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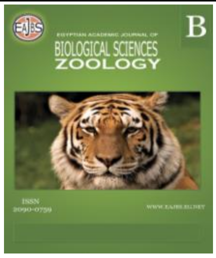

Ultrastructure Study on The Exo-Morphology of Four Species of Scorpion Inhabiting New Valley, Egypt

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ABSTRACT

Scorpions are terrestrial arthropods inhabiting deserts. Exomorghological studies play an important role in the identification of different scorpions' species. The current study deals with the ecomorphology of four species of scorpions belong to the family buthidae (*Leiurus quinquestriatus*, *Androctonus amoreuxi*, *Orthochirus innesi* and *Buthacus leptochelys*) inhabiting New Valley governorate, Egypt. Random samples were implemented during the period from April to July, 2017 and July to September, 2019. Scorpions were obtained from the area under rocks, gap of soil, leaf litter, under bark, and within vegetation. Morphological characteristics of these species were done using a scanning electron microscope (SEM). In conclusion, the differences among four species of studied scorpions were noticed in the dorsal carapace, respiratory pores of the third segment of mesosoma, pectinal teeth count between females and males, last segment of metasoma and telson. In all scorpions' species studied, one venom pore was detected except in *Androctonus amoreuxi* two venom pores in telson were observed.

INTRODUCTION

Scorpions have a problem of public health in several countries all over the world. They were mentioned as the most ancient arachnids on the earth (El-Hennaway, 2001). The scorpion activity period in Egypt is warm months and these environmental conditions explained the nature of scorpions (Hmimou *et al*., 2008). Salama and Sharshar (2013) were interested in study on scorpions from different regions in Egypt. Saleh *et al* (2017) studied the geographical distribution and diversity of the scorpion fauna in Egypt.

Scorpions are among the most successful terrestrial arthropods inhabiting deserts worldwide (Polis and Yamashita, 1991). Behaviorally, they avoid environmental extremes by retreating to shelters (e.g., burrows, rock crevices) and by a reduced and largely nocturnal surface activity. In Egypt, there are about 26 species of scorpion, included 13 genera, and 4 families; Buthidae, Scorpionidae, Diplocentridae, and Euscorpiidae (El-Hennaway, 1985). The most three important orders of Arachnida are Araneae (spiders), Scorpiones (scorpions), and Acarina (ticks and mites) (Mullen and Stockwell, 2002; Isbister *et al*., 2003; Ozkan and

Karaer, 2004). The most important species of Buthidae family are *Buthotus tamulus, Leiurus uinquestriatus*, *Androctonus crassicauda*, *Androctonus australis*, *Tityus serrulatus* and *Centruroides suffuses* (Minton, 2010) About 30 out of more than 1,300 described species worldwide have venom potent enough to be considered dangerous to human beings (Fet *et al*., 2000; Brownell and Polis, 2001). Among the most dangerous genera are *Leiurus*, *Para-buthus*, *Tityus*, *Centruroides*, and *Androctonus* (Lucas and Meier, 1995). Scorpions lack antennae, and thermoreceptors have not been found on the legs, so their thermoreceptors are located on another peripheral appendage. The pedipalps (claws) are one of the scorpion's peripheral appendages; these structures are responsible for catching and holding prey during predation (Danielle, 2013). Scorpion chelae are used for several functions in prey handling, defence (Casper, 1985), (Harington, 1978). The pedipalps are covered with many sensory hairs; function similarly to the antennae of insects (Fet *et al*., 2003).

Dark-colored species of *Orthochirus* are common in the deserts from North Africa to Central Asia (Fet and Lowe, 2000). The pedipalps are usually the first part of the scorpion to come into contact with the prey; which is gripped immediately with the chelae. Scorpions possess comb-shaped sensory organs called pectines on their mid-ventral surface (Cloudsley-Thompson, 1955). The pectines are sexually dimorphic; where the males having considerably larger pectines than the females. Gaffin and Brownell (1997) showed that male scorpions use their pectines to detect female pheromones. Each pectin consists of a flexible spine from which extend numerous teeth.

Scorpions have many variations in the details of body surface and structure of the external exoskeleton between different species. The taxonomy and diversity of scorpion fauna of Egypt were studied by Badry *et al* (2017). Whereas Abd-el-Wahab (1957) studied the morphological and histological description of the male genital system of the scorpion *Buthus quinquestriatus*. Venom and the composition of the poisonous gland were studied by Salama and Sharshar (2013). Abd El-Atti *et al* (2020) described the morphology, histology, histochemistry and ultrastructure of the venom gland of *Leiurus quinquestriatus*. In the medical field, the potential anti-inflammatory effects of the Egyptian scorpion (*Androctonus amoreuxi*) was reported by Hassan (2019). Toxicological and morphological characterization of scorpions (*Leiurusquin questriatus* and *Androctonus crassicauda*) were done in Luxor, Egypt by Nafie *et al* (2020). The previous author also studied the antimicrobial activity of venom against bacteria and fungi. *Leiurus quinquinquestriatus* and Egyptian cobra venoms have an effective therapy for breast and prostate cancer especially in the cases of metastasis (Omran, 2003).

