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Identification key for Echis snakes (Serpents: Viperidae) of the East, South East, and South West of Iran

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Abstract

Presented data are on the morphological characters of medically important *Echis* viper (Merrem, 1820) from three populations collected from Sistan- Baluchistan (Zabol)-Southeast, South Khorasan (Ghaen)-East and Khuzestan (Aghajari)-Southwest of Iran. Using 6 morphological, 14 meristic and 4 morphometric characters for morphological comparison. Results show the three snake populations are differentiated on the bases of cluster analysis of morphological and morphometric characters ($P \leq 0.05$) and populations of Zabol and Ghaen are closely related as illustrated by dendrogram. The number of ventral scales, subcaudal scales, dorsal scales, and the oblique lateral scales in the three viper's population were also significantly different ($P \leq 0.05$). On the bases of this study identification key was codified for *Echis* of East, SouthEast and South West populations of Iran.

Reviewing the *Echis* viper populations across their distribution ranges, may leads us to finds their differences and recommend the use of venoms of different populations to improves the antivenin's efficacy and helps the better treatment of envenomation.

Keywords: *Echis* viper, morphology, venom proteins, electrophoresis, Iran

1. Introduction

Echis viper (Merrem, 1820) is from Viperidae family. It is an invasive venomous snake with a vast global distribution and massive populations, which featured this taxon as one of the world's most dangerous snakes. There are many debates on taxonomy of *Echis*. Klemmer^[1] recognized only two species: *Echis carinatus* in most of the range and *E. coloratus* in Arabia. Joger^[2, 3] added *E. pyramidum* for southwestern Arabia. Cherlin^[4] postulates that the genus *Echis* originated from the Irano-Turanian region in Asia and then radiated to the west across Mesopotamia, then forking to Oman and to northwestern Arabia and Africa. Cherlin^[4] distinguished three subgenera within the genus *Echis*: *Echis*, *Turanechis* and *Toxicoa*, which are not congruent with current molecular and morphological data^[5]. Later Cherlin^[4] recognized three species within *Echis* group, as defined, *E. carinatus* (southern India, Sri Lanka), *E. sochureki* (northern India to Pakistan), and *E. multisquamatus* (Central Asia, Iran). Auffenberg and Rehman^[6] found clinal variation across the range of the group, and considered all populations as subspecies of *E. carinatus*, and Lenk *et al.*^[7] found very low levels of divergence between *E. carinatus sochureki* from Pakistan and *E. multisquamatus* from Turkmenistan. By contrast, other researchers argue on the taxonomy of *Echis* vipers recognized ten to twelve species with 20 subspecies for this taxon^[8, 9, 10]. On the bases of comprehensive study by Stümpel and Joger^[11] and also Khan^[12] on the taxonomy of *Echis* from the East Asia (Near East) and Southwest Asia (Middle East), the number of species known to be active in Southeast Asia should be changed from 2 to 6 species. These species are classified into three different groups, including Asian, African, and an Arabic variety, and a variety of snakes were discovered in southern Arabia. *Echis carinatus* group (Asian group): *E. carinatus sochureki* (Oman, UAE, Iran, Central Asia, Afghanistan, and Pakistan). *Echis coloratus* group (Arabian group): *E. coloratus* (Egypt, Arabian Peninsula) and *E. omanensis* (Oman, UAE). *Echis pyramidum* group (one of two African groups): *E. pyramidum* (Egypt, Sudan, East Africa), *E. khosatzkii* (Western Oman, Yemen) *E. sp. (cf. borkini)* (Yemen, SW Saudi Arabia) and *E. borkini* was originally described as a subspecies of the East African *E. varia* by Cherlin^[4]. Studies on the *Echis* from Iran revealed two species *E.c. pyramidum* and *E.c. sochureki* exist in the southern and central parts of Iran^[13].

Bagheri and kami ^[14] reported two subspecies of *Echis carinatus multisquamatus* Cherlin, 1981 from the eastern and northeast regions of Iran and *Echis carinatus sochureki*, Stemmler, 1969 from southern of Iran. In the present study we try to make an identification key for *Echis* vipers that are used in our institute for production of antivenin in order to find if there are variation between populations of different geographical area of Iran.

