

## New data on *Montivipera kuhrangica* (squamata: serpentes: Viperidae)

Oraie, H.<sup>1,2\*</sup> and Khosravani, A.<sup>2</sup>

<sup>1</sup>Department of Zoology, Faculty of Science, Shahrood University, Shahrood, Iran

<sup>2</sup>Department of Biodiversity, Institute of Biotechnology, Shahrood University, Shahrood, Iran

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The genus *Montivipera* comprised of two recognized clades in light of molecular as well as morphological data (Stümpel & Joger, 2009; Rastegar-Pouyani *et al.*, 2014; Stümpel *et al.*, 2016; Behrooz *et al.*, 2018). The first, the ‘*xanthina*-complex’, are endemic to Asia Minor, Syria and Lebanon. The second clade, the ‘*raddei*-complex’, comprises populations over a wide range from north-western and western Iran to the southern half of Armenia, south of Azerbaijan, Nakhchivan and eastern Turkey. (Nilson & Andrén, 1986; Behrooz *et al.*, 2018).

*Montivipera kuhrangica* Rajabizadeh, Nilson and Kami, 2011, described from the Kuhrang area, (Chaharmahal and Bakhtiari Province, Iran). It is one of the least known species in the *raddei* complex of the genus. Taxonomically, it was identified a subjective junior synonym of *M. raddei* on the basis of DNA barcoding data (Rastegar-Pouyani *et al.*, 2014). However, two separated multigene phylogenetic analysis considered it as a valid species (Stümpel *et al.*, 2016; Behrooz *et al.*, 2018).

In a mountainous area of NW Fereidonshahr, Isfahan Province (Fig. 2), we collected one *Montivipera* specimen (Fig. 1) at altitudes above 2900 m (32.969074 N, 50.060461E). Moreover, two executed samples were gotten from obscure places around Fereidonshahr by rural people. We retained standard morphological characters used by Oraie *et al.* (2018). Measurements, except body and tail lengths, were taken with Vernier calipers with an accuracy to the nearest 0.01mm. Ventral scales were counted according to Dowling (1951). The numbers of dorsal scale rows are given at one head length behind the head, at mid-body, and at one head length before vent respectively. Values for symmetric head characters are given in the left/ right order.

The total genomic DNA was extracted from ethanol-preserved tissue samples using the tissue DNA extraction kit (Aron-Gene, Isfahan, Iran) following the manufacturers’ instructions. Amplification conditions for the partial mitochondrial COI gene with the primers RepCOI F and RepCOI R (Nagy *et al.*, 2012) consisted of 36 thermal cycles as described by Rastegar-Pouyani *et al.* (2014). The amplification products were sequenced on an automated sequencer ABI 3730XL (Codon Genetic Group, Iran) according to standard protocols. The mitochondrial COI haplotypes have been deposited in GenBank (HAC\_687: MK814534; HAC\_688: MK814535; HAC\_689: MK814536). Published COI sequences for the Iranian species of *Montivipera* (Rastegar-Pouyani *et al.*, 2014) were retrieved from NCBI (<http://www.ncbi.nlm.nih.gov>) and a data set with a final sequence length of 629 nucleotides were generated. The mean genetic distances were estimated by using the Mega X (Kumar *et al.*, 2018).

Our molecular and morphological analyses bring us to the decision that newly found specimens belongs to *Montivipera kuhrangica*. Morphologically, the newly found specimens consistent with the description of type-specimen (Rajabizadeh *et al.*, 2011), except for a lower count of Loreal scale (5/5 vs. 8/8) and their lower number of Infralabial scales that are in contact with anterior chin-

shield (2/3 vs. 5/4). More comparisons are provided in Table 1. Molecular results indicate a very close genetic connection (less than 0.004 %) between the newly found specimens and *M. kubrangica* (Table 2). As expected, the phylogenetic trees come from different reconstruction methods (MP, ML, and BI) are identical in branch patterns presented in Rastegar-Pouyani *et al.* (2014).

**TABLE 1.** Morphological characters of newly found specimens of *Montivipera kubrangica* and its comparison with type specimen.

characters	HAC 687(Male)	HAC 688 (Male)	HAC 689 (Female)	<i>Montivipera kubrangica</i> *
SVL	560.5	490.5	590.0	610.0
Tal	51.88	45.23	54.55	57.0
Tal/SVL	0.0925	0.0922	0.0924	-
HL/SVL	0.0427	0.0524	0.0504	-
HW/SVL	0.0395	0.0385	0.0359	-
HH/SVL	0.0165	0.0198	0.0137	-
DBN/SVL	0.0105	0.0128	0.0109	-
IOD/SVL	0.0170	0.0198	0.0196	-
SL/SVL	0.0116	0.0110	0.0145	-
Pre	2	2	1	-
Ven	166	163	164	163
Scd	35	33	34	37
Sq1	23	23	23	23
Sq2	23	23	23	23
Sq3	18	17	16	17
Can	3/3	3/3	3/3	3
Interocular	7	7	8	-
InCanSup	44	53	55	44
Blspl	25	24	28	-
Spl	10/9	10/9	10/10	9/11
Ifi	9/9	11/10	12/11	13/13
Incir	13/14	16/15	16/15	16/13
Outcir	17/16	16/18	18/17	16/18
Lor	5/5	5/5	5/5	8/8