To the best of the present authors' knowledge, previous studies on scorpions inhabiting New Valley, Egypt are scarce. So, the present study was focused on the study of ultrastructure characterization, of four species of scorpions in New Valley, Egypt namely (*Leiurus quinquestriatus*, *Androctonus amoreuxi*, *Orthochirus innesi* and *Buthacus leptochelys)*.

MATERIALS AND METHODS

Collecting:

Samples were collected randomly by professional hunters, during the period from April to July in 2017 and July to September 2019. A random search was done during the survey. Scorpions were found under rocks, gap of soil, leaf litter, under bark, and within vegetation.

Scanning Electron Microscope (SEM):

The specimens of scorpions were isolated and fixed in 3% glutaraldehyde buffered with 0.1 M sodium phosphate (pH 7.2) for two hours. The specimens were washed four times in sodium phosphate buffer (PBS) and postfixed in 1 % osmium tetroxide (OsO_4) in the same buffer for 2 hours at +4 ºC. After washing in PBS, the specimens were dehydrated in a graded ethanol series (40%–100% ethanol). The last stages of dehydration were performed with propylene oxide. The specimens were dried and coated with a thin layer of gold. The materials were examined at (JEOL TEM – T200) at the Electron Microscopy Unit (EMU) of Assiut University.

RESULTS

The current study revealed that scorpions have many variations between different species and between females and males in count pectinal teeth.

1-Leiurus quinquestriatus:

Chelicera:

It is a small, very sharp claw-like structure that protrudes from the mouth. It is provided with specialized setae. It has a movable finger provided with denticles. The movable finger rests in a groove of the cheliceral basal segment, which provided with protrusion like structures (coarse granules) (Fig.1a) (Table 1).

Pedipalp Chela:

It has elongated fingers with carinae or keel (granular ridges). It has sharp denticle margins with different setae (Fig.1b) (Table 1).

Carapace: Its dorsal side is provided with one pair of median eyes. It has regular mediolateral ocular carinae. The anterior surface of the carapace has a concave shape (Fig.1c) (Table 1).

The respiratory pore of the third segment of mesosoma (spiracles):

Oval and relatively large. The third segment of mesosoma is provided with setae. It has protrusion like structures distributed on the ventral side. Also, it has teeth on its edge (Fig.1d) (Table 1).

Genital Operculum:

In females, it consists of two plates. It is relatively wider in females than in males. Pectinal teeth in females ranged between 29:32. In male genital operculum consists of two plates. It is relatively narrow in males than in the female. Pectinal teeth in males ranged between 33:37. In both female and male pectins are provided with sensilla (sensory hair) (Fig.1e, f, g, and h) (Table 1).

Last Segment Of Metasoma:

Cylenderical in shape with dorsal coarse granuls. It has few numbers and short setae, rough edge provided with strong spinoid granules on the dorsal side (Fig.1i) (Table 1).

Telson: It has an internal terminal venom pore ending in a quite sharp tip provided with setae (Fig.1j and k) (Table 1).

2-*Androctonus amoreuxi*:

Chelicera:

It was explained previously in the *Leiurus quinquestriatus***.** It is a constellation array's arranged in longitudinal ridges (Fig. 2a) (Table 1).

Pedipalp Chela:

It was explained previously in the *Leiurus quinquestriatus* (Fig. 2b) (Table 1).

Carapace:

Its dorsal side is provided with a pair of median eyes. It has an irregular distribution of the different size of mediolateral ocular carinae. The anterior surface of the carapace has a concave shape (Fig. 2c) (Table 1).

The respiratory pore of the third segment of mesosoma (spiracles):

Oval in shape. And the third segment of mesosoma provided with setae and a smooth edge on the ventral side (Fig. 2d) (Table 1).

Genital Operculum:

In females, it consists of two plates. It is relatively wider than in males. Pectinal teeth ranged between 23:25. Pectinal teeth in males ranged between 32:35. Both female and male pectins are provided with sensilla (sensory hair) (Fig. 2e and f) (Table 1).

Last segment of metasoma:

Cylindrical in shape with dorsal coarse granules. It has relatively long setae and a rough edge provided with strong spinoid granules on the dorsal side (Fig. 2g) (Table 1).

Telson: It has a lateral venom pore and rounded tip of telson provided with setae (Fig. 2h and i) (Table 1).

3- *Orthochirus innesi*:

Chelicera:

It was explained previously in the *Leiurus quinquestriatus* (Fig. 3a) (Table 1).

Pedipalp Chela:

It was explained previously in the *Leiurus quinquestriatus* (Fig. 3b) (Table 1).

Carapace:

The dorsal side is provided with one pair of median eyes and bears the different size of mediolateral ocular carinae. Whereas the anterior surface of the carapace has a concave shape (Fig. 3c) (Table 1).