2. Materials and Methods

A total of 4 morphometric characters, 14 meristic characters (counting traits) (Table 1) and 6 multivariate qualitative characters (morphological traits) were evaluated in 21 samples of *Echis* snakes. Samples were collected from three areas of Zabol (Sistan and Baluchistan Province), South East Iran, Ghaen (Southern Khorasan Province), East of Iran and Aghajari (Khuzestan province) southwest of Iran. Morphological characters were measured according to Bagherian and Kami ^[14] and Latifi ^[15]. Normality of qualitative characters were estimated by Shapiro-Wilk and also Kolmogorov-Smirnova and for Non-parametric data were tested by Kruskal-Wallis. Data were analyzed and compared by ANOVA, SPSS (vs15) and cluster analysis and dendrogram was illustrated to show the relationship between the populations.

3. Results and Discussion

3.1. Morphology of characters

In this study, we studied the morphological traits of the *Echis* populations collected from three provinces: Sistan and Baluchistan (southeast), Southern Khorasan (East) and Khuzestan (southwestern) in Iran (Figures 1-3). The results of 6 morphological, 14 meristic and 4 morphometric characters in 21 snakes from three provinces showed that the Iranian *Echis* snake was relatively small in size, and the adult's body length were lesser than one meter. Head length was short, broad, pear-shaped and distinct from the neck, and muzzle were short and round, while the eyes were fairly large and protruding and the pupil was vertical. Their heads were covered with small and irregular scales, their body was relatively narrow and cylindrical. A short tail with a narrow tip, a single anal plate, and an undivided subcaudal scales was seen. Statistical analysis showed that there were significant differences between four morphometric characters of body length and tail length and head width in *Echis* snakes from three provinces ($P \leq 0.05$). In four meristic characters; there were significant differences in the number of ventral scales, subcaudals, dorsals and lateraloblique scales in the three populations ($P \leq 0.05$). There were no significant differences in the other ten meristic characters (Tables 1).



Fig 1: Echis Snake from Sistan Baluchistan Province, south east of Iran



Fig 2: Echis Snake from South Khorasan Province, East of Iran



Fig 3: Echis snake from Khuzestan Province, Southwest of Iran

Table 1: Descriptive statistics of morphometric and meristic characters in Echis snakes from East, Southeast, and Southwest of Iran

Characters	Population	N	Mean±SE	F value
SVL	1	10	45±1.24	17.868*
	2	5	54.8±2.8	
	3	6	39.8±0.47	
	Total	21	45.8±1.4	
TL	1	10	4.2±0.16	23.627*
	2	5	5.1±0.18	
	3	6	3.3±0.4	
	Total	21	4.2±0.16	
HL	1	10	5.3±1.2	3.861*
	2	5	2.6±1.6	
	3	6	2±0.8	
	Total	21	2.3±0.8	
HW	1	10	1.7±0.7	7.898*
	2	5	2.1±1.7	
	3	6	1.5±0.1	
	Total	21	1.7±0.8	
Ventrals	1	10	1.7±1.2E2	11.115*
	2	5	1.8±2.7E2	
	3	6	1.7±0.5E2	
	Total	21	1.7±1.2E2	
Subcaudals	1	10	32.3±0.7	4.754*
	2	5	32.6±0.6	
	3	6	29.5±0.4	
	Total	21	31.5±0.5	
Dorsals Anterior	1	10	27±0.3	14.518*
	2	5	26.8±0.2	
	3	6	22.5±1.1	
	Total	21	25.6±0.5	
Dorsals mid body	1	10	32.3±0.4	13.974*
	2	5	35.4±0.6	
	3	6	30±0.8	
	Total	21	32.3±0.5	
Dorsals Posterior	1	10	21.3±0.2	16.536*
	2	5	23±0.3	
	3	6	20.5±0.3	
	Total	21	21.4±0.2	
Oblique Laterals	1	10	5.4±0.3	6.848*
	2	5	7±0.4	
	3	6	5±0.2	
	Total	21	5.7±0.3	
RSL	1	10	11.1±0.1	1.598
	2	5	11.6±0.4	
	3	6	11.1±0.1	
	Total	21	11.2±0.1	
LSL	1	10	11.1±0.1	2.234
	2	5	11.6±0.2	
	3	6	11.3±0.2	
	Total	21	11.2±0.1	
RIL	1	10	12.1±0.2	.524
	2	5	12.4±0.2	
	3	6	12±0	
	Total	21	12.1±0.1	
LIL	1	10	12±0.2	1.148
	2	5	12.4±0.2	
	3	6	12.5±0.2	
	Total	21	12.2±0.1	
RSAE	1	10	15.7±0.6	2.075
	2	5	14.6±0.5	
	3	6	16.5±0.3	
	Total	21	15.6±0.3	
LSAE	1	10	15.6±0.3	1.922
	2	5	14.6±1.3	
	3	6	16.6±0.4	
	Total	21	15.6±0.3	
Between Eyes	1	5	9±0.4	2.308
	2	5	10±0.3	
	3	6	9.6±0.2	
	Total	16	9.5±0.2	
SBES Lon R	1	5	1.2±0.2	2.533
	2	5	1.6±0.2	
	3	6	1.8±0.1	
	Total	16	1.5±0.1	

