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Surveillance study on scorpion species in Egypt and comparison of their crude venom protein profiles

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Venom;
Protein profiles

Abstract The present study aimed to focus on all the famous species of scorpions from different regions in Egypt and the attempts to use protein profiles of their venom as a simple tool for taxonomy rather than traditional morphological methods. For this purpose, total protein concentration and protein profiles using SDS–PAGE were measured and the similarity coefficients of the protein bands of different species were calculated. The present results showed that there is one species (*Scorpio maruas palmatus*) which belongs to the family Scorpionedae, and seven species which belong to the family Buthidae. Four of them fall into the genus Androctonus namely: *Androctonus crassicauda*, which is very rare in Egypt, *Androctonus australis*, *Androctonus bicolor*, and *Androctonus amoreuxi*. In the electrophoretic analysis the protein bands ranged between14 and 200 KDa. A notable find was that all scorpion venom samples examined contained two protein bands with MW of 200 and 95 KDa, except in one species. One protein band (125 KDa) is common in six species only. Based on this electrophoretic pattern the similarity indices indicate that there is inter-family, inter-genus, and inter-species variation between different scorpion samples. The closest species were *A. amoreuxi* and *A. australis*. This study proposes variation in venom protein composition which was measured qualitatively and quantitatively among different scorpion species collected from different regions in Egypt, which throws light on its importance and enables the researchers to consider it a guideline in taxonomy.

Introduction

The three most 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, 2004a). Scorpions are terrestrial arachnids that are easily recognized by their characteristic elongated body and segmented tail ending in a bulbous sac and a stinger (telson). There are approximately 1500 scorpion species worldwide (Bucherl, 1971; Ozkan and Filazi, 2004; Ozkan and Karaer, 2004b). Scorpion venom, which has lethal and paralytic effects,

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is a secretion composed of water, salts and simple, low-molecular-weight proteins (Calderon-Aranda et al., 1993; Ozkan et al., 2006a,b,c). It is a unique defense and feeding weapon. Scorpions also employ this valuable tool in several sophisticated ways: for subduing prey, deterring predators, probably during mating, and for protection against humans (Balozet, 1971; Ismail et al., 1994; Inceoglu et al., 2006). Scorpion envenomation remains a major health problem in many tropical and subtropical countries (Goyffen et al., 1982; Meddeb-Mouelhif et al., 2003; Theakston et al., 2003; Ozkan et al., 2006a,b,c).

Previous literature on scorpion taxonomy recognizes 16 extant scorpion families (some including extinct genera or species): Bothriuridae, Buthidae, Chactidae, Chaerilidae, Diplocentridae, Euscorpiidae, Hemiscorpiidae, Heteroscorpionidae, Luridae, Liochelidae, Microcharmidae, Pseudochactidae, Scorpionidae, Superstitioniidae, Troglotayosicidae, and Urodacidae (ICZN, 2001; Fet et al., 2000; Lourenco, 1998, 2000; Prendini, 2000, 2001, 2003a,b; Soleglad and Fet, 2003). All scorpions hazardous to humans belong to the Buthidae family (Goyffen et al., 1982).

Scorpion venoms are complex mixtures of components, where peptides and proteins play a fundamental role. Most scorpion venom peptides are composed of 20–75 amino acid residues, whereas the few known proteins (enzymes) contain 120–370 residues (Zlotkin et al., 1978; El-Henawy, 1992; Possani and Rodríguez de la Vega, 2006). There are 100 peptide components obtained by analysis of proteins in one scorpion venom (Batista et al., 2004; Diego-Garcia et al., 2005). Fet et al. (2000) revealed that, there are more than 1500 different species of scorpions known in the world, and 50 species have been characterized either by proteins or peptides (Rodríguez de la Vega and Possani, 2004, 2005). Possani and Rodríguez de la Vega (2006) estimated fewer than 350 components of scorpion venom peptides.

Pimenta et al. (2003) was the first using the concept of the 'venom', and the taxonomic protein profile which was found in the venom gland. Proteomic analysis of venom components can produce a valuable fingerprint that can be used as a useful reference tool in taxonomy and as a complementary method to morphology and behavioral characterization for species identification and classification of related specimens (Newton et al., 2007). Only a few attempts have been carried out on the abundant scorpions of Egypt (Fet, 1997; Fet et al., 2000; Fet and Soleglad, 2005). The latter authors actively involved in systematics, faunistics, taxonomy of scorpions, and then they published the Catalog of the Scorpions of the World (1758–1998) (2000), listing about 1300 species and 170 genera of the existing scorpions, plus about 100 fossil species.

The most previous studies were brief and focused on morphology and phylogeny of few types of scorpion species in Egypt neglecting variation in protein components of venom in each species. Therefore, the present study was focusing on the biodiversity of scorpion species in Egypt and applying an additional technique of protein profile for taxonomy.

Materials and methods

Sites of collection

Scorpions were collected by professional hunters during the period from April to October in 2012. 250 scorpions were collected from each geographical wild infested area in Egypt (Aswan, Sinai, Baltim, Borg El-Arab, Marsa-Matrouh) as shown in Fig. 1, then placed in glass containers according to the geographical areas. The scorpions were anesthetized and classified according to Scorpion Files (2003) following Fet and Soleglad (2005).

Venom samples

Twenty telsons from each collected species of scorpions were used. Venom solution was prepared using the maceration method in which telsons were removed from anesthetized scorpions at the point of their articulation with the last caudal segment as previously described by Ozkan et al. (2006a,b,c) and Ozkan and Filazi (2004). All telsons were weighed. Then, they were ground to a fine powder which was dissolved in physiologic saline solution (PSS; 0.9% w/v NaCl) and kept at 4 °C for 72 h. The venom solution was centrifuged at 10000*g* for 10 min at 4 °C. Supernatant was removed and immediately lyophilized and stored at −20 °C until use.

