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SEXTUAL DIMORPHISM IN *Gloydius halys* (VIPERIDAE: CORTALINAE) OF CENTRAL ALBORZ MOUNTAINS OF IRAN

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ABSTRACT

We described the taxonomic relationship of pitvipers population of Central Alborz mountains of Iran. The morphological data on *Gloydius halys* snakes; 8 female and 11 male snake from three Lar, Taleghan and Gachsar regions of Central Alborz Mountains Iran, showed sexual dimorphism. The results of independent samples test showed that body length (SVL) in male was 48 ±10 cm that was more than SVL in female, 43 ± 6 cm (P≤0.05), head length (HL) in male was 2.2 ± 0.3 cm and more than females, 1.9±0.2 cm at P=0.01. The mean height of the head (HH) was 0.95 ± 0.04 cm and more than 0.52 ± 0.70 cm, respectively (P = 0.04). The size of the Sq front in male snakes was 22.6 ± 0.8 and 21.7 ± 0.7 in female (P = 0.02), Ciruocular right in male was 5.6 ± 0.5 and in female snake 5.13 ± 0.3 (P = 0.02), the size of the CG trait for male and female was respectively 9.6 ± 0.8 and 11.1 ± 0.8 at P = 0.007, the size of the ventral scales number for males was 153.7 ± 4.2 and for females 148 ± 5.9, P = 0.03, and the subcaudal for male was 37.18 ± 1.5 and females 32.7±1.9, (P≤0.05). Also, in the multivariate analysis of the main components, the separation of male and female samples is evident. In the geographical variation, there was a significant difference in the seven characters between different populations, and in the hierarchical clustering analysis, the separation of three populations of Lar, Taleghan and Gachsar was observed. Result reveals the geographical differences in *Gloydius halys* of Central Alborz mountains of Iran and tow populations from Lar and Takht –E-Solyman have close relations and somehow distinct from halys of Gachsar population and dimorphism was observed in female and male of all three populations.

Keywords: *Gloydius halys;* sex dimorphism; morphology; Iran pitviper; geographical variation.

ABBREVIATIONS

Svl : Snout-vent length,
TL : Tail length,
HL : Head length,
HW : Head width,
Supl : Supralaibial scales,
Infrl : Lower labial scales,
PrOc : Pre ocular scales,
PoOc : post ocular scales,
CircuOc : Circumocular scales,
CG : Gular,
A ch infrl : Between chin and lower labial,
Vent : Ventral,
Scd : Subcaudal,
Temp : Temporal.

INTRODUCTION

Herpetologists classify pit vipers in Viperidae family and Crotalinae subfamily. All species of this family are venomous and dangerous for humans, and often produce hemotoxic or cytotoxic venom, and have therapeutic and research value as a biological product in medical and biomedical fields. The *G. halys* [1] is a pitviper occurs in Russia, the eastern Ural Mountains to Siberia, Iran, Mongolia to the north and center of China, as well as the southern Riviera of Japan. According to Gloyd and Conant [2], the location of the type specie is salt lake near Lugaskoi Sawod (factory) in Upper Yenisey (Siberia, Russia), and according to a review by Bour [3] in the Naryn or Ryn Peski desert, it is near the border with Russia and Kazakhstan.

The Caucasian snake is only pitviper of Iran and its distribution rang is in different parts of the Alborz mountains (Semnan, Tehran, Alborz, Qazvin, Gilan, Mazandaran, Golestan, North Khorasan and Khorasan Razavi provinces) [4,5,6] and is habitant in various habitats, including tropical forests with a height of 3,000 meters above sea level and high steppe mountains [7].

