, 1971), while Letzkus *et al.* (2006) and Frasnelli *et al.* (2010) investigated the anatomical differences in the number of sensilla between the right and the left antennae of the honeybee using SEM.

The aim of the present study is to compare between the existing honey bee subspecies in Egypt and whether there is any phylogenetic relationship exist among them.

MATERIALS AND METHODS

Samples of worker honey bee *A. mellifera*, of three subspecies studied were collected at the age of 72 hours old from Entomology Department, Faculty of Agriculture, Ain Shams University, Cairo, Egypt.

For scanning electron microscopy study; heads, antennae and lancets were dissected from honey bee workers, fixed in glutaraldehyde and buffered overnight (pH 7.1) at room temperature. Next day, specimens were washed three times, 10 minutes each, in the buffer solution (pH 7.1) before transferring to distilled water. Specimens were then picked up using a fine brush and attached to a scanning stub using Ruban Adhesive pressure sensitive tap.

The stubs were placed on stub holder and cooled rapidly by plunging into liquid nitrogen. Then they were transferred to a freeze dryer for 3 - 4 hours at -60°C . Phosphorous pentoxide was kept in the vacuum chamber to absorb excess moisture.

Finally, the specimens were coated with gold in Sputter Coater in which gold was evaporated from an electrode in an atmosphere of argon. This was carried out three successive times, one minute each, using a current of 40 mA (Ahmed, 1987). Specimens were then viewed with scanning electron microscope (JOEL SEM). Different types of sensilla organs and setae were examined and photographed at proper magnifications.

The numbers of each sensilla organ type were counted at fixed-size areas ($70 \times 60 \mu\text{m}$) over four different flagellomeres (2nd, 3rd, 9th & 10th), calculated and expressed as the mean numbers and mean measurements (Gupta, 1992; Naik *et al.*, 1995 and Stort & Rebutini, 1998). The mean surface areas (μm^2) were determined

according to formula of Maurizio (1954).

Morphometrical analysis of numbers and dimensions of antennal segments and antennal sensory organs was carried out. SPSS-17 Program was used to draw phylogenetic dendrogram of the three subspecies.

RESULTS AND DISCUSSION

I- Surface structure of the antennae:

The antennae of the three honey bee workers subspecies, the Egyptian *A. m. lamarckii*, the Carniolan *A. m. carnica* and the Italian *A. m. ligustica* are of the geniculate type consisting of a scape, a pedicel, and a flagellum (Fig. 4). Near the base of the antenna there is a slight constriction which is the basal ball, covered with a field of simple sensilla, the basal ball fits into the antennal socket (Fig. 5). The diameter of the antennal socket of *A. m. lamarckii* is 0.34 mm and it is equal in diameter in both *A. m. carnica* and in *A. m. ligustica* measuring 0.33 mm. The distance between the two antennal sockets in *A. m. lamarckii* is 0.30 mm, in *A. m. carnica* is 0.25 mm and in *A. m. ligustica* is 0.26 mm. The length of the groove between the two antennal sockets were 0.84 mm in *A. m. lamarckii*, 0.87 mm in *A. m. carnica* and 0.90 mm in *A. m. ligustica* (Figs. 1, 2 & 3) (Table 1).

Table 1: Lengths of antennal parts (mm); scape, pedicel and different flagellomeres in three subspecies; *A. m. lamarckii*, *A. m. ligustica* and *A. m. carnica*.

Antennal part	<i>A. m. lamarckii</i>	<i>A. m. ligustica</i>	<i>A. m. carnica</i>
Scape	1.38	1.30	1.25
Pedicel	0.19	0.21	0.20
1 st flagellomere	0.28	0.29	0.23
2 nd flagellomere	0.17	0.23	0.18
3 rd flagellomere	0.33	0.38	0.32
4 th flagellomere	0.33	0.35	0.29
5 th flagellomere	0.30	0.33	0.29
6 th flagellomere	0.29	0.31	0.27
7 th flagellomere	0.26	0.29	0.27
8 th flagellomere	0.31	0.27	0.32
9 th flagellomere	0.29	0.23	0.23
10 th flagellomere	0.26	0.31	0.27

The scape:

The scape is proportional to the total antennal length and it is closely similar in the three subspecies. Their lengths are 29.22%, 29.38% and 30.80% of total antennal length in the Egyptian, the Carniolan and the Italian honey bee workers, respectively. The anterior surface of the scape is imbricate and is covered with simple setae. The sculpture of the scape is different among the three subspecies of *A. mellifera*; in *A. m. lamarckii* it looks like hexagonal ornamentation close to each other. On the other hand, in *A. m. ligustica* it looks like ripples and in *A. m. carnica* it takes the lenticels shape (Figs. 6, 7 & 8).

The pedicel:

The pedicel is pivoted and approximately as long as it is broad. It has an imbricate surface and covered with trichodean sensilla and simple setae. At its base there is a small patch of sensilla (Fig. 9). No recognized or specialized features differentiate between the three subspecies.

The flagellum:

The flagellum is subdivided into 10 flagellomeres, beginning proximally (Table 1 & Fig. 30). Except for the terminal one, each flagellomere is a slightly flattened cylinder. The surface of flagellomeres is highly sculptured in an imbricate to alveolate pattern. The relative length to width of each flagellomere varies both among the flagellomeres and among the three subspecies. The last flagellomere had a blunt roundish end (Fig. 4). The first 2 flagellomeres are dominated by setae than the rest of antennal flagellomeres (Fig. 10). The last flagellomere had a blunt roundish end. Sensillae are similarly distributed over flagellomere 3 to 8. Distribution of sensillae over 9 & 10 flagellomere is different from other flagellomeres. The numbers of sensillae over flagellomeres 2, 3, 9 & 10 are recorded (Table 2 & Fig. 31).

Table 2: Mean total numbers of different types of sensilla carried on the 2,3,9 and 10 flagellomere of antenna studied in the three subspecies; *A.m. lamarckii*, *A. m. ligustica* and *A. m. carnica*.

Types of sensilla	<i>A. m. lamarckii</i>	<i>A. m. ligustica</i>	<i>A.m. carnica</i>
Trichodea A	77	33	29
Trichodea B	18	7	24
Trichodea C	106	42	48
Trichodea D	4	4	5
Basiconica	9	8	8
Placodea	136	70	61
Campaniformia	2	7	20
Ampullacea	1	2	6
Coeleconica	2	6	13
Ceolecocaptiular	1	0	2
Setae	3	20	1
Chaetica	0	1	0

Main types of flagellar sensilla:

Eight main types of sensilla are recorded on the antennae of three subspecies of the *A. mellifera* workers; *A. m. lamarckii*, *A. m. carnica* and *A. m. ligustica* (Figs. 11, 12 & 13).