The respiratory pore of the third segment of mesosoma (spiracles):

Oval in shape. The third segment of mesosoma is provided with different sizes of setae and with a rough edge on the ventral side (Fig. 3d) (Table 1).

Genital operculum:

In females, it consists of two plates that are relatively wider than in the male. Pectinal teeth ranged between 14 :16 in the female. In the male, it ranged between 17:18. Both female and male pectins are provided with sensilla (sensory hair) (Fig. 3 e and f) (Table 1).

Last segment of metasoma:

It is cylenderical in shape with dorsal coarse granules. It has relatively long setae and a rough edge provided with strong spinoid granules on the dorsal side (Fig. 3g) (Table 1).

Telson: It has a small terminal venom pore. It is provided with setae (Fig. 3h and i) (Table 1).

4- *Buthacus leptochelys*:

Chelicera:

It was explained previously in the *Leiurus quinquestriatus* (Fig. 4a) (Table 1).

Pedipalp Chela:

It was explained previously in the *Leiurus quinquestriatus* (Fig. 4b) (Table 1).

Carapace:

Its dorsal side is provided with one pair of median eyes and separated by ocular diameters. It has a different size of mediolateral ocular carinae. The anterior surface of the carapace has a concave shape (Fig. 4c) (Table 1).

The respiratory pore of the third segment of mesosoma:

Oval in shape. The third segment of mesosoma has a smooth edge and is provided with relatively short setae (Fig. 4d) (Table 1).

Genital operculum:

In female, it consists of two plates. It is relatively wider in female than in male. Pectinal teeth in females ranged between 22:26. In males ranged between 27:31. Both female and male pectines are provided with sensilla (sensory hair) (Fig. 4e and f) (Table 1).

The last segment of metasoma:

It has dorsal coarse granules with a smooth edge. It has relatively very long setae

(Fig. 4g) (Table 1).

Telson: It has two subterminal venom pores with a narrow end in a quite sharp tip. It is provided with setae (Fig. 4h and i) (Table 1).

Table 1: Description of some organs of the four species of scorpions *Leiurus quinquestriatus, Androctonus amoreuxi, Orthochirus innesi* and *Buthacus leptochelys*.

Measurement	*Leiurus quinquestriatus*	*Androctonus amoreuxi*	*Orthochirus innesi*	*Buthacus leptochelys*
Chelicera	1- Movable finger with denticles. 2- Protrusion (coarse granules). 3- Specializes setae	1- Movable finger with denticles. 2- Protrusion (coarse granules). 3- Specializes setae	1- Movable finger with denticles. 2- Protrusion (coarse granules) 3- Specializes setae	1- Movable finger with denticles. 2- Protrusion (coarse granules). 3- Specializes setae
Pedipalp chela	1- Cylindrical, with elongated finger. 2- Dentate margins. 3- Granular ridge. 4- Setae.	1- Cylindrical, with elongated fingers. 2- Dentate margins. 3- Granular ridge. 4- Setae.	1- Cylindrical, with elongated fingers. 2- Dentate margins. 3- Granular ridge. 4- Setae.	1- Cylindrical, with elongated fingers. 2-Dentate margins. 3- Granular ridge. 4- Setae.
Carapace	1- Ocular granules. 2- Anterior carapace with concave in shape. 2- One pair of median eyes.	1- Ocular granules. 2- Anterior carapace with concave in shape. 3- One pair of median eyes.	1- Ocular granules. 2- Anterior carapace with concave in shape. 3- One pair of median eyes.	1- Ocular granules. 2- Anterior carapace with concave in shape. 3- One pair of median eyes.
Respiratory pore of third segment of mesosoma	1- Oval respiratory pore. 2- With protrusion. 3- Rough edge. 4- Provided with setae.	1- Oval respiratory pore. 2- Without protrusion 3- Smooth edge. 4- Provided with Setae.	1- Oval respiratory pore. 2 Without protrusion. 3- Rough edge 4- Provided with setae.	1- Oval respiratory pore. 2- Without protrusion. 3- Smooth edge 4- Provided with setae.
Genital operculum	1- Pectinal teeth in female ranged between 29:32. 2- Pectinal teeth in male ranged between 33:37. 3- Sensilla (sensory hair)	1- Pectinal teeth in female ranged between 23:25. 2- Pectinal teeth in male ranged between 32:35. 3- Sensilla (sensory hair).	1- Pectinal teeth in female ranged between 14:16. 2- Pectinal teeth in males ranged between 17:18. 3- Sensilla (sensory hair).	1- Pectinal teeth in female ranged between 22:26. 2- Pectinal teeth in males ranged between 27:31. 3- Sensilla (sensory hair).
Last segment of metasoma	1- Cylenderical in shape. 2- Coarse granules. 3- Spinoid granules. 4- Setae.	1- Cylenderical in shape. 2- Coarse granules. 3- Spinoid granules. 4- Setae.	1- Cylenderical in shape. 2- Coarse granules. 3- Spinoid granules. 4- Setae.	1- Cylenderical in shape. 2- Coarse granules. 3- Without spinoid granules 4- Setae.
Telson	1- Terminal venom pore. 2- Quite sharp tip. 3- Setae.	1- Lateral venom pore. 2- Rounded tip. 3- Setae	1- Terminal venom pore. 2- Narrow ending. 3- Setae	1-Two sub terminal venom pores. 2- Quite sharp tip. 3- Setae.