Recently, 13 species of Gloydius are introduced: *Gloydius blomhoffii* [8], from China, Korea, Japan and the Far East of Russia [9]; *Gloydius brevicaudus* [10]; from China, North Korea and South; *Gloydius halys* [1] from Azerbaijan and Iran to several Central Asian countries to Eastern Siberia, Mongolia and China, *Gloydius himalayanus* [11], Northeastern Pakistan, along the Southern hills of Himalaya to Northern India and Nepal, *Gloydius intermedius* [12], according to Orlov and Barabanov [13], is limited to the Far East of Russia, Northwest China, and the Korean Peninsula, however, according to Gloyd and Conant [2], and Ananjeva et al. [14] *G. intermedius* is distributed in Southeastern Azerbaijan, all over North of Iran, South of Turkmenistan and Northwestern Afghanistan to Northwest China and Mongolia, while *G. saxatilis* is described by Orlov and Barabanov [13] as the synonym of *G. intermedius*, from Eastern Siberia to Northeastern China North and South Korea; *Gloydius lijianlii* species [15], are native to the island of Dhishan, China; *Gloydius liupanensis* species [16]; Only in its locus of type in the Lipan mountains of China; *Gloydius monticola* [17] is known only from the mountains in Northern Yunnan, China*; Gloydius shedaoensis* [18] is native to Shedao Island, China; *Gloydius strauchi* [19] known from the Tibetan plateau (Tsinghai = Quinghai) and the West (Szechwan = Sichuan), China; *Gloydius tsushimaensis* [20] is native of Tsushima Island of Japan; and *Gloydius ussuriensis* [21] from the Far East of Russia to the Northeast of China to North and South, also to Quelpart Island.

Uetz and Hosek [22] classified the *G. halys* into eight subspecies: *Gloydius halys affinis* [23]; *G. h. boehmei* [24]; *G.h. caraganus* [25]; *G. h. caucasicus* [26]; *G. h. Cognatus* [25]; *G.h.halys* [1]; *G. h. Mogoi* [3]; *G. h. Stejnegeri* [27] and *G. h. caucasicus* [26] in Southeast Azerbaijan (Talesh mountains), South Turkmenistan (Kopt Dog Mountain), Northern Iran and Northwest Afghanistan [26,28,29,30,14]. Morphological studies in eight Gloydius species show a considerable variation in the size and other characters of this taxon [2,13].

The report of Ramezani et al. [31] on the morphological study of the Caucasian viper in Gorgan, Iran showed that the *Gloydius halys* have a triangular head, a visible neck, a nose located above, and somewhat prominent. The eye pupil is horizontal. The 3 identified specimens had 23 dorsal scales.

The body color was brown from the back of the neck to the end of the body with some small brown spots. The sides are bright brown with dark spots, with a distance between them and the entire ventral surface is much darker with small brown spots. There is a brown chocolate line from the back of the eye to the end of the mouth. Under threat, this snake is ready to bite with a twist and forward facing body, and like rattlesnake tail sounds to scare the enemy, but it moves slowly and calmly. Found around the forests, meadows, steppe areas and near residential areas in Gorgan [31].

Comparison of morphological characters in *Gloydius halys caucasicus* populations from Western, Central and Eastern Alborz provinces of Iran by Khani et al. [32] showed that these three populations are different, and the Western Alborz population has the most distinction with Eastern Alborz. The distinctive characters included sudcaudals, interior and posterior dorsal scales, supralabials, gular, snout length- width ratio, and snout- head length ratio [32].

The Iranian *G. halys* specimens formed a cluster with *G. halys* from Kazakhstan, but molecular phylogeny did not strongly support this relationship [33]. The separation of Iran pit viper from *G. halys* was not supported by monophyletic criteria or genetic distance [33]. The distribution of Iranian *Gloydius* is currently restricted to the Alborz and Kopet Dagh Mountains, supporting this hypothesis [4].

Despite the intensive reviews on taxonomy of pit vipers, still revision is needed to clarify the relationships among Gloydius species. So in this study morphological status of pitviper populations of Central Alborz Mountains of Iran was identified.

MATERIALS AND METHODS

Morphological Characters

Caucasian snake samples, 8 females and 11 males were collected from Lar (Tehran-Mazandaran), Taleghan (Alborz province), and Gachsar (Alborz province), Centeral Alborz mountains, Iran, and fixed in alcohol after being cured. The 10 morphological and 24 meristic characteristics were studied and recorded in the forms based on the identification keys [34,4]. The results were analyzed and compared by ANOVA, SPPS v.15. Cluster analysis was used and dendrogram was drawn.

RESULTS

Morphology of Central Alborz mountains *G. halys*

The *Gloydius halys* snakes from Central Alborz Mountains of Iran are shown in Figs. 1-3. Their morphological characters are presented in the table1 and compared on the bases of sexes of male and female.