1- Non-innervated hair (seta):

The shape of setae varies from long, smooth, hair-like structures with sharp, tapered tips, to flattened, saber-like forms with or without longitudinal furrows (Fig. 14).

2- Peg-like sensillum (sensillum basiconicum)

It is found in the form of a straight peg having a blunt or nearly flattened tip (Fig. 15).

3- Sensilla Chaetica:

A simple sensory receptor consisting of one cell or a few cells, especially a spoon – like sensilla is projecting through the cuticle of antennae (Fig. 16). This type only recorded in antennae of *A. m. ligustica*.

4- Pore plate (sensillum placodeum):

Sensilla placodea are oval to nearly circular discs. When oval, the longest dimension is parallel to the long axis of the antenna. They are regularly distributed. Their number range from 1000 to 1250 per flagellomere arranged in 25 lines (Fig. 10). Under high power, these sensilla have slight radial furrows surrounding 7-10 μm pitch (Fig. 17). Flagellomere 1 and 2 are vacant from that type of sensilla.

5- Pit organs (sensillum ampullaceum and sensillum coeloconicum):

Pit organs appear as holes of different diameters in the antennal surface, within

which there is a peg that sometimes protrudes slightly. Under high power, the peg has furrows following meridian lines. These types are similar in scanning electron microscope, except that the diameters of sensilla coeloconica (5-7 μm) are greater than those of sensilla ampullaceal (3-5 μm) and the former has dome-shaped structure surrounding the peg. Pit sensilla are located in the three subspecies (Figs. 18 & 19).

6- Sensilla Campaniformea:

These sensilla are known to be mechanoreceptors of insects. The term campaniform refers to the bell shape appearance of these sensory structures when viewed in cross section (Fig. 20).

7- Sensilla coelecapitular:

This type is exhibiting a cuticular apparatus with a mushroom like protrusion in a pit on the antennal surface (Fig. 21). Sensilla coelecapitular is demonstrated in *A. m. lamarckii* and in *A. m. carnica* but was not observed in *A. m. ligustica*.

8- Sensilla trichodea A, B, C & D:

Sensillum trichodeum A: This sensillum is thin and tapering, slightly too markedly s-shaped (Fig. 22).

Sensillum trichodeum B: This sensillum is thin at the base and tapers to a very fine point; the distal portion is strongly arched, and the degree of curvature varies among sensilla, as well as among taxa (Fig. 23).

Sensilla trichodea C & D: These sensilla are long hairs with rounded to slightly sharpened tips, and are sometimes lightly curved away from the antennal surface (Figs. 24 & 25).

II- Surface structure of the sting:

Only females (workers and the queen) possess a sting. Part of the female genital apparatus is transformed into a sting, connected to two poison glands (Fig. 26). The sting is composed of:

- 1- A stylet resulting from the fusion of the genital pieces of the 9th segment, enlarged at the base and possessing two grooves internally.
- 2- Two lancets: genital pieces of the 8th segment. The venom canal is situated between the 2 lancets. Each lancet carries numbers of barbs (teeth) pointing opposite to the direction of stinging. The barbs differ in numbers and shapes among the workers of three subspecies, 10 barbs in *A. m. lamarckii* and 9 in *A. m. carnica*, and 7 in *A. m. ligustica* (Figs. 27, 28 & 29).
- 3- Two poison glands: The dorsal acid gland, "y" shaped, is the only one really venomous. It is linked to a bulky reservoir by a long canal. An alkaline gland with a non-toxic secretion, which serves to lubricate the lancets and to increase the pH of the venom, enhancing its toxicity also located (Fig. 26).

The present observations showed that there is a direct proportional relationship between length of flagella (Table 1 & Fig. 30) and the number of carried sensilla (Table 2 & Fig. 31). The general characters such as the length of antennal parts, length of different sensilla types and other characters are used to compare between the three subspecies *A. m. ligustica*, *A. m. lamarckii* and *A. m. carnica* (Table 3 & Fig. 32). Data were analyzed using the recent statistical analyzer SPSS-17 program for the morphometrical variation among the used subspecies, to show phylogenetical relationship among them. The dendrogram illustrated in Figure (33) separated the Egyptian subspecies *A. m. lamarckii* from the other two subspecies with a highly genetic distance of 0.25 between them. The Italian *A. m. ligustica* and Carniolan *A. m. carnica* subspecies were grouped into one main cluster with a genetic distance of 0.01 between them.

Table 3. Mean dimensions (mm) of morphometrical characters of three subspecies; *A.m. lamarckii*, *A. m. ligustica* and *A. m. carnica*.

Character	<i>A. m. lamarckii</i>	<i>A. m. ligustica</i>	<i>A. m. carnica</i>
Socket diameter	0.34	0.33	0.33
Distance between sockets	0.30	0.26	0.25
Length of the groove between	0.84	0.90	0.87
Scape length	1.38	1.30	1.25
Scape diameter	0.26	0.27	0.27
Pedicel length	0.19	0.21	0.20
Pedicel diameter	0.16	0.19	0.18
Flagellum length	2.88	3	2.7
Flagellum diameter	0.21	0.229	0.227
Trichodea A length	0.015	0.012	0.014
Trichodea B length	0.016	0.011	0.011
Trichodea C length	0.012	0.011	0.011
Trichodea D length	0.016	0.014	0.014
Basiconica length	0.015	0.011	0.009
Placodea diameter	0.014	0.015	0.016
Campaniform diameter	0.005	0.006	0.006
Ampullacae diameter	0.005	0.003	0.004
Coeleconica diameter	0.007	0.005	0.005
Coelocapitular diameter	0.006	0.00	0.008
flagellar setae length	0.011	0.011	0.013
Chaetica length	0.00	0.012	0.00
Lancet length	2.20	1.39	1.20
Lancet barbs number	10	7	9

DISCUSSION

The honeybee antenna functions as an odour, mechano and thermal receptor (Wigglesworth, 1981). Each antenna consists of one segmented scape, a pivoted pedicel and a long slender flagellum, which is composed of 10 flagellomeres in female queen and workers. Sensory organs, or sensilla, on the antennae of honeybees can be distinguished into seven different types; basiconica, campaniformea, placodea, and trichodea type A, B, C and D (Argen, 1977 and Chapman, 1982). But the present study recorded twelve types of sensilla. Beside the previous types sensilla; sensilla ampullacea, coeloconica, coelocapitular, non-innervated hair (seta) and sensilla chaetica were recorded. Sensilla placodea are located on the last eight segments of the antennae. They register air pressure and have olfactory functions (Crane, 1990 and Gupta, 1992).

