

Preliminary analysis of dorsal pattern variation and sexual dimorphism in *Montivipera latifii* (Mertens, Darevsky and Klemmer, 1967) (Ophidia: Viperidae)

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Submitted on: 2011, 20th July; revised on: 2011, 14th October; accepted on: 2011, 2nd December.

Abstract. In this study, sexual dimorphism and dorsal patterns were investigated in Latifi's viper (*Montivipera latifii*) from Iran. Sexual dimorphism was evaluated in 13 males and 15 females using 12 morphological characteristics. Despite the low sample size, the results showed that both sexes significantly differ in the number of subcaudal scales, the number of outer circumocular scales and tail length. In a limited area, the Lar National Park, three different dorsal patterns were observed (n=26 specimens): about 50% displayed a complete zigzag dorsal pattern, 15% of the individuals displayed a striped dorsal pattern, and about 35% had an incomplete zigzag dorsal pattern. These findings confirmed partially results from former published studies. Finally, we hypothesised that the four pattern described in *M. latifii* could be a combination of only two genetically define dorsal marks.

Keywords. *Montivipera latifii*, dorsal pattern, sexual dimorphism, genetic basis.

INTRODUCTION

Montivipera latifii (Mertens et al., 1967) is one of the four endemic vipers of Iran (Rastegar-Pouyani et al., 2008) and is a member of the *M. raddei* group. A particularity of this species is a high level of polymorphism of the dorsal pattern. So far, four different patterns have been identified in *M. latifii*. Most of the specimens present a darker vertebral line connecting with a varying number of alternating or opposing blotches that, together, represent an intensive and well-developed zigzag pattern (Bare; Fig. 1C). Very occasionally, the dorsal pattern can consist of a one-to-two scales width, straight vertebral stripe, without additional blotches (Stripy; Fig. 1A). Occasionally, the central vertebral line

is missing and only the alternating blotches remain, resulting in a diffuse spotty or blotchy pattern (Spotted; Fig. 1B). Mertens et al. (1967) reported a unique specimen with no pattern (Plain). Such kinds of patterns seem to be extremely rare in *M. latifii* because, to the best of our knowledge, no other mentions of them have been recorded. In a study carried out by Andr n and Nilson (1979) on the variability of the dorsal pattern in *M. latifii* in Lar Valley, no specimen with a completely uniform colour was observed, whereas 59% of the individuals presented a well-developed zigzag pattern, 33% had spots or a blotchy pattern and only 8% had a vertebral stripe. Additional detailed information about the morphological description and diagnosis of this species have been given in the following publications: Mertens et al. (1967), Andr n and Nilson (1979), Nilson and Andr n (1986), Latifi (2000), Mallow et al. (2003). Current knowledge of the distribution of *M. latifii* suggests that this species is restricted to a small area in the central Alborz mountain range (Fig. 2; Rajabizadeh, 2008; Behruz et al., 2009). Consequently, this species has been listed in IUCN red list as “Endangered” (<http://www.iucnredlist.org>).

Sexual dimorphism (SD), defined as the phenotypic difference between males and females of a species, is a frequent phenomenon in animals including reptiles (Andersson, 1994). Stuart-Fox and Ord (2004) regrouped three main forms of SD in reptiles; sexual size dimorphism (SSD), ornamentation dimorphism, and coloration dimorphism. Sexual size dimorphism (SSD) describes the situation where the two sexes differ in certain morphological traits, especially body length (SVL). In about two-thirds of snake species, females grow larger than males (Shine, 1998). In addition, significant dimorphism has been documented regarding relative head size, head shape and tail size (Shine, 1993). Furthermore, a general trend towards more ventrals in females and more subcaudals in males has also been documented (e.g. Pope, 1935; de Silva, 1969). Although snakes do not show as vivid colours as many sexually dichromatic lizard species, sex differences in colour are more common among snakes than has generally been appreciated (Shine, 1993).

