

Geometric morphometrics of the head shape of *Pseudocerastes persicus* (DUMÉRIL, BIBRON & DUMÉRIL, 1854) (Ophidia: Viperidae) from central Iran

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Abstract

In the present study, we focused on three populations of Persian Horned Snake, *Pseudocerastes persicus* from Iran and examined them morphologically. Geometric morphometrics is an appropriate method to evaluate the scale positions and shape in different populations of a given species in snakes. We used this method to evaluate the geographic variation among three populations of *Pseudocerastes persicus*. Based on variance and multivariate analyses, all populations from Isfahan, Semnan and Markazi provinces don't show variation in head scales. This result from morphological features confirmed the previous molecular study on these populations. We recommended examining further taxonomic characters of these populations as karyotype and allozyme features.

Keywords: Viperidae, Iranian Plateau, Horned viper, homogeneity, Geometric morphometric

1 Introduction

Genus *Pseudocerastes* has three representatives in Iran as *P. urarachnoides* Bostanchi, Anderson, Kami and Papenfuss 2006, *P. fieldi* Schmidt 1930 and *P. persicus* (Duméril, Bibron and Duméril

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1854). The species has a wide distribution range in central, southern and eastern Iran (Rajabizadeh 2018). Previously, *P. persicus* had two different subspecies as *P. p. persicus* and *P. p. fieldi*, but based on analyses of their venom composition and proteins, that was significantly different, they upgraded into full species level (Bdolah 1986; Gasperetti 1988). As is mentioned in the literatures, these species have a parapatric distribution range in Western Iran, and the Zagros Mountains play an important role in their separation (Bostanchi et al 2006). Fathinia et al (2014) revised the genus *Pseudocerastes* in Iran taxonomically using molecular phylogeny based on the Cyt b gene. According to their results, the genus divided into three distinct species genetically, but the morphological diagnostics was not clear between *P. persicus* and *P. fieldi*. As mentioned above, venom analyses confirmed these three species in the genus. Based on the molecular phylogeny results, there is some differentiation within *P. persicus* in Iran as Western Iran, southern Iran and Damghan populations. These groups show probable differences, but more sampling is needed to be clear.

Morphological studies are important in describing new taxa and populations. Morphological data can provide taxonomic identity and evolutionary relationships among taxa. Geometric Morphometrics (GM) is a method that combines shape analysis technique with the spatial distribution of landmarks. Combinations of these methods can be used as multivariate statistics and can be used to describe variation in many taxonomic groups (Claude et al. 2003). This method (GM) has recently increased in use in herpetological studies, especially for taxonomy (Kaliontzopoulou 2011). Snake morphology has limited characters, so GM could be useful to examine the shape variation of scales, their position, and other characteristics (Shine et al. 2002). . In this study, we aimed to examine some specimens of *Pseudocerastes persicus* in Iran belonging to different populations using the GM approach.

2 Materials and Methods

In total, 82 specimens of *Pseudocerastes persicus* were collected from three localities in Iran, 42 specimens from Markazi province (Saveh and Delijan), 24 specimens from Isfahan province (Najaf Abad) and 16 specimens from Semnan province (Damghan) (Fig. 1). The left view of all specimens was photographed under standard conditions using a CANON IXUS 130. Different morphological characters were measured as follows: dorsal scales, mental scales, rostral scale, interorbital scales, upper labials, lower labials, parietals, temporals, subcaudal scales, ventral scales; body length, tail length, head length, head width, eye diameter. TPS Util V. 1.52 (Rohlf 2012) was used to build a tps file from all of the photographs. This tps file is the standard file used for GM analyses. All specimens were adults. We used all of the photographs in a series analyses. TPSDIG2 V. 2.16 (Rohlf 2010) was used to digitize all landmarks (Fig. 2). Then, all data were loaded into SPSS 16.0 and MorphoJ 1.02 (Klingenberg 2008) Analysis of Variance (ANOVA), Principal Component Analysis (PCA) and Canonical Variate Analysis (CVA) were run for all specimens and populations.

