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Morphological variation among populations of *Mesalina watsonana* (Stoliczka, 1872) (Sauria: Lacertidae) in Iran

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Abstract: During extensive fieldwork surveys from August 2009 to September 2011, 60 specimens of *Mesalina watsonana* were collected from 10 localities representing 3 population groups in Iran. Owing to clear sexual dimorphism, a total of 39 males were selected and examined for various meristic and morphological characters. Statistical analyses (multivariate analyses such as principal component analysis, canonical variate analysis, and cluster analysis) were used to compare morphological variations among specimens from these 3 population groups in Iran. These groups were separated geographically, apparently due to barriers such as Dasht-e-Kavir and Dasht-e-Lut. According to the multivariate analyses, *Mesalina watsonana* can be divided into 2 major morphological groups in Iran. The Zagros group is related to 2 others; the southern and eastern groups clearly separate and show a break between themselves.

Key words: East clade, *Mesalina watsonana*, statistical analysis, 3 operational taxonomic units, Zagros clade

1. Introduction

The genus *Mesalina* Gray, 1838 contains 14 species that inhabit the dry regions of North Africa, the Arabian Peninsula, and the Iranian plateau and comprises several groups as African and Asian groups (Szczerbak, 1989; Anderson, 1999; Kapli et al., 2008; Smid and Frynta, 2012). The genus *Mesalina* and other Palearctic genera such as *Acanthodactylus*, *Ophisops*, and *Eremias* create a distinct group based on morphological characters (Arnold, 2004). The majority of *Mesalina* species occur west of the Zagros Mountains, but *Mesalina watsonana* (Stoliczka, 1872) occurs east of the Zagros Mountains throughout the Iranian plateau in Iran, Turkmenistan, Afghanistan, and Pakistan (Anderson, 1999). In most parts of Iran, *Mesalina watsonana* (Stoliczka, 1872) is abundant on hard soils and alluvial fans, and is commonly found on hillsides, in valleys, and near streams in areas where vegetation is sparse or completely absent (Rastegar-Pouyani et al., 2007); this includes most of the areas situated in the central plateau, the southern region of Iran, and the eastern and northeastern margins of the Iranian plateau. The species is abundant at lower elevations and is rarely found above 2000 m (Hosseinian Yousefkhani et al., 2013). In this study, the species has been observed at sea level (0 m) in the southern part of Iran and at up to 1711 m in Khorasan Razavi Province near Robat-e Sang.

Mesalina watsonana can be found throughout most of the Iranian plateau but does not occur in the Kavir Desert and Lut Desert. According to Rastegar-Pouyani et al. (2010), as the distributions of *Mesalina watsonana* and *Eremias persica* Blanford, 1875 in the central part of the Iranian plateau have an overlap, we can presume that those barriers affect the distribution patterns of both *Eremias persica* and *Mesalina watsonana*. Thus, we hypothesize that these deserts may represent barriers to gene flow among *Mesalina watsonana* populations, leading to morphological differences among various populations. Additionally, recent work by Smid and Frynta (2012) found that populations of *Mesalina watsonana* have multiple genetic clades within the Iranian plateau. Herein, we use multivariate analyses of meristic and mensural morphological features across Iranian populations to determine if populations separated by desert barriers are morphologically distinct or not.

2. Materials and methods

2.1. Study system

A total of 39 males were collected from most parts of the species' distribution range in Iran, except from the Khuzestan plateau (Figure 1; Table 1). The specimens from 3 distinct areas are referred to as population groups,