Protein assay

Protein content of the venom samples was determined by reading absorbance at 280 nm and expressed as mg protein/ml (Layne, 1957; Stoscheck, 1990).

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE)

SDS–PAGE (12% gel) analyses of both venom samples and their protein bands were carried out according to Laemmeli (1970). Proteins were stained with 0.1% Coomassie Blue R-250 Silver. Molecular mass standard (Sigma, S8445) was run in parallel in order to calculate the molecular weights of proteins. Then, gel was photographed and the molecular weights were calculated using Molecular Imaging Software (MIS, Kodak). A similarity matrix was constructed on the basis of the presence/absence of bands from Dice's similarity coefficient (Dice, 1945) using the formula:

$$S = 2a/2a + b + c$$

where a = number of bands shared between samples 1 and 2, b = the number of bands present in 1 but not in 2 and c = number of bands present in 2 but not in 1.

Results

Surveillance studies

During the present study eight species of scorpions were collected from different regions from the period from April to October and placed in glass containers to be examined for taxonomical identifications compared to general morphology of the scorpion as shown in Fig. 2. Scorpions were collected from five localities in Egypt (Aswan, Sinai, Baltim, Borg El-Arab, and Marsa-Matrouh) as indicated in a map (Fig. 1). The recorded species encountered were: *Androctonus bicolor*, *Androctonus australis*, *Androctonus amoreuxi*, *Androctonus crassicauda*, *Leiurus quinquestriatus*, *Buthacus arenicola*, *Orthochirus innesi*, and *Scorpio maurus palmatus*.

The collected scorpions were identified and classified into two families (Buthidae and Scorpionidae). The first seven

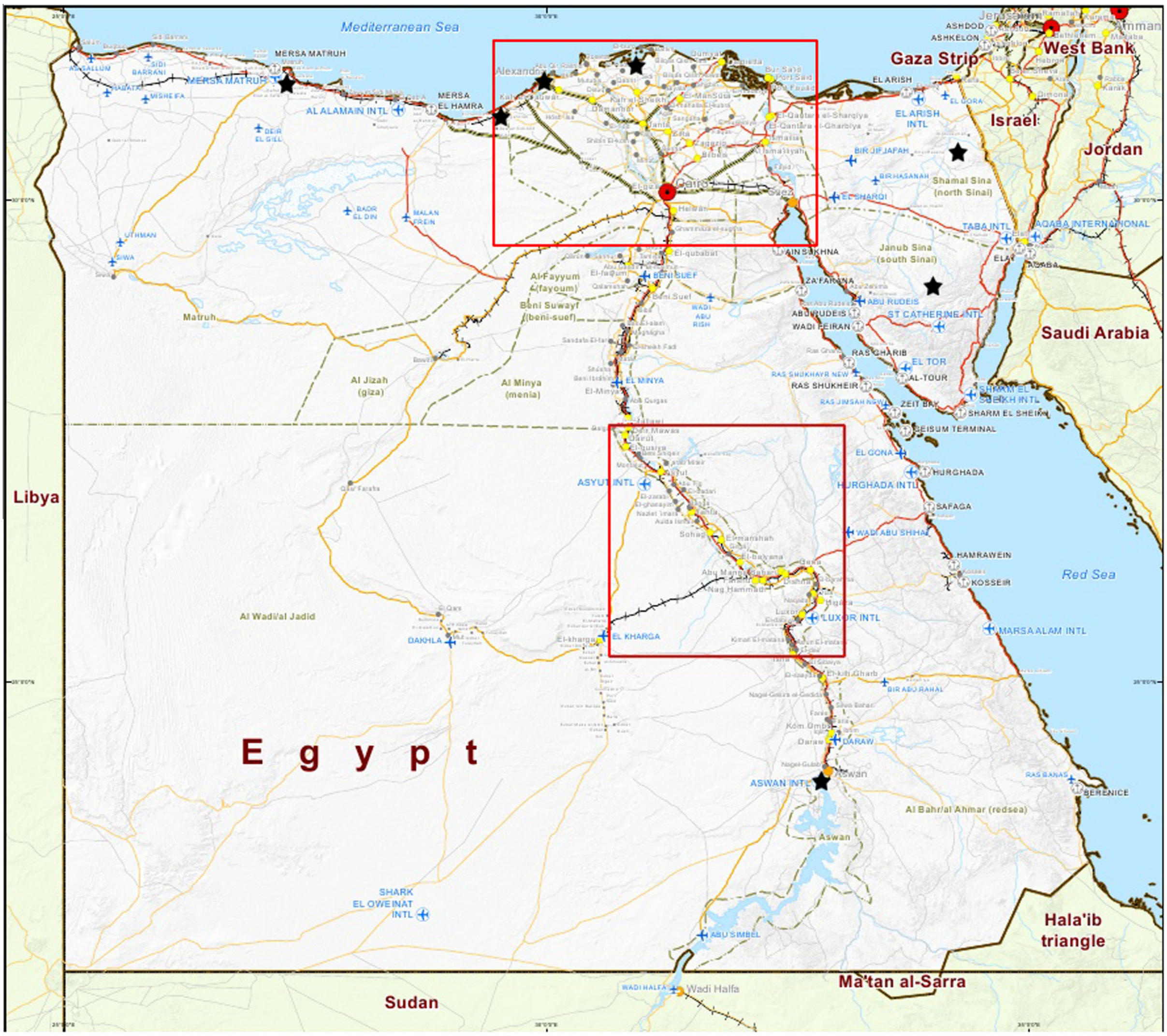

Figure 1 Map of Egypt showing the localities of collection indicated by star.

species belong to family Buthidae; four of them belong to genus *Androctonus*, one to genus *Leiurus* and the other belongs to *Buthacus*, while the last one belongs to family Scorpionidae. Number, collection area, body length and color of each collected species are displayed in Table 1.

