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Molecular and morphological analyses have revealed a new species of blunt-nosed viper of the genus *Macrovipera* in Iran

Hamzeh Oraie[1,2], Eskandar Rastegar-Pouyani[3], Azar Khosravani[2], Naeim Moradi[4], Abolfazl Akbari[5], Mohammad Ebrahim Sehhatisabet[7], Soheila Shafiei[6], Nikolaus Stümpel[8] & Ulrich Joger[8]

[1)] Department of Zoology, Faculty of Science, Shahrekord University, Shahrekord, Iran
[2)] Department of Biodiversity, Institute of Biotechnology, Shahrekord University, Shahrekord, Iran
[3)] Department of Biology, Faculty of Science, Hakim Sabzevari University, Sabzevar, Iran
[4)] Iranian Plateau Herpetology Research Group (IPHRG), Faculty of Sciences, Razi University, 6714967346 Kermanshah, Iran
[5)] Department of Venomous Animals and Antisera Production, Razi Vaccine and Serum Research Institute, Karaj, Iran
[6)] Provincial Office of Kerman, Department of the Environment, Iran
[7)] Department of Biology, Faculty of Sciences, Shahid Bahonar University of Kerman, Iran
[8)] State Natural History Museum, Brunswig, Germany

Corresponding author: Eskandar Rastegar-Pouyani, e-mail: rastegarpouyani45@gmail.com
Both first authors contributed equally to this manuscript

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Abstract. A new species of blunt-nosed viper of the genus *Macrovipera* is described from the central and southern parts of Iran on the basis of morphological and molecular examination. The mitochondrial Cytb gene was used to investigate phylogenetic relationships amongst the Iranian species of the genus *Macrovipera*. A dataset with a final sequence length of 1043 nucleotides from 41 specimens from 18 geographically distant localities across Iran was generated. The findings demonstrated that two major clades with strong support can be identified within the genus *Macrovipera* in Iran. One clade consists of individuals belonging to a new species, which is distributed in the central and southern parts of Iran; the second clade includes two discernible subclades. The first subclade is distributed in western and northwestern Iran, *Macrovipera lebetina obtusa,* and the second subclade consists of northeastern populations, representing *Macrovipera lebetina cernovi*. The new species, *Macrovipera razii* sp. n., differs from its congeners by having higher numbers of ventral scales, elongated anterior chin-shields, and lower numbers of canthal plus intersupraocular scales.

Key words. Squamata, Serpentes, Viperidae, *Macrovipera*, new species, mitochondrial Cytb, phylogeny, taxonomy, Iran.

Introduction

Blunt-nosed vipers of the genus *Macrovipera* Reuss, 1927 are widely distributed across North Africa, some islands in the eastern Mediterranean Sea, the Levant, various Middle Asian countries, ranging as far east as Kashmir in India and south to Yemen (Joger 1984, McDiarmid et al. 1999, Khan 2004, Ananjeva et al. 2006, Stümpel & Joger 2009). The genus has its main distribution area in Asia, where these snakes can be found in foothill, mountain, semi-desert and steppe habitats (Nilson & Andren 1988, Stümpel & Joger 2009). During the past three decades, much research has been done on different aspects of the biology (e.g., ecology, taxonomy, phylogeny, toxicology) of members of this genus (for instance, Joger 1984, Nilson & Andren 1988, Al Oran et al. 1998, Fatehi Hassanabad & Fatehi 2004, Stümpel & Joger 2009, Moradi et al. 2014). Nevertheless, there has been disagreement with regard to the taxonomic position of some nominal taxa of the genus *Macrovipera*. Two species are widely recognized (Uetz & Hošek 2016), although *M. schweizeri*, with its restricted distribution on a few islands in the western Cyclades, is recognized only as a subspecies of *M. lebetina* by some authors (Lenk et al. 2001; Stümpel & Joger 2009).

Currently, *Macrovipera lebetina* is the only valid species that occurs predominantly in Asia, a region where several morphotypes are found (Joger 1984, Nilson & Andren 1988, Stümpel & Joger 2009). During the last few decades, *M. lebetina* has been subdivided into several subspecies, some of which have been shown to be valid taxa whereas others have been synonymized (Nilson & Andren 1988, Stümpel & Joger 2009). In one of the most

comprehensive molecular studies on the blunt-nosed viper, the validity of four subspecies, i.e., *lebetina, obtusa, turanica* and *cernovi* was verified (Stümpel & Joger 2009, Stümpel 2012). Stümpel (2012) also identified a clade of *Macrovipera* (represented by one sample) from the mountains of southern Iran, which showed a high mitochondrial distance from typical *M. lebetina*. Further molecular studies on the Iranian populations of *M. lebetina* were not conducted until now, and two subspecies of *M. lebetina*, *M. l. obtusa* and *M. l. cernovi,* were believed to be the only representatives of blunt-nosed vipers in Iran (Rastegar Pouyani et al. 2008, Moradi et al. 2014, Safaei-Mahroo et al. 2015). A quantitative morphological study suggests that populations of *M. lebetina* in Iran could be divided into three distinct forms, with one of them being a potentially new taxon (Moradi et al. 2014). The need for a comprehensive study of the Iranian populations of *Macrovipera* is highlighted by those studies.

Here, we analysed sequences of the mitochondrial Cytb gene along with morphological data in order to test the hypothesis that some central and southern Iranian populations of *Macrovipera lebetina* could represent a new taxon.

Material and methods

Sample collection and molecular analyses

In order to examine the phylogenetic relationships within and between *Macrovipera* populations in Iran, 41 individuals from 18 geographically distant localities throughout the range were used in a molecular study (Table 1, Fig. 1). Specimens were collected within the framework of the Iranian herpetofauna project between 2002 and 2015. Many of the specimens were released in their natural habitats after blood and muscle (tail clips) samples had been taken. All tissue samples were deposited in the Hakim Sabzevari University Herpetological Collection (SUHC), Sabzevar, Iran, and kept at -20°C in absolute ethanol until DNA extraction. Genomic DNA was extracted from tissue samples using proteinase K digestion (10 Mm Tris–HCL Ph 8.0, 50 Mm EDTA PH 8.0, 10 Mm NaCl, 0.5% SDS, 0.1 M DTT, and 10 U of 10 Mgr/ML Proteinase K) followed by an Ammonium Acetate-Extraction protocol (Kapli et al. 2013). The primers F1_Cytb (5'-TGA GGC CTG AAA AAC CAC CGT TG-3') and RB_Cytb (5'- CCA TCT TCG ATT TAC AAG GAC GAT GC-3') were used for amplifying the partial sequence of the mitochondrial cytochrome b gene (Stümpel 2012). PCR was performed in a total volume of 20 µl, containing 10 µl ready-to-use Taq DNA Polymerase Master Mix RED (Amplicon, Cat Number: A180301), 1 µl of each primer (10 pM/ml), 1 µl of DNA (>50 ng/ml), and 7 µl DD water. We followed amplification conditions as described by Stümpel (2012). PCR products were subsequently purified and sequenced on an automated sequencer ABI 3730XL (Macrogen, South Korea) according to manufacturer's protocols. DNA sequences were aligned using the ClustalX program incorporated in Bioedit (Hall 1999) using default parameters. Additional Cytb nucleotide sequences of *Macrovipera lebetina* from Azerbaijan (KJ415300) and Uzbekistan (KJ415301) were retrieved from the NCBI databases (deposited by Zinenko et al. 2015) and included in the analysis. *Montivipera raddei* (KX168762.1) and *Montivipera xanthina* (KX168811.1) were chosen as outgroup taxa for all analyses, because *Montivipera* most likely is the sister-group of *Macrovipera* (Lenk et al. 2001, Wüster et al. 2008, Stümpel & Joger 2009). Genetic distances within and between recovered clades were calculated using Mega 7 (Kumar et al. 2016). A statistical parsimony network was inferred with TCS v.1.21 (Clement et al. 2000), using the default 0.95 probability connection limit. For Maximum Likelihood and Bayesian analysis, parameters of the model were estimated from the data set using JModeltest 2.1.4 (Darriba et al. 2012). The selected model under Akaike information index was GTR. Phylogenetic analyses were performed using Maximum Parsimony (MP), Bayesian Inference (BI), and Maximum Likelihood (ML). PAUP* v.4.0 (Swofford 2002) was employed for the MP, with reliability being assessed by nonparametric bootstrapping with 2000 replicates. The MP analysis was performed with all sites weighted equally, with the tree-bisection-reconnection (TBR) algorithm for branch swapping. Bayesian analyses were performed on MrBayes v.3.2.5

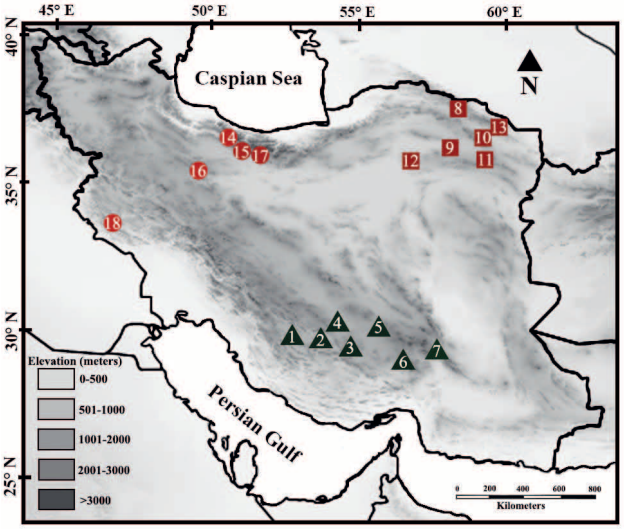

Figure 1. Sampling locations of *Macrovipera* spp. used in molecular analysis from throughout its distribution range in Iran. Information on the localities is given in Table 1. ▲ *Macrovipera razii* sp. n., ● *Macrovipera lebetina obtusa* and ■ *Macrovipera lebetina cernovi.* (1) Fars Province, Bamoo National Park; (2) Fars Province, Bakhtegan National Park; (3) Fars Province, Ghatroyeh; (4) Yazd Province, Baghe-Shadi Protected Area; (5) Kerman Province, north of Sirjan; (6) Kerman Province, Khabr National Park; (7) Kerman Province, Bab-Gorgi village; (8) North Khorasan Province, Dorbadam protected area; (9) Razavi Khorasan Province, Sabzevar to Neyshabur; (10) Razavi Khorasan Province, Mashhad; (11) Razavi Khorasan Province, Soltanabad; (12) Razavi Khorasan Province, Zaman Abad; (13) Razavi Khorasan Province, Sar Rud; (14) Gazvin Province, northern Gazvin; (15) Alborz Province, Karaj; (16) Markazi Province, Chahar Had village (17) Tehran Province, Lavasan; (18) Ilam Province.