The location of different types of sensilla on antenna of *A. mellifera* is similar to those of *A. florae*. The numbers of each type of olfactory sensilla were comparatively much less on the antenna of *A. florae* (Bhardwaj, 1974 and Gupta, 1982) than on *A. mellifera* (Dietz *et al.*, 1974).

The distribution of various types of sensillae along the antenna of *A. mellifera* is similar to that found in other *Apis* species. Sensilla trichodea type A are the most common structures on the antenna. The olfactory function of sensilla trichodea was

hypothesized by many workers (Vogel, 1921; Frisch, 1967; Schneider, 1968 and Dietz & Humphreys, 1971). However, Lacher, 1964 reported that sensilla trichodea type A did not respond to any chemical stimuli but sensilla trichodea type B responded to mechanical stimuli in *A. mellifera*. Martin and Lindauer (1966) found some of these sensilla on the distal antennal segment of *Apis* species and suggested that they may be used to decipher wax smoothness. A gustatory function for sensilla trichodea type C & D was also suggested (Esslen & Kaissling, 1976). Sensilla trichodea are the sex pheromone receptors on the male spruce budworm *Choristoneura fumigarana* (Albert *et al.*, 1974) and the male red banded leaf roller *Aroyrotaenia velutinana* (O'Connell, 1972 and 1975).

Sensilla placodea were the easiest to compare among the three subspecies as there is more comparative information available. The highest number of sensilla placodea present in the Italian *A. m. ligustica* as it is the tallest flagellum, followed by the Egyptian *A. m. lamarckii*. The shortest recorded one is the Carniolan *A. m. carnica*. The sensilla placodea have been shown to be odour receptors in *A. mellifera* (Lacher & Schneider, 1963 and Kaissling & Renner, 1968). Slifer *et al.* (1959) and Schneider *et al.* (1964) thought that sensilla basiconica participated in chemoreception in grasshoppers and moths, and the pores present in their walls are much suited for the perception of olfactant molecules.

In sensilla ampullacea and sensilla coeloconica, the surface around the pore is patterned in *A. mellifera* in contrast to the smooth one in *A. florea* (Argen, 1975). These have been shown to respond to carbon dioxide in *A. mellifera* (Lacher, 1964), temperature in *Aedes aegypti* (Davis & Sokolove, 1975) and *Periplanta americana* (Altner *et al.*, 1977) and humidity in *P. americana* (Yokohari & Taneda, 1976; Altner *et al.*, 1977 and Yokohari, 1978). Sensilla campaniformia in *A. mellifera* are probably mechanoreceptors. It was reported the presence of campaniform sensilla on cockroach legs which are mechanoreceptors and responded to the stresses on the cuticle. Campaniform sensilla are found to occur in association with ampullaceous and coeloconic sense organs and hence these may be considered sensitive to temperature, carbon dioxide and humidity or a combination of these factors (Pringle, 1938; Esslen & Kaissling, 1976; Yokohari & Tateda, 1976 and Yokohari, 1978). This speculation is based on the electrophysiological findings (Lacher, 1964).

The similarities and differences in distribution and numbers of sensilla along the antenna among the three subspecies; *A. m. lamarckii*, *A. m. ligustica* and *A. m. carnica* are recorded. Less similarity occurs in distribution if one compares *A. mellifera* with less closely related Hymenoptera.

Moist and dry hygroreceptors have been identified along with a thermal receptor in a specialized coeloconic sensillum. This sensillum comprises a cuticular, shallow depression (diameter 4 μm) having a central opening (1.4-1.5 μm) and a mushroom-shaped protrusion (1.4-1.5 μm) from the opening. The head of the protrusion is irregular in shape and is not perforated. This sensillum has been thus far referred to as a "sensillum campaniformium" (Dietz & Humphreys, 1971), henceforth, it is referred to as a coelocapitular sensillum.

Many electrophysiological studies have been conducted on insect hygroreceptors including *A. mellifera* (Lacher, 1964). Responses of locust hygroreceptors do not depend on either the relative humidity or absolute humidity (Waldow, 1970). The dry receptor, one of the hygroreceptors of the cockroach *P. americana*, is bimodal, responding to both temperature and humidity (Loftus, 1976). Finally, cockroach hygroreceptors respond to relative humidity with a mechanical transformation of the primary process of hygroreception (Yokohari & Tateda, 1976

and Yokohari, 1978).

Coeloconic sensillum described in the three subspecies studied, have stimulus conducting pores similar to those of the olfactory sensilla recorded in locust and cockroach (Steinbrech, 1969; Altner *et al.*, 1973 and 1977). Different from those found in *A. millifera*, coeloconic sensilla of the walking stick are poreless (Yokohari *et al.*, 1975; Altner *et al.*, 1978; Yokohari, 1981 and Tominaga & Yokohari, 1982). Recently, Schaller (1978) and Altner and Prillinger (1980) found additional poreless sensilla in cockroach and locust antennae, respectively, and suggested that they may be hygroreceptive.

Hygroreceptors of the honey bee may function also by a mechanism similar to that proposed for *P. americana* (Yokohari, 1978), i.e., insect hygroreceptors are receptors for relative humidity with a mechanical transformation occurring in the primary process of hygroreception.

The present study demonstrates that the hygro- and thermoreceptive organ of the honey bee is the coelocapitular sensillum. Lacher (1964) reported that a moist receptor is contained within a coeloconic or ampullaceous sensillum. A coelocapitular sensillum, however, is present in association with an ampullaceous or coeloconic sensillum. Further, Dietz and Humphreys (1971) reported that, this type of sensillum was not known to occur in the honey bee antenna. While, Lacher (1964) recorded hygroresponses from the coelocapitular sensilla. This type of sensillum was first found by scanning electron microscopy and referred to as sensillum campaniformium by Dietz and Humphreys (1971). This sensillum, however, does not resemble the true campaniform sensillum which appears externally as a dome-shaped protrusion of the cuticular surface with a tiny button at its center.

