

Scorpions of the Ha'il Region, northern Saudi Arabia, and molecular phylogenetics of two common species, *Androctonus crassicauda* and *Scorpio maurus kruglovi*

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Summary

The present work is a systematic approach to the scorpion fauna of the Ha'il region (Saudi Arabia), based on morphology. In addition, a phylogenetic study of two common species, *Androctonus crassicauda* and *Scorpio maurus kruglovi*, was carried out, based on 16S gene sequences. The purpose is to provide an updated account of the scorpion fauna, and to present a brief description on the distribution of the scorpions of this region, which has been largely neglected and remains poorly known. Eight species of scorpions were identified: seven species and one subspecies belonging to the family Buthidae, and one subspecies belonging to Scorpionidae. Geographic distribution and relative abundance of the species collected were recorded in the study area. We report the 16S gene sequence for two scorpion species, *Androctonus crassicauda* and *Scorpio maurus kruglovi*, which are the most abundant scorpions in the study area and represent the two reported scorpion families. The gene sequences of these two species were deposited into GenBank with accession numbers HM125965 and HM125964 for *A. crassicauda* and *S. m. kruglovi* respectively. 16S gene sequences from these two taxa were compared with those from other species prevalent in Saudi Arabia, retrieved from GenBank, and aligned sequences were used to construct a phylogenetic tree. The results presented provide the first molecular phylogenetic study of the scorpion fauna of Saudi Arabia. Moreover, searching the data base revealed that the 16S gene of *S. m. kruglovi* was sequenced for the first time. The goal was to evaluate the potential of 16S gene sequencing to provide better resolution of the systematic problems of the Saudi scorpion fauna, and to resolve the phylogenetic relationships among them.

Introduction

Scorpions are a highly diverse group of organisms that embraces 155 genera in 16 families with a controversial phylogenetic position within the Arachnida (Dávila *et al.* 2005). Scorpions are known as one of the most abundant and dangerous animals for humans. A number of highly venomous species inhabit Saudi Arabia (*Androctonus crassicauda*, *Leiurus quinquestriatus* and *Parabuthus leiosoma*); it is important to document their ranges and make this information available to the general public so they will be made aware of potential encounters with the dangerous scorpions in their area. Some accounts of scorpionism in Saudi Arabia have been discussed by Dittrich *et al.* (1995), Mahaba (1997), Ismail (2003) and Al-Sadoon & Jarrar (2003). A total of more than 18,000 cases of scorpion stings are recorded in Saudi Arabia each year (Al-Sadoon & Jarrar 2003). In the Ha'il region some 2240 sting accidents have been reported in about 15 months (Mahaba 1997).

Few investigations of the scorpion fauna have been made in Saudi Arabia. In a checklist to the scorpions of the Middle East, Vachon (1966) listed 22 species and subspecies thought to exist in Saudi Arabia. Later, he made the first attempt to study the entire scorpion fauna of that country (Vachon 1979). In doing so, he only provided taxonomic analyses for 14 species and subspecies of all families. Fet *et al.* (2000) gave references to as many as 25 species and subspecies and Hendrixson (2006) suggested that there are at least 17 nominal species within ten genera in Saudi Arabia. All the previous authors recorded only two scorpion species in the Ha'il region. Similarly, Sharawy and Alshammari (2009) recorded two highly venomous scorpion species, *Leiurus quinquestriatus* and *Androctonus crassicauda*, at Aja Mountain in Ha'il. Recently, Al-Asmari *et al.* (2007, 2009a,b) identified 16 scorpion species belonging to 11 genera in five regions of Saudi Arabia (in addition to five unidentified species); eight of them were recorded in the Ha'il region. El-Hennawy (2009) listed 23 species and three subspecies of scorpions in Saudi Arabia.

Several recent studies have made use of molecular data in order to explore the taxonomic and phylogenetic relationship at several taxonomic levels (Desouky & Awwad 2004; Salomone *et al.* 2007; Masta *et al.* 2009). By using molecular data, the phylogeographic approach has yielded great insights into the recent evolutionary history of many animal species (Hewitt 2000). In this respect, the mitochondrial genome has been extensively used to study phylogenetic relationships at several taxonomic levels, mainly because of its maternal inheritance, the fast evolutionary rate compared to that of the nuclear DNA, and the lack of intermolecular genetic recombination (the exchange of homologous DNA sequences between different chromosomes) (Boore 2006; Masta *et al.* 2009). It has also been shown that the 16S gene is generally conserved within the Metazoa and could be used as a simple model for comparative genomics (Simon *et al.* 1994; Dávila *et al.* 2005).

The present work is a systematic study of the scorpion fauna of the Ha'il region (Saudi Arabia), based on morphological data. In addition, a phylogenetic study of two common species (*Androctonus crassicauda* and *Scorpio maurus kruglovi*) was made, based on 16S gene sequences. The purpose is to provide an updated account of the scorpion fauna and to present a brief description on the distribution of the scorpions of this region that has been largely neglected and remains poorly known. This will be valuable to recognize new recorded species and to confirm the existence of reported ones.