Species description

S. marus palmatus

In Egypt, one species, *S. marus palmatus* (Fig. 3), described by Ehrenberg (1828) belongs to family Scorpionidae which was established by Latreille (1802). This family belongs to the largest scorpions in the world.

Description: The name *scorpio* means scorpion and the specific name *maurus* means dark or obscure. Its common name is large clawed or Israeli gold scorpion (Fig. 3A). It has a yellow color, a pentagonal sternum, very powerful, broad pedipalps and enlarged chela. The length of the adult ranges between 6 and 8 cm. It has a yellow vesicle (Fig. 3B) and dark curved aculeus.

Distribution: In the present study, this species was collected from Borg-El-Arab, and also occurred in Southern Sinai and the Western Mediterranean Coastal Desert.

There are seven species belonging to the family Buthidae which was established by Koch (1837). Buthidae is the largest family of scorpions, its members are known as thick-tailed scorpions and bark scorpions. Four species belong to genus *Androctonus* (Ehrenberg, 1828) with a common name of fattail (fat-tailed) scorpion (Figs.4–7).

A. crassicauda

Described by Olivier (1807). It is a dangerous and rare scorpion (Fig. 4). Its common name is the Arabian fat-tailed scorpion.

Description: Its general body color is black with length ranging between 9 and 9.5 cm (Fig. 4A). Color is generally reddish brown to black in the dorsal region and yellowish brown at the opithosomal ventral surface, legs end with yellow ochre color marks. Slender pedipalp with bulbous chela occurs. Metasomal segments are slightly widened backward, metasomal segments I–IV with carinae are strongly developed. Vesicles have 3 series of granules, aculeus moderately curved as long as the vesicle (Fig. 4B).

Distribution: This species was recorded in the East of Sinai.

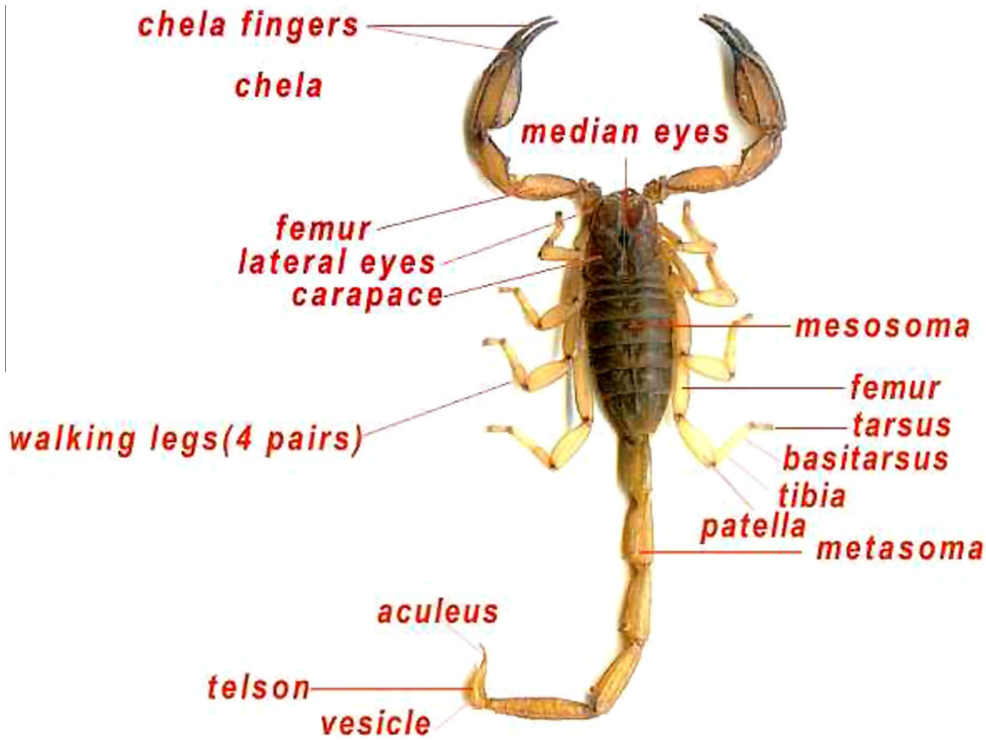

Figure 2 General morphology of scorpion.

Table 1 The parameter observed in different species of scorpions collected from different regions in Egypt.

No.	Scorpion	Collection area	Family	Color	Length (cm)
1	*Androctonus bicolor* (Ehrenberg, 1828)	Marsa-Matrouh	Buthidae	Black	8–8.5
2	*Androctonus australis* (Linnaeus,1758)	Borg-El-Arab	Buthidae	Yellow	9.5–10
3	*Scorpio maurus palmatus* (Ehrenberg,1828)	Borg-El-Arab	Scorpionidae	Yellow	6–8
4	*Androctonus amoreuxi* (Audouin,1826)	Baltim	Buthidae	Faint yellow	8.5–10
5	*Leiurus quinquestriatus* (Ehrenberg,1828)	Aswan	Buthidae	Orangish yellow	9–9.5
6	*Buthacus arenicola* (Simon,1885)	Sinai	Buthidae	Yellow	5–6.5
7	*Androctonus crassicauda* (Olivier,1807)	East of Sinai	Buthidae	Black	9–9.5
8	*Orthochirus innesi* (Simon, 1910)	Middle Sinai	Buthidae	Black	3–3.5

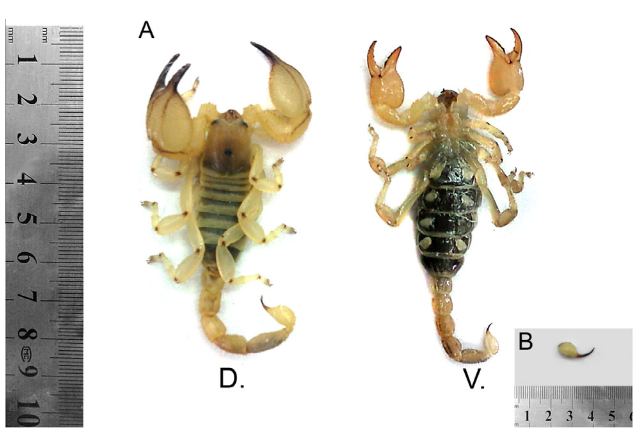

Figure 3 Dorsal and ventral view of *Scorpio maurus palmatus* (A), and enlarged vesicle with aculeus (B).