It is well established that the campaniform sensillum functions as a proprioceptive mechanoreceptor (Moran *et al.*, 1971). Thus, it is inappropriate to refer to this sensillum as a "sensillum campaniformium". This sensillum appears to be of the coeloconic type. This sensillum contains hygro- and thermoreceptors; coeloconic sensilla generally contain olfactory receptors (Altner & Prillinger, 1980).

In the present study, the sensillum referred to as coelocapitular sensillum is distinguished from the coeloconic sensillum as the latter hook-like cap is not in the center. The small protrusion of a coelocapitular sensillum is also characterized by being situated below the surface of the antenna, may be protected from harsh contact with solid components of the environment. This is explained as hygroreceptors of honey bees were shown to respond to a deformation of the sensillum, similar to that shown for cockroach hygroreceptors (Yokohari, 1978). Sensilla of similar structure have been identified as hygro- and thermoreceptors (Altner *et al.*, 1978; Tichy, 1979 and Altner *et al.*, 1981). Thermoreceptors of the cockroach are found in both the capitular sensillum (Yokohari *et al.*, 1975) and in the grooved sensillum (Altner *et al.*, 1977 and Yokohari, 1983). Structurally comparable sensilla have been described in the antennae of a great variety of beetles (McIver, 1973; McIver and Siemicki, 1976 & 1979), and the bed bug (Steinbrecht & Miller, 1976). These sensilla are inferred to be hygro- and thermoreceptors. The number of these sensilla, estimated in the present study is similar to that of the sensillum capitulum of *P. americana* (Esslen & Kaissling, 1976 and Tominaga & Yokohari, 1982). This suggests that this number of hygroreceptors may be necessary for bee worker to perceive humidity in the environment.

In the present study, twelve sensilla types were recorded on all acquired images. In particular, the putative olfactory sensilla were identified sensilla: placodea, trichodea type A to D, and basiconica with thick and tapered end. The sensilla with

non-olfactory functions: coeloconica; involved in perception of temperature, carbon dioxide and humidity, campaniformia; considered as hygro- and thermoreceptors and mechanoreceptors and chaetica; sensitive to mechanical or gustatory stimuli (Dietz & Humphreys, 1971 and Whitehead & Larsen, 1976).

Hygro- and thermoreceptive sensilla have been identified electrophysiologically in many insects (Altner & Prillinger, 1980) and their structure has been studied by scanning and transmission electron microscopy. The coeloconic sensillum described in this study similar to that observed in *L. migratoria* (Altner *et al.*, 1981) and the basiconic sensillum of *P. americana* are reported to possess stimulus-conducting pores similar to those of olfactory sensilla (Steinbrecht, 1969; Altner *et al.*, 1973, 1977 and Yokohari *et al.*, 1975).

In the present study antennae of the worker *A. mellifera* carry many non-porous sensilla and setae. Other investigators described similar structure to these sensilla to be poreless as in *Carausius morosus* (Altner *et al.*, 1978); *P. americana* (Schaller 1978; Yokohari, 1978 & 1981 and Tominaga & Yokohari 1982); *L. migratoria* (Altner *et al.*, 1981) and in *Leucophaea maderae* (Schaller, 1982). The outer cavity of the sensillum communicates directly with the exterior (Schaller, 1978). In the poreless coeloconic sensillum and cold-moist-dry sensillum, the outer cavity communicates through a cleft and electron-dense clusters (Altner *et al.*, 1978 and 1981), whereas the cavity of the capitular sensillum is completely closed (Yokohari, 1981).

The present study describes the ultrastructure of the antennal hygro- and thermoreceptive sensillum, the sensillum coelocapitulum, in subspecies *A. m. lamarckii* and *A. m. carnica* but not in *A. m. ligustica*. This type has been identified as electrophysiological organ (Yokohari *et al.*, 1982). Externally the cuticular apparatus appears as a spherical-shaped protrusion located in a central opening of a circular, shallow depression. The cuticular apparatus is set in a cylindrical pit of about 3 μm diameters. It is about 4 μm long and is slightly dilated at its head. Thus, the free space in the pit is narrow, measuring about 0.2 μm wide distally and about 0.5 μm basally. The head of the cuticular apparatus is visible externally and appears by SEM to have an irregular surface. The cuticular wall is not perforated transversely in any region, and the central part of the head is more electron dense than the other parts. Impulses were recorded from hygroreceptors and thermoreceptors in antennae of the honey bee queen, *A. mellifera* (Lacher, 1964). Recently, Yokohari *et al.* (1982) re-identified electrophysiologically the hygro- and thermoreceptors in a coelocapitular sensillum.

The present work clearly shows that, morphologically, coelocapitular sensillum belongs to the hygro- and thermoreceptive sensilla category, and not to that of proprioceptive mechanoreceptive sensilla. Some studies have shown that the hygro- and thermoreceptors can be identified electrophysiologically in structures morphologically similar to olfactory sensilla (Steinbrecht, 1969; Altner *et al.*, 1973 and Altner *et al.*, 1977). Many studies indicate that these receptors are found in the poreless sensilla (Yokohari *et al.*, 1975, 1982; Altner *et al.*, 1978, 1981; Yokohari, 1978, 1981; Schaller, 1978, 1982 and Tominaga & Yokohari, 1982).

On the other hand, Altner *et al.* (1981) found the cleft at the base of the cuticular apparatus associated with deposits of electron-dense material in the poreless coeloconic sensillum of *L. migratoria*, and discussed its possible role in stimulus transduction. In the present study, the centro-apical part of the coeloconic sensillum in worker honey bee, and may be plugged by electron-dense node, is found in all poreless hygro- and thermoreceptive sensilla. This is probably a molting pore, which can be demonstrated for the coelocapitular sensillum (Yokohari, 1981). The cuticular apparatus is situated either in the pit or beneath the bristles. This arrangement would

protect the sensillum from harsh contact with solid components of the environment. The hygroreceptors are capable of discharging in response to mechanical deformation of the sensillum (Yokohari, 1978 and Yokohari *et al.*, 1982).

In the present study, a first record was done for sensilla chaetica carried on antennae of *A. m. ligustica*. Chaetica sensilla recorded also in the males of Indian meal moth *P. interpunctella*, the chaetica sensilla responsible for mechanoreceptors (Ahmed *et al.*, 1991).