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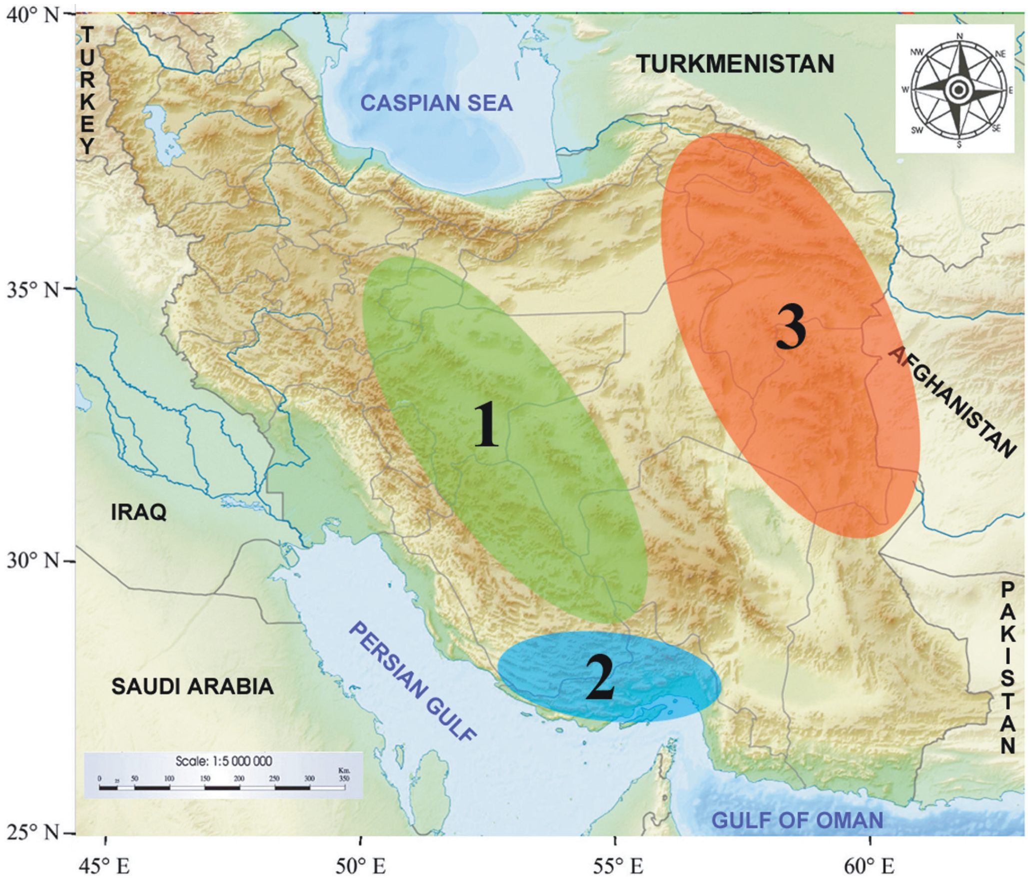

Figure 1. Geographic distribution of 3 OTUs of *Mesalina watsonana* used in this study.

also called operational taxonomic units (OTUs), and are numbered 1–3 (Table 2). All specimens were collected during 7 trips from August 2009 through September 2011 on the Iranian plateau and are preserved in the Sabzevar University Herpetological Collection (SUHC). For the sexual dimorphism that is shown in the species (Oraei et al., 2011), we selected and examined 39 specimens of males for 19 mensural and 9 meristic characters commonly used to identify lacertid species (Table 3). Meristic characters were quantified using an Olympus loupe, and mensural characters were measured with digital calipers at up to 0.01 mm of accuracy. By collecting specimens from different parts of Iran, we observed differentiation in color and pattern among male specimens (Figure 2).

2.2. Statistical analysis

Statistical analysis was conducted with SPSS 16.0. A one-way analysis of variance (ANOVA) was used to examine the pairwise comparison of characters between populations, with the null hypothesis that character means are equal. This technique is an extension of the 2-sample t-test. Multivariate analyses include cluster analysis, principal component analysis (PCA), and canonical variate analysis (CVA). Cluster analysis was used to determine which individuals were the most morphologically similar based on the unweighted pair group method with arithmetic mean (UPGMA). PCA based on a correlation matrix of 8 characters was used to determine if populations were morphologically clustered. CVA based on 8 characters was used to determine if individuals would be assigned to the correct population group based on morphological measurements (Rastegar-Pouyani, 2005).

3. Results

Morphological character summaries are shown in Table 4, including the mean, standard error, and minimum and maximum values for each population. The results of these analyses show that the population of the species in the Iranian plateau is divided into 2 major groups: the eastern and northeastern group and the southern group. These 2 groups are related to the Zagros group.

According to the ANOVA, 8 characters show meaningful value ($P < 0.05$) (Table 5); we used these characters for PCA and CVA (SVL, HH, HW, LFL, LHL, IOR, NDS, and SDLT; see Table 3 for definitions). Cluster analysis found 2 major morphological clusters (Figure 3) that correspond with eastern and western populations of

Table 1. Locality data of *Mesalina watsonana* collected from the Iranian plateau.

