Original Article

Morphometric Indices and Venom Protein Profile in Different Populations of *Androctonus crassicauda*

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(Received 28 Jan 2019; accepted 27 Oct 2021)

Abstract

Background: *Androctonus crassicauda* is the most medically relevant animal and understanding its morphological characteristics is essential in the production of antiscorpion sera.

Methods: Adults of *A. crassicauda* were collected from different areas of Zanjan Province and the morphometric parameters and the cuticular fluorescence patterns of samples were studied. The crude venom of samples was extracted by electric stimulation, and their biochemical properties were analyzed by the SDS-PAGE method.

Results: Values of the morphometric parameters depended on sex and altitude of the area. Except for values of the pectinal organ, these parameters in females were higher than in males. No significant difference was in the number, shape, and intensity of cuticular fluorescence patterns. The body length of males in high and lowlands was 72.53±1.53 and 77.33±2.70mm, respectively. Females' body lengths in that area were 81.66±2.19 and 86.55±2.33mm, respectively. Analysis of toxin proteins showed two isotypes that the 12, 13, 15, 16, 18, and 19kDa proteins were in all areas. However, the 41 and 74kDa proteins, and 46 and 63kDa proteins were detected in low and highlands, respectively.

Conclusion: Black fat-tailed scorpion has a considerable dominancy and developing preventive programs and providing treatment facilities in studied areas are necessary. Values of the morphological parameters and venom electrophoresis patterns depended on the geographical location. Therefore, pool crude toxin is suggested for the production of effective antivenoms. Moreover, additional field complementary works in the geographic information system based niche modeling and mass fingerprinting of scorpion venoms are suggested for screening effective isotypes.

Keywords: *Androctonus crassicauda*; Black fat-tailed scorpion; Morphometric parameters; Scorpion venom SDS-PAGE; Scorpion venom extraction

Introduction

Scorpions are potentially fatal venomous animals, and their envenomation is a major public health problem in the world. Buthidae is the largest family of scorpions with worldwide distribution and contains the most dangerous species (1). The Black fat-tailed scorpion, *Androctonus*, is a large and ancient group of Buthidae scorpions that has successfully adapted to various ecological conditions and occurs in different habitats. The overall body plan of this group has changed slightly and its systematics at the species level remains poorly defined. At present, eight species and 17 subspecies are reported within the *Androctonus* genus and among them, *A. crassicauda* is the most dangerous, causing many human deaths (2-5). Populations of this scorpion have a widespread distribution in the Middle East and Africa. The wide distribution of this species mostly in subtropical and temperate regions, between 23º–38º latitudes, indicates it's adapting to various ecological conditions and has different types (6). Despite intensive research performed on external morphology in differ-

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ent species of scorpions, few investigations have been conducted on *A. crassicauda*, and the characterization of some parameters of this species such as reproductive apparatus, is still unknown (7, 8).

In the past decades, scorpion biology has been revolutionized using ultraviolet (UV) lamps (black lights) for their detection in the field and morphological surveys (9, 10). These animals are strongly fluorescent when illuminated with long UV light. Molecules associated with the cuticular fluorescence of scorpions have been identified as the beta-carbon and 4-methyl, 7-hydroxy coumarin. The fluorescence exhibited by the tegument and the intensity of the emitted light is depended on the amount of these compounds in the cuticle. However, the biological function of fluorescence has not been definitively demonstrated (6, 11, 12). No difference in fluorescence spectra between genders of species is detected. However, statistical significance between the two sympatric species and subspecies of some scorpions was observed previously (13). Black scorpions have a protected body form with black coloration and current morphological methods, using white light, cannot be accurate enough to identify the minor morphological differences in these scorpions. Therefore, studying fluorescence patterns could provide complementary information to the morphological study of *A. crassicauda*.

Scorpion venom is the other most important item in the study of scorpions. It is a secretion compound of water, salt, and low molecular weight peptides with 13–76 amino acid residues. It resembles typical short and long-chain toxins specific for ion channels and receptor target cells. Biochemical analyses of scorpion toxins show about 44 different eluting fragments, from which 30 fragments are completely separated. These fractions are unique defense, feeding weapons, and effective semiochemicals that modulate their behavior. Scorpions apply these compounds in several sophisticated ways, for subduing prey, and deterring predators, probably during mating and frequently for deferring against humans (6). Molecular and biochemical characterization of *A. crassicauda* venom in Anatolia, southeast of Turkey, represented at least 44 different fractions with toxicity to mice and insects. The analysis of toxin in this scorpion identified 80 distinct molecular mass compounds varying from 267–44551 Da peptides that could modify K and Na channels. To date, only eight peptides have been identified from *A. crassicauda* venom in that region, three of which have been fully sequenced (*Acra1*, *Acra3*, *Acra4*), and one (*Acra2*) is partially sequenced, and the other four are putative (*Acra5-8*) (14, 15, 16). However, due to the increment of new strategies of proteome analysis and gene cloning from transcriptomes, the venom variation in different areas, and the number of identified components may increase significantly (17).