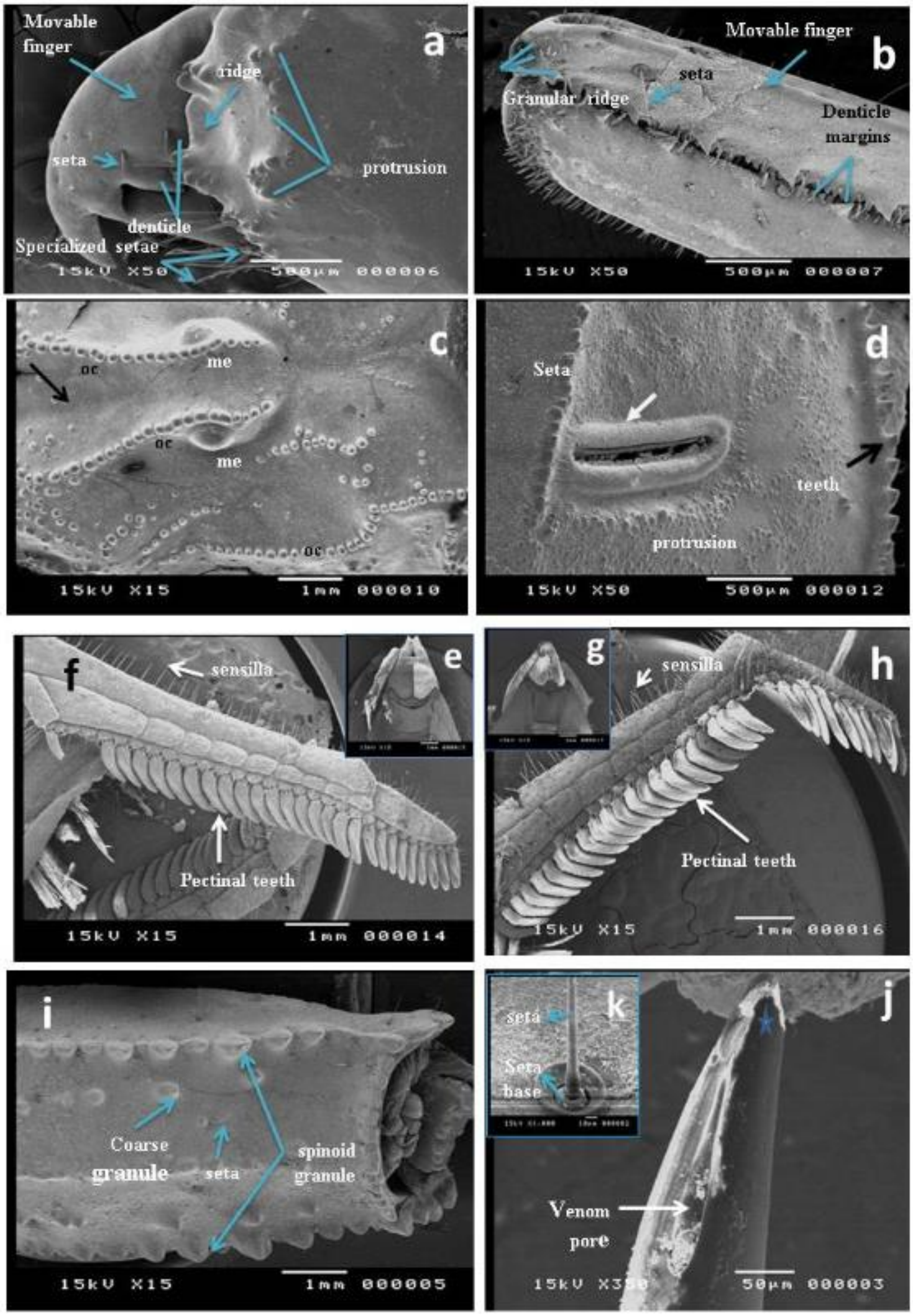

Fig. 1: Scanning electron microscope of *Leiurus quinquestriatus*, showing
(a): chelicerae with (movable finger), (denticles), (ridge), (Specialized setae) and protrusion (coarse granules). **(b):** Pedipalp chela with (denticle margins), (granular ridge), (setae) and (movable finger). **(c):** Dorsal side of carapace with ocular carinae (oc), median eyes (me) and concave shape (arrow). **(d):** Oval respiratory pores of third segment of mesosoma (whit arrow), (protrusion), (setae) and teeth (black arrow). **(e):** Female genital operculum, **(f):** Pectinal teeth and (sensilla). (**g**)**:** Male genital operculum**, (h):** Pectinal teeth (arrow) and (sensilla). **(i):** The last segment of metasoma with (coarse granules), (setae) and (spinoid granule), **(j):** Venom pore (arrow) with quite sharp tip (Star). **k:** Seta and seta base.

