

RESEARCH ARTICLE

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# Phenotypic and geographic variation among populations of the *Platyceps rhodorachis-ventromaculatus* species complex (Ophidia, Colubridae) in Iran

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## Abstract

Geographical diversity appears in the phenotype of different populations of a given species and specific geographical factors are directly or indirectly involved in this variation. Due to their wide distribution range, snakes of the genus *Platyceps* Blyth, 1860 are wonderful case studies for morphological evaluation. In this study, 49 specimens of the *Platyceps rhodorachis-ventromaculatus* species complex in four geographic areas from Iran were examined using morphological characters. The results of statistical analysis did not show any sexual demorphisem except in the character of Gular scales ( $P$  value = 0.038), but did show significant variation in the number of scales and in other morphological characters among populations in this group. Generally, four morphological clusters were distinguished with Sistan and Baluchestan Province being the most distinct from each other. Additionally, there was a visible slope of changes in some morphological characters associated with latitude, so that with increasing latitude, the body size of the specimens increases.

**Key words:** *Platyceps rhodorachis*, *Platyceps ventromaculatus*, Colubridae, Morphology, Phenotypic, Iran

## INTRODUCTION

The genus of *Platyceps* Blyth, 1860 belongs to the Colubridae family and contains 29 species globally, of which 7 have been recorded in Iran (Schaetti *et al.*, 2014; Rajabizadeh, 2018; Uetz, 2020). The genus distribution is quite large and includes the Saharo-Indus biogeographical regions from Africa to East Asia and the Mediterranean region, North Africa, and the Middle East to the Nepalese highlands (Schatti, 2006; Geniez & Gauthier, 2008; Jablonski & Bragin, 2019).

*Platyceps rhodorachis* (Jan, 1863) is one of the most important species in this genus. This species has a maximum length of 1290 mm. Its body shape is cylindrical and elongated and it is found in brownish-gray, olive, gray, brown colors with a distinct spot under the eyes and dark brindled stains or without dorsal stains (Leviton *et al.*, 1992; Schätti & McCarthy, 2004). The former *Platyceps rhodorachis*



pecies complex had four subspecies, *P. r. rhodorachis* (Jan in Filippi, 1865), *P. r. subniger* (Boettger, 1893), *P. r. saharacus* (Schätti & McCarthy 2004), and *P. r. ladacensis* (Anderson, 1871). Currently, two subspecies of this species complex have been elevated to full species status. *Platyceps saharicus* (Schätti & McCarthy 2004) that cover the western part of the distribution range (North Africa, northwestern Saudi Arabia, Jordan, and Palestine) (SCHÄTTI and McCarthy, 2004; Geniez and Gauthier, 2008). Additionally, a subspecies of *P. r. ladacensis* (Anderson, 1871) from Aden (Yemen) has been elevated to full species status due to differences in ventral scales and lack of overlap of the distribution with *P. rhodorachis* and seems to have a wider distribution. It is also distributed in the northern regions of India (Perry, 1985; Schätti & McCarthy, 2004; Schatti, 2006; Perry, 2012; Шестопал, 2020; Hussain & Tantarपाल, 2021).

Currently, *P. rhodorachis* contains two valid subspecies, *P.r.subniger* (Boettger 1893) and *P.r.rhodorachis* (Jan in Filippi 1865), whose distribution in Iran has been confirmed (Latifi & Leviton, 1991; Latifi, 1991,2000; Moradi *et al.*, 2013; Schaetti *et al.*, 2014; Rajabizadeh, 2018). *Platyceps rhodorachis rhodorachis* is distributed through Iran, Afghanistan, India and northeast Iraq (Hussain & Tantarपाल, 2021; Malik *et al.*, 2021). *Platyceps rhodorachis subniger* is distributed in Iran, Eritrea and Djibouti to north and central of Somalia and adjacent Ethiopia (Latifi *et al.*, 1991; Khan, 1997; Schaetti *et al.*, 2014). *Platyceps rhodorachis kashmirensis* (Khan, 1997) has been recommended as a synonym of *P. rhodorachis* species by Schätti 2014 (Latifi *et al.*, 1991; Khan, 1997; Schaetti *et al.*, 2014).

*Platyceps ventromaculatus* (Gray, 1834) is mainly found in Afghanistan, Pakistan, India, and Iran. However, new findings indicate that the distribution of this species is more than has been documented and this species has recently been found in parts of southeastern Antalya, Turkey (Yildiz, 2011; Bhandari *et al.*, 2021). The *Platyceps ventromaculatus* species complex includes three subspecies: *P. v. ventromaculatus* (Gray, 1834), *P. v. bengalensis* (Khan & Khan, 2000), and *P. v. indusai* (Khan & Khan, 2000). Pattern and color differences are evident in the subspecies, but *P. v. bengalensis* and *P. v. indusai* were introduced as subspecies based only on a slight difference in the number of scales (Khan, 1986; Khan & Khan, 2000; Barabanov, 2002). However, because Khan & Khan (2000) didn't specify a neotype for their new subspecies, the current status of these subspecies is unclear.