The Black fat-tailed scorpion is the most significant scorpion species in Iran and the Middle East countries. It is the main scorpion species in Zanjan Province, northwest of Iran, causing many cases of scorpionism in this area (18, 19). Due to the multifunctional role of venom in scorpions, its analysis could exhibit the variation of population groups in *A. crassicauda*. Moreover, detection of venom components in *A. crassicauda* is extremely important to produce effective antivenom and understanding of clinical symptoms of patients. Although *A. crassicauda* causes many human fatalities, the knowledge of this species is restricted and limited to past decades. In addition, despite the long history of venom research in the world, the venom of a few Iranian scorpions has investigated. Therefore, it is important to study the polypeptide electrophoretic patterns, and morphometric indices of *A. crassicauda* in distributed areas, as these characteristics, may have been applied in therapeutic management. Therefore, the aims of this study were to characterize the morphometric indices, the fluorescence pattern of ex-

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Published Online: March 31, 2022

ternal anatomy, and the polypeptide electrophoresis pattern of venom in different populations of *A. crassicauda* in Zanjan Province.

Materials and Methods

Study area

Zanjan Province is located in northwest Iran, from 35°35' to 37°15'N and 47°15' to 49°25'E (Fig. 1). The altitude in this region varies from 270m to 3400m above sea level (asl). The average maximum temperature in this area is around 27 °C, whereas the average minimum temperature stands at -19 °C. Meanwhile, the temperature rises to 32 °C on hot days; it drops to -27 °C on icy days. The average annual rainfall in the first month of spring stands at 72 millimeters, while in the second month of summer, it slips to a meager 3.6mm. The rate of humidity in the morning stands on average at 74% and at noon at 43% (https://en.climate-data.org/asia/iran/zanjan/zanjan-764536/).

Sample collection

Adults of *A. crassicauda* specimens were collected in different periods between Jun and September 2015–2016 from twenty-three localities of Zanjan Province (Table 1, Fig. 1). The collection sites are grouped in two lowlands (under 700m asl) and highlands (above 1200m asl). The geographical characteristics of sampling stations were determined by the Global Positioning System (GPS). Scorpions were caught at night by using ultraviolet light, a portable flashlight equipped with 3W 375–380nm Ultraviolet LED, detection method, and a few were captured in the daytime by rock rolling in the field. Avoiding scorpion cannibalism, captive specimens were housed in individual plastic boxes. They were kept alive in laboratory conditions (40% relative humidity, 12:12 L: D, and 24±2 °C) and fed with ground meat and living crickets and received daily water *ad labitum*. Food was given a weak after venom extraction in order to allow time for the animals to recover from stress. Dead specimens were transferred into 75–96% ethyl alcohol and kept at -20 °C for further studies. They were deposited in the scorpion collection at the Department of Medical Entomology, Zanjan University of Medical Sciences, Iran.

Morphological survey

In the laboratory, sexing and taxonomic study of the samples were conducted with the help of stereomicroscope (Olympus, SZX9) and the keys suggested by Farzanpey (20). Examination of cuticular sculpture and morphology were facilitated using white light and ultraviolet fluorescence photomicrography. Preserved specimens were attached to a dark, non-fluorescent plastic surface. Images were acquired when the light was excited by the 3W Indium Gallium Nitride (InGaN) light emitting diodes (LEDs) emit light within a narrow band in the near UV wavelength range (395–410nm) and 455–470nm in white light posited at the distance of 0.25m from the specimen. Imagines were produced using a digital camera (Nikon DS Camera DS-Fi1) fitted on a Nikon SMZ 1500 stereomicroscope. Biometric measurements were taken with a >0.001mm accuracy by using Digimizer software. (https://www.digimizer.com). Measurements of 27 morphometric characters of adult specimens were adapted from Mirshamsi et al. (21) for subsequent statistical analysis.