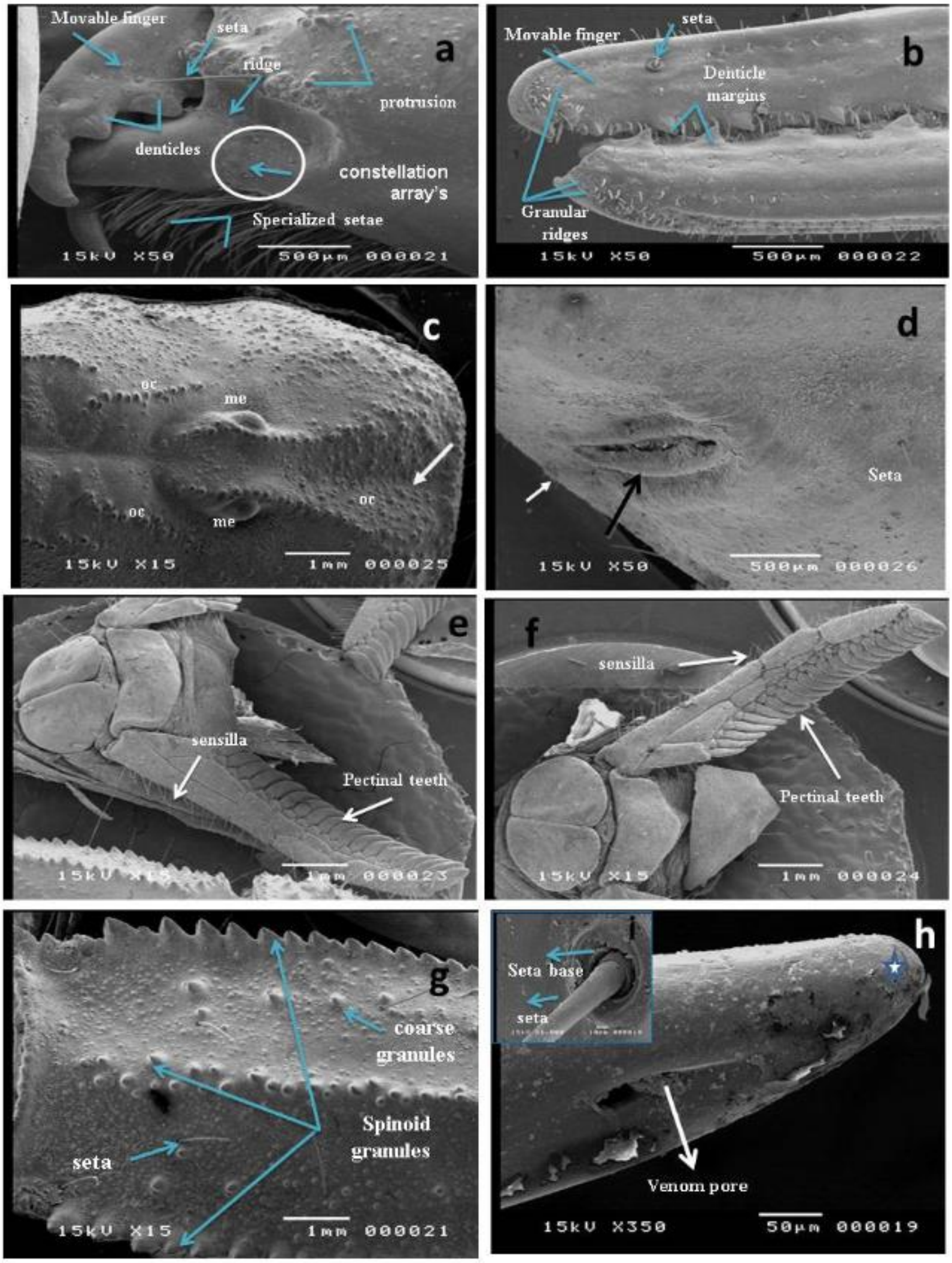

Fig. 2: Scanning electron microscope of *Androctonus amoreuxi*, showing
(a): Chelicerae with (constellation array's), (movable finger), (Specialized setae), (ridge), protrusion (coarse granules) and (denticles). **(b):** Pedipalp chela with (denticle margins), (movable fingers) (granular ridges) and (setae). **(c):** Dorsal side of carapace with median eyes (me), ocular carinae (oc) and concave shape (arrow). **(d):** Oval respiratory pore of third segment of mesosoma (black arrow), (setae) and smooth edge (white arrow). **(e):** Genital operculum in female showing pectinal teeth and (sensilla). **(f):** Genital operculum in male showing pectinal teeth and (sensilla). **(g):** The last segment of metasoma with (coarse granule), (setae) and (spinoid granules)**. h:** Venom pore (arrow), narrower rounded tip of telson (stare). **i:** Seta and seta base.