*Platyceps ventromaculatus* is morphologically very similar to the *P. rhodorachis* species complex and differs only slightly in the number of dorsal spots and the number of scales. Hence, the situation between these two species has become a species complex and *Platyceps rhodorachis-ventromaculatus* has attracted considerable attention as a polymorphic group. Nonetheless, taxonomic status of this group has not yet been resolved and has undergone many changes since its description. Perry (2012) believed that the number of ventral scales is the leading cause of changes in the length of *Platyceps rhodorachis* (Jan, 1865), but did not provide sufficient evidence to support this.

Extensive morphological variations of the number of scales, color patterns, and dorsal stripes have been reported in *P. rhodorachis-ventromaculatus*. However, four significant groups of them can be seen in Iran (Schaetti *et al.*, 2014). Although many attempts have been made to study the morphology of the *P. rhodorachis-ventromaculatus* complex group, most have been limited to a small area and based on initial identifications and this is problematic in that high morphological similarity is observed and individuals are morphologically similar, making them easily misidentified. Another problem is that species descriptions are usually based on a limited number of specimens and due, to their wide distribution range, a comprehensive view of the morphological status of *P. rhodorachis-ventromaculatus* complex group is not available (Khan, 1997; Schatti, 2006; Yildiz, 2011; Schaetti *et al.*, 2014).

We know that a species' geographical range is determined by the complex interaction of species characteristics and environmental factors limiting species distribution (Gaston, 2003); subsequently, a detailed morphological study should be performed in a range commensurate with the range of species distribution.

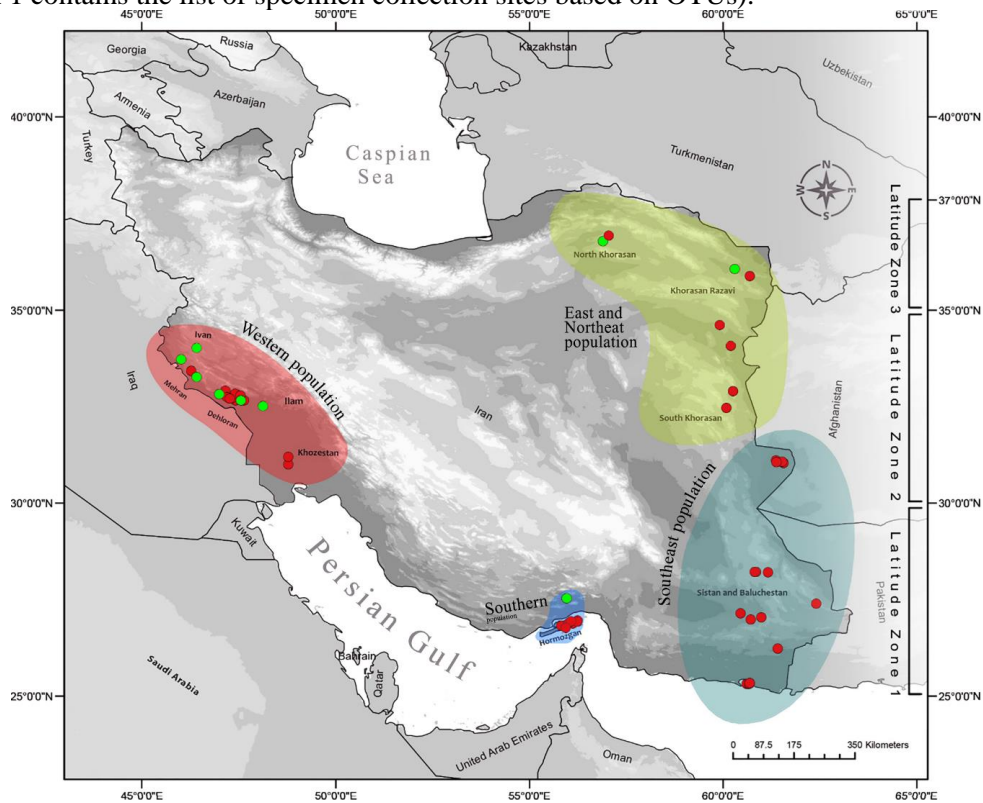
The range size has been associated with several biogeographical, environmental, and life history factors such as body size (Gaston & Blackburn, 1996), altitude (Stevens, 1989), and latitude (Böhm *et al.*,

2017). According to Bergmann's rule, the body size of animal's changes in association with latitude changes. In colder regions, the body size of a species is larger than the body size of that species in lower latitudes and warmer regions (Meiri & Dayan, 2003). Additionally, by Rapoport's rule, range size increases with increasing latitude, especially in the northern hemisphere (Böhm *et al.*, 2017). Because Iran has high geographical diversity and natural barriers such as Kopeh Dagh, and the Lut and Kavir Deserts, and Zagros Mountains from northwest to the south, and the Alborz Mountain range that extends from northwest to the northeast (Fu *et al.*, 2007; Edwards *et al.*, 2012; Le Galliard *et al.*, 2012), and based on previous surveys and our results from this research, the spread of *P. rhodorachis-ventromaculatus* complex species is highly probably influenced by these geographical barriers.