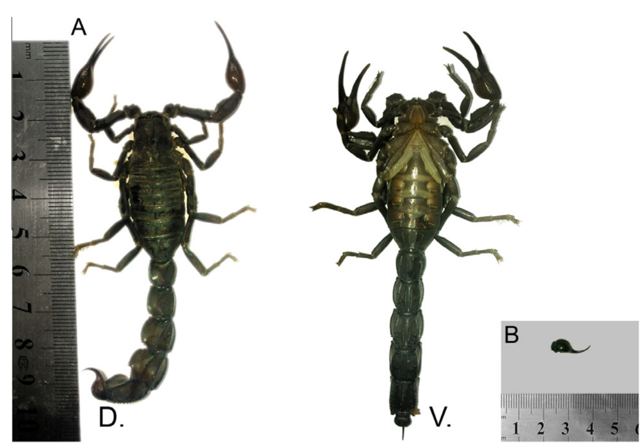

Figure 4 Dorsal and ventral view of *Androctonus crassicauda* (A), and enlarged vesicle with aculeus (B).

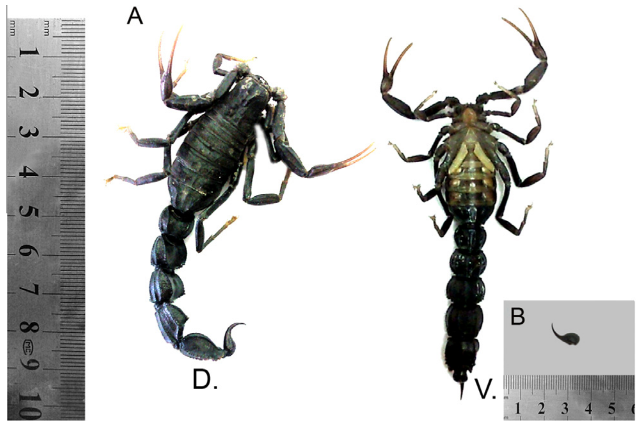

Figure 5 Dorsal and ventral view of *Androctonus bicolor* (A), and enlarged vesicle with aculeus (B).

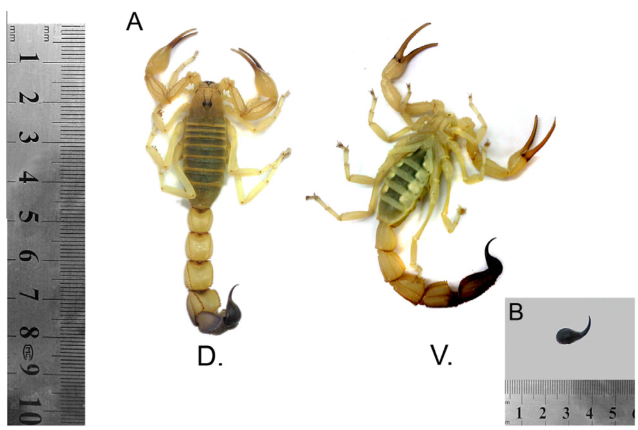

Figure 6 Dorsal and ventral view of *Androctonus australis* (A), and enlarged vesicle with aculeus (B).

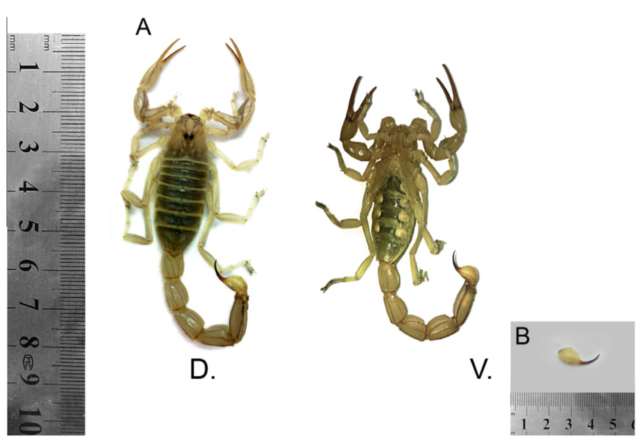

Figure 7 Dorsal and ventral view of *Androctonus amoreuxi* (A), and enlarged vesicle with aculeus (B).

A. bicolor

Described by Koch (1839), its common name is the black fat tailed or man killer scorpion (Fig. 5).

Description: This species has a black colored body with length ranging between 8 and 8.5 cm. This is often confused with *A. crassicauda* (black variety) but its pedipalp is comparatively slender and the chela is thin and elongated, not bulbous, with yellowish brown fingers (Fig. 5A). Aculeus are moderately curved and as long as the vesicle (Fig. 5B).

Distribution: This species was collected from Marsa-Matrouh.

A. australis

Described by Linnaeus (1758), its common name is the Egyptian or yellow fattailed scorpion (Fig. 6).

Description: This species is large, the adult attaining 10 cm. Its color is pale yellow more or less ochre sometimes with a darker zone on the body (Fig. 6A). Metasomal segments I–IV are yellowish, with carinae ventrally brownish on segment V and the vesicle darker. Telson with aculeus is black in color. Pedipalps yellow with rounded bulbous chela have moderately long and dark fingers, legs are pale yellow. The Metasomal segments are strongly widened backward, metasomal segments I–IV with dorsal carinae strongly marked, with spiniform granules on the posterior side. Aculeus is lightly curved and as long as the vesicle (Fig. 6B).