Although a large dorsal pattern variation was observed in *M. latifii*, practically no information on intrapopulation variation is available. The aims of this study were to i) evaluate the proportions of different dorsal patterns in the Lar Valley (Iran) and ii) to assess morphological differentiation between both sexes at a larger scale. Finally, different scenarios regarding the possible genetic aspects of the different patterns of *M. latifii* will be discussed.

MATERIALS AND METHODS

To study dorsal pattern variation in the Lar Valley populations, we gathered data from 26 specimens collected between 2006 and 2009 in surveys conducted by M. Rajabizadeh and R. Behruz and pictures taken by amateur Iranian photographers from “Gozal Darre” and Alarm in Lar Valley. For this study, all samples collected in the Lar river valley and side branches of the Lar National Park were regarded as belonging to the Lar Valley study area (Fig. 2). The direct observations and pictures allowed the dorsal pattern to be evaluated and classified into four categories (Stripy, Spotted, Bare and Plain; see above for a complete description and Fig. 1), as recognized by Nilson and Andr n (1986).

To study sexual dimorphism in *M. latifii*, a total of 28 specimens including 15 females and 13 males were examined. The sampling regrouped five specimens from Central Albert (collection of the

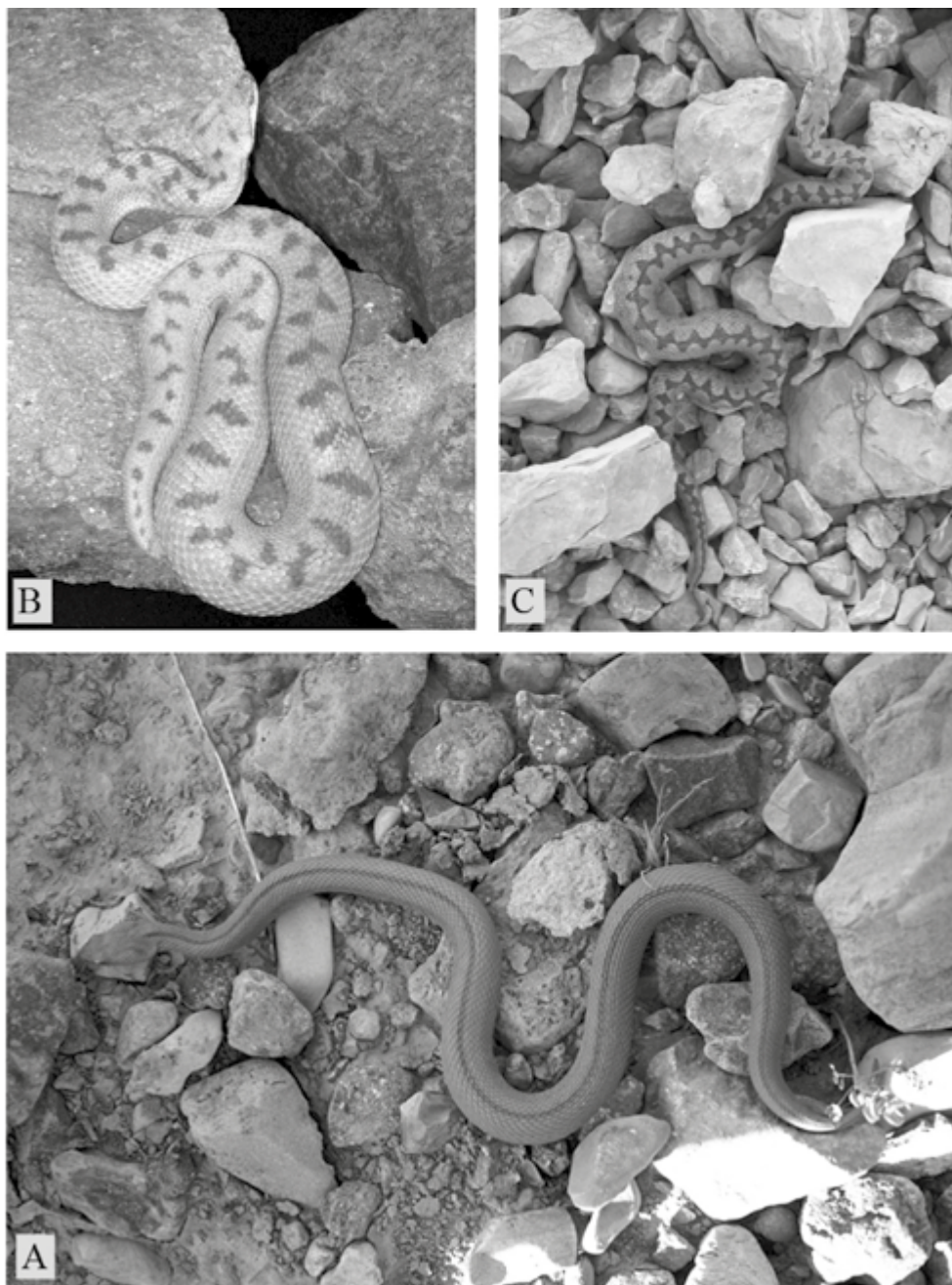


Fig. 1. The three differently coloured patterns of *Montivipera latifii* observed in Lar National Park (Iran). A: Stripy; B: Spotted (incomplete zigzag); and C: Bare (complete zigzag).

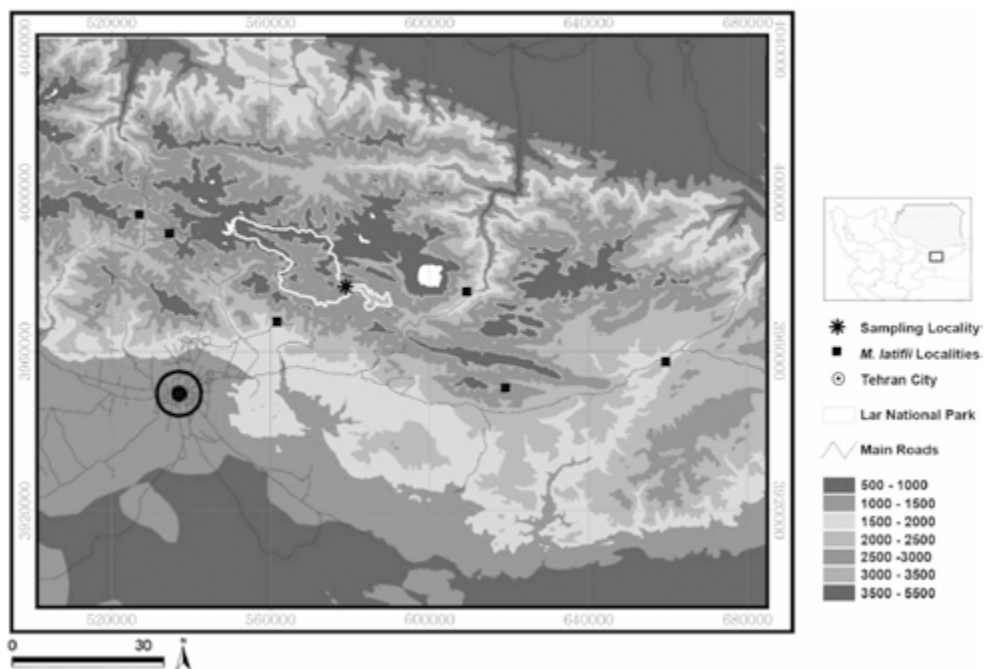


Fig. 2. Distribution of *Montivipera latifi* and the sampling locations in Lar National Park, Iran.