Material and methods

Scorpions were collected during regular trips between 2007 and 2010. In total, 406 specimens were collected from different habitats in the Ha'il district. Specimens were collected under the stones during the day, preserved in absolute ethanol and deposited in the central laboratory, Faculty of Science, Ha'il University.

Morphological identification

Morphological identification was performed according to the taxonomic keys with a zoom stereo dissecting microscope. The main references consulted for systematics and identification keys are: *Catalog of the Scorpions of the World (1758–1998)* (Fet *et al.* 2000), and the work of Hendrixson (2006).

Molecular studies

DNA extraction. Total genomic DNA was extracted from leg muscle of alcohol-preserved specimens using the CTAB buffer (0.1 M Tris-HCl pH 8.0, 1.4 M NaCl, 0.02M EDTA, 2% CTAB, 0.2% 2-mercaptoethanol) and standard phenol–chloroform/ethanol extraction (Hillis *et al.* 1996).

Amplification and sequencing. Amplification of a part of the mitochondrial large ribosomal gene (16S gene) was carried out using the universal 16S Forward Primer [5–CGA TTT GAA CTC AGA TCA–3] and reverse primer [5–GTG CAAAGG TAGCATAATCA–3] (Palumbi *et al.* 1991). PCR was performed in a 10 µL volume, where 1 µL of template DNA was mixed with 0.2 mM dNTPs, 2.5 mM MgCl₂, 0.2 pmol of each primer, and 1 unit of Taq Polymerase (Gibco). Thermocycling was performed in a PTC-100 thermocycler (MJ-Research). The cycle programme comprised an initial denaturation at 94°C for 2 min, followed by 40 cycles of 1 min at 94°C, 1 min at 48°C, and 32 s at 72°C. The cycling was ended with 10 min sequence extension at 72°C. Before sequencing, the resulting PCR products were verified on 1.5% agarose gel and purified using Qiagen gel extraction kit (Qiagen, Germany). Chromatograms were verified for accuracy, and sequence assembly was performed with Bioedit 7.0.9 (Hall 1999). The primers in the sequencing reactions were the same as in the amplification procedure. Searches for sequence similarity were performed using the Blast network service (<http://www.ncbi.nlm.nih.gov>).

Sequence alignment and phylogenetic analysis

To determine the relationship of our samples with other scorpion species, the obtained sequences were aligned with

the 16S gene of other species which were retrieved from the gene bank (<http://www.ncbi.nlm.nih.gov/genbank>). All species chosen for alignment are prevalent in the Saudi Arabian fauna. Alignment and generation of the phylogenetic tree was performed using the Clustal W program at EBI (<http://www.ebi.ac.uk/Tools/clustalw>).

Results

Study area

The Ha'il region (Fig. 1) is located in the central northern part of Saudi Arabia, between 25°35'N and 29°00'N and 39°01'E and 44°45'E. The entire region is about 1000 m in elevation. The Ha'il region covers an area of 118,322 km². It is characterized by several landscape units, such as isolated mountains, escarpments, valleys and sand-seas. The Ha'il valley extends to the north-east, whereas the Great Nofoud desert, with rolling sand dunes forms the northern part of the region. Rocky desert occurs to the west of Ha'il and covers about one-third of the province. The Ha'il region contains the most prominent mountains such as Salma, Aja, Heden, Ruman, Louf, Khashab, Aqab and others. The Shammar Mountains form a major feature and consist of two great ranges, namely Aja, made up of granite, and Salma composed of basalt. The Al-Nofoud desert, which makes up approximately one-third of the area, is composed mainly of sand dunes. The climate in the Ha'il region is generally arid to extra arid. Summer temperatures typically rise as high as 50°C in the day, with diurnal variation of about 25°C. Winter temperatures hover around freezing at night, and daytime temperatures nearly always reach 25°C in the sun. The rainfall is erratic and there is no regular pattern.

Morphological studies

A list of the scorpion taxa collected in this study is provided in Table 1. Figure 1 shows the geographic distributions of the eight scorpion taxa analysed in this work. The total number of specimens collected from this region was 406. Seven taxa belonging to the family Buthidae, and one belonging to Scorpionidae, were identified. About 44% of

	Taxon	Distribution	Number	Percent
1	<i>Androctonus crassicauda</i>	Around Ha'il airport, Hadco, Aja Mountain, Salma Mountain, Ash Shai'lania, As Shamli, Al Hulaifa	178	43.84%
2	<i>Scorpio maurus kruglovi</i>	Great Nofoud, Moqaq, Takhaeel, Al Rouda, Qafar, Ash Shai'lania, Al Hulaifa, Baqaa, Gobba, Ha'il, Aja Mountain	127	31.28%
3	<i>Buthacus buettikeri</i>	Hufair, Sarraa	37	9.11%
4	<i>Androctonus bicolor</i>	Around Ha'il airport, Salma Mountain, Umm Saanan Mountain, Sufaytt	21	5.17%
5	<i>Leiurus quinquestriatus</i>	Aja Mountain, Salma Mountain	15	3.69%
6	<i>Buthacus yotvatensis nigroaculeatus</i>	Qa'Ajlal, Al Ajajah	11	2.71%
7	<i>Compsobuthus werneri?</i>	Snaiem, Great Nofoud, Takhaeel	10	2.46%
8	<i>Apistobuthus pterygocerus</i>	Great Nofoud	7	1.72%
Totals			406	100%

Table 1: Distribution and prevalence of scorpion taxa collected from the Ha'il region.