Distribution: This species was collected from Borg-El Arab and also occurred in Marsa-Matrouh, and Sinai.

A. amoreuxi

Described by Audouin (1826), its common name is the yellow scorpion (Fig. 7).

Description: This species is also large and its length reaches 10 cm. Its color is generally yellow with dark prosomal carapace and tergites. Metasomal segments are yellowish and it has a constant width backward (Fig. 7A). The vesicle ochre occurs with yellowish aculeus at the base and is reddish and black at the end (Fig. 7B). The aculeus is a little longer than the vesicle.

Distribution: This species was collected from Baltim.

There are three other species that belong to family Buthidae (Figs. 8–10) and are described as follows.

L. quinquestriatus

Described by Ehrenberg (1828), its common name is the death stalker (Fig. 8).

Description: Its length ranges between 9 and 9.5 cm, coloration is orangish-yellow. Prosomal carapace and metasomal

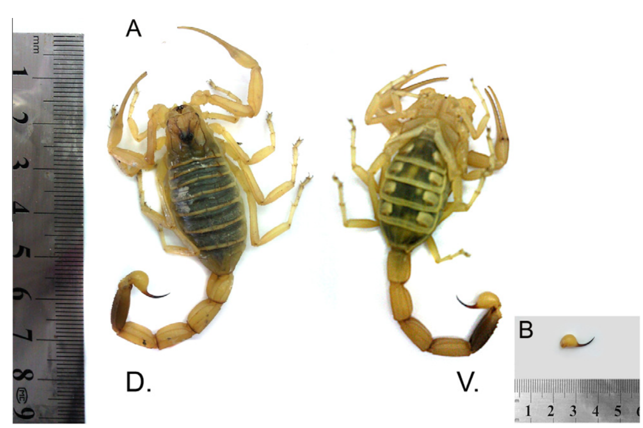

Figure 8 Dorsal and ventral view of *Leiurus quinquestriatus* (A), and enlarged vesicle with aculeus (B).

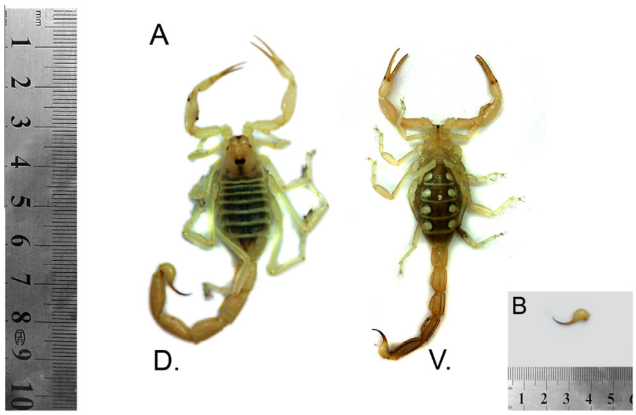

Figure 9 Dorsal and ventral view of *Buthacus arenicola* (A), and enlarged vesicle with aculeus (B).

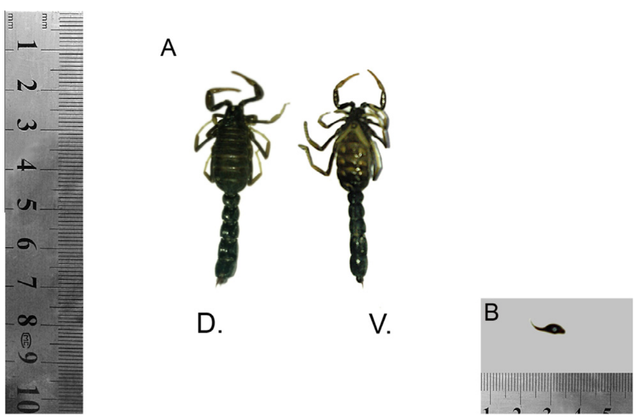

Figure 10 Dorsal and ventral view of *Orthochirus innesi* (A), and enlarged vesicle with aculeus (B).

segment V with brown color (Fig. 8A). It has elongated and gracile chela. Vesicle has yellow color with reddish and brown aculeus at the end (Fig. 8B).

Distribution: It is collected from Aswan.

B. arenicola

Described by Simon, 1885 (Fig. 9).

Description: Small to moderately sized scorpions 5–6.5 cm. It has yellow color with a very soft body, long walking legs and a slender metasoma; pedipalp ends with very gracile and elongated chelae (Fig. 9A). Cephalothorax is smooth or with very weak carinae. Telson is very smooth (Fig. 9B).

Distribution: Species was collected from Sinai.

O. innesi

Described by Simon (1910), its common name is the Egyptian pillar tailed scorpion or dwarf scorpion (Fig. 10), because it is a very small scorpion.

Description: Its length ranges between 3 and 3.5 cm (Fig. 10A). Its color is black on the dorsal side and yellowish brown on the ventral side. It has elongated prosoma. The end of legs and fingers of the chela have yellow color. Metasomal segments are without median carinae and it has a diagnostic feature when it is alive involving it being curved over the cephalothorax. Elongated and not bulbous vesicle observed (Fig. 10B).

Distribution: This species was collected from Middle Sinai.

Variations in the protein contents and profile of the scorpions' venom

The total protein content assay revealed different concentrations of protein between different scorpion species. The data obtained in Table 2, showed that total protein contents had the highest value (10.46, 9.18, 8.6 mg/ml) in *L. quinquestriatus*, *A. amoreuxi*, and *A. australis* respectively. Protein contents of *A. crassicauda* (8.01 mg/ml) showed a close ratio to those of *A. australis*. Lower protein contents were found in *A. bicolor* (7.4 mg/ml), *B. arenicola* (4.57 mg/ml) and in *S. marus palmatus* (4.37 mg/ml). On the other hand *O. innesi* showed the lowest value of all (1.7 mg/ml).