Defensive behaviour of worker honeybees is elicited by visual stimuli, such as moving a dark object, and is enhanced by alarm pheromone secreted from the alkali glands of attacked nest mates. The culminating stage of the defensive behaviour is the 'stinging response', which involves the penetration of the shaft of the sting and the release of venom from the venom sac (Boch *et al.*, 1962).

The stinging apparatus is structurally homologous to the ovipositor in Orthoptera, Hemiptera and non-stinging Hymenoptera. Its morphology has been described by various authors (Snodgrass, 1956 and Dade, 1962). The barbs carried on the lancets of sting differ in number among the three studied subspecies. They are 10, 9 and 7 in *A. m. lamarckii*, *A. m. carnica* and *A. m. ligustica*, respectively. These differences in the morphology of antennae and lancet barbs may reflect the violent and calm behavior of these subspecies.

In the present study, ultrastructure of the head features, types and numbers of the sensory organs carried on the antennae and the sting lancets are used to differentiate among the three studied subspecies of the honey bee *A. mellifera* and the bearing of these differences on their phylogenetic relationship. Twenty five different characters of the antennae, sensillar organs and sting lancets were mentioned. These characters and their opposites are listed and analyzed using SPSS - 17 program, to draw a dendrogram to show the phylogenetic relation between the three subspecies reared in Upper and Lower Egypt; *A. m. lamarckii*, *A. m. ligustica* and *A. m. carnica*.

The resulted dendrogram (Fig. 33) showed that the Italian and Carniolan subspecies are sister groups. The Egyptian subspecies lies in a separate clad distinguished by many different morphological characters including numbers, types, dimensions and distribution of antennal sensilla.