Morphometric variables

Abbreviations of morphometric characters include Bl: body length, Ca_L: carapace length, Ca_Aw: anterior width of carapace; Ca_Pw: posterior width of carapace, X: distance between anterior margin of the carapace and anterior margin of median eyes; Y: distance between anterior margin of median eyes and posterior margin of carapace; Mt_L (I–V): length of metasomal segments I–V; Mt_W (I–V): width of metasomal segments I–V; Mt_H (I–

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Published Online: March 31, 2022

V): height of metasomal segments I–V; Tl_L: telson length; Tl_W: telson width; Tl_H: telson height; Ch_L: chela length; M_L: manus length; Mf_L: movable finger length; Pl-L: pectinal left lamella length, and Pr-L: pectinal right lamella length.

Venom extraction and preparation

The crude venom was obtained by electrical stimulation of the telson of scorpion using an electro-pulse stimulator. In summary, one person held the pre-abdomen and telson of a scorpion individual with tweezers to keep it stationary and another person collected the venom from the aculeus of the scorpion. For electrical stimulation of the membrane anterior to the telson, we used the transcutaneous electrical nerve stimulation Tensmed 400 (Arman Poya Co, Iran) connected with modified tweezers to apply power. To improve stimulation and enhance electrical contact, the tweezers were lubricated with glycerin saline gel. The intensity of the stimulation current was adjusted in pulse width from 200–250μs, a pulse rate of 60–80Hz, and amplitude of 12–16v. The extracted venom was collected with a microcapillary glass tube (inner diameter 1.5mm) that was attached to a flexible silicon tube and disposable insulin syringe and transferred into a 1.5ml centrifuge tube. Crude venom was suspended in deionized water, centrifuged (12,000×g, 4 °C, 10min), and the supernatant that contained soluble venom proteins were transferred to a clean 1.5mL tube. Finally, the taken supernatants were immediately lyophilized and stored at -20 ºC until use according to their geographical origins. After milking venom (venom collection), the animals were housed in individual boxes for another milking process.

Measurement of protein concentration

Protein concentrations was determined by absorbance measurements at 280nm and expressed as mg protein/ml. For each venom type, we prepared a solution of venom with a final concentration of 5mg/mL.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of venoms

Electrophoretic analysis of venoms was performed on 18% polyacrylamide gel in the presence of SDS. All samples were dissolved in a sample loading buffer (50mM Tris–HCl, pH 6.8, 1% β-mercaptoethanol, 6% glycerol, 2% SDS, and 0.01% bromophenol blue). For the separation of proteins, samples were run on running buffer (25mM Tris, 192mM glycine, 0.1% SDS, pH 8.3). The molecular mass standard (SinaClon, PR911654) was run parallel to calculate the molecular weights of proteins. A constant electric current of 80mA was applied for 4–5 hours for the migration of proteins. After migration, the gel was stained with 0.1% Coomassie Blue R-250. The gel was then scanned, and the molecular weights of the proteins were calculated.

Results

A total of 98 samples of adult scorpions comprising *A. crassicauda*, *Mesobuthus eupeus Odontobuthus doriae*, and *Scorpio maurus townsendi* were collected from the studied areas. The black fat-tailed scorpion was the most frequent species and included about 70 % (68 scorpions) of the samples. The frequency of the rest scorpions, *M. eupeous*, *Od. doriae*, and *Scorpio maurus*, was 19%, 4%, and 3%, respectively. Samples of *A. crassicauda* were collected mainly in the summer months (Table 1).

Morphological survey

The descriptive statistics (minimum and maximum values) of morphometric characters for each sex of *A. crassicauda* in study groups are presented in Table 2. The schematic view of representative males and females in low and highlands are illustrated in Fig. 2. Analysis of morphometric parameters showed a statistical difference between sexes for the length

of pedipalps, carapace, telson, and pectinal organs. A univariate comparison of morphological parameters showed that these parameters also vary with the local altitude. Female scorpions were presented as larger than males. The values of some characters were generally large in females except for the pectinal features. Small spectrum variation for long, wide, and high metasomal 1-V characters is considered to have minimal scalar overlapping with their homologous character. The body length values of male scorpions in high and lowlands were 72.53±1.53 and 77.33±2.70mm, respectively. This parameter also varied in females in different areas and average body length reached 81.66±2.19 and 86.55±2.33mm in low and highlands, respectively. A comparison of these values showed that the body length of female scorpions in highlands overlapped with the size of males in lowlands.