Svl. Snout-vent length, TL. Tail length, HL. Head length, HW. Head width, RSL. Right Supralabial scales, LSL. Left Supra labial scales, RIL. Right Lower labial scales, LIL. Left Lower labial scales, LSAE. Left circumocular scales, SBES Lon R. Between right eye and supralabial scales. Asterisk mark comparison between characters in the three snake populations with significant differences ($P \leq 0.05$).

The body color pattern of dorsal in the Aghajari snake was brown in the background at regular intervals with black blocks and large transverse white spots, the ventral color pattern was cream or bright brown with black spots (each ventral band has 3 to 5 black spots). The color pattern of the dorsal of the Zabul and Ghaen snakes were brown background, and at regular intervals, have large white spots, and enclosed by large black spots in a zigzag, or white spots surrounded by black spots, The ventral were bright and the ventral bands were either without spots, or in case of Ghaen, each band had 1-2spots. At the lateral of Zabul and Ghaen Echis, there was a white line in the sinusoidal waves that creates a narrow, intermittent wave pattern, but Khuzestan Echis either did not have a white zigzag line, or it was discrete. In the cluster analysis, three populations were separated from each other in terms of morphological characters, and Echis population of Zabul had a close relationship with Ghaen (Tables 2 and 3, Dendrogram 1).

The pattern on the top of head of Echis snake was always distinguishable and its patterns were varied. In the samples of the Echis in this study, the pattern of head of Zabul (Fig. 4A) and Ghaen (Fig. 4B) were observed in a narrow cross shape without spots and head mark in Aghajari Echis was broad cross shape with spots (Fig. 4C). These markings vary from an arrow to a cross-shaped mark in Echis population from Iraq^[16]. These markings in previous study Iranian Saw-scaled Viper vary from the arrow mark, the broad cross and narrow cross^[16].

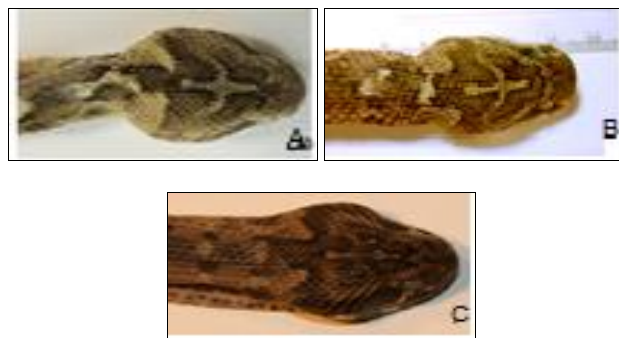


Fig 4: Pattern at the top of head in Echis Zabul (A) Ghaen (B) and Aghajari (C)

Lateral body color pattern in saw-scaled viper reported from Iraq^[16] (A): Darkground color, zigzag present, no evidence of spots row I and II; (B): Dark ground color, crescents present spots row I absent, II evident; (C): light ground color, very dim zigzag, spots rows I and II both evident.(intermediate); and in Iranian Echis reported by Rhadi *et al.*^[16] (D): Dark ground, crescents present, faint traces of spot row I and II (intermediate); (E): Dark to light ground color, bright narrow zigzag, no evidence of spot row I, faint trace of spot row II. (F): Medium ground color, crescents present, and spots of rows I and II are absent.