Table 1. List of specimens used in molecular analysis along with the localities and NCBI accession numbers. Taxon names correspond to changes proposed in this paper. ERP refers to field numbers of specimens that are now deposited in the Hakim Sabzevari University Herpetological Collection (SUHC).

Species	Voucher specimens	Locality number	Locality	Lat/long	NCBI Accession Numbers
Macrovipera razii sp. n.	SUHC (ERP)1981	1	Iran, Fars Province, Bamoo National Park	29°41'/52°38'	MF445994
Macrovipera razii sp. n.	SUHC (ERP) 1518	2	Iran, Fars Province, Bakhtegan National Park, Tolombeh-Badi region	29°33'/53°39'	MF445990
Macrovipera razii sp. n.	SUHC (ERP) 1531	3	Iran, Fars Province, Ghatroyeh, Bahrame-Goor National Park	29°20'/54°38'	MF445991
Macrovipera razii sp. n.	SUHC (ERP) 3183	4	Iran, Yazd Province, 40km West of Harat, Baghe-Shadi Protected Area	30°03'/54°14'	MF445992
Macrovipera razii sp. n.	SUHC (ERP) 1941	5	Iran, Kerman Province, 50 Km North of Sirjan	29°59'/55°36'	MF445993
Macrovipera razii sp. n.	SUHC (ERP) 2376	6	Iran, Kerman Province, Khabr National park	28°44'/56°26'	MF445995
Macrovipera razii sp. n.	SUHC (ERP) 143	7	Iran, Kerman Province, on the road of Jiroft to Bam, Around Bab-Gorgi village	29°05'/57°34'	MF445989
Macrovipera lebetina cernovi	SUHC (ERP) 696	8	Iran, North Khorasan Province, Quchan, Dorbadam protected area	37°30'/58°27'	MF445997
Macrovipera lebetina cernovi	SUHC (ERP) 975	9	Iran, Razavi Khorasan Province, on the road of Sabzevar to Neyshabur	36°05'/57°59'	MF446000
Macrovipera lebetina cernovi	SUHC (ERP) 1118	10	Iran, Razavi Khorasan Province, Mashhad	36°24'/59°13'	MF446004
Macrovipera lebetina cernovi	SUHC (ERP) 1366	11	Iran, Razavi Khorasan Province, South Neyshabur, Soltanabad	35°39'/59°11'	MF446005
Macrovipera lebetina cernovi	SUHC (ERP) 1470	12	Iran, Razavi Khorasan Province, South Sabzevar, Zaman Abad	35°35'/56°46'	MF446006
Macrovipera lebetina cernovi	SUHC (ERP) 3426	13	Iran, Razavi Khorasan Province, Kalat Naderi, Sar Rud	36°48'/59°50'	MF446007
Macrovipera lebetina cernovi	SUHC (ERP) 3427	13	Iran, Razavi Khorasan Province, Kalat Naderi, Sar Rud	36°48'/59°50'	MF446008
Macrovipera lebetina cernovi	SUHC (ERP) 3428	13	Iran, Razavi Khorasan Province, Kalat Naderi, Sar Rud	36°48'/59°50'	MF446009
Macrovipera lebetina cernovi	SUHC (ERP) 3429	13	Iran, Razavi Khorasan Province, Kalat Naderi, Sar Rud	36°48'/59°50'	MF446010
Macrovipera lebetina cernovi	SUHC (ERP) 665	–	Iran, unspecified locality in Khorasan Province		MF445996
Macrovipera lebetina cernovi	SUHC (ERP) 936	–	Iran, unspecified locality in Khorasan Province		MF445998
Macrovipera lebetina cernovi	SUHC (ERP) 937	–	Iran, unspecified locality in Khorasan Province		MF445999
Macrovipera lebetina cernovi	SUHC (ERP) 986	–	Iran, unspecified locality in Khorasan Province		MF446003
Macrovipera lebetina cernovi	SUHC (ERP) 992	–	Iran, unspecified locality in Khorasan Province	MF446001	MF446001
Macrovipera lebetina cernovi	SUHC (ERP) 993	–	Iran, unspecified locality in Khorasan Province	MF446002	MF446002
Macrovipera lebetina obtusa	SUHC (ERP) 3422	14	Iran, Gazvin Province, north Gazvin, on the road to Alamout	36°25'/50°32'	MF446014
Macrovipera lebetina obtusa	SUHC (ERP) 3423	14	Iran, Gazvin Province, north Gazvin, on the road to Alamout	36°25'/50°32'	MF446015
Macrovipera lebetina obtusa	SUHC (ERP) 3424	14	Iran, Gazvin Province, north Gazvin, on the road to Alamout	36°25'/50°32'	MF446016
Macrovipera lebetina obtusa	SUHC (ERP) 3425	15	Iran, Alborz Province, Karaj	35°56'/50°57'	MF446017
Macrovipera lebetina obtusa	SUHC (ERP) 3438	15	Iran, Alborz Province, Karaj, on the road to Baraghan	35°56'/50°57'	MF446026

Species	Voucher specimens	Locality number	Locality	Lat/long	NCBI Accession Numbers
Macrovipera lebetina obtusa	SUHC (ERP) 3439	15	Iran, Alborz Province, Karaj, on the road to Baraghan	35°56'/50°57'	MF446027
Macrovipera lebetina obtusa	SUHC (ERP) 3440	15	Iran, Alborz Province, Karaj, on the road to Baraghan	35°56'/50°57'	MF446028
Macrovipera lebetina obtusa	SUHC (ERP) 3441	15	Iran, Alborz Province, Karaj, on the road to Baraghan	35°56'/50°57'	MF446029
Macrovipera lebetina obtusa	SUHC (ERP) 3430	16	Iran, Markazi Province, northwest of Saveh, near Chahar Had village	35°21'/49°33'	MF446018
Macrovipera lebetina obtusa	SUHC (ERP) 3431	16	Iran, Markazi Province, northwest of Saveh, near Chahar Had village	35°21'/49°33'	MF446019
Macrovipera lebetina obtusa	SUHC (ERP) 3432	16	Iran, Markazi Province, northwest of Saveh, near Chahar Had village	35°21'/49°33'	MF446020
Macrovipera lebetina obtusa	SUHC (ERP) 3433	16	Iran, Markazi Province, northwest of Saveh, near Chahar Had village	35°21'/49°33'	MF446021
Macrovipera lebetina obtusa	SUHC (ERP) 3434	16	Iran, Markazi Province, northwest of Saveh, near Chahar Had village	35°21'/49°33'	MF446022
Macrovipera lebetina obtusa	SUHC (ERP) 3435	16	Iran, Markazi Province, northwest of Saveh, near Chahar Had village	35°21'/49°33'	MF446023
Macrovipera lebetina obtusa	SUHC (ERP) 3436	16	Iran, Markazi Province, northwest of Saveh, near Chahar Had village	35°21'/49°33'	MF446024
Macrovipera lebetina obtusa	SUHC (ERP) 3437	17	Iran, Trehran Province, Lavasan	35°48'/51°37'	MF446025
Macrovipera lebetina obtusa	SUHC (ERP) 1065	18	Iran, Ilam Province	33°30'/46°34'	MF446011
Macrovipera lebetina obtusa	SUHC (ERP) 2523	18	Iran, Ilam Province	33°30'/46°34'	MF446012
Macrovipera lebetina obtusa	SUHC (ERP) 2552	18	Iran, Ilam Province	33°30'/46°34'	MF446013

(Ronquist et al. 2012). Four incrementally heated Markov chains with the default heating values were used. The analysis started with randomly generated trees and ran for 8×10^6 generations in two independent runs with samplings at intervals of 100 generations. After verifying that stationarity had been reached, both in terms of likelihood scores and parameter estimation, the initial 25% of the samples were discarded as burn-in and a majority rule consensus tree was generated from the remaining post-burn-in trees. The frequency of any particular clade among the individual trees contributing to the consensus tree represents the posterior probability of that clade (Huelsenbeck & Ronquist 2001); only values equal or > 95% were considered to indicate sufficient support (Wilcox et al. 2002). An ML analysis was performed using RaxML GUI v. 0.95 (Silvestro & Michalak 2012) with the 'GTRGAMMA' option and 1000 bootstrap replicates. We used the estimates of the age of the split and separation between *Montivipera* and *Macrovipera* as the calibration point, assuming this separation occurred 15.3 million years ago (Stümpel et al. 2016).

Table 2. The 13 localities of *Macrovipera lebetina* used in morphological study.