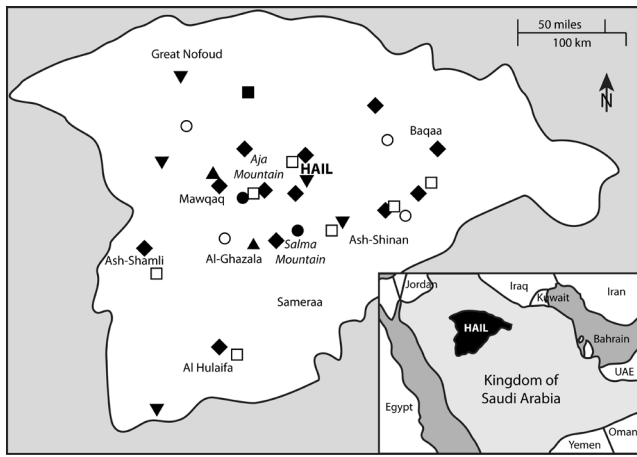


Fig. 1: Map of the Ha'il region showing the geographic distribution of the scorpion fauna. ▲ = *B. buettikeri*, ▼ = *A. bicolor*, ■ = *A. pterygocerus*, □ = *A. crassicauda*, ○ = *Compsobuthus* sp., ● = *L. quinquestriatus*, ◆ = *S. maurus*.

the collected specimens were the highly venomous *Androctonus crassicauda*. The next commonest was *Scorpio maurus kruglovi* (~31%). On the other hand, three taxa were considered scarce (<3%) in the Ha'il region: *Buthacus yotvatensis nigroaculeatus*, *Compsobuthus wernerii*? and *Apistobuthus pterygocerus* (Table 1).

Key to the studied scorpion species

[Adapted from El-Hennawy (2009) and Hendrixson (2006)]

1. Pedipalp patella without ventral trichobothria; sternum subtriangular; anterior margin of carapace not conspicuously notched..... Buthidae 2
- Pedipalp patella with one or more ventral trichobothria; sternum subpentagonal; anterior margin of carapace with distinct notch Scorpionidae: *Scorpio maurus kruglovi*
2. Metasomal segment II widely flared, much wider than other segments of metasoma *Apistobuthus pterygocerus*
- Metasomal segment II not wider than other segments...3
3. Mesosomal tergites I and II with 5 distinct carinae..... *Leiurus quinquestriatus*
- Mesosomal tergites I and II without carinae, or with at most 3 carinae..... 4
4. Carapace smooth or granulated but without distinct carinae *Buthacus* 5
- Carapace with distinct carinae.....6
5. Metasomal segments densely hirsute *B. yotvatensis nigroaculeatus*
- Metasomal segments with few setae *B. buettikeri*
6. Central lateral (centromedian) and posterior lateral (posteriomedian) carinae of carapace fused forming a continuous linear row of granules to posterior margin.... *Compsobuthus* sp. (*C. wernerii*?)
- Central lateral (centromedian) and posterior lateral (posteriomedian) carinae of carapace do not form a continuous linear row of granules to posterior margin.... *Androctonus* 7

7. Pedipalp hand slender, lighter than body colour *A. bicolor*
- Pedipalp hand broad and stout, same colour as body..... *A. crassicauda*

Family Buthidae C. L. Koch, 1837

***Androctonus crassicauda* Olivier, 1807 (Fig. 2)**

Previous records in Saudi Arabia. Vachon (1979): 31–34, figs. 1–2, Riyadh. Hendrixson (2006): 38–43, figs. 1–2, plate 1; Makkah. Al-Asmari *et al.* (2007): 833; Al-Medina Al-Munawara. Al-Asmari *et al.* (2009a): 106; Ha'il. Al-Asmari *et al.* (2009b): 618; Riyadh.

Habitat. It is found in rocky desert, mountainous areas of various soil types, or sand dune systems, where they build scrapes under large rocks or find refuge in the burrows of other animals.

Coloration. It ranges from olive-brown to reddish brown to black. Sternites yellowish brown. Metasoma and vesicle reddish brown uniformly with blackish carinae.

Size. Adults to about 105 mm in length; females generally more heavy-bodied.