In the present study, we tried to compare different populations of *P. rhodorachis-ventromaculatus* complex species in terms of morphological diversity. We also tried to study the effect of geographical barriers in Iran on the morphological variations of *P. rhodorachis-ventromaculatus* complex species.

## MATERIAL AND METHODS

A total of 49 adult specimens (25 ♂, 24 ♀) of *P. rhodorachis-ventromaculatus* species complex were examined morphologically. During fieldwork (2002-2010), specimens were collected in western, southern, northeastern, and southeastern parts of Iran by Hakim Sabzevari University and Imam Hossein University researchers (Fig. 1). All specimens were deposited at Sabzevar University Herpetological Collection (SUHC). West Zagros population includes 17 specimens, the southern population includes 9, the southeastern population includes 15, and the northeastern and eastern population contains 8 (Appendix 1 contains the list of specimen collection sites based on OTUs).



**FIGURE 1.** Collecting sites of *Platyceps rhodorachis* (red circles) and *Platyceps ventromaculatus* (green circles) in Iran. Western populations belong to Ilam, Mehran, Dehloran, Ivan, and Khuzestan. The populations of the south are from Hormozgan, and the populations of the southwest are from Sistan and Baluchestan Province, and the populations of the northeast and east are collected from North Khorasan, Khorasan Razavi, and South Khorasan.

### Characters and measurements

Specimens were identified using the valid identification keys (Habeeb & Pouyani, 2016; Rajabizadeh, 2018), then 21 morphological features were measured to investigate morphological differences in each population and to identify the presence of sexual dimorphism (Yildiz, 2011; Schaetti *et al.*, 2014).

The morphometric and meristic characters were: snout-vent length (SVL), length of tail from the posterior edge of the cloaca to end of the tail (TL), the total length of trunk from the tip of snout to the tip of tail (TOL), length and width of the head (HL, HW), length of the snout from tip of snout to preocular scale (SL), the width of snout from loreal scales area (SW), length of frontal scales (FL), length and width of supraocular scales (SCL, SCW), eye diameter in the largest horizontal position (HED), number of supralabial scales (SUP), number of gular scales (GUL), number of ventral scales (Ventrals), number of dorsal scales rows in mid-body (Dorsals), number of dorsal scales rows at the end of body before tail (DAV), number of one row of tail scales in the direction of ventral (Subcaudals). We also considered four ratio characters as tail length to total body length (TL / TOL), head width to head length (HW / HL), snout width to head length (SW / HL), snout length to head length (SL / HL).

Gender determination in snakes was performed by measuring the depth of the cloaca bag using a sexing probe (McDiarmid *et al.*, 2012). Metric characters were measured using a meter (with an accuracy of  $\pm 1.00$  mm) and digital caliper (with an accuracy of  $\pm 0.01$  mm). The meristic characters were counted under the stereomicroscope and, to reduce human error in counting traits, measurements were repeated three times for each specimen (Table 1).

### Statistical Analyses

Measured morphological datasets were analyzed using SPSS Statistics V.26. The normality of the data was assessed using descriptive analysis; the normality of metric and ratios characters was determined using the Shapiro-Wilk test and the normality of meristic characters was determined using the Kolmogorov-Smirnov test. The independent samples test (t-test) and 2-independent samples analysis (U-test) were used for normally distributed and non-normally distributed characters, respectively. The dataset is generally divided into four operational taxonomic units (OTUs) based on geographical barriers such as the Zagros Mountains in the west and the Lot Desert or separation of the mainland by sea (Fig. 1). To evaluate the significant level of characters among OTUs, analysis of variance (ANOVA) was performed for normally-distributed characters, and the Kruskal-Wallis test was used for non normally-distributed characters. Principal component analysis (PCA) and Canonical variate analysis (CVA) were performed to determine the most important characters contributing to the variance among populations (Figs 3, 4). The same process was the repeated to determine which characters varied significantly by latitudes changes and a linear regression diagram of the character changes was drawn (Appendix 9). Subsequently, PCA diagrams were drawn for significant characters in three latitudes zone (Fig. 5).