The values of the pectinal organ as other morphometric parameters depended on scorpion sex and local altitude. Male scorpions showed pectinal lengths of 9.25±0.25 and 9.43±0.32mm in high and lowlands, respectively. Despite the body length, the length of the pectinal organs in female scorpions did not vary and its value was 8.04±0.18mm in both study areas. A Survey of the genital operculum of scorpions showed that it is more oval in males and slightly triangular in females. In addition, the genital operculum is fused in females and split among males.

Carination

The emitted florescent light from different parts of scorpions and cuticular florescent patterns are illustrated in Fig. 3. No significant difference was seen in the number, shape, and intensity of emitted light from carina in the carapace, prosoma metasomal segments, telson, and pedipalps of specimens.

Scorpion venom

All scorpions were observed as being very aggressive during all keeping milking time. A total of 75µl crude venom was extracted from each scorpion sample. A colorless watery secretion was obtained during capturing followed by more viscous milky droplets or ejaculate (mucous accompanied with the venom) during stimulation and did not turn blue after milking. After centrifugation of the whole venom, the supernatant was of a more viscous form.

SDS-PAGE Analysis of the Venom

The protein profiles of *A. crassicauda* venoms were analyzed by SDS-PAGE followed by Coomassie blue staining. In these analyses, 2 different isotypes, each with a different molecular mass, were detected. The number of protein bands for the investigated scorpions in two groups was 9. Out of all protein bands, the bands of 12, 13, 15, 16, 18, 19 and 57kDa consistently appeared in all venom samples (Fig. 3). In addition to the above share proteins, two protein bands of 47kDa in lowlands and 63kDa in highlands were noticed unique in each group, and two bands of 41kDa in lowlands and 46kDa in highlands were not clearly different between two groups (Fig. 4).

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Published Online: March 31, 2022

Table 1. Frequency of collected *Androctonus crassicauda* from different areas of Zanjan Province, northwest of Iran

Studied Areas		Coordinates Latitude and Longitude		Altitude (Meters asl)	Time of collection	Frequency
District	**Locality**					
Zanjan	**Zanjan**	36.698138 N	48.514163 E	1600–2000	Sept, 2016	4
	Gavazang	36.719335 N	48.521419 E		Aug, 2016	4
	Valarood	36.716381 N	48.372671 E		Aug, 2016	4
	Sarimsaghlu	36.759271 N	48.371128 E		Sept, 2016	3
	Do Asb	36.706016 N	48.566930 E		Sept, 2015	4
	Sayan	36.647160 N	48.541718 E		Sept, 2015	3
						3
Subtotal						24
Khodabandeh	**Khodabandeh**	35.940912 N	48.144053 E	1600–1800	July, 2016	1
	Tatardeh	35.956463 N	48.102211 E		Sept, 2015	1
	Khalife	35.994119 N	47.993913 E		July, 2016	1
	Sohrevard	36.074354 N	48.432798 E		July, 2016	1
	Qeshlaq Vakil	36.144121 N	48.046618 E		July, 2016	2
	Garmaab	35.850285 N	48.198390 E		July, 2016	2
Subtotal						8
Mahneshan	**Mahneshan**	36.768985 N	47.668715 E	1200–1500	Jun, 2016	5
	Sari Aghol	36.823192 N	47.629671 E		Jun, 2016	4
	Sahand E Sofla	36.773948 N	47.541359 E		Jun, 2016	3
	Sahand E Olia	36.777957 N	47.519815 E		Sept, 2015	2
Subtotal						14
Tarom	**Chavarzagh**	36.994055 N	48.777488 E	400–700	July, 2016	2
	Daraam	37.024636 N	48.778250 E		Aug, 2016	5
	Haronabad	36.834549 N	49.025299 E		Aug, 2016	4
	Abbar	36.921868 N	48.960540 E		Aug, 2016	3
	Dastjerdeh	36.849681 N	48.943995 E		Sept, 2016	2
	Sansooz	36.835585 N	48.943389 E		Sept, 2016	4
	Tashvir	36.789542 N	49.002457 E		Sept, 2016	2
Subtotal						22
Total						68