Remarks. The genus *Androctonus* is widely distributed throughout the deserts of northern Africa, the Middle East and into India. These large-bodied scorpions, along with the genus *Parabuthus*, are easily distinguished from other buthid scorpions by their extremely heavy and robust metasomal segments. *Androctonus* can also be identified by its distinct carapacial carination: the posteriomedian and centrolateral carinae are fused to form a continuous lyriform keel.

The systematics of *Androctonus* at the species level remain problematic, and a revision of the group is needed. For example, *A. crassicauda* was misidentified as *A. australis* by Levy and Amitai (1980) but El-Hennawy (1992), and Hendrixson (2006) considered that these specimens are referable to *A. crassicauda*, and tentatively proposed that *A. australis* is not present in Saudi Arabia. Moreover, the basitarsal spur of *A. crassicauda* rarely possesses a basally bifurcated outer tooth as in *A. australis*.

Androctonus crassicauda venom has an i.v. LD₅₀ in mice of 0.32 +/- 0.02 mg/kg, which makes the scorpion among the most toxic species in the world (Ismail *et al.* 1994).

***Androctonus bicolor* Ehrenberg, 1828 (Fig. 3)**

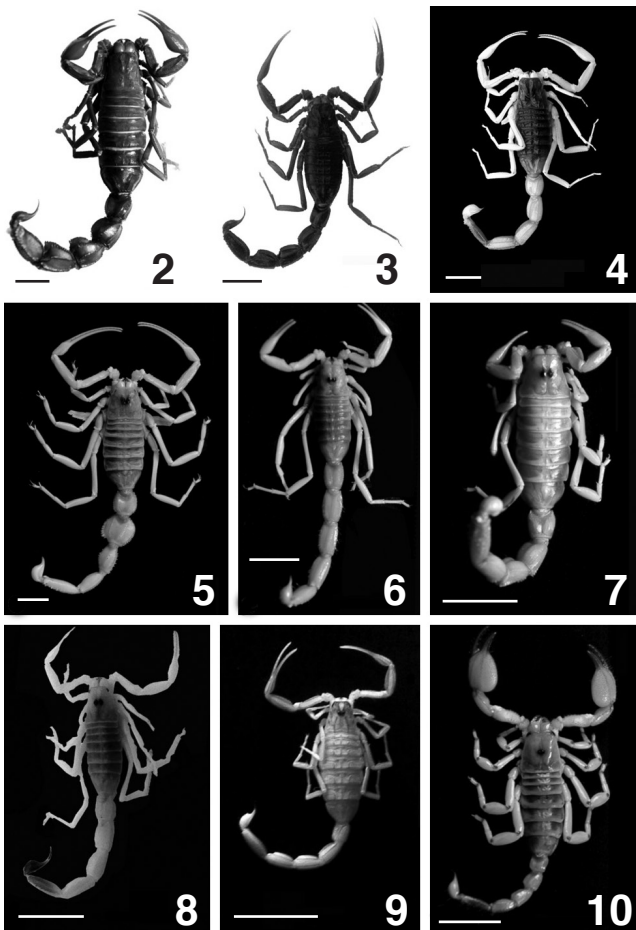
Previous records in Saudi Arabia. Al-Asmari *et al.* (2009a): 106; Ha'il. Al-Asmari *et al.* (2009b): 618; Riyadh.

Habitat. Its habitat is similar to that of *A. crassicauda*.

Coloration. The adult is dark brown to black, with end of the legs and fingers paler.

Size. Adults may reach 90 mm in length.

Remarks. *A. bicolor* is black and has an extraordinarily slender pedipalp and thick metasoma. This is quite often confused with *A. crassicauda* (black variety) but its pedipalp is comparatively slender and lighter than the body colour.



Figs. 2–10: Habitus of scorpions of the Hai'il region: **2** *Androctonus crassicauda*; **3** *Androctonus bicolor*; **4** *Leiurus quinquestriatus*; **5** *Apistobuthus pterygocercus*; **6** *Buthacus buettikeri* (♂); **7** *Buthacus buettikeri* (♀); **8** *Buthacus yotvatensis nigroaculeatus*; **9** *Compsobuthus werneri*?; **10** *Scorpio maurus kru-glovi*. Scale lines = 10 mm.

Leiurus quinquestriatus Ehrenberg, 1828 (Fig. 4)

Previous records in Saudi Arabia. Gough & Hirst (1927): 5, fig. 8; Medina. Vachon (1979): 49–50, figs. 8, 37, 46–50, 64–66; Khamis Mushayt; Mecca Road; Jeddah; Wadi Khumra; Gizan. Hendrixson (2006): 84–84, figs. 18–19, 20b–c, plates 14–15; Manfah. Al-Asmari *et al.* (2007): 833; Al-Medina Al-Munawara. Al-Asmari *et al.* (2009a): 100, 106; Al-Baha, Ha'il. Al-Asmari *et al.* (2009b): 618; Riyadh.

Size. Adults may reach 80 mm in length.