### RESULTS

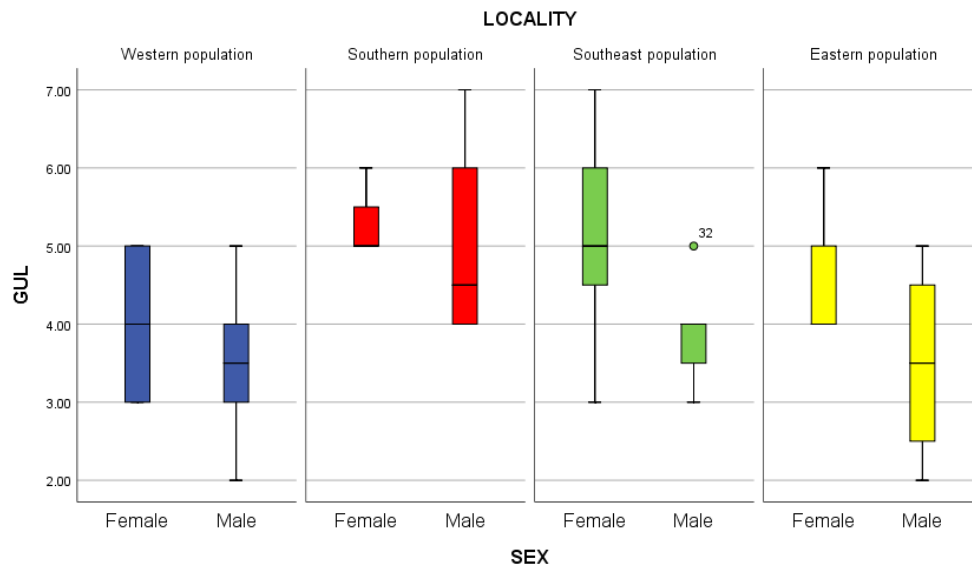
The number of Gular scales showed a significant difference value between male and female specimens, but there was no difference between males and females in the other characters measured. Therefore, this difference may be due to different reasons such as the difference in the number of males and females in OTUs (e.g., in the population of southern Iran) or due to the age difference of the specimens. For this reason, we did not consider sexual dimorphism and examined Gular independently among OUTs (Fig. 2).

**TABLE 1.** Means $\pm$ 1 SE and ranges for descriptive parameters of 25 morphometric, meristic, and ratio characters measured in two species groups of *P. rhodorachis* and *P. ventromaculatus* collected from four geographic areas in Iran.

Characters	Western Group (n=17)		Southern group (n=9)		Southeast group (n=15)		Eastern group (n=8)		P value
	Mean $\pm$ 1 SE	Range	Mean $\pm$ 1 SE	Range	Mean $\pm$ 1 SE	Range	Mean $\pm$ 1 SE	Range	
<b>SVL</b>	593.94 $\pm$ 17.49	511-702	679.89 $\pm$ 34.48	479-852	390.13 $\pm$ 22.23	224-500	710.13 $\pm$ 22.09	638-800	<b>0.000</b>
<b>TL</b>	254.35 $\pm$ 7.59	207-306	212.56 $\pm$ 18.04	105-263	159.13 $\pm$ 13.8	60-264	251.38 $\pm$ 15.74	179-295	0.000
<b>TOL</b>	849.65 $\pm$ 24.67	731-1007	895.22 $\pm$ 49.99	619-1117	544.07 $\pm$ 35.93	286-750	963 $\pm$ 30.24	850-1088	<b>0.000</b>
<b>HL</b>	16.16 $\pm$ 0.47	13.7-19.5	19.31 $\pm$ 1.08	14-26	12.44 $\pm$ 0.57	8.8-16	19.23 $\pm$ 0.77	16-23	<b>0.000</b>
<b>HW</b>	566.31 $\pm$ 21.23	224-852	13.27 $\pm$ 0.85	8-17	8.84 $\pm$ 0.71	5-13.8	12.41 $\pm$ 0.75	10-16	<b>0.000</b>
<b>RL</b>	6.25 $\pm$ 0.24	4.8-8	7.7 $\pm$ 0.58	5-11	4.42 $\pm$ 0.22	3-5.6	7.36 $\pm$ 0.5	5-9	<b>0.000</b>
<b>RW</b>	6.41 $\pm$ 0.3	4.84-8.9	7.79 $\pm$ 0.48	5-9.8	4.73 $\pm$ 0.22	3-6	7.15 $\pm$ 0.39	6-9	<b>0.000</b>
<b>TL/TOL</b>	0.3 $\pm$ 0	0.28-0.32	0.24 $\pm$ 0.01	0.15-0.26	0.29 $\pm$ 0.01	0.21-0.38	0.26 $\pm$ 0.01	0.2-0.31	<b>0.000</b>
<b>HW/HL</b>	0.65 $\pm$ 0.02	0.53-0.92	0.69 $\pm$ 0.03	0.57-0.84	0.7 $\pm$ 0.04	0.55-1.05	0.65 $\pm$ 0.03	0.56-0.8	0.426
<b>RW/HL</b>	0.4 $\pm$ 0.01	0.33-0.52	0.4 $\pm$ 0.01	0.36-0.45	0.38 $\pm$ 0.01	0.33-0.45	0.37 $\pm$ 0.01	0.33-0.42	0.321
<b>RL/HL</b>	0.39 $\pm$ 0.01	0.33-0.44	0.4 $\pm$ 0.02	0.29-0.44	0.35 $\pm$ 0.01	0.29-0.42	0.38 $\pm$ 0.01	0.31-0.42	<b>0.035</b>
<b>FL</b>	5.35 $\pm$ 0.17	4.4-7	5.73 $\pm$ 0.25	5-7.3	4.12 $\pm$ 0.19	3-5	5.89 $\pm$ 0.2	5.2-7	<b>0.000</b>
<b>SCL</b>	4.77 $\pm$ 0.2	4-6.8	5.24 $\pm$ 0.25	4.3-6.2	3.52 $\pm$ 0.16	2-4.3	5.31 $\pm$ 0.24	4-6	<b>0.000</b>
<b>SCW</b>	2.64 $\pm$ 0.14	1.94-4	3 $\pm$ 0.17	2-4	1.94 $\pm$ 0.07	1-2.2	3.24 $\pm$ 0.15	3-4	0.000
<b>HED</b>	3.47 $\pm$ 0.14	2.4-4.3	3.64 $\pm$ 0.2	3-4.7	2.73 $\pm$ 0.13	1.7-3.8	3.83 $\pm$ 0.2	3-4.8	<b>0.000</b>
<b>SUP</b>	8.94 $\pm$ 0.1	8-10	9 $\pm$ 0	9-9	8.93 $\pm$ 0.15	7-10	8.88 $\pm$ 0.13	8-9	0.951
<b>Dorsals</b>	19.31 $\pm$ 1.08	14-19	19 $\pm$ 0	19-19	18.6 $\pm$ 0.27	15-19	19 $\pm$ 0	19-19	0.202
<b>DAV</b>	12.88 $\pm$ 0.12	12-14	13.33 $\pm$ 0.24	13-15	13 $\pm$ 0.14	12-14	12.88 $\pm$ 0.23	12-14	0.257
<b>GUL</b>	3.76 $\pm$ 0.22	2-5	5.11 $\pm$ 0.35	4-7	4.53 $\pm$ 0.32	3-7	4 $\pm$ 0.42	2-6	0.024
<b>Ventrals</b>	222.35 $\pm$ 4.05	190-263	211.22 $\pm$ 3.13	195-225	204.8 $\pm$ 6	154-236	220.25 $\pm$ 6.23	196-239	<b>0.048</b>
<b>Subcaudals</b>	10.49 $\pm$ 0.55	73-121	13.27 $\pm$ 0.85	48-100	8.84 $\pm$ 0.71	77-140	12.41 $\pm$ 0.75	94-137	0.000