No.	Locality	Sample size	
		Males	Females
1	Kalate Naderi, Khorasan Province	9	18
2	Gonbade Kavoos, Golestan Province	2	4
3	Gorgan, Golestan Province	–	6
4	Karaj,Elborz Province	–	1
5	Gazvin, Gazvin Province	–	1
6	Khalkhal, Ardabil Province	1	–
7	Abhar, Ardabil Province	–	1
8	Mianeh, East Azarbaijan Province	6	4
9	Dom-Gheshlagh, West Azarbaijan Province	1	2
10	Uromieh, West Azarbaijan Province	–	1
11	Hamadan, Hamadan Province	4	–
12	Kermanshah, Kermanshah Province	1	1
13	Nahavand, Hamadan Province	1	–
Total		25	39

Morphological analyses

Material for the morphological examinations included 64 alcohol-preserved specimens from the Zoological Collections of the Department of Venomous Animals and Antisera Production, Razi Vaccine and Serum Research Institute (Table 2) along with three specimens belonging to the new species described herein, holotype (SUHC 143), paratype (SUHC 1941), and an individual from Kerman (SUHC 2377). These specimens were deposited in the Hakim Sabzevari University Herpetological Collection (SUHC), Sabzevar, Iran. Long-term preservation of alcohol-preserved specimens at the Razi Vaccine and Serum Research Institute is the main obstacle in using them for molecular

surveys in this study. The following morphological characters were measured on the right side of each specimen using Vernier calipers with an accuracy to the nearest 0.01 mm: snout–vent length (SVL), measured from tip of snout to vent; tail length (TL) from vent to tip of tail; head length (HL) from tip of snout to angle of jaws; head width (HW); head height (HH); snout length (SL), distance between rostrum to the anterior margin of eye; distance between nostrils (DBN); interocular distance (IOD).

In addition to the metric continuous variables, the following meristic variables were collected by the same person (HO) using a stereomicroscope: Number of preventral scales (PreV); number of ventral scales (Ven); number of subcaudal scales (Scd); number of dorsal scale rows, counted across at 10% of the body length, approximately one head length behind the posterior end of head (Sq1); number of dorsal scale rowsacross mid-body (Sq2); number of dorsal scale rowsat 90% of the body length, approximately one head length before the anal scute (Sq3); number of apical scales (Ap); number of canthal scales (Can); number of canthal + intersupraocular scales (InCanSup); number of scales between oculars at level of the centre of the eye (Interoculars); number of scales between the last supralabials across the head (Blspl); number of supralabials (Spl); number of sublabials (infralabials) (Ifl); number of inner circumocular scales (inCir); number of outer circumocular scales (around eye and supraocular scales) (outCir); number of loreals (Lor); number of supraoculars (Supraoc).

A final dataset comprising 109 individuals, including some data from Moradi et al. (2014), was compiled for statistical analyses. To this end, all Iranian individuals of *Macrovipera* spp. were categorized into three groups, i.e., *Macrovipera lebetina cernovi*, *Macrovipera lebetina obtusa*, and "potentially new", following morphological keys. The latter included the holotype and paratype specimens of the new species along with samples that were named as an unknown species by Moradi et al. (2014).

Our data met the assumptions appropriate for parametric analyses. Statistical analyses were performed using the SPSS (ver. 16.0, SPSS Inc., Chicago, IL, USA), PAST (ver. 3.18), and S-PLUS (ver. 8.0, Halcyon Software, Inc., Cambridgeshire, UK) software applications. The eight metric and 17 meristic characters were analysed independently. We used ANOVA to detect the degree of differentiation between the three prior groups. In order to avoid effects of body size on the analysis, we calculated the residual values of each metric character as a function of SVL. The Principal Components Analysis (PCA) and canonical variable analysis (CVA) based on a variance-covariance matrix of meristic data were used to test our null hypothesis. The significance level for all the statistical tests were set at $p = 0.05$.

Nomenclatural acts

The electronic edition of this article conforms to the requirements of the amended International Code of Zoological Nomenclature, and hence the names contained herein are available under that Code. This published work and the nomenclatural acts it contains have been registered in ZooBank (www.zoobank.org), the online registration system of the ICZN. The LSID (Life Science Identifier) for this publication is: urn: lsid: zoobank.org: pub:149C66E9-455C-4EB3-8071-FB5F68CAA57C. The electronic edition of this work has also been published in a journal with an ISSN, has been archived, and is available from the following digital repository: www.salamandra-journal.com.

Results

Molecular analyses

We generated a total of 41 new sequences that were deposited in GenBank (Table 1). A dataset with the final sequence length of 1045 nucleotides (148 nt variable; 124 nt parsimony informative; 895 nt conserved) was generated for Cytb. No indel was found in the aligned sequences. Translation to protein did not reveal any unexpected stop codons, supporting the assumption that our Cytb dataset coded for functional mitochondrial Cytb and does not contain nuclear copies. Nucleotide composition analyses revealed 36 haplotypes, a haplotype diversity of 0.989, and an average of 0.436 for the GC content.

To resolve the phylogenetic structure of *Macrovipera* populations in Iran based on Cytb sequences, phylogenetic trees were calculated under MP and ML criteria, as well as by Bayesian inference. All phylogenetic analyses showed branch patterns that were identical in their general structure. Therefore, only the (ML) is presented in Fig. 2. Corrected genetic divergence (K2-p distances) between the clades as revealed by phylogenetic analysis along with the outgroup taxa are shown in Table 3. Average genetic divergences between clades were from 3.9 to 15.8% (see Table 3).

The phylogenetic trees provided strong support for the monophyly of *Macrovipera* populations relative to the outgroup taxa, *Montivipera raddei* and *Montivipera xanthina* (Fig. 2). In support of our hypothesis, according to the phylogenetic tree (Fig. 2), the existence of two distinct lineages of *Macrovipera* (Clade A and B) was discovered with high statistical support, MP (100%), PP (1), and ML (100%). There is more than 10% genetic divergence (K2-p distances) between Clades A and B (Table 3). This finding implies a deep divergence between *Macrovipera* populations in Iran and reveals an entity (Clade A) that is new to the Iranian herpetofauna. Clade A was distinct with highest support (Fig. 2) and includes populations that are distributed widely in the central and southern parts of Iran (Fig. 1). Within-group genetic divergence in this group is 0.7 %.

Another major lineage (Clade B) with an extensive distribution range from western to northeastern Iran is divided into sublineages B_1 and B_2 with high support values (Fig. 2). Genetic distance (K2-p distances) between Subclades B_1 and B_2 is less than 4 % (Table 3). Subclade B_1 is a cluster of individuals from various populations native to

western and northern Iran, represented by numbers 14, 15, 16 and 17 (Fig. 1). Interestingly, a specimen from Azerbaijan (KJ415300) was nested within Subclade B_1 with close relationships to a northern Saveh population (number 16 in the map; see Fig. 1). It represents haplotype 4, which occurred in northern Saveh with three representatives (ERP 3434, ERP 3435 and ERP 3436). The northern Saveh population shows the greatest haplotype diversity among the studied populations of Subclade B_1 in Iran, as is illustrated by numbers 8, 9, 10, 11, 12 and 13 (Fig. 1). Haplotype network reconstruction using TCS1 provides support for the hypothesis that northern Saveh could be the ancestral region of Subclade B_1.

Subclade B_2 includes populations distributed mainly in northeastern Iran, with weak phylogenetic structure. There is just 0.2 % within-group genetic divergence in this group. This subclade consists of a specimen from Uzbekistan (KJ415301), which is placed in a basal position, with a high value of support.

Morphology

Descriptive parameters of metric and meristic characters and the results of our Analysis of Variance (ANOVA) are presented in Table 4. We used t-tests to investigate the degree of sexual dimorphism between male and female specimens. Although there is no significant difference between males and females except in ScD, multivariate analyses were conducted for each sex separately. The Principal Components Analysis (PCA) based on a variance-covariance matrix of meristic data was used to explore differ-