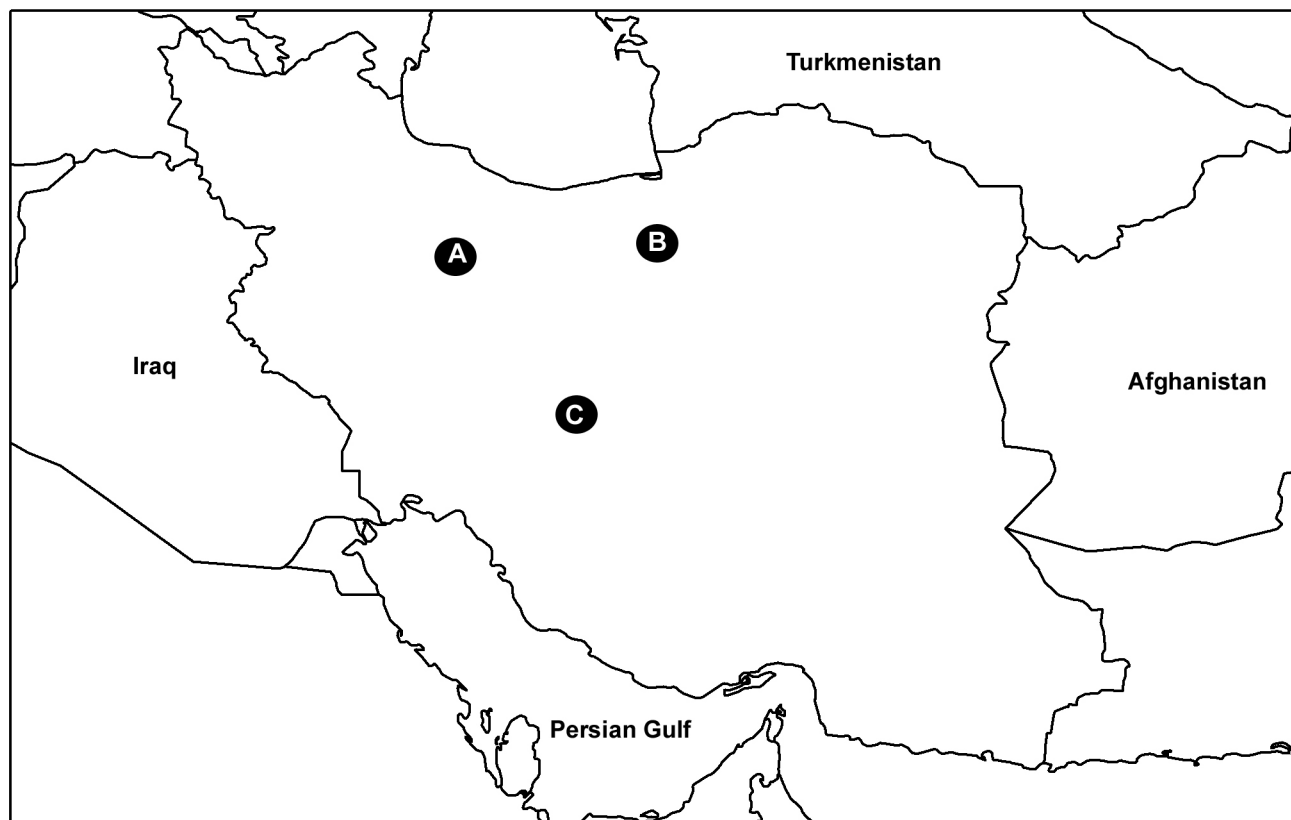


Figure 1. Map of Iran and locality of samples collection. A) Markazi province; B) Damghan, Semnan; C) Isfahan, Najafabad.