According to the analysis of variance (ANOVA), 12 out of 21 characters differed significantly ( $P \leq 0.05$ ) among four groups of *Platyceps rhodorachis-ventromaculatus* species complex in Iran (Bold characters in Table 1), with all normally-distributed characters showing significance in the one-way ANOVA. We also performed the Kruskal-Wallis test for nine non normally-distributed characters and found TL, SCW, and Subcaudals showed significant values (Table 2).

The lowest means metric values are related to the samples from Sistan and Baluchestan Province in southeastern Iran. In meristic characters, some of the specimens related to the populations from the southeast and west of Zagros showed a significant difference in the number of rows of dorsal scales with other specimens (Table 1). A principal component analysis (PCA) indicated that the first three components explained 86.42% of the cumulative variance and that SVL, TL and Ventral scales are highly diversified characters (Table 3).



**Figure 2.** Box diagram showing the difference between the number of Gular scales in male and female *P. rhodorachis-ventromaculatus* specimens collected from 4 regions of Iran.

**TABLE 2.** Means and ranges for the abnormal character descriptive of 8 morphometric, meristic, and ratio characters measured in adult specimens, collected from four geographic areas across their distribution in Iran. Bold values refer to significant differences in characters among groups.

Characters	Mean	Asymp. Sig.	Kruskal-Wallis H	Range
<b>TL</b>	217.041	<b>0.000</b>	21.776	60-306
HW/HL	0.671	0.463	2.570	0.53-1.05
SW/HL	0.390	0.300	3.663	0.33-0.52
<b>SCW</b>	2.591	<b>0.000</b>	27.280	1-4
SUP	8.939	0.860	0.756	7-10
Dorsals	18.878	0.069	7.089	15-19
DAV	13.000	0.376	3.106	12-15
<b>Subcaudals</b>	109.875	<b>0.000</b>	22.823	48-140

Canonical variate analysis (CVA) showed that the first three functions explained 100% of the variance (Table 4) and, in the scatter plot of the first function relative to the third function, the OTUs are entirely separated from each other (Fig. 4).

Principal component analysis and Canonical variate analysis for 15 significant characters measured from adult specimens of *P. rhodorachis-ventromaculatus* species complex in Iran showed that the four populations studied are morphologically different from each other.