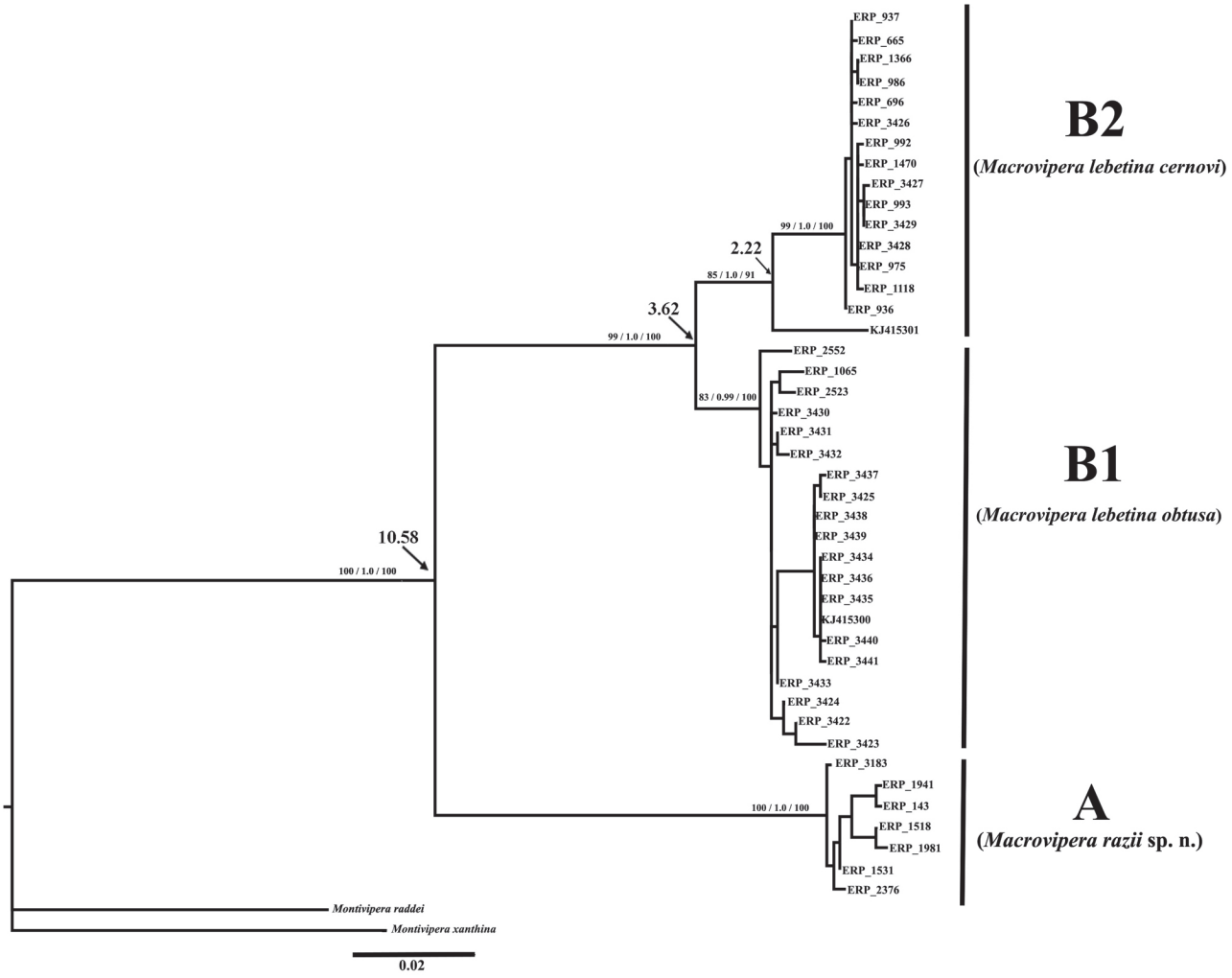

Figure 2. Phylogenetic relationships among the *Macrovipera* spp. populations included in the analysis. Individuals of *Montivipera raddei* and *Montivipera xanthina* were used as outgroup taxa. Phylogenetic analyses of maximum parsimony (MP), maximum likelihood (ML), and Bayesian inference (BI) produced trees with the same topology with regard to the major lineages. Only the ML tree is presented. Numbers close to the branches are ML bootstrap supports (1000 replicates), posterior probabilities of BI, and MP bootstrap supports (1000 replicates). A, B1 and B2 indicate the major clades. The arrows indicate the estimated times of divergence of the major clades (in MA). Information on the sample number is given in Table 1.

Table 3. Corrected genetic distances (Kimura 2 parameters) among *Macrovipera razii* sp. n., two subspecies of *M. lebetina,* along with *Montivipera raddei* and *M. xanthina* as outgroup taxa inferred from Cyt-b gene.

n	species	1	2	3	4	5	6
1	*Montivipera raddei*						
2	*Montivipera xanthina*	0.099					
3	*Macrovipera razii* sp. n.	0.158	0.149				
4	*Macrovipera lebetina cernovi*	0.151	0.161	0.114			
5	*Macrovipera lebetina obtusa*	0.141	0.143	0.105	0.039		
6	*Macrovipera lebetina* from Uzbekistan	0.142	0.145	0.106	0.028	0.043	
7	*Macrovipera lebetina* from Azerbaijan	0.140	0.143	0.104	0.041	0.006	0.044

ences between Iranian populations of *Macrovipera lebetina* and the new species described herein at multivariate level. The first two principal components address 58.04 and 61.60 % of the total variation in males and females, respectively (Table 5). The newly described species is obviously separated from other populations, morphologically supporting the hypothesis that it is indeed new (Fig. 3). In order to further evaluate variation, the three clusters of meristic characters indicated by the PCA were used as a priori groups for a CVA of these traits (Table 6). The classification results and predicted group membership showed that, in total, 84.38and 88.64% of the originally grouped males and females, respectively, were correctly classified (Fig. 4 and Table 7).

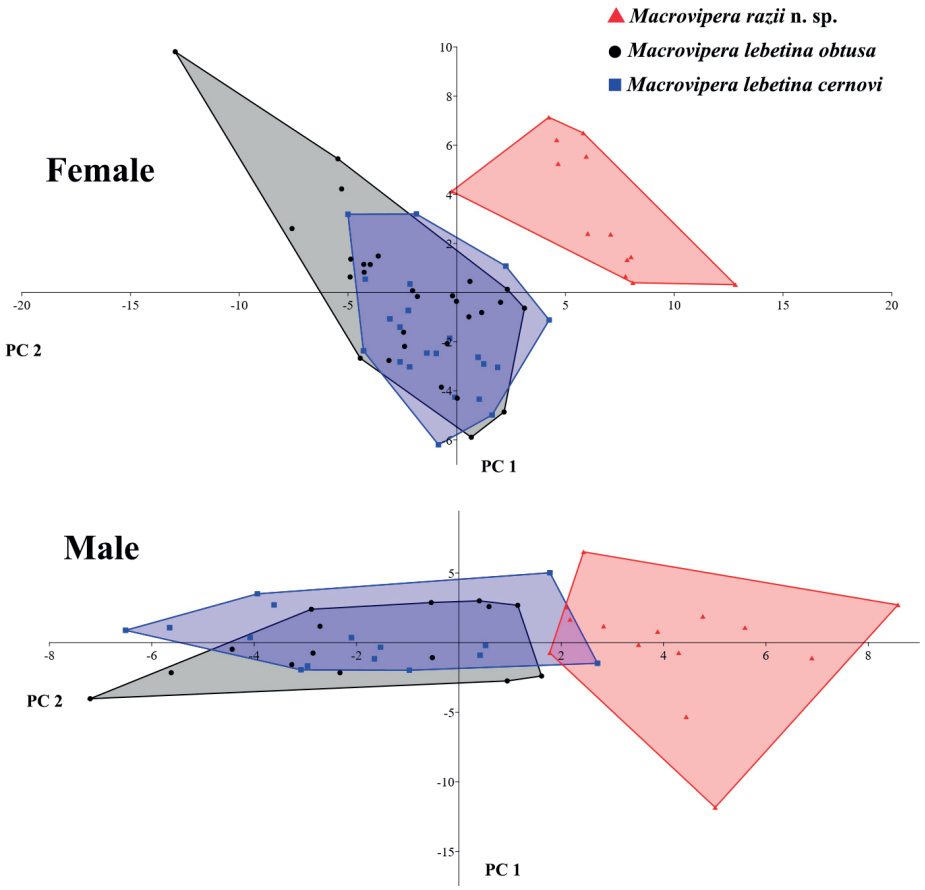

Figure 3. Ordination of individuals on the first two principal components based on meristic characters in males and females, respectively. The first two principal components address 58.04 and 62.16 % of the total variation in males and females, respectively. The new species, *Macrovipera razii* sp. n., is obviously separated from Iranian populations of *M. lebetina*. ▲ *Macrovipera razii* sp. n., ● *Macrovipera lebetina obtusa*, and ■ *Macrovipera lebetina cernovi*.

Table 4. ANOVA comparison of meristic and morphometric characters including minimum, maximum, mean and standard deviation (SD) in *Macrovipera* spp. in Iran.