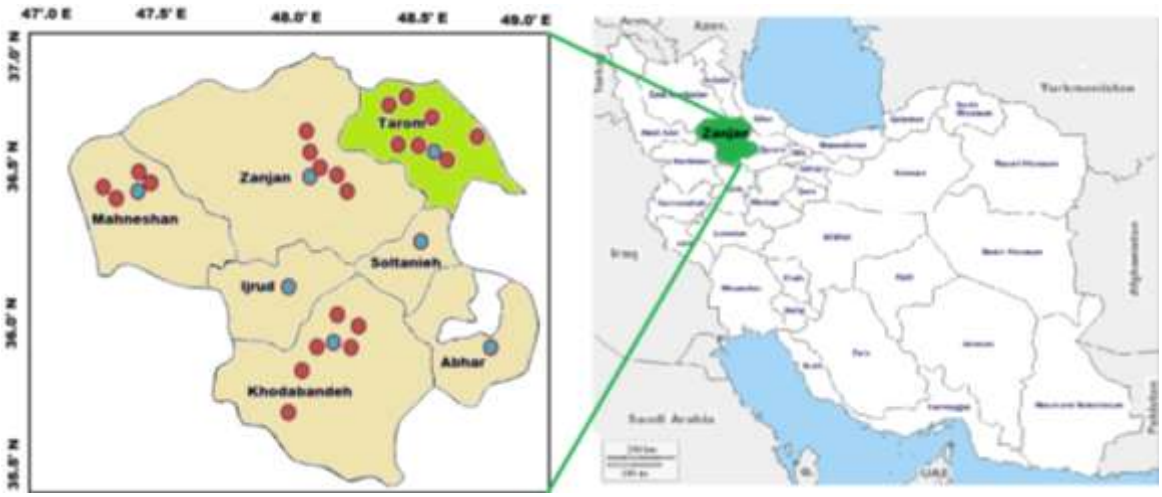

Fig. 1. Map of study areas in Zanjan Province. Locality of collection sites (●), district center (●) lowlands (■) and highlands (■)

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Published Online: March 31, 2022

Table 2. Minimum (min) and maximum (max) values of the morphometric indices (with 95% confidence interval) in specimens of *Androctonus crassicauda* collected from low and highlands of Zanjan Province

Parameter	Lowlands (400-700m asl)				Highlands (1200-2000m asl)			
	♂(n=27)		♀(n=19)		♂(n=12)		♀(n=10)	
	min	max	min	max	min	max	min	max
BL	72.03	82.63	81.98	91.12	69.53	75.53	77.36	85.96
Ca- L	8.50	9.32	9.29	10.93	8.38	9.01	9.29	10.49
Ca-Aw	4.23	5.08	4.87	5.62	4.44	4.93	5.04	5.55
Ca-Pw	8.60	9.69	9.99	11.96	8.55	9.13	9.98	11.21
Mt(I)-L	5.51	6.22	5.85	7.06	5.56	6.24	5.70	6.47
Mt(I)-W	3.58	4.27	3.78	4.80	3.70	4.37	4.05	4.41
Mt(I)-H	4.20	5.10	4.11	5.27	4.16	4.74	4.40	4.94
Mt(II)-L	6.47	7.14	6.86	7.79	6.34	6.97	6.75	7.45
Mt(II)-W	4.17	5.17	4.48	5.67	4.47	5.12	4.69	5.21
Mt(II)-H	4.31	5.13	4.29	5.66	4.28	4.86	4.42	4.99
Mt(III)-L	6.66	7.48	7.14	8.01	6.49	7.06	6.94	7.62
Mt(III)-W	5.11	6.13	5.14	6.29	5.13	5.68	5.33	6.07
Mt(III)-H	4.59	5.34	4.73	5.94	4.56	5.20	4.90	5.50
L Mt(IV)-	7.52	8.59	7.88	9.22	7.62	8.17	7.89	8.60
Mt(IV)-W	5.66	6.67	5.46	6.85	5.54	6.16	5.64	6.43
Mt(IV)-H	4.77	5.42	4.73	5.74	4.56	5.19	5.14	5.48
Mt(V)-L	7.33	8.48	8.37	9.64	7.58	8.21	7.65	8.68
Mt(V)-W	5.26	6.20	5.19	6.57	5.28	5.85	5.42	6.13
Mt(V)-H	2.97	3.46	3.05	3.69	2.92	3.40	3.29	3.72
Tl-L	8.01	9.32	9.23	9.81	7.71	8.72	8.83	9.98
Tl-W	2.94	3.51	3.38	4.22	3.02	3.39	3.63	3.91
Tl-H	2.82	3.33	3.04	3.64	2.64	3.06	3.19	3.58
X	3.44	3.82	3.86	4.49	3.47	3.73	3.94	4.41
Y	5.12	5.73	5.47	6.67	5.01	5.44	5.49	6.18
Ch- L	14.69	15.64	16.41	18.60	14.64	15.93	15.83	17.79
M-L	6.36	7.01	6.91	8.16	6.49	7.24	6.81	7.71
Mf-L	10.43	11.37	11.99	13.02	10.19	11.13	11.56	12.59
Pl-L	8.64	9.89	7.11	8.83	8.94	9.92	7.35	8.36
Pr- L	8.52	9.77	7.25	8.90	8.94	9.88	7.69	8.39