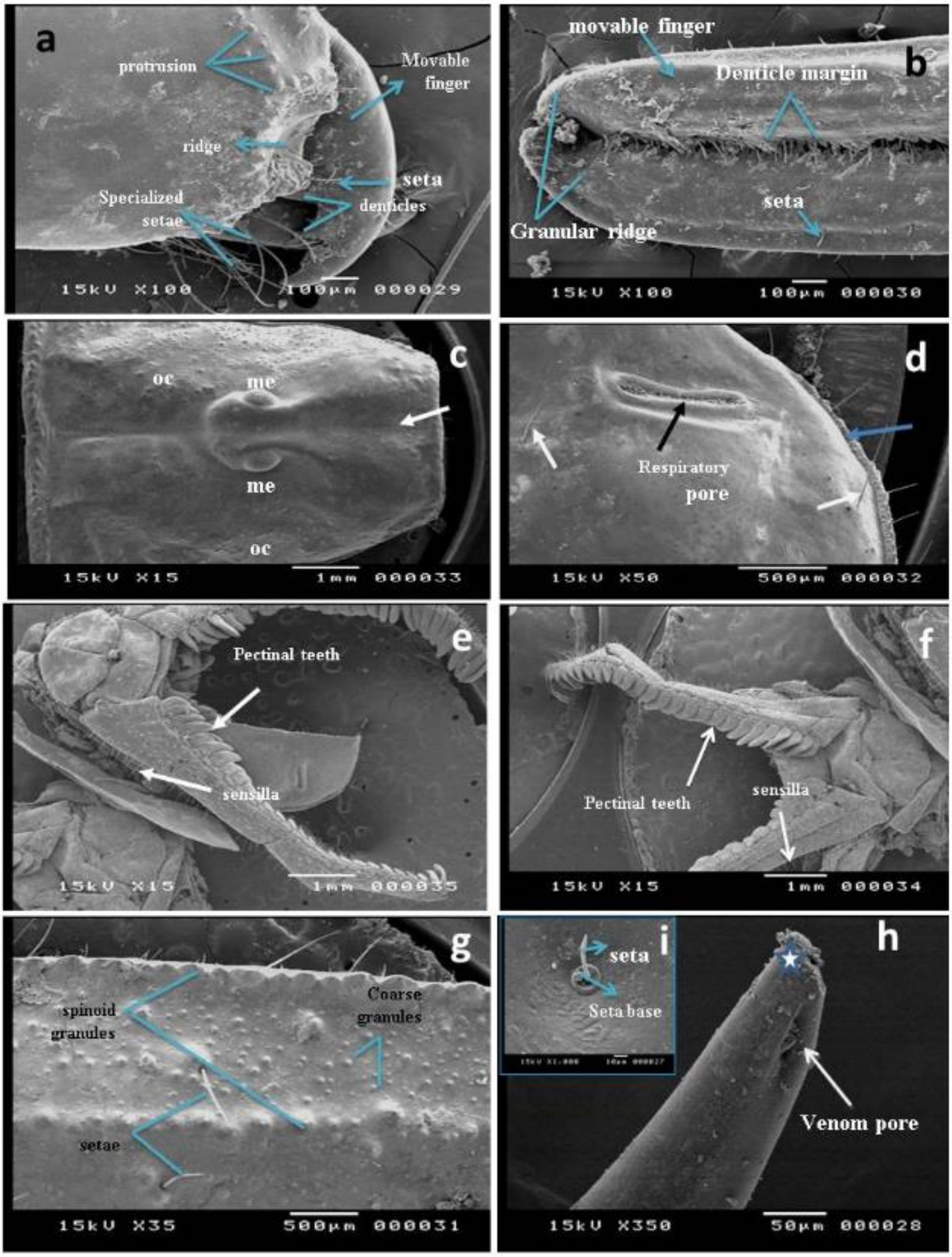

Fig. 3: Scanning electron microscope of *Orthochirus innesi* showing
(a): Chelicerae with (movable finger), (denticles), (specialized setae), (ridge) and protrusion (coarse granules). **(b):** Pedipalp chela with (movable fingers), (setae), (denticle margin) and (granular ridge). **(c):** Dorsal side of carapace with one pair of median eyes (me), ocular carinea (oc) and concave shape (arrow). **(d):** Oval respiratory pore of third segment of mesosoma (black arrow), seta (white arrows) and rough edge (blue arrow). (**e**)**:** Female genital operculum showing pectinal teeth and (sensilla). **(f)**: Male genital operculum showing pectinal teeth and (sensilla). **(g):** The last segment of metasoma with (coarse granule), (setae) and (spinoid granules). **h:** venom pore (arrow) with narrow telson ending (stare). **i:** Setae and setae base.