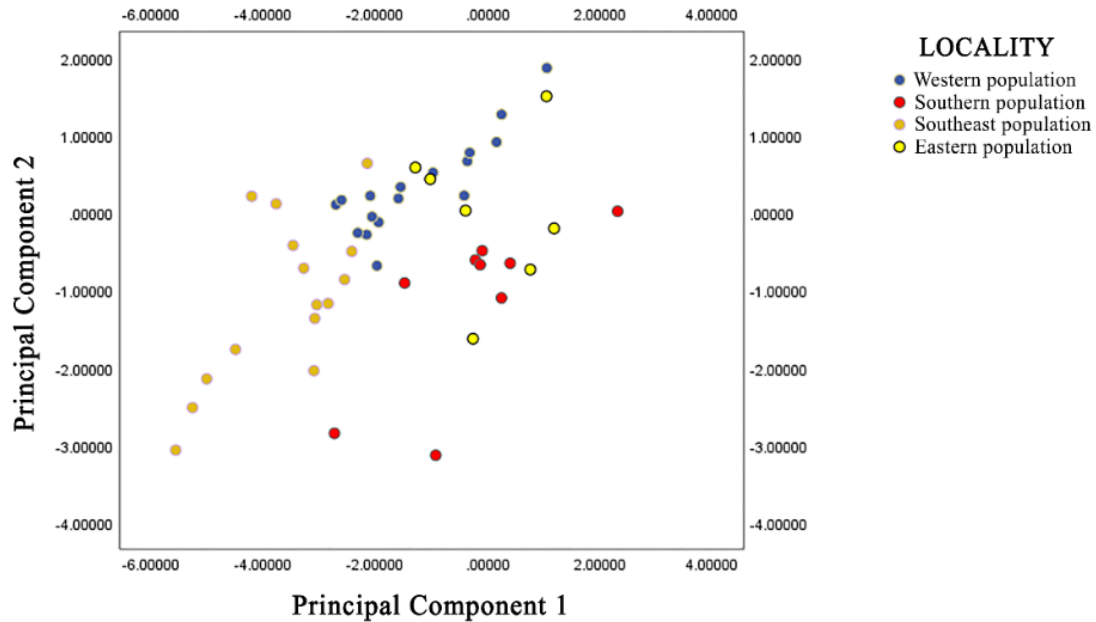
We also performed CVA for metric and relative characters. The results of a Wilks' Lambda test showed that the first three functions comprise 100% of the total variance (Appendix 7) (Appendix 5).

**TABLE 3.** Factor loadings of the first three Principal Components (PCs) elicited from a correlation matrix of 15 metric, ratio, and meristic significant characters measured in adult specimens.

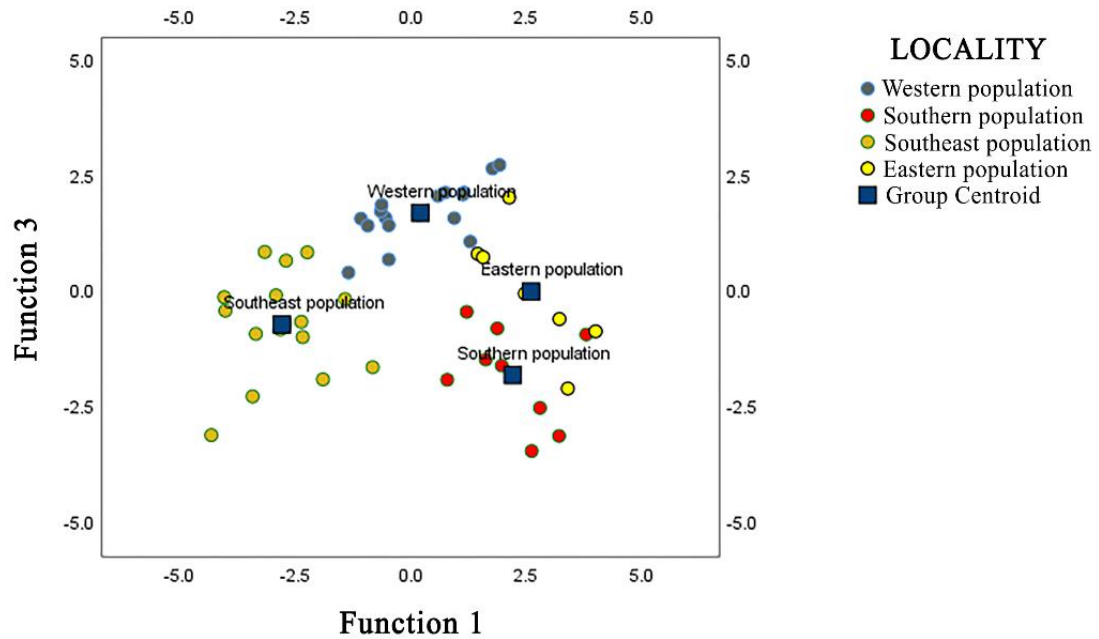
Characters	PC1	PC2	PC3
SVL	0.953	-0.067	-0.179
TOL	0.848	0.443	-0.065
TL	0.114	0.961	0.147
HL	0.937	-0.074	-0.101
HW	0.886	0.190	0.254
SL	0.945	-0.302	-0.075
SW	0.908	-0.104	-0.134
FL	0.530	0.474	0.346
SCL	0.784	-0.145	0.367
SCW	0.714	0.357	0.227
HED	0.612	0.721	-0.269
Subcaudals	-0.287	0.692	0.515
Ventrals	-0.051	-0.608	0.782
TL/TOL	-0.368	0.899	0.230
SL/HL	0.811	-0.519	0.046
Eigen values	48.038	27.990	10.402
Cumulative variance %	48.04	76.02	86.42

**TABLE 4.** Function loadings of the first three canonical variate analyses elicited from a correlation matrix of 15 metric, ratio, and meristic significant characters measured in adult specimens.