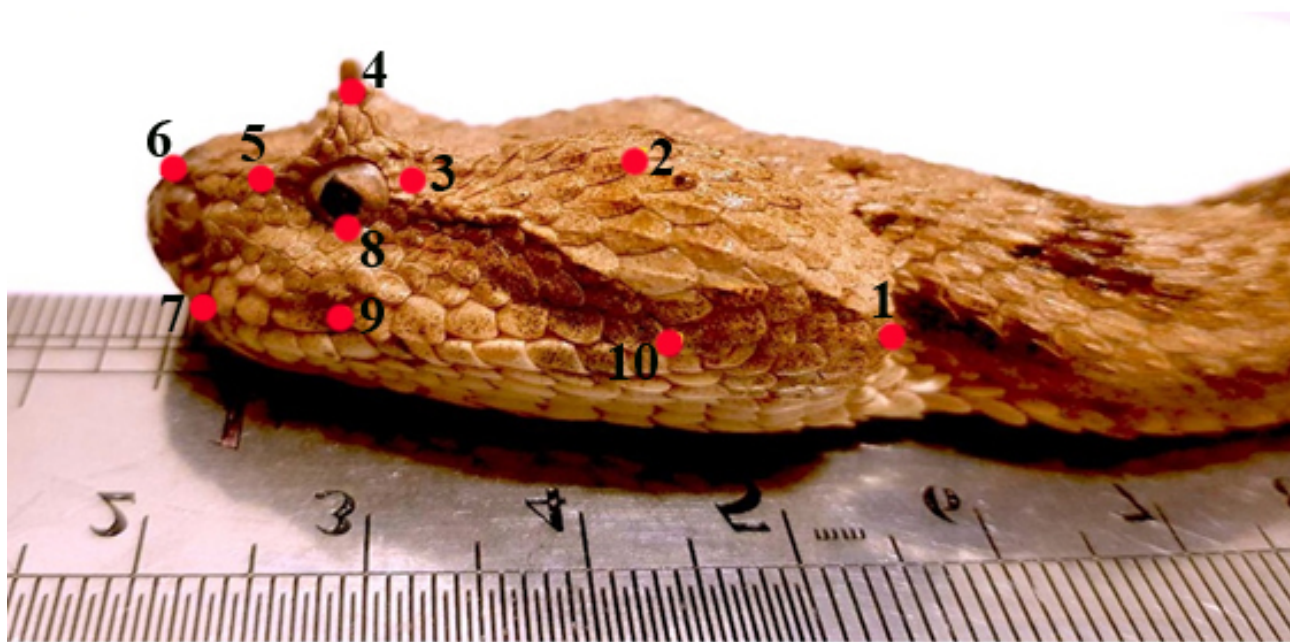


Figure 2. Left view of the *Pseudocerastes persicus* head and landmarks were used in this study.

3 Results

First, we measured centroid size (CS) among three populations of *Pseudocerastes persicus* by MorphoJ 1.02. The populations did not show any significant differences in CS (Markazi population CS mean: 26.629; Isfahan population CS mean: 26.984; Semnan population CS mean: 26.542), but, generally, the Semnan population has smaller CS than the other groups (Fig. 3).