Fig. 1. Lar Caucasian snake's dorsal (A) and ventral pattern (B)

Sexual Differences in Metric and Meristic Characters in *Gloydius halys*

Morphological study in eight female and eleven male halys snakes from Central

Alborz mounts, Lar, Taleghan and Gachsar areas reveals sexual dimorphism on the bases of 10 main characters. The results showed that average body length (SVL) was 48 ± 10 cm in males and 43 ± 6 cm ($P \leq .05$) in females; head length (HL) in males was 2.2 ± 0.3 cm that was more than females, 1.9±0.2cm; mean height of the head (HH) was 0.95 ± 0.04 cm in males and more than in females, 0.25 ± 0.70 cm ($P = 0.04$). The size of the Sq front in males was 22.6 ± 0.8 and 21.7 ± 0.7 in females ($P = .02$), Ciruoc-R in males was 5.6 ± 0.5 and in female snakes 5.5 ± 0.3 ($P = 0.02$), the size of the CG was 9.6 ± 0.8 for males and 11.1 ± 0.8 for females ($P = .007$), the number of ventrals for males 153.7 ± 4.8 and females 148 ± 5.9, ($P = .03$) and the mean scd scales for males and females was respectively 37.18 ± 1.5 and 32.7 ± 1.9 and differ at $P \leq .05$, (Tables 1& 2).

Fig. 2. Takht-e- Solyman Caucasian snake's dorsal (A) and ventral pattern (B)

Fig. 3. Gachsar Caucasian snake's dorsal (A) and ventral pattern (B)

Main Distinctive Components Analysis

The first four components have obtained 89.41 percent of the total variance (Table 3). SL, ventral and dorsal pattern have the greatest effect on the formation of the first component. The Sq front and Circuoc-R had the greatest effect on the formation of the second component, and the TL and CG had the greatest effect on the formation of the third component and the HH and Scd had the greatest effect on the formation of the fourth component (Table 4). The figure of the main components indicates the sex morphological differences in the pit viper of Iran on the bases of first and second components that obtained 73.1% of the total variance (Table 2, Fig. 4). The results of single-variable analysis (independent t-test) of sex dimorphism also showed that all examined characters were significantly different in males and females (Table 1). Also, in the analysis of the multivariate analysis of the main components, the separation of male and female samples was evident (Fig. 4).

Geographical Differences among *Gloydius halys* Central Alborz Populations

The geographical variation was observed on the bases of significant difference of seven characters of SVL, HL, HH, Sq front, Circuo-Right, CG and ventral between three populations (Table 5). In the hierarchical clustering analysis, the separation of three populations of Lar, Takht-E-Solyman and Gachsar is also apparent (Fig. 5), may because of the genetic distances that is under study.

Hierarchical cluster analysis shows the separation of the three populations of Lar, Takht-E-Solyman and Gachsar, and shows the intra-species variation also specially between Gachsar and two other populations (Fig. 5).

Table 1. Descriptive statistics of morphometric and meristic characters in *Gloydius halys* from Central Alborz Mountains of Iran (Mean±SD)

Characters	Male		Female	
	N	Mean	N	Mean
TL	11.00	5.9±0.7*	8.00	5.1±0.4*
HH	6.00	0.59±0.04*	8.00	0.52±0.07*
SL	6.00	2±0.2*	8.00	1.7±0.2*
SVL	11.00	48±10.6*	8.00	43.9±6.8*
HL	11.00	2.2±0.3*	8.00	1.9±0.2*
HW	11.00	1.4±0.1	8.00	1.3±0.1
SW	6.00	0.65±0.08	8.00	0.6±0.03
Sq.Front	11.00	22.6±0.8	8.00	21.7±0.7
Sq.Mid	11.00	22.9±0.3	8.00	23±0
Sq.Hind	11.00	17.9±1	8.00	17.2±0.7
Sq.Anal	-	-	5.00	36±1.6
SupL.Right	11.00	7.9±0.8	8.00	7.9±0.3
SupL.Left	11.00	7.8±0.8	8.00	7.7±0.4
InfrL.Right	11.00	11±0.6	8.00	10.9±0.6
InfrL.Left	11.00	11±0.6	8.00	11.2±0.4
PrOc.Right	6.00	2.5±0.5	8.00	2.1±0.3
PrOc.Left	6.00	2.5±0.5	8.00	2.1±0.3
PoOc.Right	6.00	1.5±1.6	8.00	1.6±1
PoOc.Left	6.00	1.5±1.6	8.00	1.6±1
CircuOc.Right	11.00	5.6±0.5	8.00	5.1±0.3
CircuOc.Left	8.00	5.9±0.3	1.00	6±0
CG	6.00	9.7±0.8*	8.00	11.1±0.8*
A.ch.infrl.Right	6.00	1±0.1*	8.00	0.25±0.7*
A.ch.infrl.Left	6.00	1±0.1*	8.00	0.25±0.7*
Po.ch.infrl.Right	6.00	2±0	8.00	1.8±0.3
Po.ch.infrl.Left	6.00	2±0	8.00	1.8±0.3
Vent	11.00	153.7±4*	8.00	148.3±6*
Scd	11.00	37.2±1*	8.00	32.7±1.9*
Tem.A.Right	6.00	2±0	8.00	2±0
Tem.A.Left	6.00	2±0	8.00	2±0
Tem.P.Right	6.00	4±0	8.00	4±0
Tem.P.Left	6.00	4±0	8.00	4±0