Museum number	N	E	Altitude	Locality
SUHC 838	27°17′	056°28′	0 m	Hormozgan Province–on the road from Bandar-e Abbas to Minab
SUHC 836	27°17′	056°28′	0 m	Hormozgan Province–on the road from Bandar-e Abbas to Minab
SUHC 1082	31°06′	061°34′	400 m	Sistan and Baluchestan Province–Bonjar–between Zabol and Doost Mohammad
SUHC 856	26°46′	056°04′	0 m	Hormozgan Province–Qeshm Island–Souza city
SUHC 455	29°14′	054°22′	1689 m	Fars Province–Neyriz–on the road to Ghetroyeh
SUHC 448	28°37′	054°20′	1103 m	Fars Province–Darab–near Tolrigi
SUHC 443	28°27′	054°14′	1111 m	Fars Province–From Fasa–on the road to Lar–Khosouyeh
SUHC 447	29°14′	054°22′	1689 m	Fars Province–Neyriz–on the road to Ghetroyeh
SUHC 1061	34°44′	060°48′	795 m	Khorasan Razavi Province–Taybad–2 km E of Taybad on the road to Dogharoun
SUHC 1067	35°32′	059°11′	1711 m	Khorasan Razavi Province–Torbat-e Heydaryieh- Robat Sang
RQP 317	34°36′	050°49′	840 m	Qom–Kamarkouh region
RQP 316	34°36′	050°49′	840 m	Qom–Kamarkouh region
RQP 314	34°36′	050°49′	840 m	Qom–Kamarkouh region
RQP 294	34°38′	051°10′	849 m	Qom–Dolat Abad
RQP 185	34°29′	051°07′	883 m	Qom–Jannat Abad
RQP 313	34°36′	050°49′	840 m	Qom–Kamarkouh region
RQP 186	34°29′	051°07′	883 m	Qom–Jannat Abad
RQP 145	34°20′	050°34′	1369 m	Qom–Dastjerd
RQP 147	34°20′	050°34′	1369 m	Qom–Dastjerd
RQP 288	34°38′	051°10′	849 m	Qom–Dolat Abad
Live specimen	36°20′	056°45′	850 m	Semnan Province–between Shahroud and Sabzevar
RQP 315	34°36′	050°49′	840 m	Qom–Kamarkouh region
RQP 199	34°29′	051°14′	870 m	Qom–Jannat Abad
RQP 110	34°29′	051°14′	870 m	Qom–Jannat Abad
RQP 185	34°29′	051°14′	870 m	Qom–Jannat Abad
SUHC 769	33°15′	058°51′	1401 m	Southern Khorasan Province–Aryian shahr (Sedeh)
SUHC 614	34°59′	058°03′	1176 m	Khorasan Razavi Province–near Bardaskan
SUHC 613	34°59′	058°03′	1176 m	Khorasan Razavi Province–near Bardaskan
SUHC 564	35°30′	059°13′	1100 m	Khorasan Razavi Province–Torbat-e Heydaryieh
No tag	36°10′	057°05′	967 m	Khorasan Razavi Province–near Sabzevar
SUHC 566	35°06′	059°42′	1180 m	Khorasan Razavi Province–Roshtkhar
SUHC 887	34°18′	051°52′	889 m	Isfahan Province–Kashan
SUHC 884	34°18′	051°52′	889 m	Isfahan Province–Kashan
SUHC 119	28°42′	057°53′	900 m	Kerman Province–Jiroft
SUHC 120	28°42′	057°53′	900 m	Kerman Province–Jiroft
No tag	34°11′	056°29′	980 m	Yazd Province–Tabas–Robat-e Goor
No tag	34°11′	056°29′	980 m	Yazd Province–Tabas–Robat-e Goor
No tag	34°46′	057°22′	1080 m	Yazd Province–between Tabas–Tapeh Taq
No tag	34°46′	057°22′	1080 m	Yazd Province–between Tabas–Tapeh Taq

Table 2. OTU numbers and localities of *Mesalina watsonana* in Iran.

OTUs	Locality
1	Zagros group (Qom, Kerman, Fars, Kashan)
2	Southern group (Qeshm, Bandare Abbas)
3	Eastern and northeastern group (Khorasan, Semnan, Tabas, Zabol)

Table 3. Mensural and meristic characters that were used in the study.

Characters	State definition
SVL	Snout–vent length (from tip of snout to anterior edge of cloaca)
TL	Tail length (from posterior edge of cloaca to tip of tail)
LHF	Trunk length (distance between hindlimb and forelimb)
HL	Head length (from tip of snout to the posterior edge of tympanum)
HH	Head height (maximum distance between upper head and lower jaw)
HW	Head width (distance between posterior eye corners)
LFL	Length of forelimb (from top of shoulder joint to tip of fourth toe)
LHL	Length of hindlimb (from hip joint to tip of fourth toe)
LFO	Length of femur (from hip joint to top of knee)
LA	Length of tibia (from top of knee to beneath wrist)
EL	Length of eye (distance from anterior corner to posterior corner to its posterior)
RED	Snout length (from tip of nostril to anterior corner of eye)
EED	Distance between posterior edge of eye to tympanum
NL	Length of neck (distance between posterior edge of tympanum and shoulder joint)
TD	Tympanum diameter (largest size)
IOR	Interorbital distance (largest size)
LV	Length of cloaca crevice (largest size)
LBT	Length of widest part of tail base
LWB	Length of widest part of belly
NSL	Number of labial scales anterior to the center of eye on the right side of head
NIL	Number of scales on the lower labial region
NGS	Number of gular scales in a strait median series
NCS	Number of collar scales
NEE	Number of scales between posterior edge of the eye to tympanum
NVS	Number of transverse series of ventral scales counted in straight median series between collar and the row of scales separating the series of femoral pores
NDS	Number of dorsal scales across midbody
SDLT	Number of subdigital lamellae on fourth toe (defined by their width, the one touching the claw included), counted in a bilateral way
NFP	Number of femoral pores, counted in a bilateral way