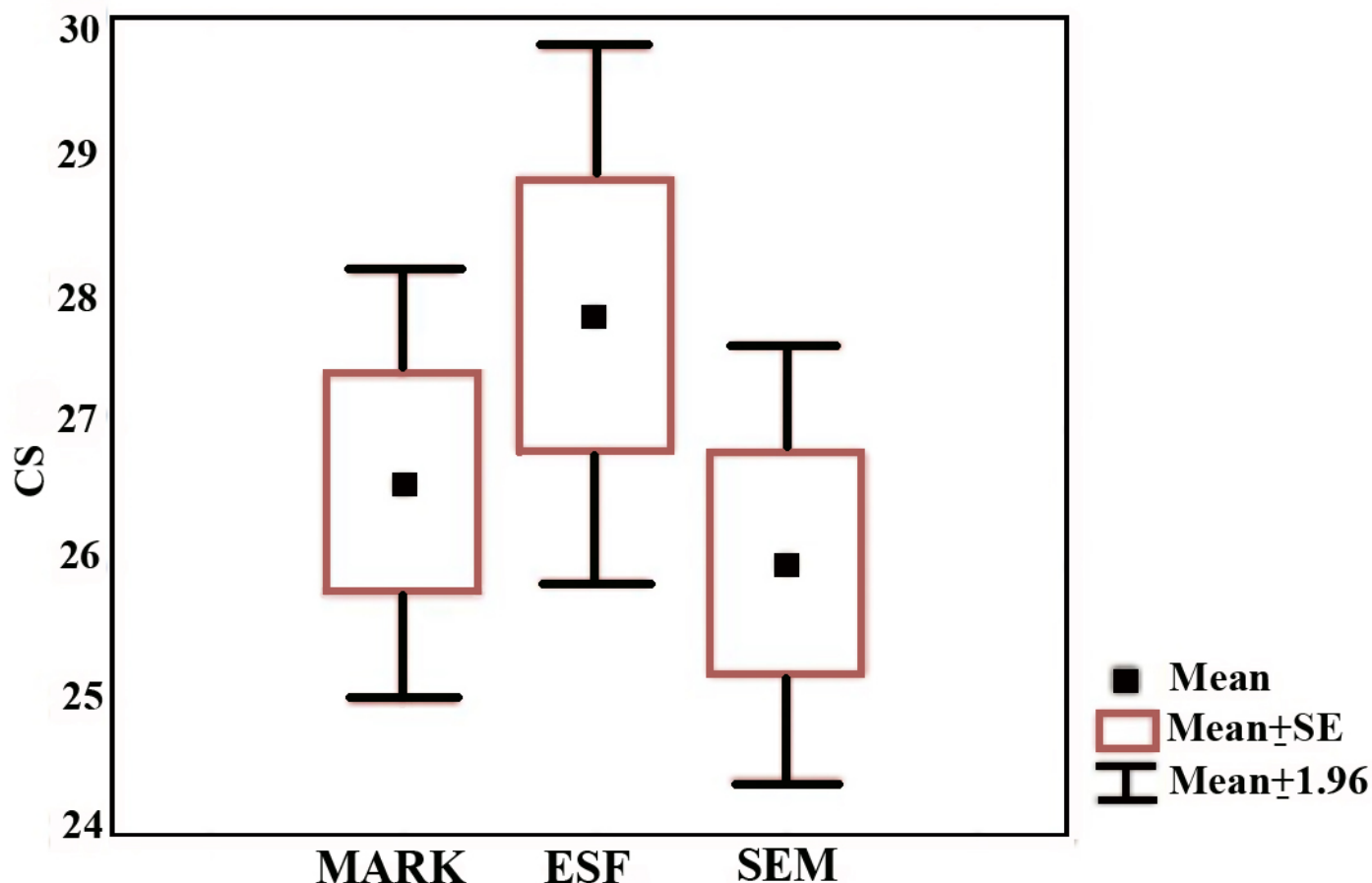


Figure 3. Result of the CS analysis. The Semnan population has the smallest size. (MRK: Markazi; ESF: Esfahan and SEM: Semnan)

The result of ANOVA post hoc (Tukey test) analysis similar to the CS analyses (Table 1), indicated that there were no differences between the populations (P values > 0.05). Then, we used PCA to evaluate the variation between all specimens. To this, first we used two PCs on the upper head scales and covered 42.66 % of the total variance (Fig. 4). CVA shows that there is little variation among populations and relevant parameters are as follows: Wilk's Lambda: 0.5134, $P < 0.0488$. The CVA plot on three populations of *P. persicus* explained that the first two CVs cover 100% of the total variance. The P value is near 0.05 and indicated low variability among populations and a high degree of overlap between groups (Fig. 5).

Table 1. *P* value of Tukey analysis among three populations of *Pseudocerastes persicus* in Iran. All groups are not significant (*P* value > 0.05).