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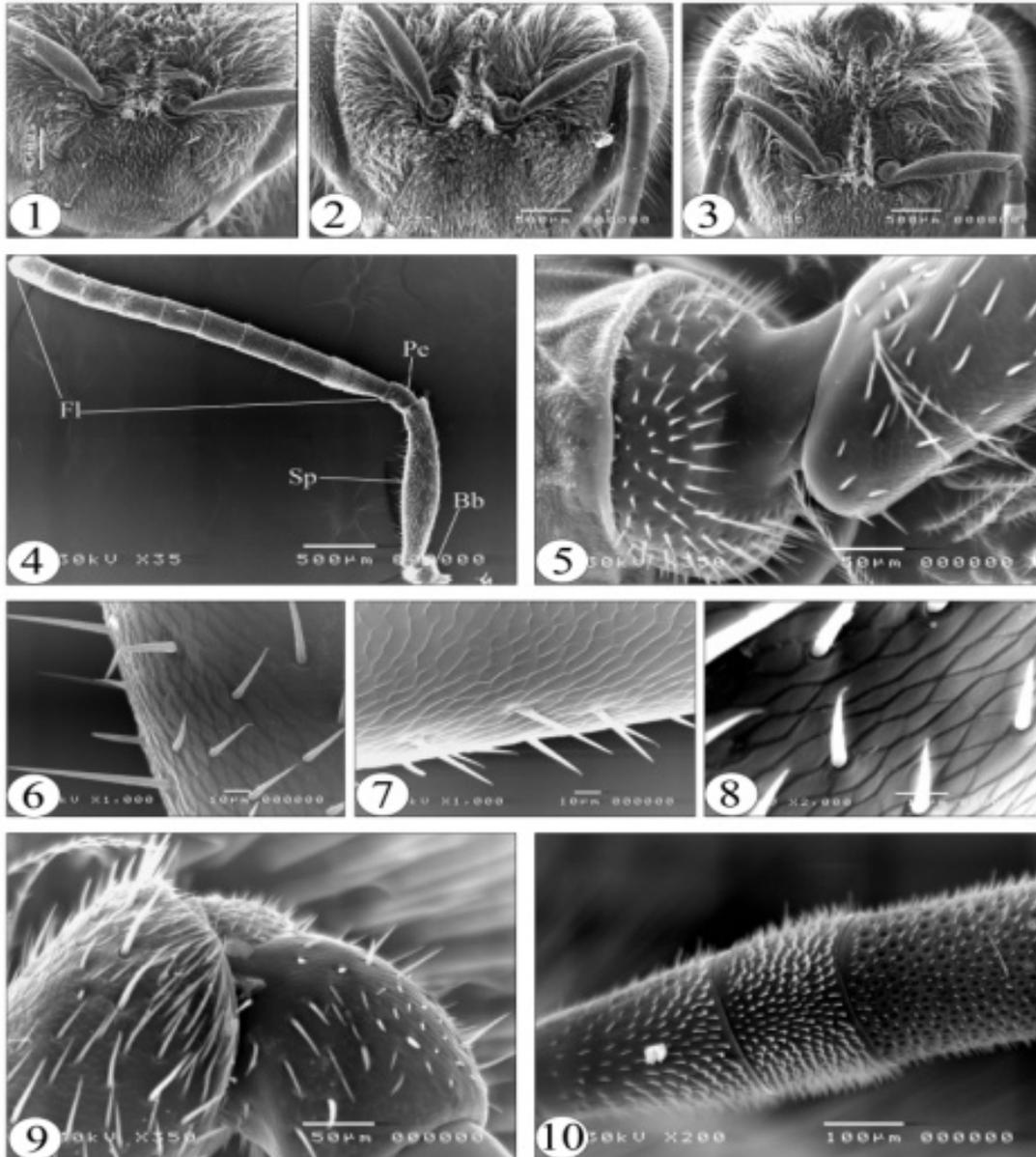
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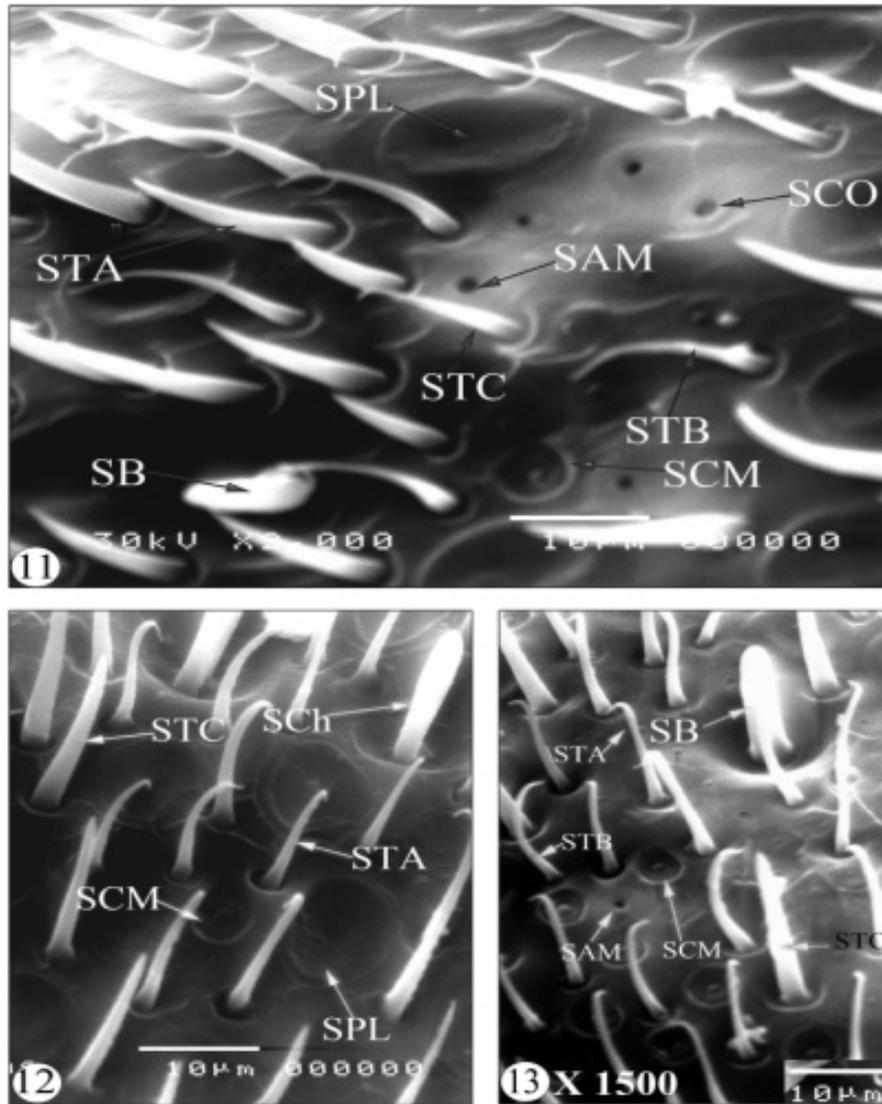
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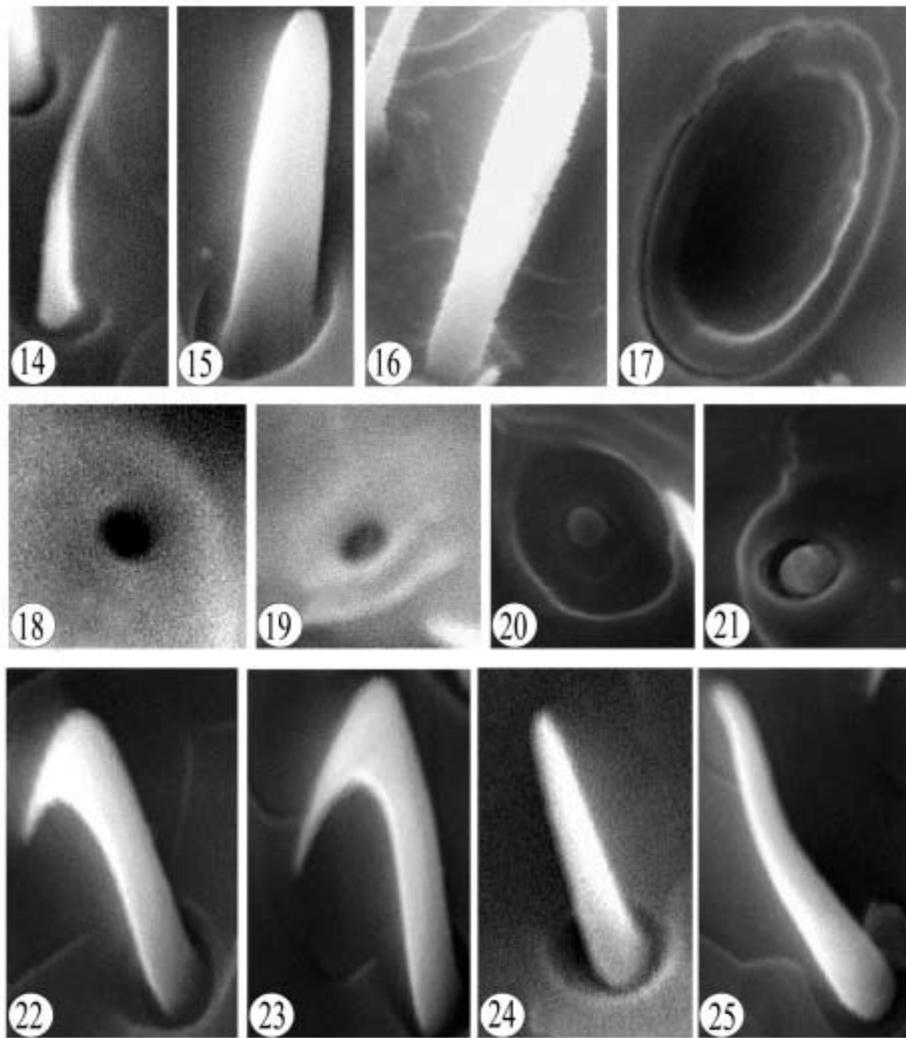
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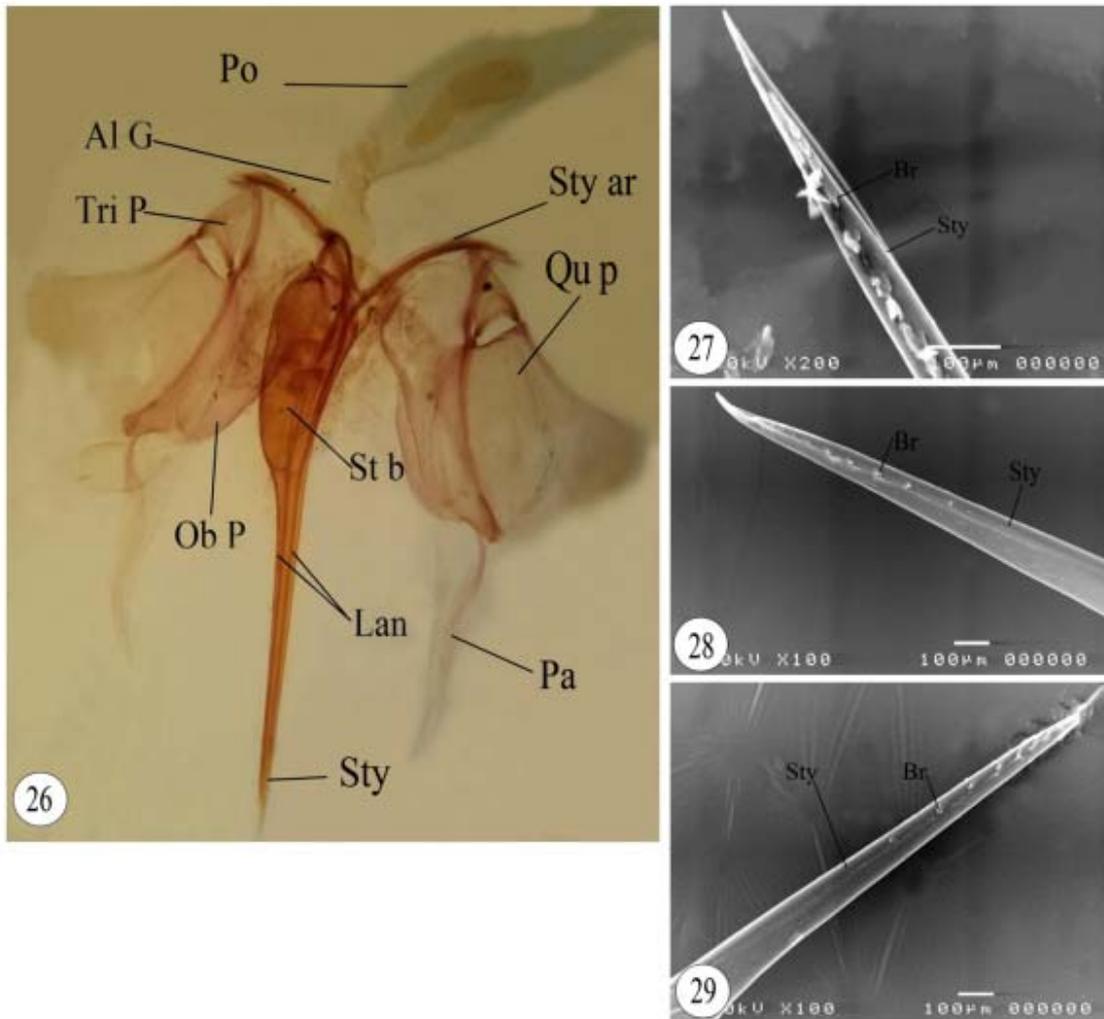
Figs. 1-10: Figs 1-3. Structure of the heads of worker honey bee: in *A. m. lamarckii* (Fig. 1); *A. m. ligustica* (Fig. 2); *A. m. carnica* (Fig. 3). Fig. 4. Morphology of an antenna carrying sensilla, basal ball (Bb), flagellum (Fl), pedicel (Pe) and scape (Sp). Fig. 5. basal ball of scape with field of simple sensory sensilla and imbricate surface sculpturing of scape. Figs 6-8. Parts of the imbricate surface of the scape, which is covered with simple sensilla. Note, the surface sculpture in three subspecies of *Apis mellifera*; the hexagonal forms compact with each other in *A. m. lamarckii* (Fig. 6); the ripples shape in *A. m. ligustica* (Fig. 7) and the Lenticels shape in *A. m. carnica* (Fig. 8). Fig. 9. The pivoted imbricate pedicel, covered with trichodea sensilla and simple setae. Note the small patch of sensilla at the base. Fig. 10. The first three antennal flagellomeres showing difference in sensillar distribution, types and length.