Habitat. This scorpion is found in dry habitats/desert areas on different substrate types (but not sand dunes). The species hides in small natural burrows or under stones.

Coloration. Almost entirely yellow with olive carapace and mesosoma; metasomal segment V noticeably darker than other segments.

Remarks. This is one of the world's most dangerous scorpions, with very potent venom. It causes several deaths each year. LD₅₀ value of 0.16–0.50 mg/kg. (Simard & Watt, 1990).

Apistobuthus pterygocercus Finnegan, 1932 (Fig. 5)

Previous records in Saudi Arabia. Finnegan (1932): 92; Uruq Dhahiqah, Shena. Vachon (1979): 34–35, figs. 4–5;

Uraq (SE Arabia); Dhahiga; Shannah; Andhur; Al Khardj; Dhahran. Hendrixson (2006): 44–46, figs. 3, 6, plate 2; Safura Desert; Shawala, Camp. Al-Asmari *et al.* (2009a): 106; Ha'il.

Habitat. Widespread on sand dunes throughout the Great Nofoud.

Coloration. Base colour uniformly yellow to orange-brown with some dusky markings around median eyes; metasoma yellow to yellow-orange.

Size. Adults may reach 110 mm in length.

Remarks. *Apistobuthus* is a remarkable genus of scorpions that is readily identified by the form of its metasomal segment II which is widely flared, much wider than other segments of metasoma, large size, greatly elongated pedipalp chela fingers and very high pectinal tooth counts. The genus presently contains two species that are endemic to the Middle East: *A. pterygocercus* from the Arabian Peninsula (Vachon 1979) and *A. susanae* from south-western Iran (Lourenço 1998). *A. pterygocercus* was rare in the present studied area. This idea is supported by a lack of representative specimens in museum collections and also by limited references in the literature. According to Hendrixson (2006) this is likely attributed to the fact that the genus is strictly psammophilous (i.e. confined to sand dune systems), such that conventional rock-rolling techniques are not useful in adequately sampling populations.

Buthacus buettikeri Hendrixson, 2006 (Figs. 6–7)

Previous records in Saudi Arabia. Hendrixson (2006): 47–52, 59, figs. 4–6, plates 3–4; Umad-Dabah. Khashm Dhibi. Addar. Wadi Maraum. Jabal al-Ghamdiyah. Kijat. Makkah By-pass km 91.5.

Habitat. Sandy desert with sparse bushes.

Coloration. Base colour of body, legs and pedipalps orange-yellow; male with slight dusky markings on carapace and tergites.

Size. Adults male may reach 70 mm in length. Females are shorter and more robust.

Variations. Females (Fig. 7) larger (to nearly 70 mm in length), with more robust metasomal segments and very strong, coarse, lobate denticles on metasomal segments II and III; and with aculeus longer than vesicle; pectinal tooth counts 24–25 in males, 15–22 in females.

Remarks. Hendrixson (2006) reported *Buthacus buettikeri* as a new species which is widespread in Saudi Arabia. He distinguished it from *Buthacus leptochelys* reported by Vachon (1979) by the enlarged lobate denticles on the ventral submedian carinae of metasomal segments II and III and by the form of the telson and aculeus, which are clearly more slender and elongated in *B. buettikeri*. Recently, this species was also reported in the United Arab Emirates by Lourenço & Leguin (2009).

Buthacus yotvatensis nigroaculeatus Levy, Amitai & Shulov, 1973 (Fig. 8)

Previous records in Saudi Arabia. Vachon (1979): 36–38, figs. 6, 8; Damman; Al Khardj. Hendrixson (2006): 52–56,

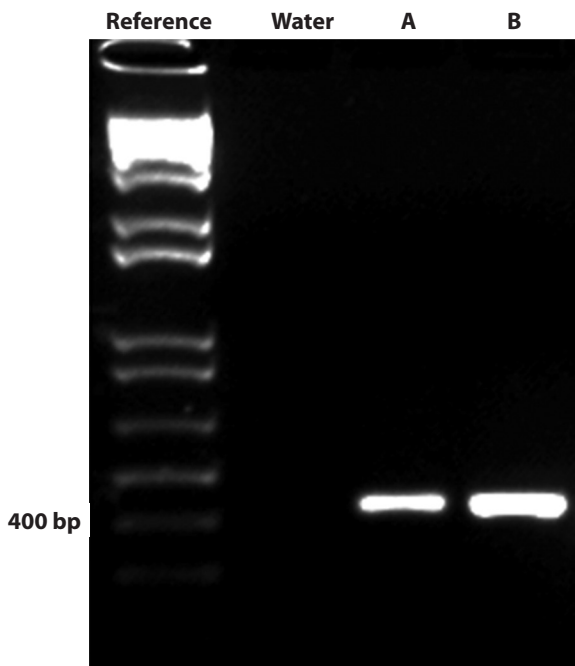


Fig. 11: PCR products verified by electrophoresis in agarose gel using 100-bp ladders. Lane Reference: 100-bp ladders; Lane Water: Negative control; Lane A: *A. crassicauda*; Lane B: *S. m. kruglovi*.

figs. 6–7, plate 5; Riyadh; vicinity of Riyadh; Aqabat al-Uhuraytan, c. 80 km NW of Riyadh; Riyadh. Al-Asmari *et al.* (2009b): 618; Riyadh.