Population	Markazi	Isfahan	Semnan
Markazi			
Isfahan	0.643		
Semnan	0.939	0.535	

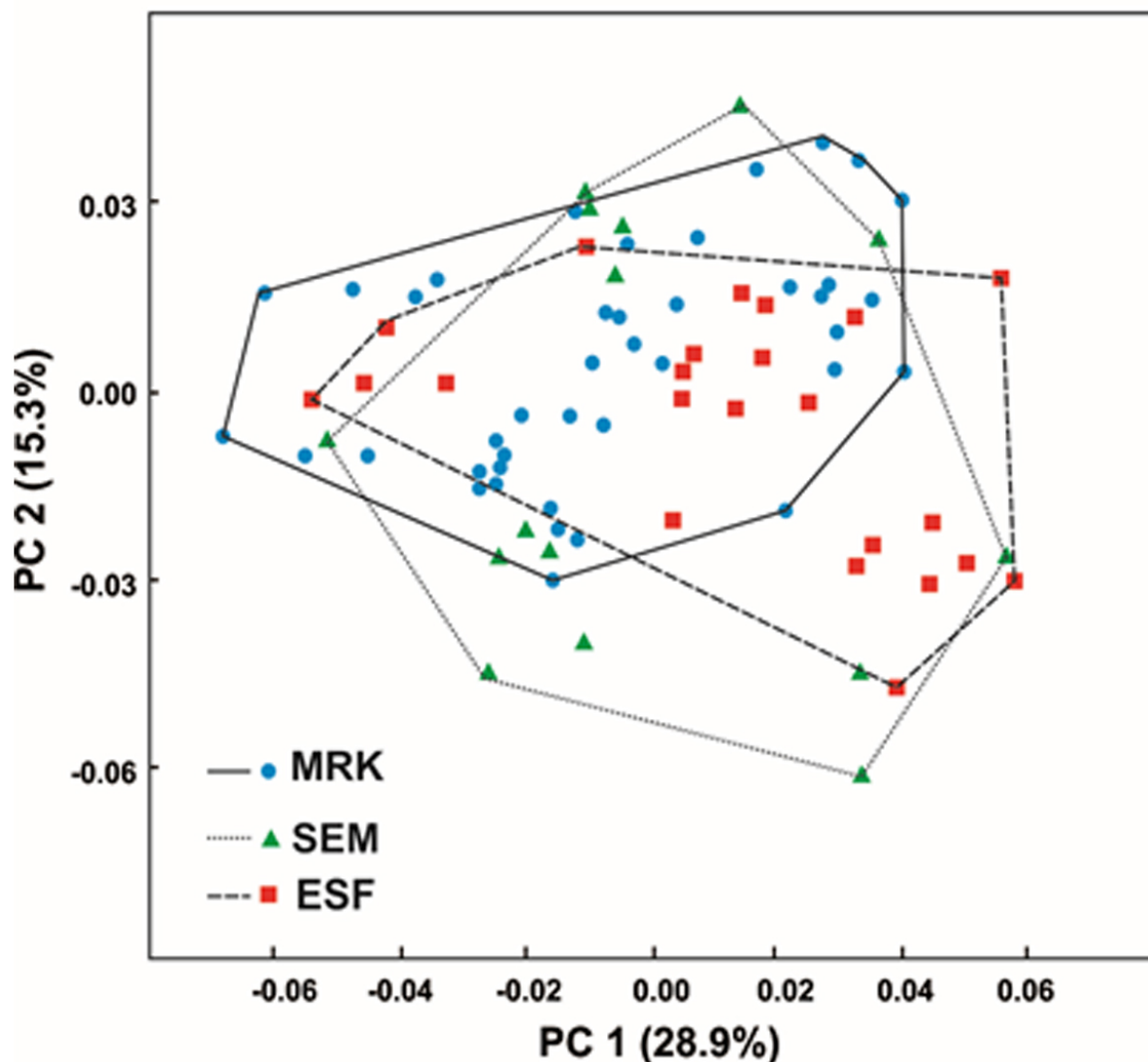


Figure 4. The graph of the first two PCs of all populations (PC1 against PC2).)

To compare the population relationships based on shape, average procrustes distances were used, but no differences were observed among populations. The result is shown as a UPGMA tree based on the upper head scales (Fig. 6).

Finally, to check the allometry (relationship between size and shape), we used a regression analysis. However, after removing the allometry, all populations overlapped each other.