Character	Sex	*M. lebetina obtusa* (Males = 15, Females = 30)		*M. lebetina cernovi* (Males = 15, Females = 22)		*Macrovipera razii* sp. n. (Males = 14, Females = 13)		ANOVA statistics	
		Mean±SD	Min.–Max.	Mean±SD	Min.–Max.	Mean±D	Min.–Max.	F	Sig.
SVL	F	961.67±171.39	660–1370	1052.50±137.66	770–1270	921.15±177.27	550–1155	3.23	.046
	M	1013±187.36	720–1260	1065.33±192.35	695–1290	1081.43±185.1	660–1360	.53	.594
Tal/SVL	F	.127±.012	.102–.153	.141± .016	.118–.176	.134± .012	.114–.155	6.415	.003
	M	.139±.009	.119–.155	.140±.010	.116–.156	.131±.016	.102–.162	2.884	.067
HL/SVL	F	.0444±.005	.021–.052	.046±.002	.041–.050	.047±.003	.041–.053	3.511	.036
	M	.043±.003	.039–.051	.044±.004	.034–.050	.043±.003	.040–.051	.181	.835
HW/SVL	F	.028±.004	.022–.040	.032±.003	.025–.039	.025±.002	.021–.028	16.79	.000
	M	.029±.003	.022–.035	.029±.005	.023–.039	.026±.004	.021–.037	3.076	.057
HH/SVL	F	.014±.002	.011–.019	.015±.002	.012–.018	.015±.002	.014–.019	3.443	.038
	M	.013±.001	.011–.016	.015±.002	.013–.020	.014±.001	.012–.018	4.342	.019
DBN/SVL	F	.008±.000	.006–.010	.009±.001	.007–.012	.009±.001	.009–.011	3.905	.025
	M	.008±.000	.007–.010	.009±.000	.008–.012	.009±.000	.008–.011	5.196	.010
IOD/SVL	F	.016±.001	.013–.022	.017±.002	.014–.019	.017±.001	.015–.020	.525	.594
	M	.016±.002	.013–.020	.016±.001	.013–.020	.016±.002	.013–.020	1.005	.375
SL/SVL	F	.012±.001	.010–.015	.013±.001	.011–.014	.013±.001	.012–.015	2.121	.128
	M	.012±.001	.010–.014	.013±.001	.012–.015	.012±.001	.011–.014	1.884	.165
Pre	F	3.17±1.26	1–5	3.23±1.06	1–5	4.69±.63	3–5	9.71	.000
	M	2.93±1.03	2–5	3.53±1.12	1–5	4.00±1.41	1–5	2.90	.066
Ven	F	167.57±2.11	164–172	167.27±1.69	164–171	175.54±1.76	171–179	93.26	.000
	M	168.33±2.71	164–173	167.67±2.22	164–172	173.64±2.20	171–178	26.7	.000
Scd	F	43.83±4.14	29–50	45.55±2.74	40–49	46.15±3.31	42–52	2.51	.089
	M	46.67±2.29	43–50	46.80±2.11	44–52	47.00±4.20	35–53	.05	.956
Sq1	F	22.53±2.04	18–25	22.05 - 1.84	19–25	24.38±1.26	23–27	6.89	.002
	M	22.67±1.88	19–25	23.20±1.52	20–25	24.35±.92	23–25	4.74	.014
Sq2	F	24.60±1.16	22–27	24.82±1.14	23–29	25.15±.555	25–27	1.24	.295
	M	24.47±1.36	20–26	24.87±.74	24–27	25.07±.62	24–27	1.48	.241
Sq3	F	19.27±.87	18–21	19.23 - .68	18–21	19.00±.000	19–19	.65	.523
	M	19.13±.92	18–21	19.27±.80	18–21	18.71±.72	17–19	1.78	.182
Can	F	2.87±.68	2–4	3.14±.64	2–4	2.15±.38	2–3	10.48	.000
	M	3.33±.82	2–5	2.73±.59	2–4	2.35±.49	2–3	8.28	.001
Interocular	F	11.50±1.10	9–14	10.86±.83	10–13	11.46±.97	9–13	2.89	.064
	M	11.40±1.05	10–13	11.47±.64	10–12	11.07±.73	10–12	.93	.402
Blspl	F	26.03±1.47	23–30	25.64±1.76	23–30	25.92±.95	24–27	.45	.637
	M	26.33±1.34	24–28	26.07±2.21	23–30	25.36±1.08	24–28	1.37	.266
Spl	F	10.37±.67	9–12	10.32±.57	9–11	10.46±.52	10–11	.227	.797
	M	10.27±.59	9–11	10.40 ±.50	10–11	10.86±.66	10 –12	3.96	.027
Ifi	F	13.17±.74	12–14	13.36±.73	12–15	14.54±.87	13–16	15.08	.000
	M	12.80±.86	11–14	13.20 ±.77	12–15	14.00±.96	13–16	7.15	.002
Incir	F	16.17±1.23	13–19	14.41±1.14	13–17	15.08±1.19	13–17	14.18	.000
	M	16±1.13	14–18	15.13±1.88	13–19	15.57±1.09	14–18	1.39	.261
Outcir	F	22.10±1.42	19–25	21.82±1.84	19–26	22.77±2.16	18–25	1.24	.295
	M	22.13±1.55	20–25	22.67±1.11	21–24	22.71±1.94	19–25	.63	.537
Lor	F	13.53±2.09	9–17	14.95±1.99	11–20	15.77±1.36	13–18	7.11	.002
	M	14.47±1.50	12–17	15±1.60	12–17	15.21±1.62	12–17	.87	.426

Table 5. Factor loadings of the first three principal components (PCs) extracted from a variance-covariance matrix of meristic characters.

Character	PC1		PC2		PC3	
	Male	Female	Male	Female	Male	Female
Pre	0.115	0.097	0.073	0.188	-0.375	0.011
Ven	0.933	0.754	-0.109	0.475	0.293	0.151
Scd	0.068	0.609	0.968	-0.724	0.157	0.063
Sq1	0.170	0.086	0.037	0.403	-0.555	0.131
Sq2	0.068	0.013	0.015	0.074	-0.152	-0.076
Sq3	-0.055	-0.012	-0.014	-0.020	0.044	-0.029
Can	- 0.091	-0.051	0.006	-0.116	0.200	-0.026
Interocular	-0.020	0.030	0.037	0.073	-0.109	0.042
Blspl	-0.190	-0.007	-0.046	-0.022	0.308	0.111
Spl	0.029	0.020	0.015	0.003	-0.019	-0.009
Ifi	0.134	0.090	-0.055	0.092	-0.156	-0.047
Incir	0.010	-0.044	-0.065	0.089	-0.201	0.152
Outcir	0.056	0.062	0.130	0.024	-0.436	-0.471
Lor	0.095	0.151	0.128	0.093	-0.121	-0.829
Proportion of Variance	0.35	0.39	0.22	0.21	0.09	0.09
Cumulative Proportion	35.51	39.61	58.04	61.60	67.90	71.48

Taxonomy

Macrovipera razii sp. n.

(Figs 5–8)

ZooBank: LSID:urn:lsid:zoobank.org:pub:149C66E9-455C-4EB3-8071-FB5F68CAA57C

Holotype: SUHC 143, male, collected at 105 km on the road from Jiroft to Bam near Bab-Gorgi village and Valley, Kerman Province, 29°05'054" N, 57°34'120" E; altitude 3150 m; collected 3–4 June 2004 by Eskandar Rastegar Pouyani.

Paratype: SUHC 1941, female, Iran, Kerman Province, Pariz, 50 km north of Sirjan, collected by Naeim Moradi, 25 April 2010

Description of holotype (Figs 5–7): A viper with a robust and massive body. Snout–vent length (SVL) 1110 mm, and tail length (TaL) 160 mm. Length of head from tip of rostral to end of lower jaw 46.92 mm, and width of head at widest point 34.76 mm. The head is broad and triangular with a bluntly rounded snout, well distinct from neck, covered with small, imbricate and strongly keeled scales. Dorsal side of head with 55 small, imbricate and strongly keeled scales (canthal + intersupraocular scales), and 10 small scales between supraoculars (intrasupraoculars). Eye bordered by a complete circumocular ring of 15 scales including one large supraocular. This circle is bordered by 21–22 scales in an outer ring. Loreals 15, of which 5 are in contact with the nasal scale. One nasorostral scale, partly fused with nasal. Two canthal scales (supranasals) above nasal, one apical scale above rostral. Twelve supralabials on each side (4th largerst, its length greater than width), and 13 infralabials more or less equal in size. Anterior chin-shields more than three times longer than the posterior ones, 3–4 infralabials in contact with anteriormost chin-shield. 175 ventral scales, which are followed by 47 divided subcaudal scales. Anal scute entire. 24 rows of dorsal scales, strongly keeled, around anterior dorsum (one head length behind head), 25 rows at mid-body, and 17 rows around posterior body (one head length before anal scute). Dorsum shiny black throughout, belly dark, anterior part of ventral face with irregular light blotches.

Table 6. Standardized canonical coefficients of all individual specimens of *Macrovipera* spp. Iran analysed. The three groups resulting from the principal component analysis (PCA) were used as a priori groups for a canonical variable analysis of meristic characters (see text for an explanation of the PCA results).

Character	CV1		CV2	
	Male	Female	Male	Female
Pre	0.222	-0.247	0.274	0.0317
Ven	1.449	-1.366	-1.012	0.309
Scd	0.074	-0.290	0.050	-0.899
Sq1	0.392	-0.337	0.163	0.326
Sq2	0.120	-0.073	0.194	-0.073
Sq3	-0.121	0.043	0.132	0.018
Can	-0.199	0.135	-0.284	-0.169
Interocular	-0.092	-0.034	0.079	0.334
Blspl	-0.230	0.001	-0.067	0.248
Spl	0.141	-0.025	0.022	0.228
Ifi	0.276	-0.220	0.129	-0.082
Incir	-0.031	0.075	-0.517	0.923
Outcir	0.102	-0.129	0.284	0.173
Lor	0.145	-0.277	0.265	-0.660
Eigenvalues	3.32	6.06	0.50	0.73
Percentage	86.91	89.24	13.09	10.76

Table 7. Group memberships in the studied individuals of *Macrovipera* spp. from Iran. The three groups resulting from the principal component analysis (PCA) were used as a priori groups for a canonical variable analysis of meristic characters (see text for an explanation of the PCA results).

	Sex	*M. lebetina obtusa*	*M. lebetina cernovi*	*M. razii* n. sp.	Total
M. lebetina obtusa	F	23	6	0	29
	M	11	3	1	15
M. lebetina cernovi	F	4	18	0	22
	M	1	14	0	15
M. razii sp. n.	F	0	0	13	13
	M	0	0	14	14

Paratype (Fig. 8): Body shape and coloration are very similar to that of the holotype. One large supraocular scale; 2/2 canthal scales; 10 interocular scales in a line between the eyes; 1 apical scale; 15 inner circumocular scales on each side, including suparaocular; 19/19 outer circumoculars on each side; 10/11 (l/r) supralabials; 12/13 (l/r) infralabials; dorsal scale rows 24/25/19; 172 ventrals: anal scute entire; 47 pairs of subcaudals. Snout–vent length 1110 mm, tail 180 mm. Length of head 50.84 mm and width of head at widest point 34.73 mm.