Habitat. Found throughout sand dune systems (southern parts of the Ha'il region).

Coloration. Base colour orange-yellow to olive-yellow; metasomal segment V and telson black.

Size. Adults to approximately 70 mm in length.

Remarks. There was a great deal of variability in Coloration within this subspecies (Vachon 1979). Hendrixson (2006) reported some specimens from Riyadh possessed a black telson, and other specimens from eastern Saudi Arabia showed no signs of melanism. Aside from colour, no significant taxonomic characters appear to distinguish the two forms.

***Compsobuthus weneri*? Birula, 1908 (Fig. 9)**

Previous records in Saudi Arabia. *C. weneri* ssp.?: Vachon (1979): 40–42, figs. 10–11, 58–60, Khamis Mushayt. Al-Asmari *et al.* (2007): 831; Jazan. Al-Asmari *et al.* (2009a): 100, 106; Al-Baha, Ha'il. Al-Asmari *et al.* (2009b): 618; Riyadh.

Habitat. Distributed throughout the mountainous areas and sand dunes system.

Coloration. Base colour of entire body yellow-orange, pedipalps and legs essentially yellow to orange-yellow; black underlying pigment surrounding median eyes

Size. The collected juveniles reached 43 mm in length.

Remarks. Before Vachon (1949) described *Compsobuthus*, its species had been placed in *Buthus* (Leach, 1815). *Compsobuthus* is widely distributed throughout large portions of Africa and Asia. The taxonomy of this group is poorly understood and is the subject of much debate among scorpion systematists. The recent catalogue of world

Taxon	Country of Origin	Collector	GenBank Accession Number
Buthidae			
<i>Androctonus crassicauda</i>	Saudi Arabia	Desouky & Alshammari	HM125965
<i>Leiurus quinquestriatus</i> (Ehrenberg, 1828)	Oman	Lowe	AY226174
<i>Vachoniolus globimanus</i> Levy, Amitai & Shulov, 1973	Oman	Lowe	AY226179
<i>Apistobuthus pterygocercus</i> Finnegan, 1932	Oman	Lowe	AY226178
<i>Buthacus yotvatenensis</i> Levi, Amitai & Shulov, 1973	Oman	Lowe	AY226173
Scorpionidae			
<i>Scorpio maurus kruglovi</i>	Saudi Arabia	Desouky & Alshammari	HM125964
<i>Scorpio maurus fuscus</i>	Israel	Prendini, Crowe & Wheeler	AY156569
<i>Scorpio maurus palmatus</i>	Egypt	Prendini, Crowe & Wheeler	AY156570

Table 2: Data on the 16S gene of the scorpions studied and of other species retrieved from GenBank.

scorpions (Fet *et al.* 2000) listed 15 species (one polymorphic) in the genus, but since the time of its publication, several new species have been described (Lourenço 1999, 2001; Lourenço & Vachon 2001; Lowe 2001; Kovařík 2003). Hendrixson (2006) identifies five species of *Compsobuthus* in Saudi Arabia, including three new species and one new country record.

Family: Scorpionidae Peters, 1862

***Scorpio maurus kruglovi* Birula, 1910 (Fig. 10)**

Previous records in Saudi Arabia. Vachon (1979): 57, figs. 40, 42, 45, 51–53; Wadi Hanifa. Al-Asmari *et al.* (2007): 833; Al-Medina Al-Munawara. Al-Asmari *et al.* (2009a): 106; Ha'il. Al-Asmari *et al.* (2009b): 618; Riyadh. *S. m.* ssp.; Hendrixson (2006): 108, figs. 27, 28, plate 21; Al-Baha,

Habitat. *Scorpio* digs burrows in a variety of soil types. The burrow generally has a crescent-shaped opening and an enlarged bottom.

Coloration. Base colour golden sandy yellow, the carapace and mesosomal segments are light to dark brown.

Size. Total length 50 to 80 mm.