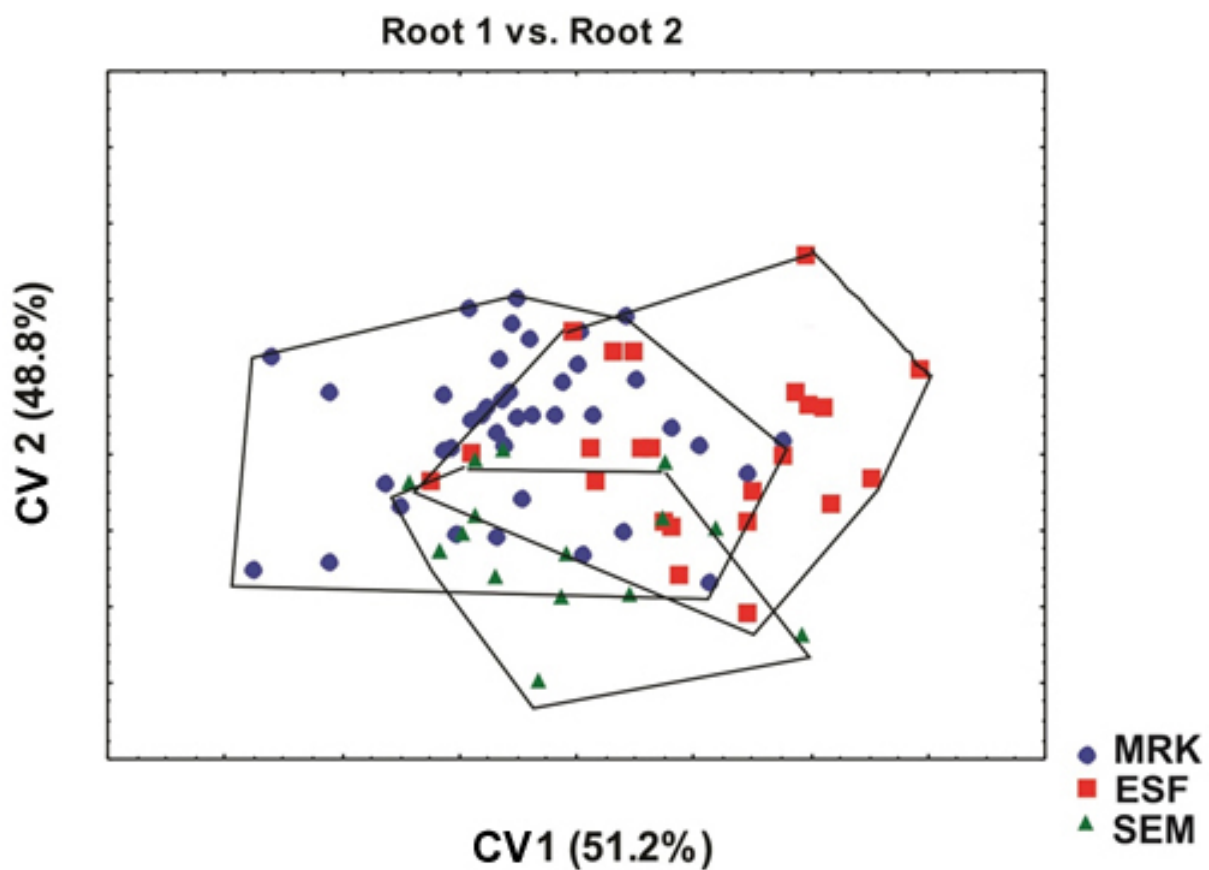


Figure 5. CVA of three populations of *Pseudocerastes persicus* in Iran.