\* Rajabizadeh *et al.* 2011

Our data provide first evidence on the occurrence of *Montivipera kubrangica* from a high-altitude area of Isfahan province, more than 60 km far from its type locality. Probably more extensive range for the *M. kubrangica* as a sky-island taxon in high-altitude habitats of central Zagros Mountain is expected (Behrooz *et al.*, 2018). Further field work is needed to discover more populations in neighbours' environments. On the other hand, our morphological data confirm some degree of intraspecific variation in scalation of *M. kubrangica*, despite very close genetic relationships between examined specimens. It could be another example of this argument that morphological data are not necessarily represented by molecular data (e.g. Sheikh *et al.*, 2019).



**FIGURE 1.** *Montivipera kuhrangica* Rajabizadeh, Nilson, and Kami, 2011 in its natural habitat, at altitudes over 2900 m in mountainous area of NW Fereidonshahr, Isfahan Province (32.969074 N, 50.060461E).

**TABLE 2.** Corrected genetic divergence (Kimura 2 parameters) among Iranian species of the genus *Montivipera* and *Marovipera lebetina* as out-group taxon

1- <i>Marovipera lebetina</i>	1	2	3	4	5	6
2- <i>M. albicornota</i>	0.09197					
3- <i>M. r. kurdestanica</i>	0.09268	0.00689				
4- <i>M. r. raddeii</i>	0.09149	0.00942	0.01162			
5- <i>M. latifi</i>	0.09653	0.01443	0.01747	0.01773		
6- <i>M. kuhrangica</i>	0.09642	0.01430	0.01734	0.01772	0.01461	
7- Newly Found Specimens	0.09642	0.01430	0.01734	0.01772	0.01789	0.00319



**FIGURE 2.** Natural habitats of the newly found specimen of *Montivipera kubrangica* in the environs of Fereidonsahr.

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#### **LITERATURE CITED**

Behrooz, R., Kaboli, M., Arnal, V., Nazarizadeh, M., Asadi, A., Salmanian, A., Montgelard, C., 2018. Conservation below the species level: suitable evolutionarily significant units among mountain Vipers (The *Montivipera Raddei* complex) in Iran. *Journal of Heredity* 109, 416-425.

Dowling, H.G., 1951. A proposed standard system of counting ventrals in snakes. *British journal of herpetology* 1, 97-99.

Kumar, S., Stecher, G., Li, M., Knyaz, C., Tamura, K., 2018. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Molecular Biology and Evolution* 35, 1547-1549.

Nagy, Z.T., Sonet, G., Glaw, F., Vences, M., 2012. First large-scale DNA barcoding assessment of reptiles in the biodiversity hotspot of Madagascar based on newly designed COI primers. *Plos One* 7, e34506.

- Nilson, G., Andren, C., 1986. The Mountain Vipers of the Middle East - the *Vipera Xanthina* Complex (Reptilia, Viperidae). Bonner Zoologische Monographien 20. Zoologisches Forschungsinstitut Und Museum Alexander Koenig, Bonn.
- Oraie, H., Rastegar-Pouyani, E., Khosravani, A., Moradi, N., Akbari, A., Sehhatisabet, M.E., Shafiei, S., Stümpel, N., Joger, U., 2018. Molecular and morphological analyses have revealed a new species of blunt-nosed viper of the genus *Macrovipera* in Iran. Salamandra 54, 233-248.
- Rajabizadeh, M., Nilson, G., Kami, H.G., 2011. A new species of mountain viper (Ophidia: Viperidae) from the Central Zagros mountains, Iran. Russian Journal of Herpetology 18, 235- 240.
- Rastegar-Pouyani, E., Oraie, H., Khosravani, A., Kaboli, M., Mobaraki, A., Yousefi, M., Wink, M., 2014. A re-evaluation of taxonomic status of *Montivipera* (Squamata: Viperidae) from Iran using a DNA barcoding approach. Biochemical Systematics and Ecology 57, 350–356.
- Sheikh, Y., Oraie, H. & Rastegar-Pouyani, E., 2019. Morphometric versus genomic evidence. The systematic status of *Eryx miliaris* (PALLAS, 1773), from sistán, east Iran. Herpetozoa 31, 133 - 144.
- Stümpel, N., Rajabizadeh, M., Avcı, A., Wüster, W., Joger, U., 2016. Phylogeny and diversification of mountain vipers (*Montivipera*, Nilson *et al.* 2001) triggered by multiple Plio-Pleistocene refugia and high-mountain topography in the near and Middle East. Molecular Phylogenetics and Evolution 101, 336-351.
- Stümpel, N., Joger, U., 2009. Recent advances in phylogeny and taxonomy of near and Middle Eastern vipers - an update. Zookeys 31, 179-191.