SVL. snout-vent length, TL. tail length, HL. head length, HW. head width, Supl. supralaibial scales, Infrl. lower labial scales, PrOc. pre ocular scales, PoOc post ocular scales, CircuOc. circumocular scales, CG Gular, A ch infrl. Between chin and lower labial, Vent. Ventral, Scd. subcaudal, Temp A. anterior temporal, Temp P. posterior temporal

Table 2. Independent t-test between male and female snakes

Independent samples test						
Characters	**Variances**	**Levene's test for equality of variances**		**t-test for equality of means**		
		F	**Sig.**	**t**	**df**	**Sig. (2-tailed)**
TL	Equal variances assumed	1.125	0.304	2.7	17	0.015
HH	Equal variances assumed	2.424	0.145	2.298	12	0.04
SL	Equal variances assumed	0	1	2.844	12	0.015
Sq.Front	Equal variances assumed	0.18	0.676	2.482	17	0.024
CircuOc.Right	Equal variances not assumed	6.882	0.018	2.597	16.995	0.019
CG	Equal variances assumed	0.143	0.712	-3.265	12	0.007
Vent	Equal variances assumed	1.453	0.245	2.285	17	0.035
Scd	Equal variances not assumed	3.512	0.078	5.488	12.695	0
Ventral Pattern	Equal variances not assumed	8.024	0.011	2.967	14.135	0.01
Dorsal Pattern	Equal variances not assumed	77.143	0	3.416	7	0.011

TL. tail length, HH. head height, SL. Snout length, CircuOc. Circum ocular scales, CG Gular, Vent. Ventral, Scd. Subcauda

Table 3. Values of principal component analysis of variances

Total variance explained						
Component	**Initial Eigenvalues**			**Extraction sums of squared loadings**		
	Total	**% of Variance**	**Cumulative %**	**Total**	**% of Variance**	**Cumulative %**
1	6.028	60.283	60.283	6.028	60.283	60.283
2	1.283	12.834	73.117	1.283	12.834	73.117
3	0.941	9.41	82.527	0.941	9.41	82.527
4	0.688	6.883	89.41	0.688	6.883	89.41
5	0.403	4.027	93.438	-	-	-
6	0.277	2.769	96.207	-	-	-
7	0.167	1.671	97.877	-	-	-
8	0.119	1.191	99.068	-	-	-
9	0.051	0.513	99.581	-	-	-
10	0.042	0.419	100	-	-	-
Extraction Method: Principal Component Analysis				-	-	-

Table 4. Values for the first to fourth components in the PCA test

Component Matrix				
Characters	**Component**			
	1	**2**	**3**	**4**
TL	0.59	0.266	**0.717**	0.099
HH	0.769	-0.032	-0.008	**0.479**
SL	**0.904**	0.191	0.007	-0.109
Sq. Front	0.692	**0.633**	-0.171	-0.04
CircuOc. Right	0.752	**0.522**	-0.246	-0.06
CG	-0.758	0.198	**0.516**	0.029
Vent	0.751	-0.443	-0.105	0.233
Scd	0.724	-0.412	0.117	**-0.484**
Ventral pattern	**0.892**	-0.111	0.174	-0.295
Dorsal Pattern	**0.877**	-0.292	0.125	0.235

TL. tail length, HH. head height, SL. Snout length, CircuOc. Circum ocular CG Gular, Vent. Ventral, Scd. Subcaudal