The electrophoretic analysis of different scorpion species was shown in Table 3 and Fig. 11. A total of eleven bands of protein which ranged between 14 and 200 KDa were noticed in the venom samples. Common bands found in the protein profile were 200, 125, 95 KDa. A protein band with a molecular weight of 200 KDa was represented in all samples except in *B. arenicola*. Also a 125 KDa protein band was found in all samples except in *A. crassicauda* and *O. innesi*, but with variable density which reached its high density in *A. bicolor* and *A. australis*. On the other hand, a protein band with a molecular mass of 95 KDa was absent only in *A. crassicauda* venom. In addition, four protein bands (188, 90, 16, 14 KDa) were specific to only one scorpion venom, *A. crassicauda*. Finally, three protein bands (60, 50, 40 KDa) were detected only in *A. crassicauda* and *O. innesi* venom samples and occurred in high intensities.

The similarity coefficient "*S*" was based on the number of protein profiles separated by SDS–PAGE (Table 4). The results obtained revealed that the highest similarity ($S = 0.9$) was obtained between *A. australis* and *A. amoreuxi*, followed by the similarity between *A. bicolor* and both *A. australis* and *L. quinquestriatus* (S = 0.83). *A. amoreuxi* showed relative similarities ($S = 0.7$) with *A. bicolor*, *L. quinquestriatus*, *S. marus palmatus*, and *B. arenicola*. Low similarity values were obtained between *A. crassicauda* and most of the species, which reach its lowest one ($S = 0.12$) with *A. amoreuxi*. Furthermore, there is no similarity ($S = 0$) between *A. crassicauda* and *B. arenicola*.

S. marus palmatus which represents the second family in this study (family: Scorpionidea) reached its highest similarity ($S = 0.74$) with *A. amoreuxi*. Moreover, it shows moderate similarity ($S = 0.66$) with *A. bicolor*, *A. australis*, *L. quinquestriatus*, and *B. arenicola*.

Table 2 Total protein contents of the different species of scorpion in Egypt.

Specimen	Total protein contents (mg/ml)
Androctonus bicolor	7.4 ± 0.1
Androctonus australis	8.6 ± 0.053
Scorpio marus palmatus	4.37 ± 0.04
Androctonus amoreuxi	9.18 ± 0.13
Leiurus quinquestriatus	10.64 ± 0.04
Buthacus arenicola	4.57 ± 0.04
Androctonus crassicauda	8.01 ± 0.02
Orthochirus innesi	1.7 ± 0.3

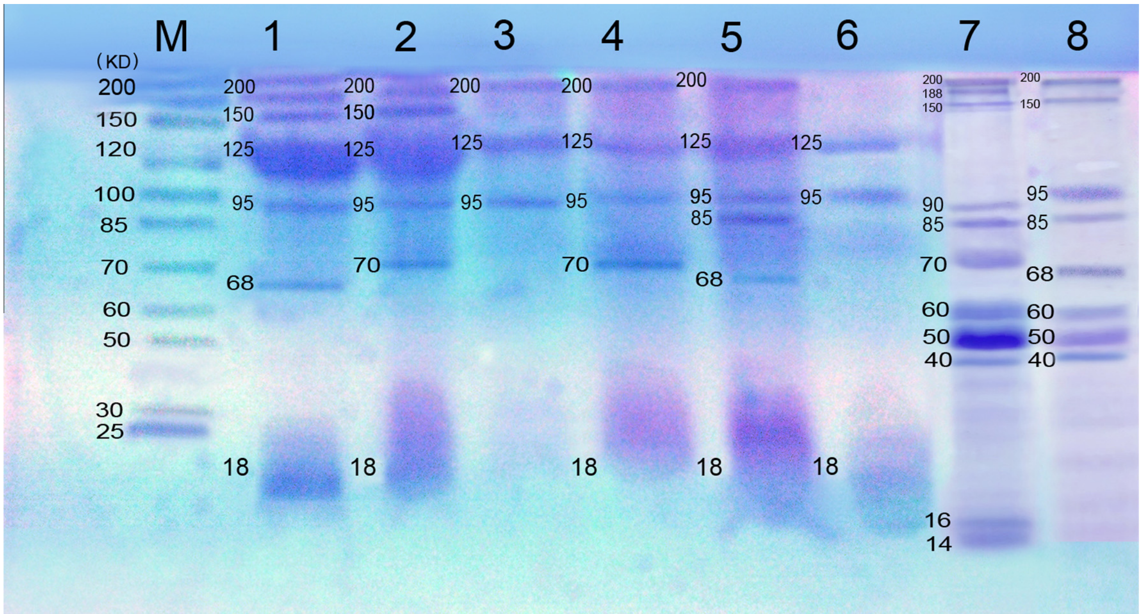

Figure 11 Protein profiles of crude venom of different scorpion species; M: protein marker, Lane 1: *Androctonus bicolor* venom, Lane 2: *A. australis* venom. Lane 3: *Scorpio maurus* palmatus venom. Lane 4: *A. amoreuxi* venom. Lane 5: *Leurius quinquestriatus* venom. Lane 6: *Buthacus arenicola* venom. Lane 7: *A. crassicauda* venom. Lane 8: *Orthochirus innesi* venom.

Table 3 Protein profile of scorpion's venom collected from different localities of Egypt using SDS–PAGE analysis.