Mesalina watsonana. The eastern cluster is closely related to Afghan populations of *Mesalina watsonana* because the eastern clade is close to the Afghanistan border and Afghan basins (Khorasan and Seistan regions), and the western cluster consists of populations that inhabit the Zagros foothills areas of Qom, Fars, Hormozgan, and Isfahan.

PCA reduced the complexity of the multivariate data into 3 principal axes that explain 91.94% of the total variation (Table 6). Principal component 1 is primarily an axis of size, and principal component 2 is heavily weighted by numbers of scales under the fourth toe (SDLT) and the number of dorsal scales (NDS). Principal component 3 is weighted by NDS. A plot of principal components

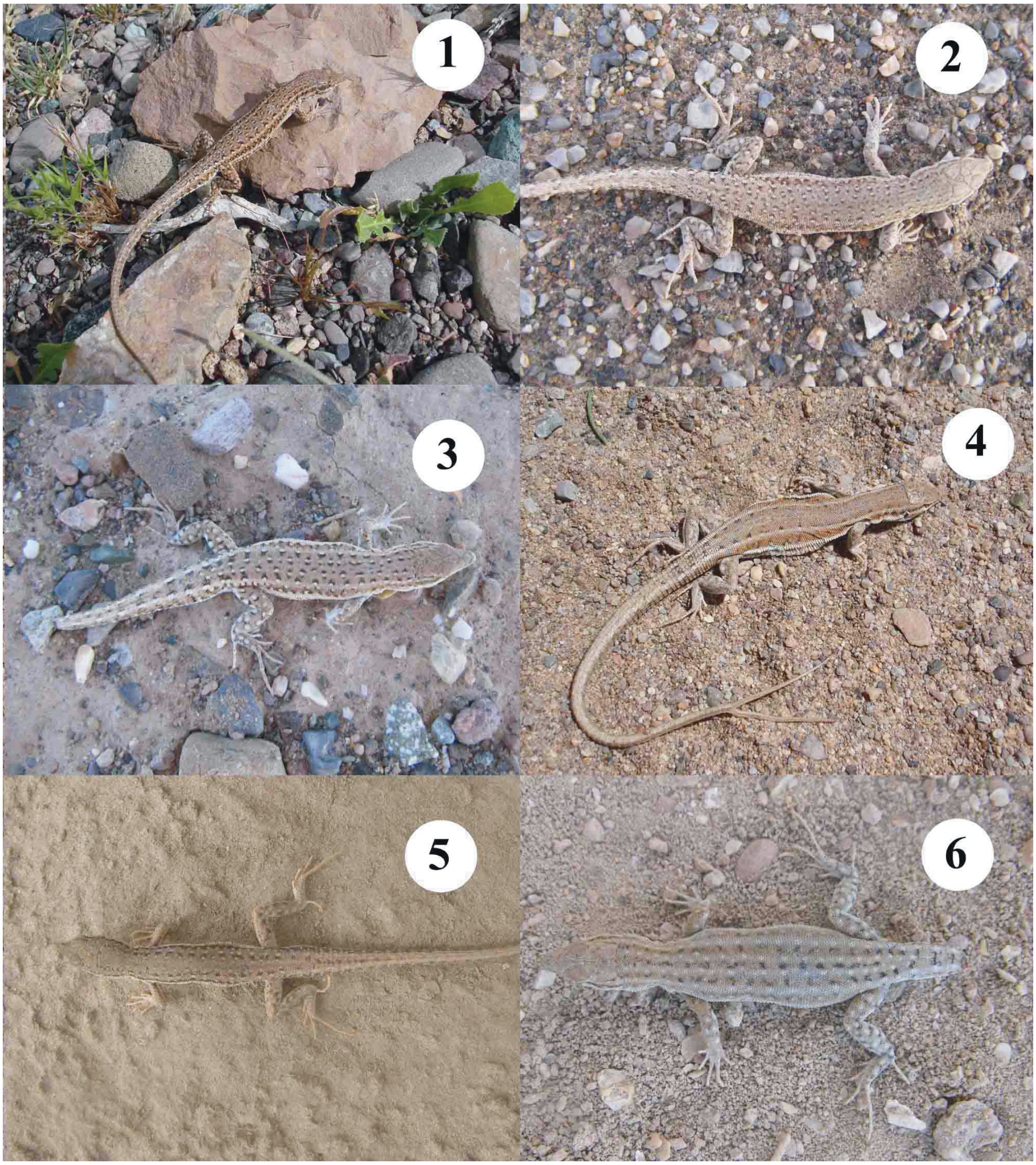

Figure 2. Six specimens from 6 localities in Iran: 1) Torbat-e-Heydariyeh, Khorasan Province; 2) Tabas, Yazd Province; 3) Bardaskan, Khorasan Province; 4) Khaf, Khorasan Province, at the border of Afghanistan; 5) Bandar-e-abbas, Hormozgan Province; 6) Qeshm Island, Hormozgan Province.

1 and 2 (Figure 4) shows that populations of *Mesalina watsonana* in Iran have a large amount of morphological overlap. Southern populations are slightly different from populations in the east and west, and there are clear distinctions between southern and eastern clades.

CVA found similar relationships to those of the PCA results (Table 7), although the populations show slightly more morphological separation (Figure 5). However, according to these results, the southern clade separates clearly from the eastern and northeastern group.

4. Discussion

The distribution pattern of *Mesalina watsonana* suggests some possible assumptions. According to the pattern, after the Zagros uplifting about 12 MYA (Smid and Frynta, 2012), ancestral populations were trapped in the central plateau of Iran; the taxon then found a new, broad niche. The *Mesalina watsonana* population limits in the central part of the Iranian plateau are determined by the Zagros Mountains in the west, Elburz and Kopet-dagh in the north, the Mokran Mountains in the south, and Hindu

Table 4. Descriptive parameters of 28 mensural and meristic characters including maximum, minimum, mean, and standard error in *Mesalina watsonana* (males).

Characters	Eastern and northeastern clade (n = 13)		Southern clade (n = 5)		Zagros clade (n = 20)	
	Mean ± std. error	Minimum–maximum	Mean ± std. error	Minimum–maximum	Mean ± std. error	Minimum–maximum