Table 5. ANOVA test to evaluate geographical variations

Characters		**Sum of squares**	**df**	**Mean square**	**F**	**Sig**
SVL	Between Groups	1236.61	2	618.305	33.71	0.000*
	Within Groups	293.474	16	18.342		
	Total	1530.084	18			
TL	Between Groups	0.441	2	0.22	0.428	0.659
	Within Groups	8.243	16	0.515		
	Total	8.684	18			
HL	Between Groups	0.749	2	0.374	5.484	0.015*
	Within Groups	1.093	16	0.068		
	Total	1.842	18			
HW	Between Groups	0.052	2	0.026	1.581	0.236
	Within Groups	0.261	16	0.016		
	Total	0.313	18			
HH	Between Groups	0.032	2	0.016	6.73	0.012*
	Within Groups	0.027	11	0.002		
	Total	0.059	13			
SL	Between Groups	0.455	2	0.228	15.12	0.001
	Within Groups	0.166	11	0.015	1	
	Total	0.621	13			
SW	Between Groups	0.013	2	0.006	2.23	0.154
	Within Groups	0.032	11	0.003		
	Total	0.045	13			
Sq. Front	Between Groups	4.384	2	2.192	3.771	0.046*
	Within Groups	9.3	16	0.581		
	Total	13.684	18			

Characters		Sum of squares	df	Mean square	F	Sig
Sq. Mid	Between Groups	0.047	2	0.024	0.421	0.663
	Within Groups	0.9	16	0.056		
	Total	0.947	18			
Sq.Hind	Between Groups	5.021	2	2.511	3.524	0.054
	Within Groups	11.4	16	0.712		
	Total	16.421	18			
SupL.Right	Between Groups	0.639	2	0.32	0.715	0.504
	Within Groups	7.15	16	0.447		
	Total	7.789	18			
SupL.Left	Between Groups	1.608	2	0.804	1.704	0.213
	Within Groups	7.55	16	0.472		
	Total	9.158	18			
InfrL.Right	Between Groups	0.547	2	0.274	0.684	0.519
	Within Groups	6.4	16	0.4		
	Total	6.947	18			
InfrL.Left	Between Groups	1.689	2	0.845	3.297	0.063
	Within Groups	4.1	16	0.256		
	Total	5.789	18			
PrOc. Right	Between Groups	2.857	2	1.429	0	0
	Within Groups	0	11	0		
	Total	2.857	13			
PrOc. Left	Between Groups	2.857	2	1.429	0	0
	Within Groups	0	11	0		
	Total	2.857	13			
PoOc. Right	Between Groups	21.429	2	10.714	0	0
	Within Groups	0	11	0		
	Total	21.429	13			
PoOc. Left	Between Groups	21.429	2	10.714	0	0
	Within Groups	0	11	0		
	Total	21.429	13			
CircuOc. Right	Between Groups	2.232	2	1.116	7.439	0.005*
	Within Groups	2.4	16	0.15		
	Total	4.632	18			
CircuOc. Left	Between Groups	0.089	1	0.089	0.778	0.407
	Within Groups	0.8	7	0.114		
	Total	0.889	8			
CG	Between Groups	8.1	2	4.05	6.02	0.017*
	Within Groups	7.4	11	0.673		
	Total	15.5	13			
A.ch.infrl. Right	Between Groups	11.429	2	5.714	0	0
	Within Groups	0	11	0		
	Total	11.429	13			

Characters		Sum of squares	df	Mean square	F	Sig
A.ch.infrl. Left	Between Groups	11.429	2	5.714	0	0
	Within Groups	0	11	0		
	Total	11.429	13			
Po.ch.infrl. Right	Between Groups	0.129	2	0.064	0.884	0.441
	Within Groups	0.8	11	0.073		
	Total	0.929	13			
Po.ch.infrl. Left	Between Groups	0.129	2	0.064	0.884	0.441
	Within Groups	0.8	11	0.073		
	Total	0.929	13			
Vent	Between Groups	213.087	2	106.543	4.848	0.023*
	Within Groups	351.65	16	21.978		
	Total	564.737	18			
Scd	Between Groups	10.955	2	5.478	0.689	0.516
	Within Groups	127.15	16	7.947		
	Total	138.105	18			
Tem.A. Right	Between Groups	0	2	0	0	0
	Within Groups	0	11			
	Total	0	13			
Tem.A. Left	Between Groups	0	2	0	0	0
	Within Groups	0	11			
	Total	0	13			
Tem.P. Right	Between Groups	0	2	0	0	0
	Within Groups	0	11			
	Total	0	13			
Tem.P. Left	Between Groups	0	2	0	0	
	Within Groups	0	11			
	Total	0	13			
Ventral Pattern	Between Groups	2.389	2	1.195	1.099	0.357
	Within Groups	17.4	16	1.088		
	Total	19.789	18			
Dorsal Pattern	Between Groups	3.214	2	1.607	0	0
	Within Groups	0	11	0		
	Total	3.214	13			
Lateral Pattern	Between Groups	2.857	2	1.429	0	0
	Within Groups	0	11	0		
	Total	2.857	13			