Fig. 2. Schematic view of the representative male and female of *Androctonus crassicauda* in study areas. Male (A, C) and female (B, D) samples in lowland (A, B) and (C, D) highland

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Published Online: March 31, 2022

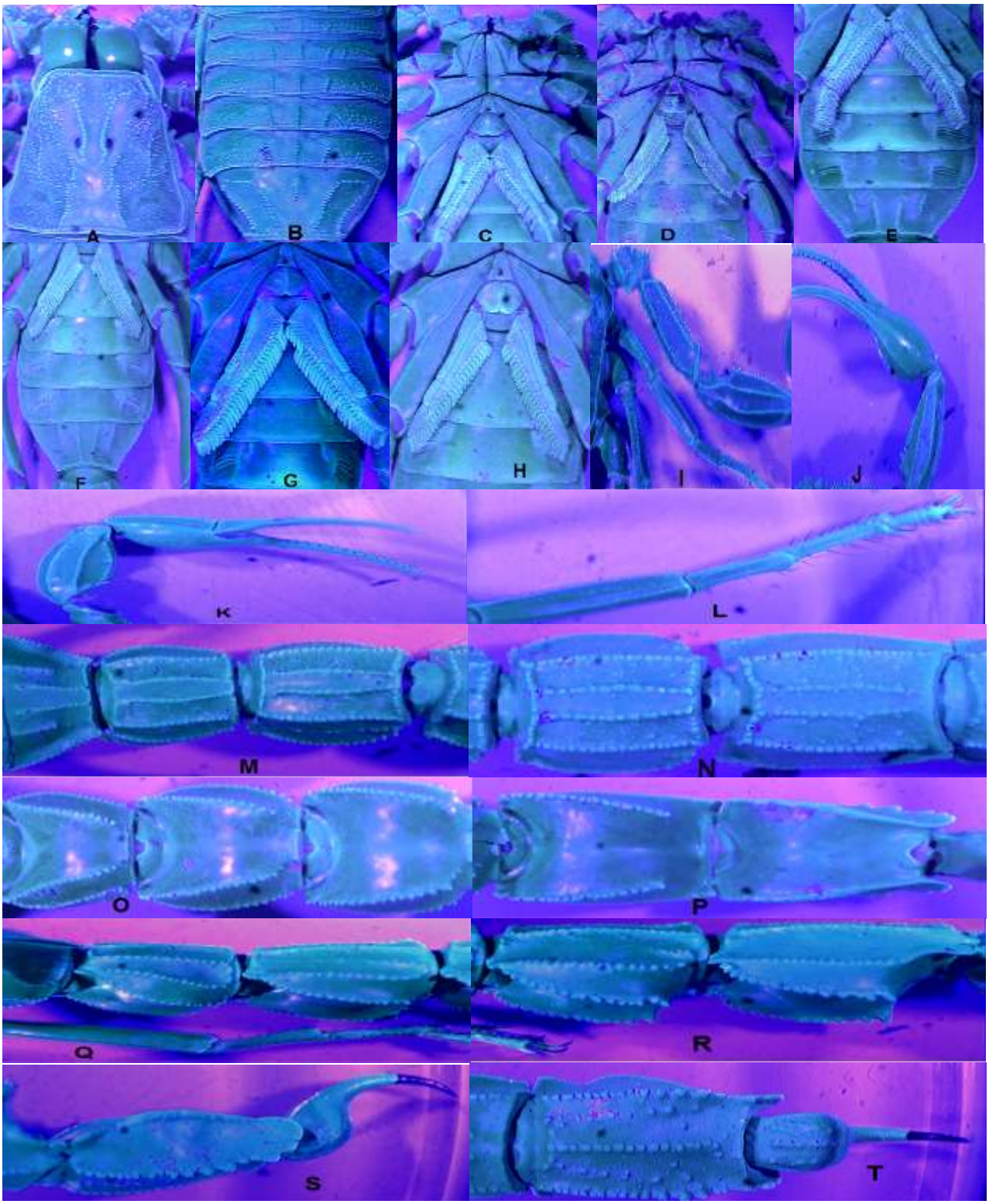

Fig. 3. Schematic presentation of external body parts in *Androctonus crassicauda* under UV fluorescence. Carapace (A); tergite (B); ventral aspects of prosoma in male (C, E, G) and female (D, F, H); legs and pedipalp (I, J, K, L); metasomal segments in ventral (M, N), dorsal (O, P), and lateral (Q, R) aspects; metasomal segment V and telson in lateral (S) and ventral (T) aspects