SVL	46.4192 ± 1.30347	32.85–50.35	40.4620 ± 1.15125	38.38–44.90	45.6340 ± 0.68328	38.52–49.70
TL	90.7367 ± 6.10270	71.83–106.51	84.3025 ± 11.30196	60.19–109.70	88.3950 ± 2.96142	74.91–99.91
LHF	20.3469 ± 0.82304	15.18–25.73	18.0960 ± 1.20016	13.78–20.83	20.5920 ± 0.59204	16.47–25.89
HL	10.8892 ± 0.32349	8.26–12.25	9.9940 ± 0.11165	9.60–10.26	10.8215 ± 0.18595	8.88–12.19
HH	5.6992 ± 0.19074	4.24–6.51	4.3520 ± 0.15561	3.84–4.72	5.2505 ± 0.12501	3.96–6.06
HW	7.7046 ± 0.26246	5.30–9.06	6.4700 ± 0.09413	6.20–6.70	7.3560 ± 0.17279	5.05–8.46
LFL	16.0608 ± 0.47271	12.87–17.96	14.2220 ± 0.46707	13.03–15.76	15.8536 ± 0.25720	13.83–17.27
LHL	29.3392 ± 0.72236	24.19–32.06	27.4240 ± 0.57310	26.41–29.61	29.9645 ± 0.37613	26.86–32.95
LFO	6.0377 ± 0.20712	4.11–7.25	6.0720 ± 0.61954	4.50–8.05	6.4065 ± 0.11893	5.29–7.23
LA	8.0823 ± 0.25350	5.98–9.16	7.3920 ± 0.15170	6.80–7.65	8.0005 ± 0.15742	6.82–8.88
EL	2.8177 ± 0.13965	2.13–4.00	2.7040 ± 0.22413	2.05–3.32	2.8070 ± 0.11639	1.82–3.70
RED	4.0038 ± 0.17315	2.44–5.03	3.8840 ± 0.34886	2.80–4.85	3.8035 ± 0.12140	2.58–4.45
EED	3.1031 ± 0.16208	1.60–3.70	2.7720 ± 0.18323	2.22–3.33	3.0045 ± 0.07335	2.51–3.86
NL	5.6531 ± 0.25753	3.41–7.29	5.3340 ± 0.39278	4.02–6.22	5.3955 ± 0.17033	3.96–6.94
TD	2.0438 ± 0.06360	1.64–2.54	1.7040 ± 0.12921	1.41–2.13	2.0415 ± 0.06607	1.37–2.50
IOR	5.3808 ± 0.16454	4.34–6.28	4.3380 ± 0.04964	4.24–4.52	5.0940 ± 0.07066	4.25–5.62
LV	4.7262 ± 0.29592	2.70–6.07	4.4420 ± 0.25879	3.69–5.08	4.5980 ± 0.22818	2.73–6.44
LBT	6.0085 ± 0.40683	2.57–7.74	5.2680 ± 0.12399	4.96–5.67	5.8425 ± 0.16642	4.44–6.91
LWB	10.3300 ± 0.55345	5.05–12.65	8.5520 ± 0.27489	7.89–9.35	9.6295 ± 0.25151	7.32–11.67
NSL	8.4615 ± 0.14391	8.00–9.00	8.0000 ± 0.00000	8.00–8.00	8.6500 ± 0.13129	8.00–10.00
NIL	7.6154 ± 0.18040	7.00–9.00	8.2000 ± 0.20000	8.00–9.00	7.7500 ± 0.19022	6.00–9.00
NGS	23.6923 ± 0.42944	20.00–26.00	24.8000 ± 1.15758	22.00–28.00	23.6500 ± 0.31014	21.00–27.00
NCS	10.9231 ± 0.30929	9.00–13.00	9.8000 ± 0.58310	8.00–11.00	10.8000 ± 0.26754	9.00–14.00
NEE	12.0769 ± 0.34828	10.00–14.00	12.4000 ± 0.81240	10.00–14.00	13.0500 ± 0.19835	11.00–15.00
NVS	27.3077 ± 0.32786	25.00–29.00	27.4000 ± 0.24495	27.00–28.00	27.4000 ± 0.24495	25.00–29.00
NDS	41.5385 ± 0.68515	36.00–45.00	39.0000 ± 2.16795	34.00–46.00	44.5000 ± 0.99604	35.00–52.00
SDLT	21.1538 ± 0.40583	18.00–24.00	24.2000 ± 0.37417	23.00–25.00	23.4500 ± 0.35891	20.00–26.00
NFP	12.1538 ± 0.35529	11.00–15.00	11.8000 ± 0.37417	11.00–13.00	12.7000 ± 0.27242	10.00–14.00