Differential diagnosis: The newly described species differs from *M. schweizeri* by its higher number of mid-dorsal scales (25 vs. 23), which however overlaps the counts in

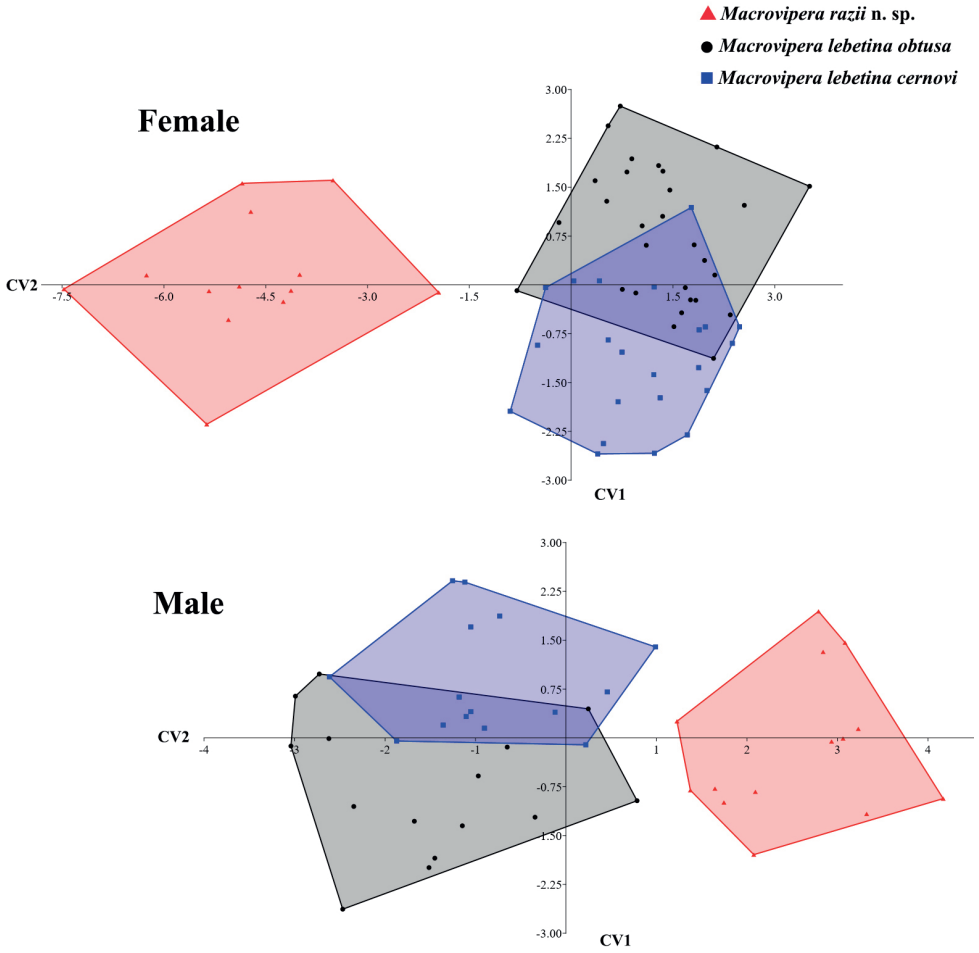

Figure 4. Ordination of the first (CV1) and second (CV2) canonical variables for meristic characters of individuals of *Macrovipera* spp. (males and females were analyzed separately). ▲ *Macrovipera razii* sp. n., ● *Macrovipera lebetina obtusa*, and ■ *Macrovipera lebetina cernovi*.

other *M. lebetina* subspecies. *Macrovipera razii* sp. n. differs from *M. lebetina* by possessing a higher count of ventrals (172–175 vs. 160–170), and by having elongated anterior chin-shields, which are more than three times longer than the posterior ones. In contrast, *M. lebetina* has square anterior chin-shields, which are less than twice as long as the posterior chin-shields (Fig. 6). Compared to *M. lebetina*, the new species has a lower number of canthal + intersupraocular scales. More comparisons are provided in Table 4. Interestingly, *Macrovipera razii* sp. n. and *M. lebetina cernovi* are similar in both possessing one large supraocular scale, which is absent in *M. lebetina obtusa* (Fig. 5). Outside Iran, the subspecies *M. lebetina euphratica* (Schmidt, 1939) differs by having supraoculars that are split up into five scales, making it clearly distinguishable from *Macrovipera razii* sp. n., which has one large supraocular scale. The latter can be distinguished from *Macrovipera lebetina lebetina* (Linnaeus, 1758) and *Macrovipera lebetina transmediterranea* (Nilson & Andrén 1988) by the higher number of ventrals (172–175 vs. 146–163 and 150–164, respectively), from *Macrovipera lebetina turanica* (Chernov, 1940) by the latter's semidivided supraoculars and a dorsal colour pattern that consists of a dark ground colour with a lighter, orange zigzag pattern.

Chromatic variation: There is considerable geographic variation in the colour pattern of *Macrovipera razii* sp. n. Despite their very close genetic relationship (Fig. 2) and weak within-group genetic divergence (0.7%), individuals from Fars Province, southern Iran, (ERP 1981, ERP 1518, ERP 1531) differ significantly in their colour pattern from the holotype and paratype (Fig. 9). Rather than having an entirely black colour, their dorsum is brownish grey with narrow crossbars, and the ventral side is a little lighter than the dorsum with small black dots. Geographic variation in colour pattern could be strongly related to landscape features and altitude, as is suggested by the holotype and paratype with their black colour occurring in high-altitude habitats with black rocks.

Habitat and ecology: Little is known about the ecology of *Macrovipera razii* sp. n. Based on our observations, this species inhabits various habitats at altitudes above 3000 m at the type locality in Kerman Province and at about 1500 m near Lake Bakhtegan (ERP 1518) in Fars Province. The type locality is located in an area with relatively dense vegetation, including *Orchis*, *Zygophyllum* and *Astragalus* spp., and lots of stones at the bases of the surrounding hills. This region has a rather cold mountain climate with long and cold winters that will see patches of snow persisting on the slopes until late May (Fig. 10A). Sympatric and/or syntopic lizard and snake species at the type locality include *Eremias lalezharica*, *Paralaudakia microlepis*, *Ablepharus pannoni-*

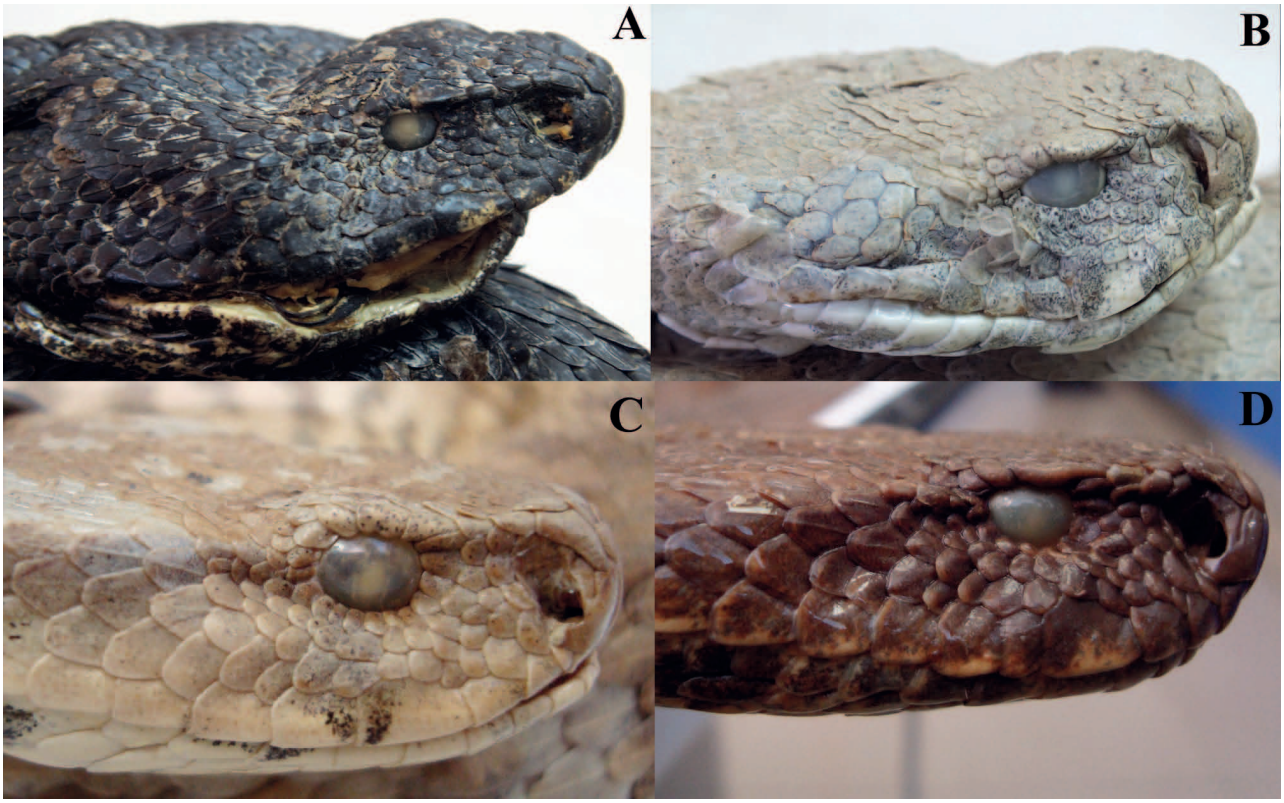

Figure 5. Comparison of the lateral aspect of the heads of Iranian *Macrovipera* spp.: large supraocular scale in *Macrovipera razii* sp. n., holotype (A); *Macrovipera razii* sp. n., ERP 1981, Bamoo National Park, Fars Province (B), and *M. lebetina cernovi*, 2228, Kalate Naderi, northeastern Iran (D), whereas supraoculars are split up into smaller scales in *M. lebetina obtusa*, 2294, Hamadan, western Iran (C).

cus, *Laudakia nupta*, *Psammophis schokari*, and *Hemorrhois ravergieri*. In our surveys, *Macrovipera razii* sp. n. was found in foothills with relatively speciose vegetation in Bamoo National Park (Fig. 10B). In contrast to the type locality, *Macrovipera razii* sp. n. was found in a much warmer and dryer habitat near Lake Bakhtegan (Fig. 10C), with a semi-desert climate and scattered shrubs due to overgrazing and a lasting drought. Harsh mountain habitats are another type of habitat which is occupied by *Macrovipera razii* sp. n. (Fig. 10D). Here, *Echis carinatus* is a sympatric viper species.