Figs. 11-13. The distribution of different types of sensilla organs; SAM: sensilla ampullaceous, SB: sensilla basiconica, SCM: sensilla campaniforme, SCO sensilla coeloconica, SPL: sensilla placodea, STA: sensilla trichodea type A, STB: sensilla trichodea type B, STC: sensilla trichodea type C and STD: sensilla trichodea type D (Fig. 11); SCh: sensilla chaetica, SCM: sensilla campaniforme, SPL: sensilla placodea, STA: sensilla trichodea type A and STC: sensilla trichodea type C (Fig. 12); SAM: sensilla ampullaceous, SB: sensilla basiconica, SCM: sensilla campaniforme, STA: sensilla trichodea type A, STB: sensilla trichodea type B and STC: sensilla trichodea type C (Fig. 13).



Figs. 14-25: The structure of sensilla types; non-innervated hair (seta) (Fig. 14), sensillum basiconicum (Fig. 15), sensillum chaetica (Fig. 16), sensillum placodeum (Fig. 17), sensillum ampullaceum (Fig. 18), sensillum coeloconicum (Fig. 19), sensillum campaniform (Fig. 20), sensillum coelecocapitular (Fig. 21), sensillum trichodeum A (Fig. 22), sensillum trichodeum B (Fig. 23), sensillum trichodeum C (Fig. 24) and sensillum trichodeum D (Fig. 25).



Figs. 26-29: Fig. 26. The sting; triangular plate (Tri P), oblong plate (Ob P), quadrate plate (Qu P), Stylet (Sty), sting bulb (St b), Alkaline gland (Al G), two lancets (Lan), Plap-like appendage (Pa), Stylet arm (Sty ar) and poison sac (Po). Figs 27-29. The barbed lancets of the sting of three subspecies workers; *A. m. lamarckii* (Fig. 27), *A. m. ligustica* (Fig. 28) and *A. m. carnica* (Fig. 29) showing Stylet (Sty) and barbs (Br).