Tehran University Zoological Museum), five specimens from Lar Valley (MR personal collection) and 18 specimens collected by M. Latifi (Razi Institute Collection); the exact collection locality of these specimens was unknown. Eight meristic and three morphometric characters were selected for this study (see Table 1 for further details). The morphometric characters were measured to the nearest 0.1 mm with a Vernier calliper. The proportions of the head and the tail sizes were calculated in order to remove the impact of individual size (strongly correlated with age) on the analyses. In order to study the sexual dimorphism, an ANOVA test was conducted for each meristic and morphometric character. In addition a Principle Component Analysis (PCA) was carried out to determine the principal characters that distinguished between the sexes. For this analysis, all morphological variables were standardized to avoid bias. All statistical analyses were performed using the SPSS statistical package (version 15; SPSS Inc., Chicago).

RESULTS

1. Dorsal Pattern

About 50% of the *M. latifi* from Lar National Park population had a complete zigzag dorsal pattern, whereas the stripy dorsal pattern was detected in about 15% of the individuals and about 35% had an incomplete zigzag dorsal pattern (Table 2). No individual with a "Plain" pattern was observed in the Lar Valley.

Table 1. List of characters used in the morphological examination of *Montivipera latifii* specimens.

Ven	Number of ventral scales (following Dowlin, 1951)
Scd	Number of subcaudal scales
inCanSup	Number of intercanthal + intersupraocular scales
Spl*	Number of supralabial scales
Ifl*	Number of infralabial scales
inCir*	Number of inner circumocular scales
outCir1*	Number of outer circumocular scales (round eye and supraocular)
outCir2*	Number of outer circumocular scales, round eye (following Nilson and Andr�en, 1986)
SVL (mm)	Snout vent length: from tip of snout to vent
TL (mm)	Tail length: from vent to tip of tail
HL (mm)	Head length: from tip of rostral to end of lower jaw
HW (mm)	Head width at the widest point
ITL*100	Ratio of tail length on total length * 100
IHL*100	Ratio of head length on total length * 100
HL.HW	Ratio of head length on head width

* Sum of left and right scales used in the analysis.

Table 2. Proportions of the different dorsal patterns of *Montivipera latifii* observed in Lar National Park (Iran).

Dorsal pattern	Number	%
Stripy	4	15.4%
Spotted (incomplete zigzag)	9	34.6%
Bare (complete zigzag)	13	50%
Total	26	100%

2. Sexual Dimorphism

Significant morphological differences were found for the number of subcaudal scales, the number of outer circumocular scales and the length of the tail of *M. latifii* (Table 3). Additionally, the proportion of the tail was marginally significant ($p = 0.053$).

Intersexual comparisons were made using a PCA. The first principal component accounted for 34.5% of the total variation, whereas the first three principal components regrouped 62.2% of the total variation (Table 4). The number of subcaudal scales and the tail length proportions had a high impact on the first axis, whereas the second principal component axis (14.7% of the variability) was mainly composed of the number of outer circumocular scales, the number of inner circumocular scales and the total number of intercanthal + intersupraocular scales.

Table 3. Evaluation of the morphometric and meristic characters measured for 28 *Montivipera latifii* individuals, including mean, standard error, minimum and maximum values. Significant differences between the sexes (*) were tested using ANOVA.