Characters	CV1	CV2	CV3
SVL	2.840	1.360	-2.459
TL	0.942	3.434	-3.063
TOL	-2.014	-3.480	4.317
HL	1.208	1.443	2.668
HW	-0.819	-0.571	-1.089
SL	-1.997	-2.603	-3.873
SW	0.012	0.337	2.032
TL/TOL	-0.646	-1.593	0.843
SL/HL	0.904	1.243	1.709
FL	-0.074	0.122	0.125
SCL	0.235	0.153	0.312
SCW	0.412	-0.200	-0.964
HED	-0.299	0.238	0.208
Ventrals	-0.201	0.143	0.400
Subcaudals	-0.105	0.740	0.130
Eigen values	4.723	1.953	0.284
Cumulative variance %	67.9	95.9	100



**FIGURE 3.** The scattered plot of the first and second principal components for 15 metric, meristic, and ratio statistically significant morphological characters in *P. rhodorachis-ventromaculatus* species complex from Iran.



**FIGURE 4.** The scattered plot of the first and second Canonical components for 15 metric, meristic, and ratio statistically significant morphological characters in *P. rhodorachis-ventromaculatus* species complex from Iran.

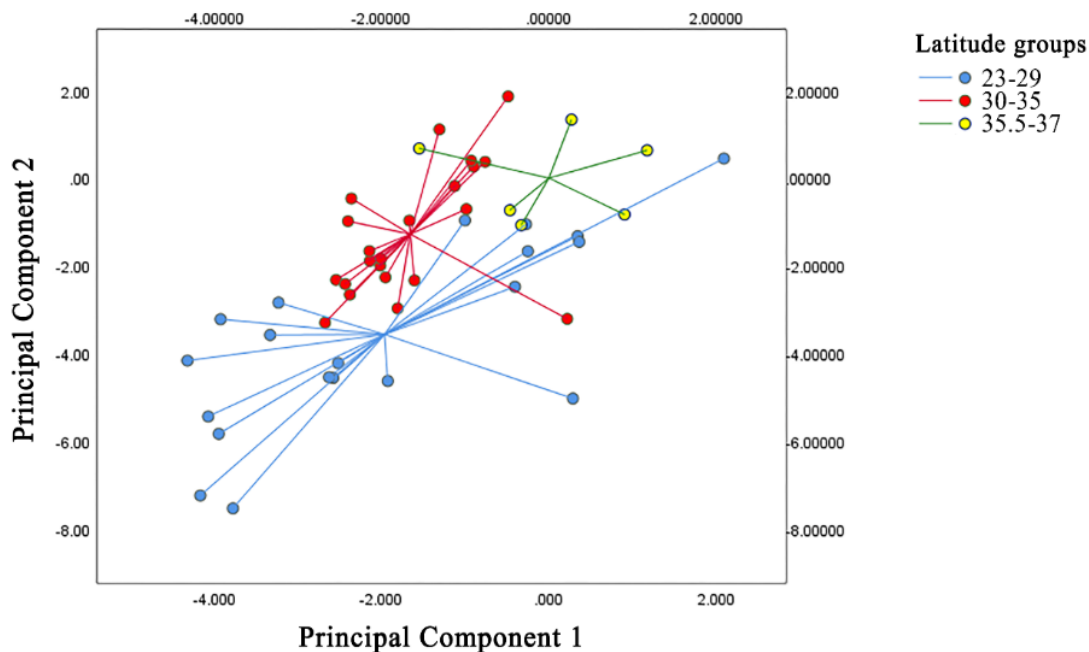


Canonical variate analysis (CVA) for meristic characters shows the same results. The number of ventral and Subcaudals scales has the most excellent effect on separating components from each other (Appendix 8). The scatter plot also clearly separates the Group Centroids (Appendix 6).

We also divided the specimens into three different groups based on latitude in Iran and investigated the diversity of morphological patterns of *P. rhodorachis* and *P. ventromaculatus* using metric, ratio, and meristic character analysis (25° N to 29° N, 30° N to 35° N and 36° N to 37° N; Fig. 1).

For this purpose, we used the ANOVA test for the analysis of normally-distributed characters (Appendix 2) and the Kruskal-Wallis test for the analysis of non-normally distributed characters (Appendix 3). Then we performed principal component analysis (PCA) on nine significant characters (Appendix 4; significance level <0.05); the scattered plot of the first and second principal components can be seen in (Fig. 3).

PCA and Linear regression plots obtained for nine significant characters in three latitude zone in Iran (Fig. 5; Appendix 9) show a significant relationship between latitude and character values.



**FIGURE 5.** Scatter plot of the first and second principal components for 9 significant characters relative *Platycephalus rhodorachis-ventromaculatus* grouped in three latitude zone in Iran.