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Published Online: March 31, 2022

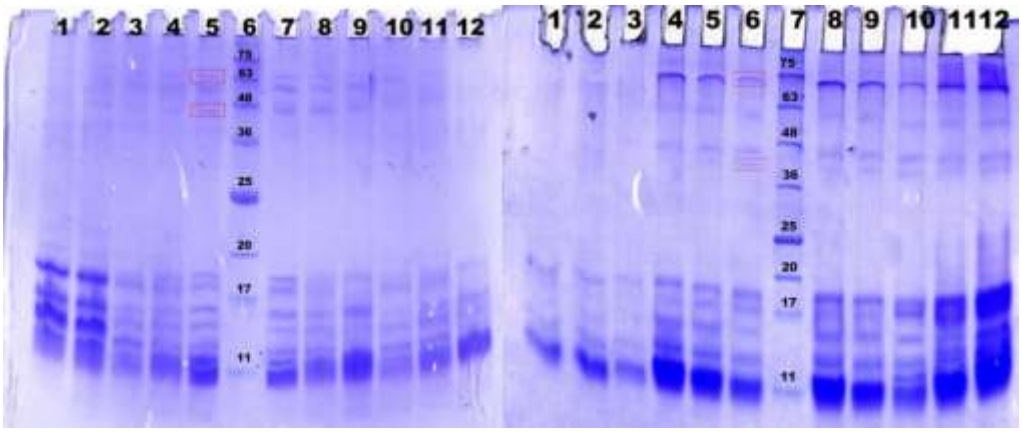

Fig. 4. Electrophoretic profile of *Androctonus crassicauda* venoms on poly acryl amide gel in the presence of SDS. Part **a**: venom obtained from captured scorpions in highlands and part **b**: venom obtained from collected scorpions in lowlands. Lanes 6 in highlands and 7 in lowland are molecular weight (kDa) markers, and the others are venom samples

Discussion

The results of the present study showed that *A. crassicauda* is a dominant scorpion in studied areas and occurs in different conditions. The appearance of this species in study areas reflects the tuning of optimal ecological conditions necessary for its survival in these areas. The presence of this scorpion in these areas calls for the authorities to take the necessary measures to prevent and cure envenomation.

The findings of this study showed that the body size of the specimens is depended on gender and local conditions (altitude). Local dependence on morphometric indices causes both sexes to have a wide range of body lengths. Although body size can distinguish the sex of the same population, geographical distance, and the overlap of the sizes of both sexes can challenge gender discrimination. Therefore, it is suggested that the values of the morphological parameters of each region must be used to analyze the samples of that region. Our data allowed us to evaluate the size variation of *A. crassicauda* with respect to local conditions. Season length is an important ecological factor that may influence the variation of deduced parameters. In high altitudes the average ambient temperature is lower, and the duration of the activity period is shorter than the lowlands. Because the black fat-tailed scorpion is a slowly developing animal, therefore, its encounter in short activity season reduces the growth period, resulting in reduced body size in highlands. Moreover, the patterns in body size variation we found in *A. crassicauda* might be adaptive under the given ecological conditions and food availability in these two lands. In the current study sexual dimorphism among scorpions was performed by morphometric parameters; length of body, carapace, pedipalp, and structure of the pectinal organ. These findings are similar to the results of studies conducted in Turkey and Fars province, southern Iran (7, 8).

Many studies have been carried out on the use of UV light to the finding of scorpions (9, 10), but the present study is the first study, in collecting of scorpions and determining the pattern of carina in different parts of the scorpion. Therefore, the protocol of this study is recommended for the study of scorpions, especially those that have dark tegument.

The findings of this study revealed that *A. crassicauda* in the study areas represented a

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Published Online: March 31, 2022

homogenous species and all the samples had one fluorescent pattern. This process may be related to the presence of selective pressure or specific ecological behavior (sex finding and mating) of specimens. Further studies could solve this problem in different situations.