Fig 5: Lateral patterns of Echis from Zabol (A) Ghaen (B) and Aghajari (C)

Variety has been reported in the ventral pattern and color in Iranian Echis. In the Echis of Zabol, GHaen and Khozestan

ventral patterns were similar to the report of Rhadi *et al.* [16]



Fig 6: Ventral patterns of Echis from Zabol region without spot (Aa) or 1 small spot (Ab), Echis from Ghaen vent with 1-2 deep and uniform spots (B), Khozestan area vent marked with 2-5 dark spots (Ca-Cb).

Ventral pattern varieties are presented in saw-scaled viper from Iran; (A) Uniform, or nearly so, no obvious pattern; (B) markings very dim small spots; (C): dim, smallspots; (D) dim, small, high intensity spots; (E): medium intensity, large markings; (F): darker, larger markings (deeply pigmented); (G): deeply pigmented, high intensity spots, dark smallmarkings; (H): dim, small spots medium intensity; (I): larger markings, dim, high intensityspots; (J): deeply pigmented, medium intensity large spots [16]. In *Echis omanensis* from Oman, Eastern Arabia, head dorsally usually uniform gray, rarely a dark X shaped marking or, in young specimens, marked by small spots; facial band covers from 36 to 44 temporal scales in males and 34–49 temporal scales in females, facial band often abruptly ends before the post-mandibular blotch, not merging with it; usually barely visible dark bands below the eye and anterior to the nasal in adults ; mostly moderately dark infralabial blotches in adults; dark, sometimes faded mid-dorsal band 5–6 dorsal scale wide connecting the dorsal blotches [17]. Morphological characters in *E. coloratus* explained by Babocsay [18], ventrals 175–204 in males, 175– 210 in females; subcaudals 45–56 in males, 40–50 in females; nasal almost never fused with the upper prenasal lower pre-nasal usually present; 3.5– 7.5 scales between the chin-shield and the pre-ventral; conspicuously elongated gulars along the two sides of the mid-line of the throat. Head dorsally usually uniform gray, mostly with an X-shaped marking, rarely spots; facial band covers from 17–38 temporal scales in males and 17–35 temporal scales in females, facial band often abruptly ends before the post-mandibular blotch, mostly merges with it; usually dark bands below the eye and and often fuse with each other or with the

facial band in adults; usually contrasting infralabial blotches in adults; usually missing or narrower mid-dorsal band [17, 18]. There were not similarity between these morphological characters with our observations. Moreover, the variation in different characters from *E. carinatus sochureki*, ventrals 154– 166 in males, 163–169 in females; subcaudals 30–34 in males, 27–33 in females; scale-rows between the eye and the supralabials 1–2 [18], that also somehow differs from our results.

In Jordan, Echis genus is represented by the single species *Echis coloratus*, abundant in the steep, dry rocky hillsides of the mountains which surround the Jordan Valley and Wādī ‘Araba [17, 18]. Morphological study of this taxa by Amr & Disi [20] show, Head very distinct from neck, Three to four scale rows between the eyes and upper labials 12 – 15 UL, 13 – 15 LL. Nostril is in a single or divided nasal and a series of scales separates the nasal from rostral. Scale rows 31 – 37, VS 152 – 205, CS 44 – 57. Anal scale entire, subcaudals single. Males are larger than females and have longer tails. Maximum length 83 cm. Coloration, ground color quite variable: yellowish-gray or brownish-gray, but may be reddish-brown or pink in areas of red sandstone or granite. On the back, there is a row of grayish-white, elongate rhomboid blotches or crossbars with dark edges. Head without distinctive marks, except a brownish gray band from the nostril to the edge of the mouth. The light dorsal blotches may have a narrow dark border. The pattern of orientation of the blotches and crossbars varies even on the same animal. On the side of the body, there is a row of brownish blotches [19]. These morphological characters is also different from our results.