## DISCUSSION

Differentiation of four Operational Taxonomic Units (OTUs) based on the distinction between morphological characters in Iran determined that SVL is the leading cause of diversity among populations. SVL in both PC and CV analyses had the highest effect on population separation. SL in PCA and TOL in CVA are determining factors of the degree of separation of populations. The most prominent character with the most impact on morphological variation in snakes (i.e., snout to vent length) shows a severe clinical change (Table 1).

The southeastern population of Sistan and Baluchestan Province showed significant differences with other populations; the differences detected in this morph do not seem irrelevant. For example, the significant difference observed in latitude changes showed high alignment with morphological results and confirms morphological separation to some extent.

Morphological differences between the populations of the West Zagros Mountains and the Southeast with the rest of the populations can be considered due to the existence of geographical barriers in the distribution range of these populations. One of these barriers is the Zagros Mountains and the impact of this barrier on reptile diversity in Iran has been demonstrated many times. Zagros, which belongs to the southern part of the third Mesozoic orogeny in Iran, was created by the opening of the Red Sea with the movement of Arabian plates and now acts as a natural barrier against Iranian reptile species (Falcon, 1974; Kafash *et al.*, 2020). Usually, the western and eastern populations of the Iranian reptile fauna are separated by the Zagros Mountains (Haftlang & Lang, 2003).

The populations of Sistan and Baluchestan Province (southeast population) separated from other populations by the presence of the Dasht-e Lut in the West; the results of PCA and CVA approve this claim to a large extent. The southeastern population shows significant quantitative differences in morphological characters. Also, according to the CVA diagram (Fig. 4), the southeastern population is separated from other populations.

Multivariate analyzes indicate the separation of populations and indicated that the southeastern population of Sistan and Baluchestan Province is more separated from other populations (Fig. 4). As the possibility of establishing a link between the eastern OTU and the southern and western OTUs seems unlikely, meristic characters in the *Platyceps rhodorachis-ventromaculatus* complex are probably conserved and not a good choice for studying the biogeographical effects on the phonetic diversity in Jan's Cliff Racer snakes. Snout length (SL) and Snout to vent length (SVL) had the most significant effect on OTUs separation (Appendix 7).

If we consider the above assumption, the variations observed in the southern population related to Qeshm Island could be a result of the greater diversity in the ecology and climate of the island compared with the mainland. This is a justification for the overlap of metrics (e.g., TOL, SVL, TL in Appendix 9) among these two populations (Simberloff, 1970; Kreft *et al.*, 2008).

Meristic characters (e.g., number of supralabials and suboculars, longitudinal position of the third reduction) show overlap among all examined populations (Appendix 9), which means the characters may not be affected by origin, gender, age, or macroecological conditions (Schaetti *et al.*, 2014).

Our impression is that the presence of this polymorphism in *P. rhodorachis-ventromaculatus* complex species, along with meristic characters stability, may indicate a phenotypic response to environment selection due to factors such as thermoregulation and response to bioclimatic parameters such as altitude and latitude (Henderson, 1997).

PCA results investigating the effects of latitude change on significant characters separated the groups into three main clusters (Fig. 5); this represents character changes based on latitude differences and largely confirms Bergmann's rule in the *P. rhodorachis-ventromaculatus* complex (Sand *et al.*, 1995).

In Iran, increasing latitudes correspond with decreasing average temperatures (Dastorani & Poormohammadi, 2012). Although there is evidence that snakes and other reptiles do not follow Bergmann's rule in other areas, the results of this study show that the metrics of the *P. rhodorachis-ventromaculatus* complex in Iran increase with increasing latitude (Pincheira-Donoso *et al.*, 2008; Feldman & Meiri, 2014).

The results of linear regression for the significant metric characters show the same results. The metric values increase with increasing latitude in the SVL, TOL, and TL characters (Appendix 9).

Non-observance of this relationship in the Qeshm Island population, which is located in latitude one (Fig. 1), as mentioned earlier, is due to the island ecosystem's variability compared to the mainland (Parker, 1949; Boback, 2003). The pattern is present among the other three populations, and as the latitude increases, so do the metric characters (Appendix 9). In meristic characters, this incremental pattern is less or not present at all; it seems that the number of scales is less affected by environmental factors and latitude.

Therefore, we conclude that the eastern population of Iranian *Platyceps rhodorachis-ventromaculatus* complex species in some characters show an intermediate between the populations of

western Zagros and southern Iran. However, the population of Sistan and Baluchestan Province in all cases shows a significant difference from other populations. This discrepancy appears to be due to the separation of Sistan and Baluchestan by Iran's Central Desert Natural barrier. Differences in metric characters in *P. rhodorachis-ventromaculatus* complex species can be seen based on latitudes, but it seems that meristematic characters do not change under the influence of latitude and have a fixed value.

Systematic studies have shown that diversity among specimens representing the same species is influenced by ecological and genetic factors (Sexton *et al.*, 2009). Genetic studies in the *Platyceps rhodorachis-ventromaculatus* complex in Iran are needed to assess whether significant morphological differences are observed in this group affected by cline changes. Further studies in this field will also help assess the extent of morphological plasticity and taxonomic differences between populations with a broader perspective.

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