In the current study, the electrophoretic protein patterns of the black fat-tailed scorpion venom were presented in the range from 12 to 74kDa. Among these proteins, six bands 12, 13, 15, 16, 18 and 19kDa appeared in all samples. Analysis of electrophoretic patterns also indicated that this scorpion possesses other proteins whose molecular weight was more than 40kDa and varied in different strains of a scorpion. Previews studies indicated that venom proteins of *A. crassicauda*, collected from Sanliurfa and Mardin provinces (Southeastern Anatolia) of Turkey, were in six bands with 12, 15, 29, 35, 53, and 58kDa. They showed that 35, 53, and 58kDa proteins played an important immunogenic role in the production of antivenom against this scorpion (22, 23).

Scorpion venoms in the present study were documented into two groups according to their molecular sizes. These groups include the short and long chain neurotoxins with molecular size below 20kDa and the target aging peptides with up 40kDa weight. Among these groups more studies are conducted about neurotoxins, however, there is scant information on the other study groups, and the studies have been limited to their toxicity and immunogenic. The low molecular weight biogenic amines (histamine, dopamine, nor-adrenalin, etc.) found in samples are involved in local reactions and their release from a single sting can lead to systemic reactions. They can act on blood vessels and nerve endings inducing swelling, redness, pain, and itching. The major toxic effects of venom are attributed to the presence of large peptides (4, 6). These peptides can cause damage to the cell membrane, leading to the release of enzymes from lysozymes and mast cell granules, resulting in cytolysis. Additionally, they can act as neurotoxic provoking hyper-excitability. Thus, the individual variability in venoms is extremely important for evaluating the venom yield and the resulting toxicity after a scorpion sting. Further studies are needed to evaluate the toxicity of venoms and the median effective dose (ED_{50}) of scorpion anti-venom against envenomation (24) in various populations of this scorpion in the future.

Analysis of electrophoresis peptide patterns in the present study indicated that *A. crassicauda* venom possesses different peptides both with neurotoxins and target aging according to the electrophoretic protein patterns. Polymorphisms of protein contents also have been observed in the venom of individual *A. australis hector* and *A. mauretanicus* (25, 26). In addition, differences in venom content have been described in other species of scorpions; *M. tamulus* (27), and *Leiurus quinquestriatus* (28). Abdel-Rahman et al. (29) suspected that a combination of local environmental conditions, geographical separation, and genetic separation may play a major role in the intra-specific variation of venom of *Scorpio maurus palmatus*. Moreover, Ozkan and Ciftci (22) indicated that variation of protein bands detected in the venom of captive male *M. gibbosus* from the same biotope in Turkey might result from the physiological condition of scorpions. The peptide variation in different populations of deduced scorpions might result from adaptive radiation to environmental conditions. Nevertheless, this might represent only the tip of the iceberg and the number of novel toxins will still be expanding in the future. Moreover, differences in the band pattern of separated protein in venom samples clearly suggest the existence of genetic variation among the scorpion strains of different regions in the study areas.

Results of this study showed that the molecular pattern of the venom proteins varies in geographical areas. It should be noted that this variation may have a significant role in the venom toxicity of geographical or ecolog-

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Published Online: March 31, 2022

ical groups of scorpions. Due to the effectiveness and toxin neutralizing capacity dependence on intraspecific variations of this scorpion, it is recommended that pooling of the numerous venoms from population groups is used in the production of antivenoms. Obviously, before mixing the crud venoms, it is necessary to identify the biochemical fractions and toxic and immunogenic proteins of each venom in different populations. Moreover, further studies are recommended for the mass fingerprinting of scorpion venoms for barcoding, chemotaxonomy, and screening of effective isotypes in the future.

Conclusion

Different populations of black fat-tailed scorpions have the same fluorescence patterns. However, morphometric parameters and venom electrophoretic patterns of this scorpion vary in different situations. Based on these findings the morphometric parameters of each region are suggested to analyze the samples of that area. Moreover, additional complementary field works in the GIS ecological niche model together with the mass fingerprint of scorpion venoms are recommended for a screening of effective isotypes in future studies.

Acknowledgment

This work was supported by research project no. A-12-84-8 offered by Vice-Chancellor for Research and Technology of Zanjan University of Medical Sciences. The authors are grateful to Mr Taghiloo and Mr Mohammadi for their valuable assistance during the surveys and samplings.

Ethical consideration

Ethical approval for this study was obtained from the Ethics Committee at Zanjan University of Medical Sciences (Iran).

Conflict of interest statement

Authors declare that there is no conflict of interest.

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