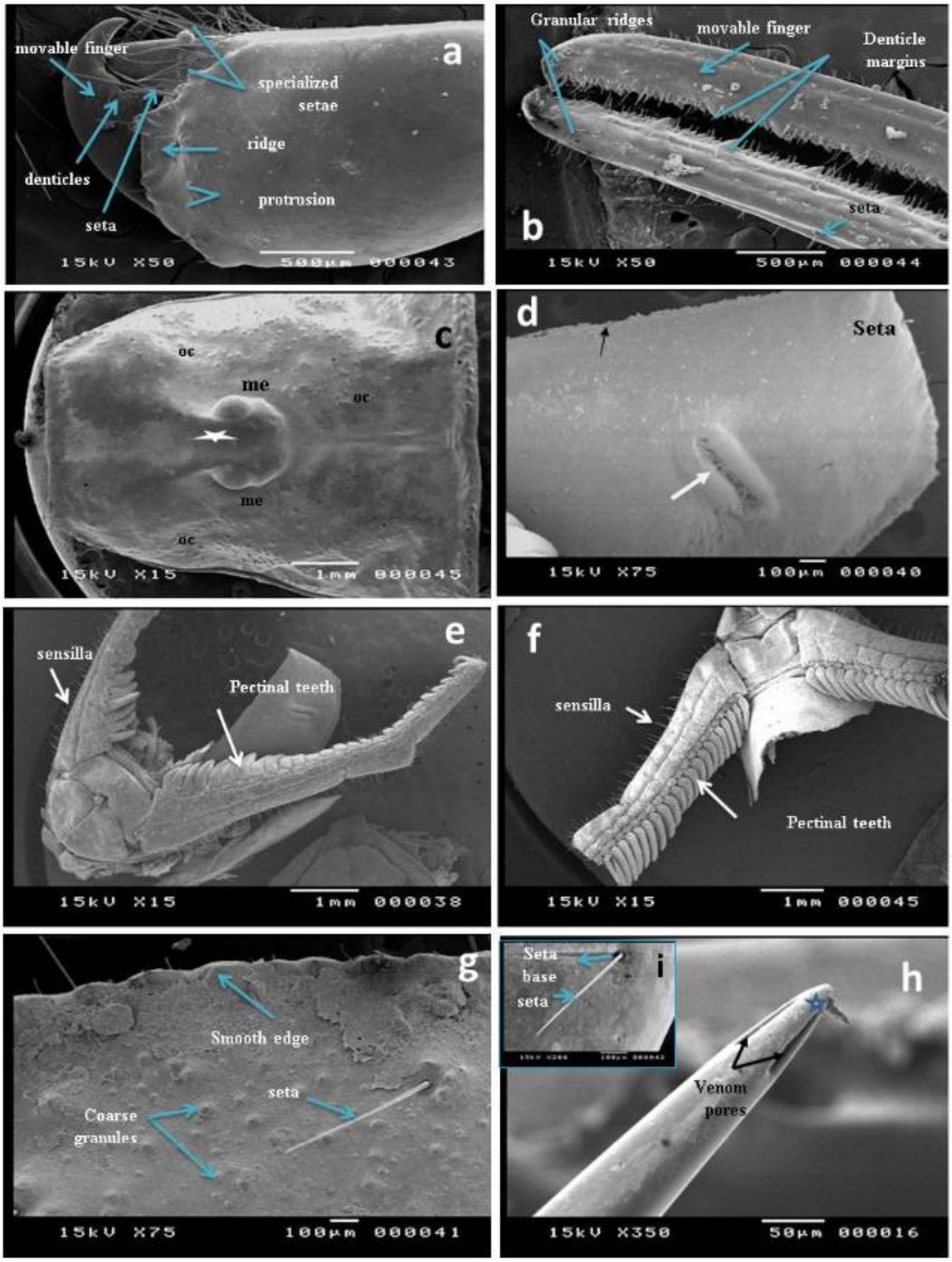

Fig. 4: Scanning electron microscope in *Buthacus leptochelys* showing
(a): Chelicerae with (denticles), (movable finger), (ridge), protrusion (coarse granules) and (specialized setae). **(b):** Pedipalp chela with (denticles margins), (setae), (granular ridges) and (movable finger). **(c):** Dorsal side of carapace with median eyes (me), ocular diameters (stare) and ocular carinea (oc). **(d):** Oval respiratory pores of third segment of mesosoma (white arrow), (setea) and smooth edge (black arrow). **(e):** Female genital operculum showing pectinal teeth and (sensilla). **(f):** Male genital operculum showing pectinal teeth and (sensilla). **(g):** The last segment of metasoma with (coarse granule), (smooth edge) and (setae). **h:** Two venom pores, narrow end (stare), **i:** Seta and seta base.