All observed individuals of *Macrovipera razii* sp. n. were active during the morning and were mostly collected in the spring months. This may in part be due to the timing of our expeditions that were conducted in months with moderate weather conditions. Our results from forced regurgitation during the spring months indicated that this species feeds on birds such as *Ammoperdix griseguları*s.

Distribution: All our specimens of *Macrovipera razii* sp. n. were collected from localities in central and southern Iran (Table 1, Fig. 1). This species might occur in other prov-

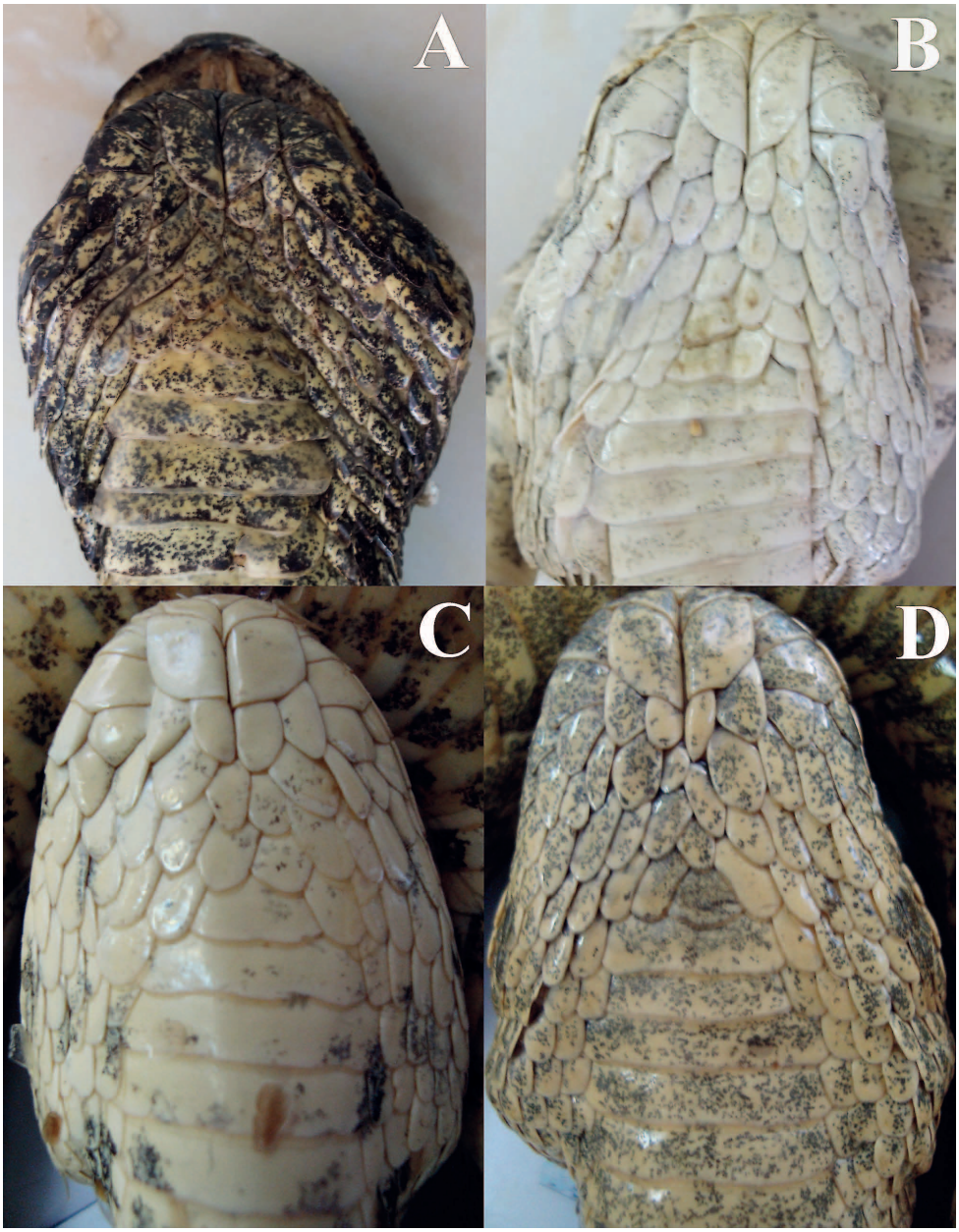

Figure 6. Comparison of the gular region of Iranian *Macrovipera* spp.: Elongated anterior chin-shields in *Macrovipera razii* sp. n., holotype (A) and a specimen (ERP 1981) from Bamoo National Park, Fars Province (B), whereas anterior chin shields are square in *M. lebetina obtusa* (2294) from Hamadan, western Iran (C) and *M. lebetina cernovi*, (2228) from Kalate Naderi, northeastern Iran (D).

inces of Iran, too, however. It is at present considered to be endemic to Iran.

Etymology: The specific epithet is a noun in the genitive case, in honour of Abu Bakr Muhammad ibn Zakariyya al-Razi (854–925 CE), a Persian polymath, physician, alchemist, philosopher, and important figure in the history of medicine. *Macrovipera* is one of the most medically important snakes in Iran, and historically, physicians like him have been involved in snake bite therapy. We propose "Razi's Viper" as a standard English name.

Discussion

This is one of the first attempts of understanding the geographic distribution of genetic variation within the genus *Macrovipera* in Iran. Our results produced congruent phylogenetic trees, in which all the representatives of *Macrovipera* analysed formed a well-supported group (Fig. 2). This finding concurs with other studies on viper phylogeny and supports a sister-group relationship between the genera *Macrovipera* and *Montivipera* (comp. Lenk et al. 2001, Wüster et al. 2008, Stümpel & Joger 2009).

Our morphological and molecular studies provide strong evidence for the occurrence of two major clades within the genus *Macrovipera* in Iran (Figs 5–7, Table 3). The first clade is a novel taxon, *Macrovipera razii* sp. n., with at least seven known (see Molecular analyses) representatives from central and southern Iran (Fig. 1), and the well-known blunt-nosed viper, *M. lebetina*, is the second. There is no consensus criterion for the amount of genetic distance between reptile species, particularly vipers. However, *Macrovipera razii* sp. n. and the Iranian populations of *M. lebetina* are quite distinct from each other genetically (K2p-distance: > 10% for Cytb Table 3). Such amount of variation is comparable with thresholds for setting species boundaries in the Viperidae (e.g., Fathinia et al. 2014) and other Iranian reptiles (e.g., Ahmadzadeh et al. 2013, Rastegar-Pouyani et al. 2012). Morphological results strongly support our molecular data, as this new species has a higher number of ventral and preventral scales, elongated anterior chin-shields, and a lower number of canthal plus intersupraocular scales (Table 4).