SVL. snout-vent length, TL. tail length, HL. head length, HW. head width, Supl. supralaibial scales, Infrl. lower labial scales, PrOc. pre ocular scales, PoOc post ocular scales, CircuOc. circumocular scales, CG Gular, A ch infrl. Between chin and lower labial, Vent. Ventral, Scd. subcaudal, Temp A. anterior temporal, Temp P. posterior temporal

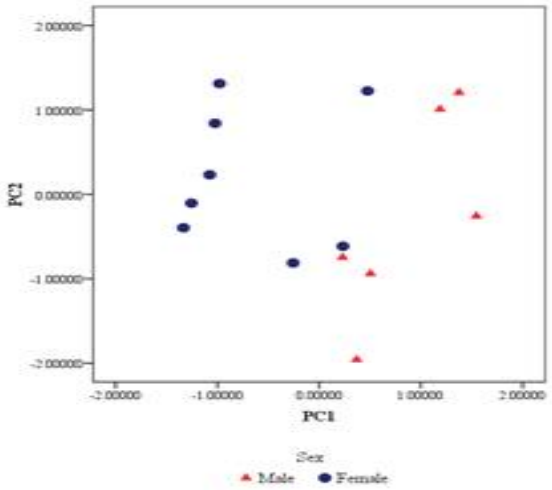

Fig. 4. The main components analysis between males and females

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A L Y S I S * * * * * * * * * * * * * * * * * * *


 Dendrogram using Single Linkage

                      Rescaled Distance Cluster Combine

   C A S E      0         5        10        15        20        25
 Label     Num  +---------+---------+---------+---------+---------+

        2   11
        2   14
        2   13
        2   15
        2   12
        1    1
        1    5
        1    2
        1    3
        1    4
        3   17
        3   19
        3   16

        3   18
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Fig. 5. Dendrogram from a cluster analysis of morphological and meristic characters between Caucasian snakes of Lar, Takht-E-Solymon and Gachsar populations

DISCUSSION

In the review of taxonomy within the pitvipers a very different picture emerged recently and there have been notable divergent and morphologically convergent in Asian taxa [35]. Here data provides morphological evidences for relevant taxonomical judgment on the three populations of pitvipers from Central Alborz mounts. There are several published materials on pitviper's taxonomy. Upon Ramazani et al. [31] report, halys pitvipers of Gorgan- Iran have triangular head in shape, neck was distinguished, nose was upward and somewhat prominent and eye pupil was horizontal. The 3 identified specimens had 23 dorsal scales. The body color from back of the neck to end of the body was brown with some light brown spots. The laterals are light brown with dark spots, with a distance between them and the entire ventral surface is much lighter with small brown spots. There is a brown chocolate line from back of the eye to end of the mouth. Under the threat, this snake is ready to bite with a twisting and forwarding body, and the tail sounds like a rattle snake to scare the enemy, but it moves slowly and calmly. They habitat in the forests, meadows, steppe areas and in Gorgan city it is found near residential areas [31]. Guo et al. [36] discussed the status and validity of several species of Gloydius vipers on the basis of the comparative morphology of the skull. A phylogenetic hypothesis based on morphological characteristics showed that this genus could be divided into three groups: brevicaudus group consisting of *G. brevicaudus* and *G. ussuriensis*; intermedius group including *G. intermedius*, *G. saxatilis* and *G. shedaoensis*; Strauchi group including *G.strauchi*, *G. liupanensis* and *G. qinlingensis* [36]. However, other authors have different suggestions. Morphological study in *Gloydius halys caucasicus* populations from three regions of Western, Central and Eastern Alborz by Khani et al. [32] showed that there are different in subcaudal scales, front and hind dorsal scales, supralabial scales, glurals, snout length – width ratio, snout length to head length ratio. These variations cause distinction between these populations and the western Alborz snake has the most distinction with Eastern Alborz. On the basis of other report, meristic characters of *G.h. caucasicus* form Golestan and Mazandaran provinces of Iran including: Supra labials scales in male and female, 14.5±0.1 and 14.7±0.1; infralabial scales in male and female, 22±0.3 and 21.8±0.3; circuocular scales in male and female, 12.4±0.2 and 12.1±0.1; preocular scales in male and female, 4.5±0.2 and 4.2±0.1; gular in male and female, 5.4±0.2 and 6±0.2; CG in male and female 9±0.3 and 8.6±0.4; anterior chine and infralabial scales in male and female, 7.4±0.1 and 7.5±0.1; posterior chin and infralabial anterior scales in male and female, 4±0.1 and 3.8±0.1; posterior chin and infralabial posterior scales in male and female, 6±0.05 and 6±0.1; anterior temporal in male and female, 4.6±0.1 and 4.6±0.2; posterior temporal in male and female, 8.7±0.2 and 8.4±0.2 were respectively reported [37] that differ with our findings. Wagner et al. [33] study on *G.halys* from Alia-Pamir ranges showed the *G. halys* complex, is characterized by the following character combination: It is a slender and moderately stout small snake, up to 479 mm total length. The head has nine symmetrical plates on the upper head, 7 supralabial and 8-9 infralabial scales. Body scales in 20-22 rows around midbody, 143-156 ventral and 35-45 usually paired subcaudal scales. The cloacal plate not divided. The general coloration consists of various different tones of olive, tan and brown, having a distinct head, but an indistinct body pattern with, excluding the tail, 26-29 transverse cross