Protein bands (KDa)	Lane 1 *A. bicolor*	Lane 2 *A. australis*	Lane 3 *S. maurus*	Lane 4 *A. amoreuxi*	Lane 5 *L. quinquestriatus*	Lane 6 *B. arenicola*	Lane 7 *A. crassicauda*	Lane 8 *O. innesi*
200	+	+	+	+	+		+	+
188							+	
150	+	+					+	+
125	+	+	+	+	+	+		
95	+	+	+	+	+	+		+
90							+	
85					+		+	+
70		+		+			+	
68	+				+			+
60							+	+
50							+	+
40							+	+
18	+	+		+	+	+		
16							+	
14							+	
Total no. of protein bands	6	6	3	5	6	3	11	8

A. bicolor: *Androctonus bicolor*
A. australis: *Androctonus australis*
S. maurus: *Scorpio maurus palmatus*
A. amoreuxi: *Androctonus amoreuxi*
L. quinquestriatus: *Leiurus quinquestriatus*
B. arenicola: *Buthacus arenicola*
A. crassicauda: *Androctonus crassicauda*
O. innesi: *Orthochirus innesi*

Discussion

Scorpions are arthropods their length ranges from 1.3 to 22 cm, and they are easily recognizable because of their morphological structure (Ozkan and Karaer, 2003; Ozkan and Filazi, 2004). Scorpions live mostly under stones in the daytime to protect themselves from high temperature during hot seasons.

The most recent taxonomic endeavors at the higher systematics (family level) have been adopted by the Scorpion Files (2003), that followed Fet and Soleglad (2005), and were applied in this study. In the present study specimens were

Table 4 Matrix of similarity index of different electrophoregrams of protein subunits of different scorpion species.

	A. bicolor	*A. australis*	*S. marus palmatus*	*A. amoreuxi*	*L. quinquestriatus*	*B. arenicola*	*A. crassicauda*	*O. innesi*
A. bicolor	1							
A. australis	0.83	1						
S. marus palmatus	0.66	0.66	1					
A. amoreuxi	0.72	0.9	0.74	1				
L. quinquestriatus	0.83	0.66	0.66	0.73	1			
B. arenicola	0.66	0.66	0.66	0.75	0.66	1		
A. crassicauda	0.26	0.34	0.34	0.12	0.23	0	1	
O. innesi	0.57	0.42	0.36	0.3	0.61	0.18	0.63	1

A. bicolor: *Androctonus bicolor*
A. australis: *Androctonus australis*
S. maurus: *Scorpio maurus palmatus*
A. amoreuxi: *Androctonus amoreuxi*
L. quinquestriatus: *Leiurus quinquestriatus*
B. arenicola: *Buthacus arenicola*
A. crassicauda: *Androctonus crassicauda*
O. innesi: *Orthochirus innesi*

collected from many localities: Aswan, Middle and South Sinai, Baltim, Borg-El-Arab, Marsa Matroh, and revealed a wide diversity of scorpion species in Egypt with two families and a minimum of eight species. The most abundant scorpion family was Buthidae. Buthidae is the largest family of scorpions, containing about 80 genera and over 800 species. Its members are known as, thick-tailed scorpions and bark scorpions. Few Buthidae scorpions are among the larger scorpions. The largest members are found among *Androctonus* (fattail scorpions), which is one of the most dangerous groups in the world. They are found throughout the semi-arid and arid regions of the Middle-East and Africa (Hendrixon, 2006). They are a moderate sized scorpion, attaining lengths of 10 cm (just under 4 inches) and their common name is derived from their distinctly fat metasoma, or tail, while the Latin name originates from Greek and means "man killer. The most commonly found species were *A. bicolor*, *A. australis*, *A. amoreuxi* and *A. crassicauda*. The last one is very rare in Egypt and was found only in South Sinai. There were another three species encountered which belong to family Buthidae: *L. quinquestriatus*, *B. arenicola* and *O. innesi*.

Three buthid species are black. *A. crassicauda* (Olivier, 1807) which is called the Arabian fattail scorpion, found in North Africa and the Middle East and rare in Egypt. It is mildly aggressive. According to Ozkan and Filazi (2004) and Vignoli et al. (2003), *A. crassicauda* can be found in the Sinai Peninsula (Egypt), across the entire Middle East (Southeastern Anatolia – Turkey), Arabian Peninsula and Armenia. *A. bicolor* (Ehrenberg, 1828), man killer or black fattail scorpion which is mostly confused with *A. crassicauda*, but its pedipalp is slender with reddish or brown fingers. This scorpion is known to be highly irritable and aggressive. The last black scorpion is *O. innesi* (Simon, 1910), Egyptian pillar tailed scorpion or dwarf scorpion, which is a small sized scorpion. It is known from Egypt, Libya, Tunisia, Algeria, Sudan, Palestine, Israel, Jordan, Lebanon, Syria, Iraq, Kuwait, Qatar, Saudi Arabia (El-Henawy, 1992; Kovarik, 1992) and Morocco (Kovarik, 1995). The present survey recognized the presence of four yellow scorpions belonging to the family Buthidae; *A. australis* (Linnaeus, 1758) yellow fattail scorpion, this is one of the world's most dangerous scorpions, with very potent venom (Gaban, 1997). It has a very thick and powerful cauda (Abroug et al., 2000). Overall coloration is yellow, with the pincers sometimes darker. The last segments of the cauda are sometimes darker than the rest of the cauda. *A. amoreuxi*, yellow scorpion (Audouin, 1826). *L. quinquestriatus* (Ehrenberg, 1828). It is known by several names including the Egyptian Scorpion, five-keeled gold scorpion and Arabian death-stalker (Ben-Abraham et al., 2000). This scorpion found in dry habitats hides in small natural burrows or under stones, and is considered as one of the most common species of desert faunas in certain regions of Sudan, especially around Khartoum and Omdurman (Cloudsley-Thompson, 1961, 1963) as well as Israel (Levy et al., 1970). This sample is distinguished by a dark colored last metasomal segment V.

The last yellow scorpion in the family Buthidae is *B. arenicola* (Simon, 1885) which is small sized, and its cephalothorax is very smooth and with very weak carinae.