DISCUSSION

Scorpions have many problems of public health all over the world, especially in north-Saharan Africa. Approximately 1500 species of scorpions are described. Only about 25 species worldwide are considered dangerous to humans Al *et al*. (2009).

In the present study, sensory organs or chemosensory such as (sensilla and setae) which scattered on the surface of (Telson, chelicera, claw and the last segment of metasoma) were observed in the four studied species of scorpions. This result is supported by Gaffin and Brownell (2001) who indicated that sensory organs are common in all arachnids, and in scorpions, chemosensory setae are scattered all over the animal's body. Root (1990) indicated that some setae are chemoreceptors but the majorities are mechanoreceptors. In scorpions, short and curved chemosensory setae are scattered all over the body (Fet *et al*, 2003).
Scorpions telson is used in defence (Van der Meijden *et al*., 2013; Carlson, McGinley and Rowe, 2014).

In the current study, the tip of the telson is quite sharp in (*Leiurus quinquestriatus* and *Buthacus leptochelys*), rounded in (*Androctonus amoreuxi*) and narrow in (*Orthochirus innesi*). Furthermore, the venom is carried by one pore in (*Leiurus quinquestriatus, Androctonus amoreuxi* and *Orthochirus innesi*) or two venom pores in (*Buthacus leptochelys*) where the two venom pores are situated on each side of the tip of telson. The sting or aculeus, which is a part of the scorpion venom apparatus, is situated on the final segment of the metasoma which is called the telson. This result is in accordance with Brownell and Polis (2001) who showed that the sting, or aculeus, is a part of the scorpion venom apparatus, which is situated on the last segment of the metasoma, which bears types of sensory setae on its surface.

In the present study, the structure of the constellation array appeared in chelicerae of scorpion *Androctonus amoreuxi* only. This structure was observed and described in many scorpion species by Fet *et al*. (2006). It is well known that the chelicerae are composed of three basal segments; the first is called coxa, the second and third segments are called tibia (fixed finger) and the tarsus (movable finger), respectively. The fixed and movable fingers are provided with various denticles used to break down the harder tissues of prey items (Vachon, 1963), which has taxonomic importance (San Martín and Cekalovic 1972). Vyas (1974) mentioned 18 muscles in the moving finger of a scorpion chelicera.

The present study illustrated that the structure of chela of pedipalp is formed from a movable finger and fixed finger which is provided with dentate margins, and granular ridge. Danielle (2013) mentioned that scorpion's chelae are one of the scorpion's peripheral appendages. These structures are responsible for holding prey during predation and catching. In the present study, it was observed that the granular ridge is found on the chela of pedipalp of studied scorpions. The fixed and movable fingers of the pedipalp's chelae are set along their cutting edge with granules that are found in various patterns (Stahnke, 1970). Scorpion has darkened or granular linear ridges, called "keels" or carinae on the pedipalp segments, which are important taxonomically (Polis, 1990).

Seed and Hughes (1997) indicated that chela has a fixed finger and movable finger in predatory crabs which feed on mollusks and other hard and slow-moving prey. They added that scorpions feed on soft-bodied prey such as spiders and insects, the chela shape in scorpions, as it is in many crabs.

The present study showed that chela of pedipalp is elongated and cylindrical. Whereas crabs possess a strong and large chela for crushing prey (Hughes, 2000). Scorpionids may use one chela as a shield while using the other to pinch the attacker (Newlands, 1969).

In the present study, the number of pectinal teeth differs in males and females in different species where the number of pectinal teeth in male exceeds that of the female.

Furthermore, different sensory hairs were observed on the pectines of different species. Farley (2001) indicated that chemosensory hairs can be found on the ventral surface of pectines. Karatas (2003) used pectinal tooth count to discriminate between sexes and to identify species.

The present study showed that the pores of spiracles are oval and provided with granules and with a rough or smooth edge of mesosoma. The spiracle openings of scorpions are known as book lungs. The spiracle openings may be slits, circular, or oval according to the species of scorpion (Polis, 1990). The spiracle openings allow the entry of air into the chamber (Vyas and Laliwala 1972).

In the present study, the dorsal carapace showed a distribution of granules surrounding the margin of the carapace and contained one pair of median eyes. Schliwa and Fleissner (1980) indicated that the median eyes have lenses and a daily fluctuation of light intensity sensor and may be capable of image formation The single pair of median eyes are situated on either side of the carapace and three lateral eyes are found on one side and two or four on the other side (Savory, 1977). The median eyes of *Euscorpius carpathicus* are composed of a lens and a vitreous body (Bedini 1967). Schliwa and Fleissner (1980) cleared up the importance and biological role of the lateral eyes which differ from those of the median eyes. The lateral eyes are highly sensitive and can detect differences in brightness even at very low light intensities, median eyes provided with relatively high acuity and good spatial discrimination.

Conclusion:

The present study recorded the difference between four species of scorpions by using a scanning electron microscope (SEM) which included venom pore, pectinal teeth count between male and female, respiratory pore of the third segment of mesosoma and carapace.

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