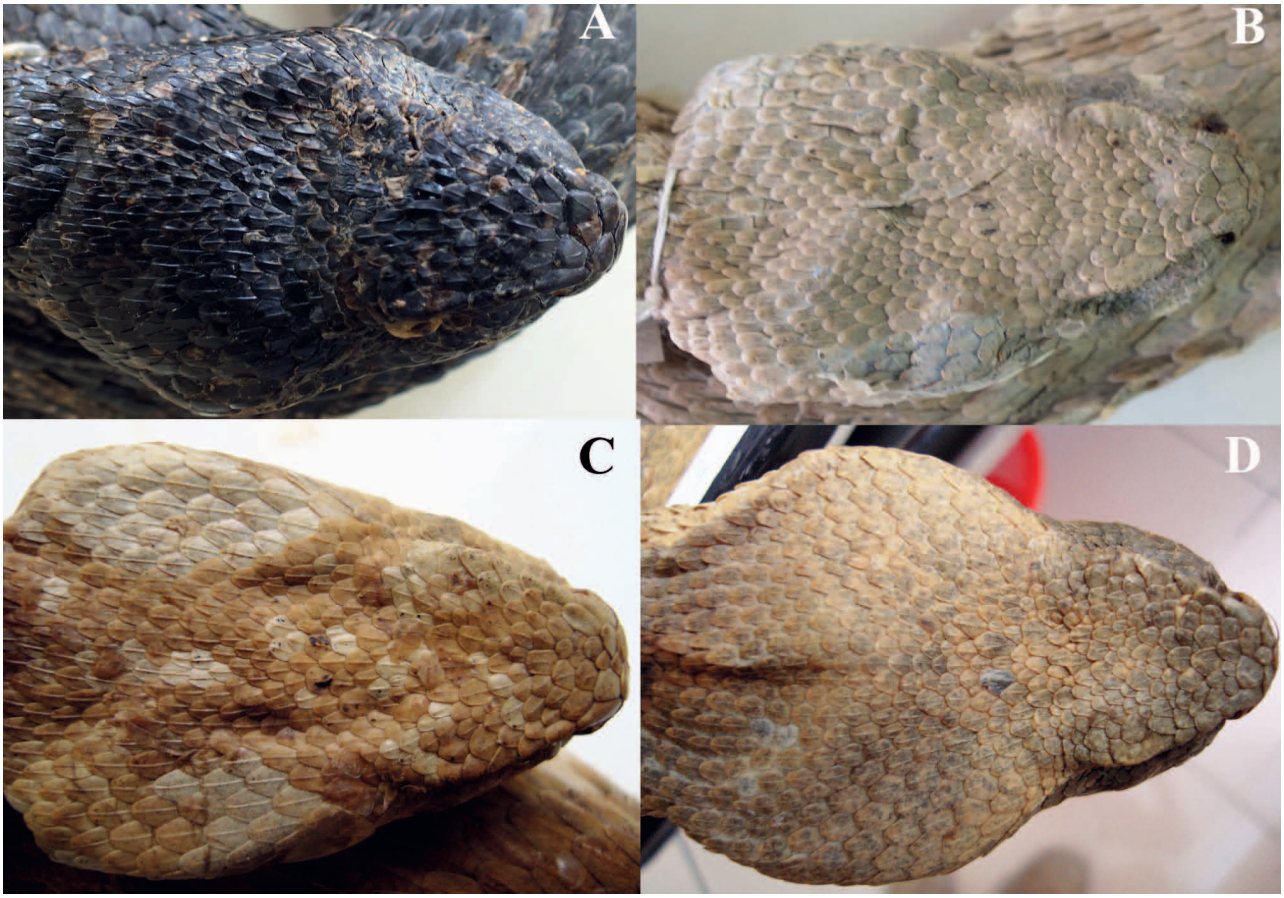

Figure 7. Comparison of dorsal head scales of Iranian *Macrovipera* spp.: Dorsal head scales are strongly keeled in the holotype (A) of *Macrovipera razii* sp. n., versus an individual (ERP 1981) from Bamoo National Park, Fars Province that has weakly keeled scales (B). Prominent keels on the dorsal scales of *M. lebetina obtusa* (2294) from Hamadan, western Iran (C), whereas *M. lebetina cernovi* (2228) from Kalate Naderi, northeastern Iran (D) has moderately keeled scales.

Figure 8. *Macrovipera razii* sp. n., paratype (adult female ERP 1941) from Pariz, 50 km north of Sirjan, Kerman Province (Photo: N. Moradi).

Figure 9. *Macrovipera razii* sp. n. (ERP 1981) in its natural habitat in the Bamoo National Park, Fars Province, southern Iran (Photo: H. Oraie).

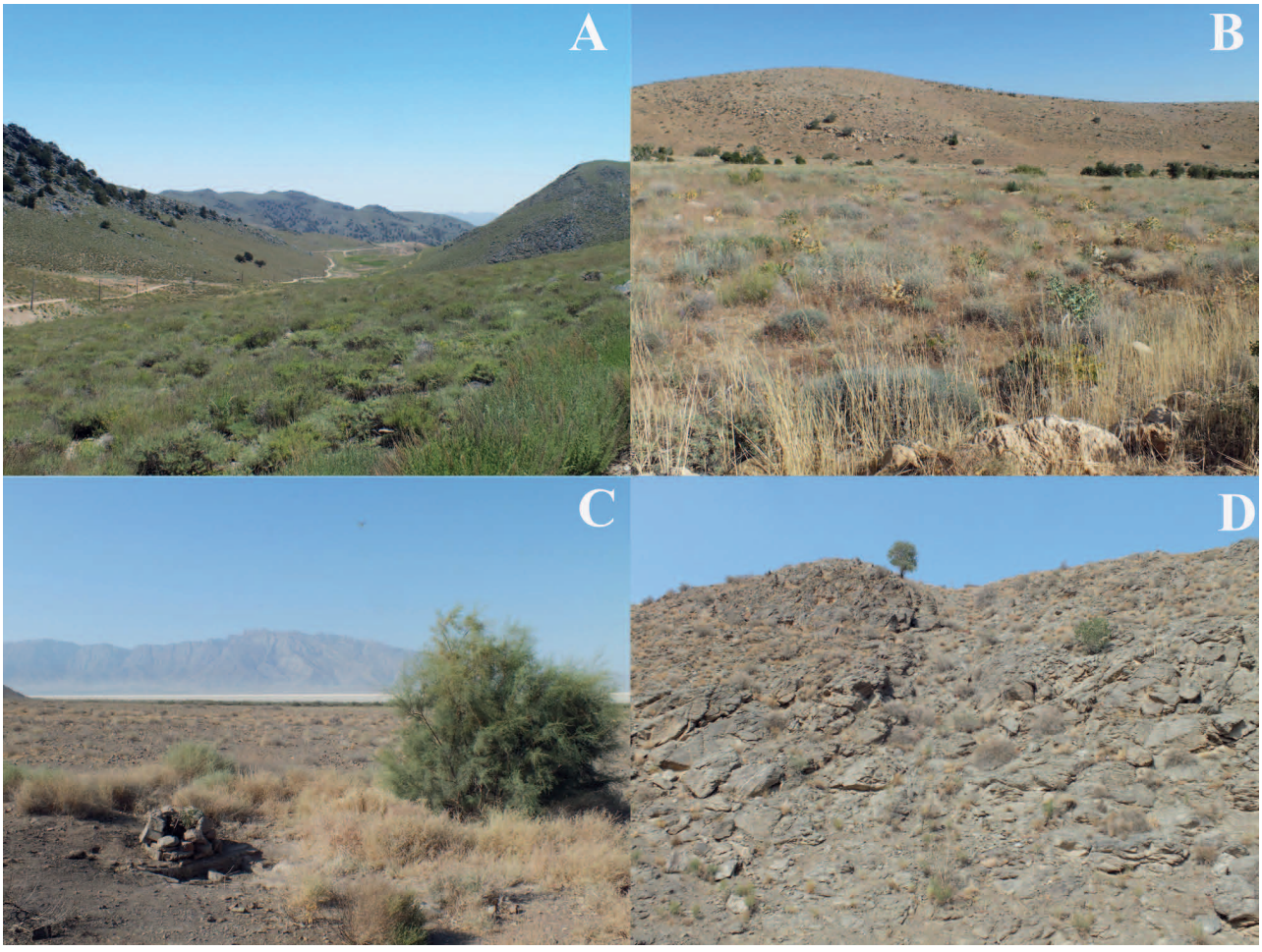

Figure 10. Various types of natural habitats of *Macrovipera razii* sp. n., type locality, 105 km on the road from Jiroft to Bam near Babgorgi village and Valley, Kerman Province (A); at the Fill Spring in Bamoo National Park, Fars Province (B); at Tolombeh Badi, Bakhtegan Lake protected area, Fars Province (C); at Ghatroyeh, Bahrame Goor National Park, Fars Province (D). (Photos: H. Oraie).

In addition, our results provide evidence of the divergence among Iranian populations of *M. lebetina*, which are separated from each other by 3.9% genetic difference in Cytb sequences (Table 3) as well as by several morphological characters (Table 4). By having its supraoculars split up into smaller plates, *M. lebetina obtusa* could be easily differentiated from *M. lebetina cernovi* (Chikin & Szczerbak 1992), and they are geographically isolated, too. Based on our results, all the western and northwestern populations of *M. lebetina* in Iran agree with the description of *M. lebetina obtusa*, but *M. lebetina cernovi* is exclusive to northeastern Iran (Fig. 1).

The high values of genetic divergence suggest that *Macrovipera razii* sp. n. has been genetically isolated from the other studied populations of *M. lebetina* for a long period of time. According to our calibrated molecular clock, their splitting may have occurred around 10.5 Mya (Fig. 2; yet only 6.6 Mya after Stümpel 2012). If our new dating is correct, this separation most likely coincided with the rise of the Zagros Mountains in the late Miocene some 12–10 Mya (Abdrakhmatov et al. 1996). However, much research still needs to be done to understand the effects of historical events on *Macrovipera* spp. in Iran and neighbouring areas. A high level of haplotype diversity in a population in northern Saveh, and the close relationship of a specimen from Azerbaijan (KJ415300) both indicated that *M. lebetina obtusa* was apparently affected by climatic oscillations during the Quaternary at least in its northern range. Multiple extinction and colonization processes may have occurred in this species during the Quaternary (e.g., Avise 2000, Hewitt 2000). These results (TCS network) may reflect that the Saveh Region play a role as a potential glacial refuge for *M. lebetina obtusa* during the Pleistocene glaciations. This hypothesis could be investigated in further phylogeographic studies.

The discovery of *Macrovipera razii* sp. n. and its restricted range within Iran highlight the need for an IUCN Red List assessment to determine its conservation status. *Macrovipera razii* sp. n. could be an interesting animal for addressing some evolutionary questions on how ecological conditions influence colour pattern and also the emergence of similar morphological characters such as large supraocular scales in two distinct lineages, *M. lebetina cernovi* and *Macrovipera razii* sp. n.

Key to the species and subspecies of the genus *Macrovipera* in Iran

1a– Elongated anterior chin-shields more than three times longer than the posterior ones, ventral scales 172–175 ..... *Macrovipera razii* sp. n.

1b– Square anterior chin-shields less than twice as long as the posterior ones, ventral scales less than 172 ..... *Macrovipera lebetina* (Linnaeus, 1758)

2a– Supraocular entire or divided and larger than circumoculars; snout dark (one third of the anterior part of the head); dorsum reddish brown or dark with large open-centre blotches that may sometimes be connected and each dorsal blotch encircling 16.66 ± 2.78 pigmented scales at mid-body ..... *Macrovipera lebetina chernovi* (Chikin & Szczerbak, 1992)

2b– Three supraoculars, of the same shape as the other circumoculars; dorsum brown or dark brown with rectangular dark markings, arranged in a zigzag pattern, with each dorsum blotch encircling 19.29 ± 1.16 pigmented scales at mid-body ..... *Macro-* ..... *vipera lebetina obtusa* (Dwigubsky, 1832)

(modified from Chikin & Szczerbak 1992, David et al. 1999, Joger 1984, and Moradi et al. 2014)

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