Table 5. The ANOVA-based intragroup comparison of 8 meristic and mensural characters in whole groups of populations of *Mesalina watsonana*.

Character	Sum of squares	df	Mean square	F	P
SVL	135.317	2	67.658	5.049	0.012
HH	6.607	2	3.303	9.557	0.000
HW	5.505	2	2.752	4.326	0.021
LFL	13.083	2	6.541	3.557	0.039
LHL	25.934	2	12.967	3.202	0.053
IOR	3.927	2	1.963	11.137	0.000
NDS	150.638	2	75.319	4.844	0.014
SDLT	53.426	2	26.713	12.073	0.000

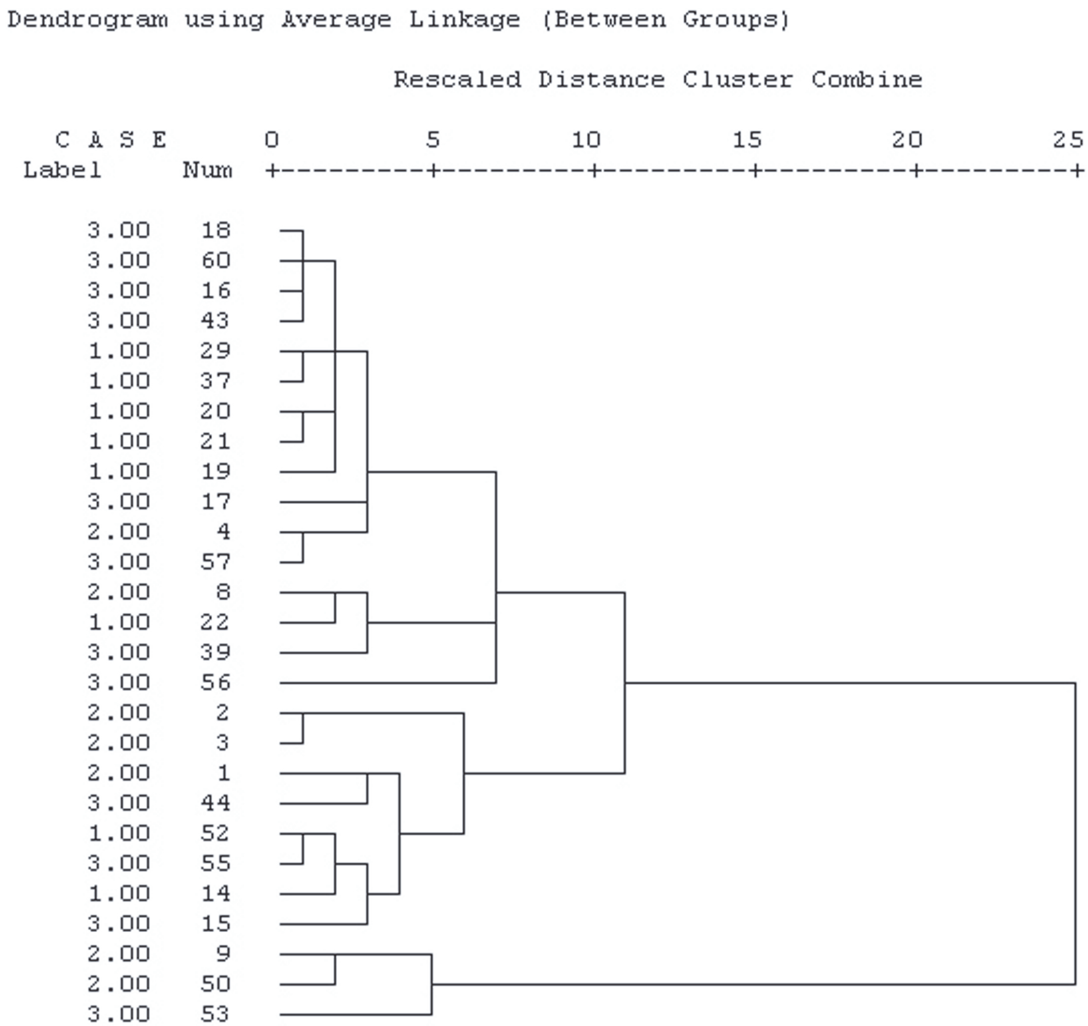

Figure 3. Dendrogram resulting from cluster analysis of 3 OTUs of *Mesalina watsonana*.

Table 6. Factor loadings on first 3 principal components elicited from a correlation matrix of 8 morphological characters for 39 males of all 3 OTUs of the *Mesalina watsonana.*

Characters	PC1	PC2	PC3
SVL	0.913	0.121	–0.228
HH	0.873	0.193	–0.297
HW	0.949	0.210	0.002
LFL	0.916	–0.195	0.036
LHL	0.928	–0.097	0.254
IOR	0.875	0.288	0.135
NDS	–0.223	0.743	0.615
SDLT	0.410	–0.721	0.526
Eigenvalues	5.177	1.299	0.879
Accumulated percent of trace	64.71	80.95	91.94