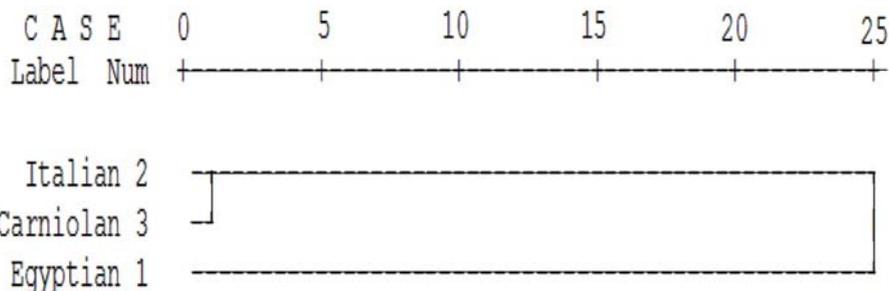
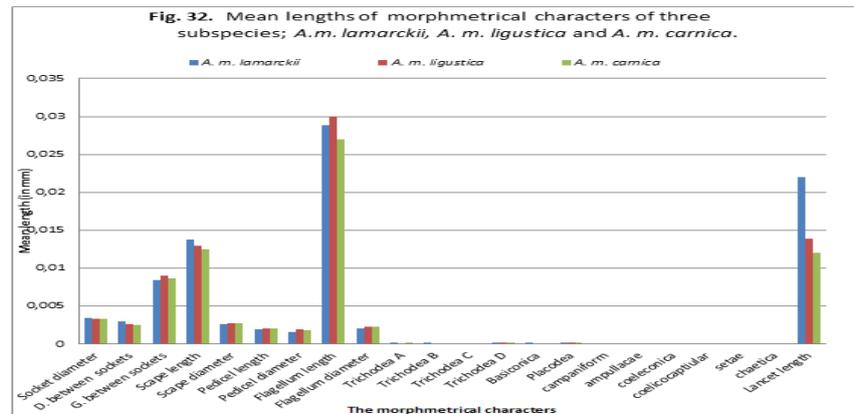
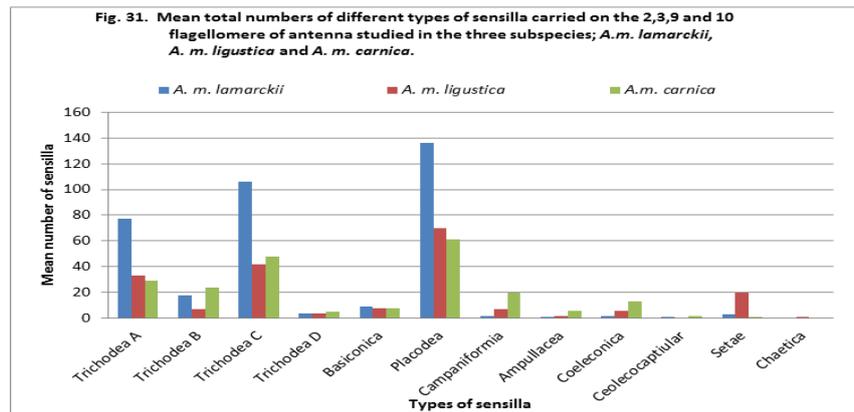
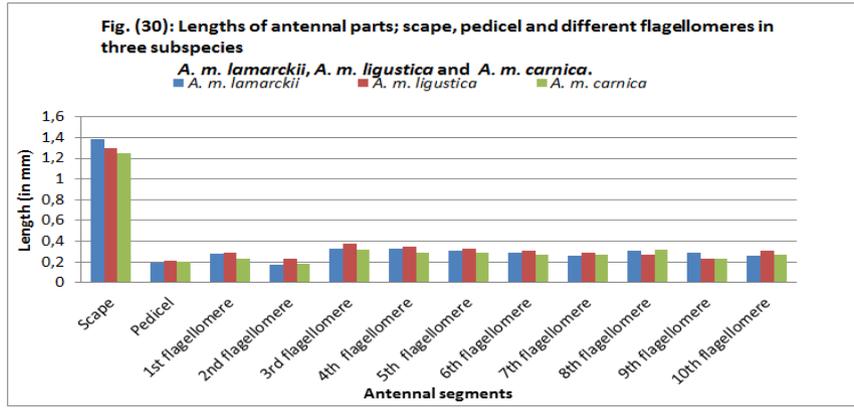


Fig. 33: Dendrogram demonstrates the phylogenetic relationship among the three subspecies of the honey bee worker, *Apis mellifera* based on the pooled results derived from mean lengths of morphometrical characters of the three subspecies; the Italian *A. m. ligustica*, the Carniolan *A. m. carnica* and the Egyptian *A. m. lamarckii*.

ARABIC SUMMERY

دراسة بالميكروسكوب الإلكتروني على قرون الإستشعار ورمح آلة اللسع لشغالات ثلاث نويغات من نحل العسل
أبيس ميليفيرا ل. (رتبة: غشائية الأجنحة - فصيلة: أبيدي)

خلف الله صابر أحمد - سعدية محمد البرماوى - هبه زكريا الجوهري - عبير مصطفى بيومي
قسم العلوم البيولوجية والجيولوجية - كلية التربية - جامعة عين شمس

تم استخدام الميكروسكوب الإلكتروني الماسح لدراسة الشكل الظاهري لقرون الإستشعار لشغالات ثلاث نويغات من نحل العسل : المصري *أبيس ميليفيرا* لاماركى والكرنيولى *أبيس ميليفيرا كارنيكا* والإيطالى *أبيس ميليفيرا ليجويستينا* والتي كانت جميعها من النوع المرفقى ، تم تسجيل إثني عشر نوعاً من أعضاء الحس على قرون الإستشعار فى النويغات الثلاثة وهى : بلاكوديا ، بازيكونيا ، ترايكوديا أنواع (أ ، ب ، ج ، د) ، أمبيولاسيا ، سيلوكونيا ، سيلوكوكابتولار ، سيتا ، كامبانيفورميا وأخيراً النوع كاتيكا الذى سجل للمرة الأولى على قرون إستشعار نحل العسل ، كما تمت دراسة أعداد وتوزيع الأنواع المختلفة من أعضاء الحس على قرون الإستشعار ، وكان النوع ترايكوديا الأكثر إنتشاراً على جميع قطع قرون الإستشعار، واقتصر وجود بلاكوديا على القطع الثمان الطرفية ، والنوعان أمبيولاسيا وسيلوكونيا على القطع الخمس الطرفية بينما النوع كامبانيفورميا فكان مقتصراً على القطع السبع الطرفية.

كما إختلف عدد الأشواك على الرمح فى آلة اللسع بين النويغات الثلاث ، فكانت ١٠ فى النويغ المصرى و ٩ فى الكرنىولى و ٧ فى الإيطالى.

وقد فصل الدندروجرام الناتج النويغ المصرى عن النويغان الأخران بمسافة وراثية ٠,٢٥ ، وجمع النويغان الإيطالى والكرنيولى فى مجموعة واحدة بمسافة وراثية بينهما بلغت ٠,٠١ .