Remarks. *Scorpio* is one of the oldest reported genera. Linnaeus (1758) placed five species into the first scorpion genus, *Scorpio*; all but one species, *S. maurus* Linnaeus, 1758, were later transferred into other genera. It gets its name from *scorpio* which means “scorpion” and *maurus* meaning “dark”. This species presently contains 19 subspecies which are distributed over much of North Africa and

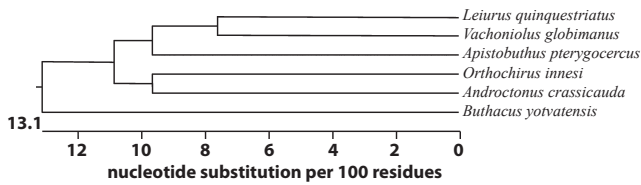


Fig. 12: Phylogenetic tree of the 16S gene from *A. crassicauda* and that of other buthid scorpions retrieved from GenBank.

the Middle East. It is widely distributed in Saudi Arabia and many subspecies have been recorded including: *S. m. arabicus* (Pocock, 1900), *S. m. kruglovi* (Birula, 1910), *S. m. palmatus*, (Ehrenberg, 1829), *S. m. yemenensis* (Werner, 1929) and *S. m. fuscus* (Ehrenberg, 1829) (Vachon 1979; Sissom 1994; Hendrixson 2006; Al-Asmari *et al.* 2009a). According to Sissom (1994) and Hendrixson (2006), many of these subspecies are virtually indistinguishable and their status needs to be investigated further. Wilson Lourenço (personal communication) has now studied the African subspecies and intends to raise several subspecies to species rank.

This species is 60–80 mm long, and the different subspecies (19 subspecies described in Fet *et al.* 2000) vary in colour. Most have a yellow to red-brown colour (the tips of the claws are usually dark red-brown), but some subspecies (e.g. *S. maurus fuscus*) are dark brown or blackish in colour. The different subspecies can be separated by colour variations, but not always. It is possible that some of the subspecies represent true species, but a thorough revision of the genus is necessary before any conclusions can be drawn.

Molecular data

The PCR products of the amplified 16S gene of *A. crassicauda* and *S. m. kruglovi*, as representatives of the two scorpion families reported in this study, are shown in Fig. 11, and their sequences listed below. The length of the 16S partial gene sequence of *A. crassicauda* was found to be 390 bp. Nucleotide composition is clearly biased towards AT with a nucleotide composition of 11.0% guanine, 37.1% adenine, 35.0% thymine, and 16.9% cytosine. On the other hand, the length of the 16S partial sequence of *S. maurus kruglovi* was 391 bp with a nucleotide composition of 12.3% guanine, 31.5% adenine, 37.40% thymine, and 18.7% cytosine. The gene sequences of these two taxa have been deposited into GenBank with accession numbers HM125965 and HM125964 for *Androctonus crassicauda* and *Scorpio maurus kruglovi* respectively (<http://www.ncbi.nlm.nih.gov/nucore>). The sequenced portion of the 16S gene of both taxa was aligned. The percentage identity

between them was found to be 68.3 % and the divergence was 41.4%.

Androctonus crassicauda, Ha'il, Saudi Arabia; 16S ribosomal RNA gene, partial sequence, mitochondrial; DNA extract from leg muscle; 390 bp

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TAGGTCGACAGACCTCCTTTATTCCGCTTTTTCGCGCA
AAAGGTATTTTATTCAACATCGAGGGTCATAAACTC
ACTTGTGCGATTAGAAGCTTTCTAAGTGAATTAAGCT
GTTATCCCTAAAGTAACTTAATTTCTTTTTTAAAATT
TTTAGGTATTAACAAGCCCGTTAATTTTTTAAAG
GAAGTCTAATTTCCCTCCACCGCCCCAGTGTAATATT
TTTCCTTTTAAATAAAAACAAAGAAATATAAAGTTT
TATTAGGGTCTTCTTGTCTAAAAGTCTTATTTTAGCT
TTTACTAAAAGTAAATTCATTTCCCTCATAAACAGT
TCTCTTAGGTACTCCTTCATTCCAGTCTCAATTAAA
AGACTAATGATATGCTACTTTGCCCAA
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Scorpio maurus kruglovi, Ha'il, Saudi Arabia; 16S ribosomal RNA gene, partial sequence, mitochondrial; DNA extract from leg muscle; 391 bp