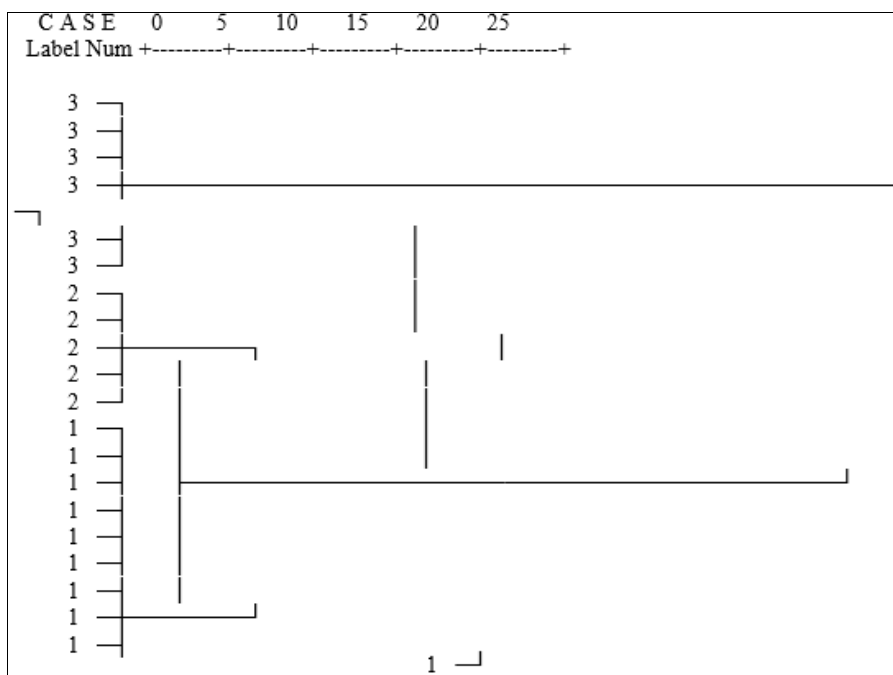
Table 2: Analysis of the normalization of qualitative data related to body pattern and color

Characters	Kolmogorov-Smirnov			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Dorsum Color	.446	21	.000	.570	21	.000
Ventral Spots	.446	21	.000	.570	21	.000
Lateral Sinus Line	.446	21	.000	.570	21	.000
Ventral Color	.348	21	.000	.640	21	.000
Color of Ventral Spots	.446	21	.000	.570	21	.000
Color Pattern on Head	.446	21	.000	.570	21	.000

The qualitative traits of the pattern and body color of the snake were significantly different among the studied populations ($P < 0.05$).

Table 3: Nonparametric test, Kruskal-Wallis, for qualitative traits of the pattern and body color among the populations of Echis snake

Statistics/Characters	Dorsum Color	Ventra Spots	Lateral Sinus Line	Ventra Color	Color of Ventral Spots	Color Pattern On Head
Chi-Square	20.000	20.000	20.000	20.000	20.000	20.000
df	2	2	2	2	2	2
Asymp. Sig.	.000	.000	.000	.000	.000	.000



Dendrogram using Average Linkage (Between Groups), Rescaled Distance Cluster Combine. 1= South east population, 2=East population, 3= South west population

Dendrogram 1: Dendrogram of morphological characters the pattern and color of the body of three different populations of Echis of Iran

According to the results of this study, three populations were distinguished on the bases of the morphological data analysis. The Zabol population was closely related to the Echis of Ghaen, and the identification key for Echis from Sistan Baluchistan (Zabol) Southeast, Southern Khorasan (Ghaen) East and Khuzestan (Aghajari) Southwest of Iran were codificated as follow.

The identification key for Echis from three populations of South Khorasan, Sistan -Baluchistan and Khuzestan, Iran

1. With sinusoidal continuous white line in the body lateral, and a narrow cross-shaped mark on head without spot 2
 - Without a sinusoidal continuous white line in the body lateral or, if present, discrete crescent, and a broad cross-shaped mark on head with spot 4
2. Each ventral plate either without spot or with 1-2 dark spots and body length less or more than 60 cm 3
 - Each ventral plate has 3-5 dark spots and body length up to 60 cm 4
3. Dorsal scales in the middle of the body less than 34 rows and body length up to 60 cm Population of Sistan Baluchistan (Zabol)
 - Dorsal scales in the middle of the body 31-37 rows and body length more than 60 cm Population of South Khorasan (Ghaen)
4. Dorsal scales in the middle of the body 28-33 rows and body lengths up to 60 cm Population of Khuzestan (Bushehr)
 - Dorsal scales in the middle of the body 29-32 rows and body lengths up to 60 cm Population

of Khuzestan (Aghajari)

4. Conclusion

Echis from three populations Southwest, Southeast and East of Iran were distinguished on the bases of the morphological data. The Southeast population was closely related to the Echis of East.

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