The second family is Scorpionidea. This family consists of 17 genera and 265 species, and has representatives in Africa, Asia, Australia, North America, Central and South America and is represented in the present survey by *S. marus palmatus* scorpion (Ehrenberg, 1828), large clawed or Israeli gold scorpion (Warburg and Elias, 1999). This species is often found in desert habitats, but can also be found in dry forests. It is a burrowing species, which inhabits different types of substrates. The burrows are often 20–70 cm deep (the bottom of the burrow is usually enlarged). The scorpion will hibernate in the burrow during winter (Rutin, 1996). This specimen is distinguished by its bright yellow colored body and much enlarged chela.

Scorpion venom contains short neurotoxin polypeptides consisting of simple, low-molecular-weight proteins which have lethal and paralytic effects (Isbister et al., 2003). Approximately 100,000 distinct peptides are estimated to be present in scorpion venom but only a limited number of these peptides have been described so far (Possani et al., 1999). In this investigation, total protein contents and SDS–PAGE analysis of different species collected from different localities in Egypt, showed differences between the protein compositions and concentrations of different scorpion's venom.

Total protein contents reach their highest value in *L. quinquestriatus*, *A. amoreuxi*, followed by *A. australis* venom. *O. innesi* showed very low protein concentration. Latifi and Tabatabai (1979) reported that the mean amount of venom obtained by electric stimulation was 0.3 mg per scorpion *A. crassicauda*, whereas by maceration of telsons, 0.5 mg per scorpion was obtained. Ozkan et al. (2007) reported that protein content of venom obtained from the same scorpion was 7.34 mg/ml.

SDS–PAGE analysis showed that, there are 11 protein bands obtained from protein profiles of scorpion venoms, with molecular masses ranging between 14 and 200 KDa. Ucar and Tas (2003), showed that crude venom of *Mesobuthus gibbous* consisted of 19 protein bands with molecular masses ranging between 6.5 and 210 KDa by SDS–PAGE. A total of 50 protein bands with molecular masses ranging between 22 and 100 KDa were detected from the venom of the same scorpion (Ozkan and Ciftci, 2010).

Nine protein bands with MWs ranging between 12 and 162 KDa obtained from venom of *A. crassicauda* (Ozkan et al., 2007).

Rodríguez-Ravelo (2013) revealed that venom of Cuban scorpion *Rhopalurus junceus* has Molecular masses varied from 46.6 to 197.55 KDa.

In the current work, bands with molecular weights of 200, 125, 95 KDa are dominant in most of the scorpion species venom, but with variable density. A protein band with MWs of 125 KDa reached its high density in *A. bicolor* and *A. australis*. Four bands with MWs of 188, 90, 16, and 14 KDa are considered as a specific band for *A. crassicauda* venom, which is considered very rare in Egypt. Low molecular mass bands (14 and 16 KDa) found in *A. crassicauda* in the present work may be responsible for its toxicity because of the presence of phosolipase represented by those bands. The previous results agreed with Rodríguez-Ravelo (2013) which detect the precence of phosolipase in the venom of the Cuban scorpion *Rhopalurus junceus* and was represented in bands with molecular mass of 14 to 19 KDa. Ozkan and Ciftci (2010) detected one protein band with MW of 68 KDa in all samples of *Mesobuthus gibbous* venom and this is due to genetic factors variation.

The present study reports the variability in different species of venom assessed by SDS–PAGE. One of the factors that may influence venom toxicity and cause variable results is environmental conditions. Abdel-Rahman et al. (2009) demonstrated that variations in the geographic, sexual status and individual markers of *S. marus palmatus* occur with different biotopes elucidating venom variation. Also, different geographical areas affected proteomic analysis of venom components of Cuban scorpion *Rhopalurus junceus* (Rodríguez-Ravelo, 2013). Ozkan et al. (2006a,b,c) reported that venom samples extracted by maceration showed different toxicities according to the weight and size of the telsons, and this difference was caused by the different maturity stages of the animals and thus venom toxicity was related to the scorpion size.

The similarity indices in the present study showed that there is inter-family, inter-genus, and inter-species variation between different scorpion species based on bands obtained from the protein profiles. Inter-family variation represented by the similarity between *S. maurus palmatus*, which considered the only species found in family Scorpionedae and the other species in family Buthidae. The closest species to *S. maurus palmatus* is *A. amoreuxi* ($S = 0.74$), despite their differences in morphology.

The inter-genus variations were very obvious in the family Buthidae. *L. quinquestriatus* has a major similarity ($S = 0.83$) with *A. bicolor* in protein profiles. *L. quinquestriatus* could be confused morphologically with *A. australis* and *A. amoreuxi*. Furthermore, *B. arenicola* show moderate similarity with *A. amoreuxi* and *O. innesi* has similarities with *A. crassicauda*.

For the initial view *A. crassicauda* can be confused with *A. bicolor* because of their black color. However, they show minor overall similarity ($S = 0.26$). *A. crassicauda* shows the lowest similarity indices between all *Androctonus* species. *A. australis* shows the closest species similarity with *A. amoreuxi* and *A. bicolor* ($S = 0.9$ and 0.83 respectively). All of these results represent the inter-species similarity variation, regardless of their morphological resemblance. The similarity coefficient data of venom and DNA obtained from different species of *S. marus palmatus* collected from different regions in Egypt were used to construct separate dendrograms, which grouped together the Southern Sinai populations and these were some distance away from the Western Mediterranean Coastal Desert population (Abdel-Rahman et al., 2009). Furthermore the study of Badhe et al. (2006) suggests the existence of genetic variation among the different strains of red scorpion *Mesobuthus tamulus* in western and southern India.

Finally, it is necessary to identify and survey the most famous species of scorpion in Egypt. Then, comparison between their qualitative and quantitative variation in the protein of their venoms to become a guide line for more researchers to study scorpion taxonomy anchored on accurate technique.

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