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TAAGCGACAGACTTCCTTTTCTCCTATTGCATGAAT
TAGGTATTTAATCCAACATCGAGGTTTCGCAAACCTC
TAATGATGATAAGAAGCTCTAAAAAAGAATTACGCT
GTTATCCCTGCAGTAACTTATTTTTCTTTAGGAATT
TCCTGATTTGACAAAAGTATTATCACAATATATAA
ATAAAATTTTATTACCTACTGCCCCAGTAAAACAAA
TTTTAATCATTTTAAGAACAATGACCTAGTTTGTA
AGTTTTGACAGGGTCTTCTCGTCCCTAAAAAAAT
TTTAGCTTTTTTACTAAAATATAAAATTTGAAATATT
ATTACAAGACAAAATTACCTAGTCTAACCATTCATA
CCAGTCTAAAATTAAGACTAATGATTTAG
```

The 16S gene from *A. crassicauda* was compared with that from five buthid species (prevalent in the Saudi Arabian fauna) retrieved from the database. The GenBank accession numbers of the 16S genes of these species are shown in Table 2. Aligned sequences were used to construct the phylogenetic tree (Fig. 12). The phylogenetic analyses revealed that *A. crassicauda* and *Orthochirus innesi*, and *Leiurus quinquestriatus* and *Vachoniolus globimanus* are closely related to each other, while *Buthacus yotvatensis* is placed in a separate clade.

Similarly, the 16S gene from *Scorpio maurus kruglovi* was compared with that from two other *Scorpio maurus* subspecies (*S. m. fuscus* and *S. m. palmatus*) retrieved from the database (Fig. 13) and aligned sequences were used to construct the phylogenetic tree that separates *S. m. kruglovi* from the other two subspecies, which were found to be closely related to each other.

Discussion

Previous studies on the scorpion fauna of Saudi Arabia have revealed that it is not highly diverse in comparison to a number of places of smaller area. El-Hennawy (2009) listed 23 species and three subspecies of scorpions in Saudi Arabia. Israel, Palestine and Sinai, for example, contain 20 species and subspecies (Levy and Amitai 1980) despite their smaller combined area compared to that of Saudi Arabia.

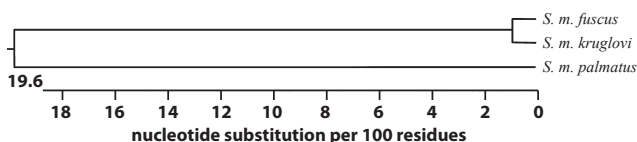


Fig. 13: Phylogenetic tree of the 16S gene from *S. m. kruglovi* and that of two other *Scorpio maurus* subspecies retrieved from GenBank.

This low scorpion diversity in Saudi Arabia may be attributed to the fact that vast areas throughout the country, including the Ha'il region, have not been adequately sampled. If ultraviolet-light detection techniques (Williams 1968), are employed instead of conventional collecting techniques such as rock-rolling, the records of scorpions will inevitably increase. Regarding the Ha'il region, Vachon (1979) recorded only two species in the region, while Al-Asmari *et al.* (2009a) recorded eight taxa there. The current work also recorded eight taxa, two of which are new records for the region. This means that ten scorpion species are now known for this region. It is not unreasonable to speculate that this region will harbour a much greater number of species since it supports the greatest diversity of habitat types.

According to Hendrixson (2006) the majority of Saudi Arabian scorpion taxa are not adequately studied; most genera are relatively problematic and require major revision. Many of the morphological characters used by previous authors to differentiate species have been found to be highly variable, subjective and of limited taxonomic use. In recent years, molecular markers have become mainstream in taxonomic studies, and the 16S gene is now routinely used in molecular systematic for resolving species-level phylogenies in different animal groups as mammals (Burk *et al.* 2002), insects (Mahendran *et al.* 2006), and scorpions (Fet *et al.* 2003; Ben Othmen *et al.* 2006; Salomone *et al.* 2007; Masta *et al.* 2008, 2009; Borges *et al.* 2010) due to their lack of recombination. Nucleotide sequences of this gene serve as an estimate of species divergence.

The results presented in this article provide the first molecular systematic study of the scorpion fauna of Saudi Arabia based on partial sequence analysis of mitochondrial DNA (16S gene). Searching the GenBank database revealed that the 16S gene of *S. m. kruglovi* was sequenced for the first time. The goal was to evaluate the potential of 16S gene sequencing to provide better resolution of the systematic problems of the Saudi Arabian scorpion fauna, and to resolve the phylogenetic relationships among them. Here, we report the 16S gene sequence for two scorpion species (*A. crassicauda* and *S. maurus kruglovi*). These two taxa are the most abundant scorpions in the study area, representing the two reported scorpion families, and constitute a major concern for identification. The 16S gene from these two taxa was compared with that from other species prevalent in the Saudi Arabian fauna, retrieved from the database, and aligned sequences were used to construct a phylogenetic tree. The phylogenetic tree of buthid scorpions separates them into: black scorpions; *A. crassicauda* and *Orthichirus innesi* in one cluster (yellow scorpions), *Leiurus quinquestriatus* and *Vachoniolus globimanus* in another cluster, while *Apistobuthus pterygocercus*, which possesses some highly derived features (flared metasomal segments), is separated alone. However, Fet *et al.* (2003) made the first molecular phylogeny of Buthidae and found that *Androctonus* and *Leiurus* could be more closely related to each other than to any other genus represented in their study, This is consistent with the data on their venom (Loret & Hammock 2001). On the other hand, The present phylogenetic tree of scorpionids separate *S. m. kruglovi* from the other *S. m. subspecies*. Many of *S. m.* subspecies are virtually indistinguishable and their status needs to be investigated (Hendrixson 2006). In

future, a combined morphological and molecular (DNA) phylogenetic analysis including more *S. m.* subspecies will further clarify the systematic problem of these subspecies and may help in raising some of them to species rank. In agreement with Fet *et al.* (2003), we are convinced that only the collaborative efforts of many scorpion taxonomists from many parts of the world will bring sufficient knowledge to clarify scorpions phylogenetic relationship.

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