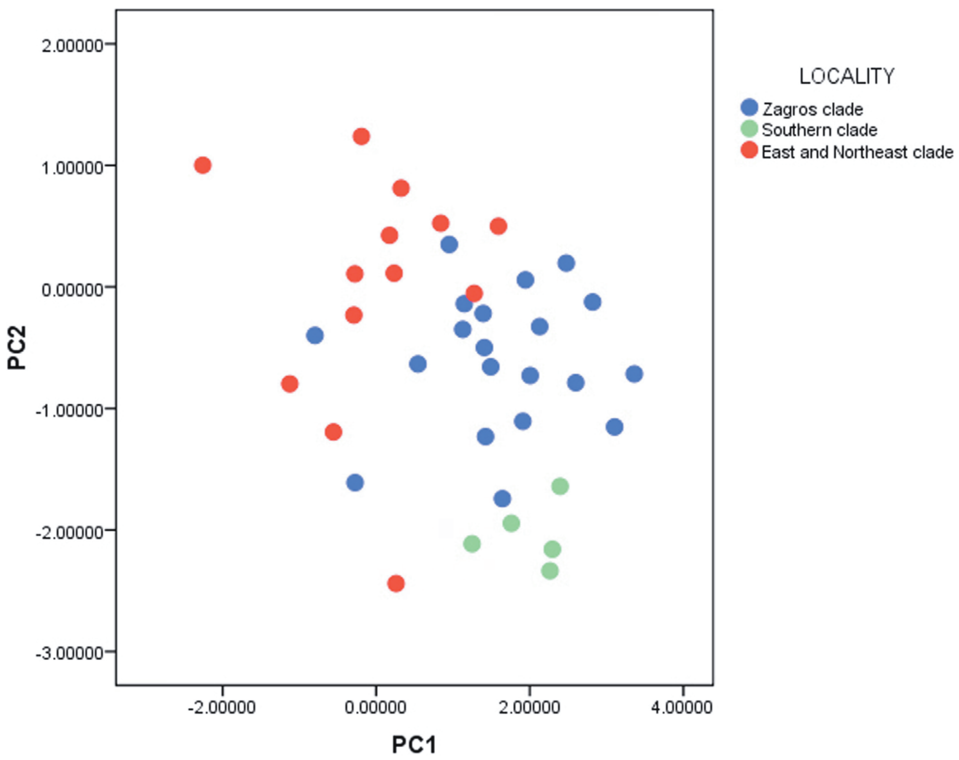

Figure 4. Ordination of the individuals of all localities on the first 2 principal components of 8 characters.

Kush in the east (Smid and Frynta, 2012). Our assumption suggests that these populations diversified with dispersal and vicariance from west to east. According to Smid and Frynta (2012), the southern clade is separated from the Zagros and eastern clades, and our study confirms the molecular data. According to the results of the study, the southern group shows a relationship with 2 other clades, the eastern and northeastern and the Zagros clades. The eastern and northeastern group shows a distinct separation from the Zagros group. We can conclude that the southern group is ancestral and divided into 2 major clades in the Zagros and the east. These clades have many internal populations that have gene flow between their populations. For example, in the eastern group, we have the Zabol and central Khorasan populations that are very far from each other, but some populations in the Qaen, Herat, and Farah areas in Iran and Afghanistan are situated between these groups. Intermediate populations exchanged genes between them. As we know, Semnan Province has a great desert (Dasht-e-Kavir) that plays a role as a barrier