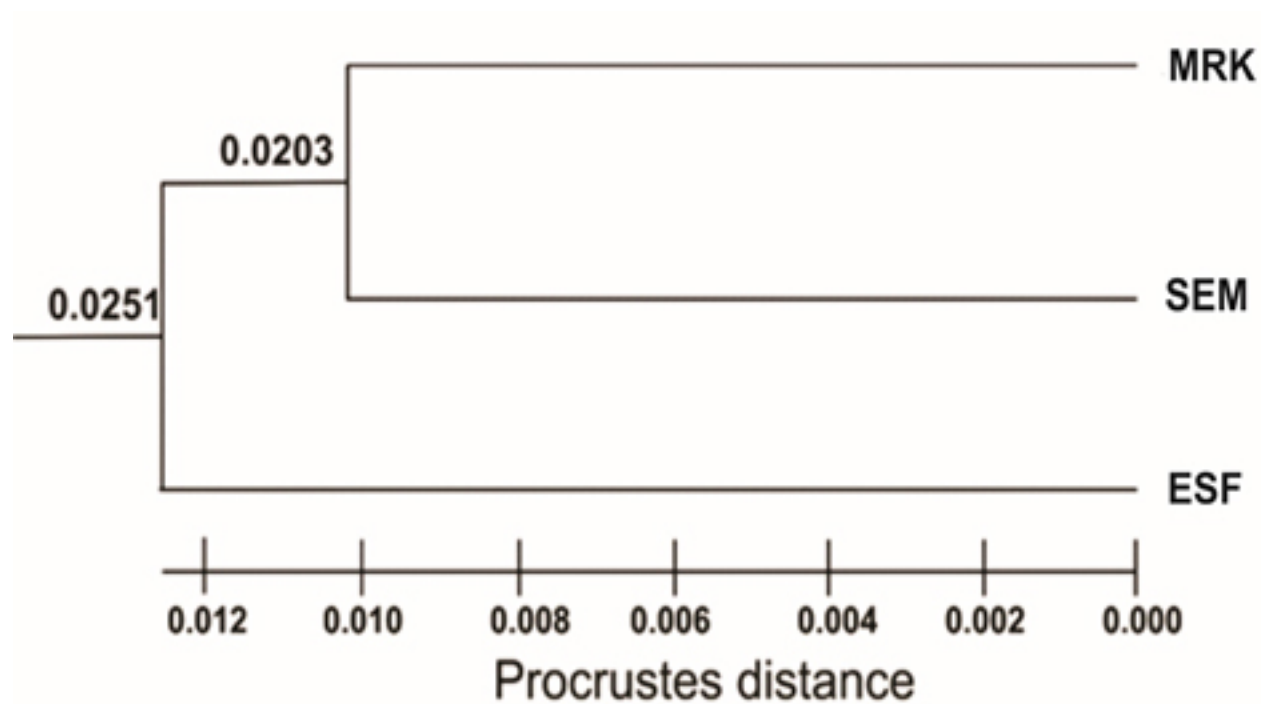


Figure 6. UPGMA tree based on the procrustes distance of the upper head scales.

4 Discussion

In the present study, we examined three populations of Persian Horned Viper (*Pseudocerastes persicus*) in Iran using the GM method. The species has two distinct subspecies on the world: *P. p. persicus* and *P. p. fieldi* (Latifi 2000). However, several authors believe that these traditional subspecies must be elevated to the full species level as *P. persicus* and *P. fieldi* (Bostanchi et al. 2006; Fathinia and Rastegar-Pouyani 2010). They referred to the distinct morphological character as dorso-lateral scale rows that strongly keeled in *P. persicus* (Gasperetti 1988; Bostanchi et al. 2006). Generally, these two subspecies are isolated from each other with long geographical distances and the Zagros Mountains playing an important role as a barrier. *P. fieldi* is distributed in the western Zagros Mountains of the Mesopotamian plate and the near east, while, *P. persicus* has a wide distribution in eastern Zagros along the Iranian Plateau (Bostanchi et al. 2006; Fathinia and Rastegar-Pouyani 2010). The molecular phylogeny of the genus *Pseudocerastes* in Iran (Fathinia et al. 2014) shows that the species has low genetic variation as represented by 2 to 4 % of corrected genetic distance (K2p distance). In this analysis, the researchers used only three populations of *P. persicus* from Damghan, Yazd and Ilam regions. Our GM result confirms their phylogenetic tree and there is little variation among the Iranian Plateau populations of *P. persicus*.

This homogeneity among long distance populations of the species influenced by the stable environmental conditions in central Iran as *Mesalina watsonana* and *Agamura persica* (Hosseinian Yousefkhani et al. 2013; Hosseinian Yousefkhani 2019). The central Iranian Plateau has a stable environmental condition from many years ago, so the reptile species that expanded into this region have not been forced to adapt to different conditions and therefore they remain more homogeneous (Fathinia et al. 2014). We highly recommend an integrative study contains ecology, allozym and karyotype approaches on the species to clarify their taxonomic status in Iran.

Conflict of interests

There are no conflicts of interest between the authors.

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