bands, which are not extending to the sides of the body. These characters were very different from *G. halys* of central Alborz ranges population. *Gloydius halys caucasicus* [26] fauna of Russia and adjacent countries. A moderately snout length of viper was up to 660 mm of total length. Supralabial scales 7-8, rarely 9. Dorsal scales was 23 (rarely 25) rows around midbody; ventral scales 142-169; subcaudal scales paired, 31-46. Body with 33-42 dark transverse bands, each 4-6 scales wide and extending down to scale row. Other species of Gloydius genus is *Gloydius intermedius* [12], *Trigonocephalus intermedius,* according to Orlov and Barabanov [13] the species is restricted to area from Russian Far East through Northeastern China to the Korean peninsula. It is large viper of the genus, up to at least 800 mm total length. Supralabial scales 7-8. Dorsal scales in 23, very rarely 21, rows around midbody; ventral scales 148-175; subcaudal scales paired, 34-52. Body with 28-45 dark transverse bands, each 3-6 scales wide and extending down to scale row 2 or 1. These identification differ from our findings.

Wagner et al. [33] proposed the elevation of the Caucasian pit viper from subspecies to species rank. This was later accepted by Shi et al. [38]. Moreover, Shi et al. [39] argued for elevation of two other subspecies *G*. *h*. *cognatus* and *G*. *h*. *stejnegeri*, to the full species rank, which was later reaffirmed by Shi et al. [38]. Data reported by Asadi et al. [40] on the *G. h. caucasicus*, based on two mtDNA (cyt b and ND4) and one nDNA (c-mos) genes support the incursion of the species from Northeastern Iran to the Western end of the Alborz, and then toward Transcaucasia via two directions: Northern and Southern slopes of the Alborz mountains. Furthermore, they have suggested that *G. h. caucasicus* could be considered as a distinct and should be further referred to as *G. caucasicus* (Nikolsky, 1916) [40]. Another study on the phylogenetic relationships of *G. caucasicus* from North-East of Iran (Khorasan province) and Central Alborz (Lar and Gachsar area) Iran using 629 bp of the Cytb gene concluded that the species belongs to the *G. halys/G. intermedius* complex, within which controversial phylogenetic relationships still remain under debate [41].

CONCLUSION

This revision clarify the distributions ranges of *Gloydius halys* and relationships between the groups. There were geographical differences in *Gloydius halys* of Central Alborz mountains and tow populations from Lar and Takht –E-Solyman have close relations and somehow distinct from halys of Gachsar population and dimorphism was observed in female and male of all three populations.

ETHICS APPROVAL CONSENT TO PARTICIPATE

We have followed the ethical standards of the committee on animal experimentation according to our Institutional Ethics Committee (RVSRI Ethics Committee) and National committee (ISIRI 7216-2) [42].

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between all authors. First author managed the literature searches,

wrote the project, performed the methods and statistical analysis and wrote the manuscript. Second author managed the analyses of the study. Third author managed the methodological task. Fourth author helped in results analyses.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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