between western and eastern reptiles, but we saw the species in that region. Two assumptions can be offered about distribution around the desert. One hypothesis is extension of the species from Qom and Tehran, and the other is from Khorasan. If the taxon chose 2 ways of dispersing, some populations went to Khorasan and some to Qom. These assumptions suggested to us that some regions in Semnan Province are contact zones between western and eastern populations of *Mesalina watsonana*. The hypothesis needs to be confirmed by intermediate specimens in Semnan Province between the Khorasan and Qom populations of the species.

Comprehensive data from Afghanistan and Pakistani specimens in the future can resolve these issues.

Table 7. Standardized canonical coefficients for the specimens (males) of all 3 OTUs of the *Mesalina watsonana*.

Characters	CV 1	CV 2
SVL	–0.373	0.455
HH	–0.359	0.572
HW	0.307	0.564
LFL	–0.036	0.667
LHL	–0.067	0.754
IOR	–0.577	0.817
NDS	–0.044	0.146
SDLT	0.605	0.796
Eigenvalues	1.699	0.107
Accumulated percent of trace (%)	94.1	100.00

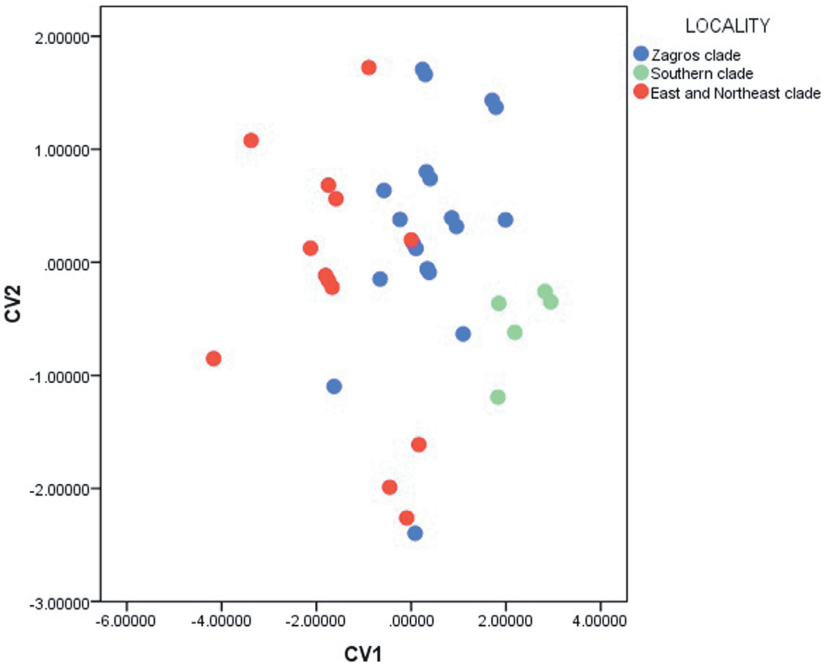

Figure 5. Ordination of the individuals of all localities on the first 2 canonical variates of 8 characters.

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