	Males (n=13)		Females (n=15)		df	F	Sig.
	Mean ± Se	Range	Mean ± Se	Range			
Ven	165.30 ± 0.71	160 - 169	164.26 ± 0.86	157 - 169	1	0.833	0.370
Scd	36.48 ± 0.33	34 - 38	32.40 ± 0.74	29 - 39	1	22.640	<0.001*
inCanSup	38.69 ± 1.36	30 - 48	39.66 ± 1.00	30 - 47	1	0.342	0.564
Spl	18.61 ± 0.18	18 - 20	18.80 ± 0.29	18 - 22	1	0.263	0.612
Ifl	23.23 ± 0.69	15 - 25	23.73 ± 0.25	22 - 26	1	0.512	0.480
inCir	26.38 ± 0.50	24 - 30	27.26 ± 0.58	24 - 31	1	1.281	0.268
outCir1	35.61 ± 0.69	32 - 41	37.53 ± 0.44	34 - 40	1	5.702	0.024*
outCir2	27.92 ± 0.47	26 - 32	29.00 ± 0.37	27 - 31	1	3.235	0.084
SVL	526/38±23.48	397-658	469.40±29.16	168-637	1	2.221	0.148
TL	53.14±2.00	38.70-64.0	44.24±2.64	17-58.0	1	6.788	0.015*
HL	25.85±0.78	20.50-30.0	23.72±1.11	11.70-28.40	1	2.293	0.142
HW	18.05±0.54	14.80-20.60	16.51±0.97	7.30-22.0	1	1.729	0.200
lTL*100	9.09 ± 0.15	7.97 - 9.95	8.66 ± 0.14	7.74 - 9.72	1	4.127	0.053*
lHL*100	4.49 ± 0.77	3.85 - 4/91	4.73 ± 0.14	4.09 - 6.32	1	2.034	0.166
HL.HW	1.43 ± 0.02	1.28 - 1.53	1.59 ± 0.12	1.24 - 3.14	1	1.392	0.249

Table 4. Scores of the first three main components (PC1 to PC3) of the Principal Component Analysis for morphological and meristic measurements of 28 *Montivipera latifii* individuals.

Variable	Component		
	PC1	PC2	PC3
Zscore(Ven)	0.386	-0.116	0.063
Zscore(Scd)	0.705	-0.113	-0.297
Zscore(inCanSup)	0.262	0.540	-0.109
Zscore(Sup)	-0.097	0.026	0.730
Zscore(Ifl)	0.049	-0.349	0.570
Zscore(inCir)	-0.009	0.508	-0.119
Zscore(outCir1)	-0.338	0.803	0.223
Zscore(outCir2)	-0.279	0.806	0.275
Zscore(lTL*100)	-0.104	0.213	-0.854
Zscore(lHL*100)	-0.858	0.047	-0.174
Zscore(HL.HW)	-0.302	-0.280	-0.147
Eigenvalue	5.18	2.21	1.94
Percent variability (%)	34.54	14.73	12.93
Cumulative percent (%)	34.54	49.28	62.21

DISCUSSION

Although the number of *M. latifii* individuals examined was limited, the preliminary results are in agreement with the general pattern of intersexual differences in snakes. Indeed, a higher number of subcaudal scales and a longer tail length were observed in males in comparison to females, confirming the previous observation of Nilson and Andrén (1986). The higher number of subcaudal scales in males is often associated with a proportionally longer tail due to the occurrence of the two hemipenises.

Based on the observations made in Lar Valley, it was found that the Plain coloration is absent or very rare in *M. latifii*. A large proportion of the individuals had a normal Bare pattern with a complete zigzag, whereas the Stripy pattern, although not very frequent, was regularly observed. A previous study by Andrén and Nilson (1979) provided similar proportions of the different dorsal patterns, based on newborns from two females. The occurrence of the Plain pattern seems to be very rare and, to the best of our knowledge, only one observation was reported. Probably, it could correspond to an anecdotal observation of a complete lack of dorsal pattern, as reported in *Vipera aspis* (Mebert et al., 2011) and *V. seoanei* (Brodmann, 1987).

Former studies on dorsal patterns of *M. latifii* reported four distinct dorsal patterns (Nilson and Andrén, 1986). It can be hypothesised that the most common pattern, Bare, may be a combination of Stripy and Spotted patterns. In such circumstances, the species would only present three distinct dorsal patterns (Spotted, Stripy and Plain), while the Bare pattern would be a combination of the two other patterns (Spotted + Stripy). Thus, the dorsal pattern could be limited to the occurrence or the lack of two parameters, a dorsal line (Stripy) and a dotted pattern (Spotted). The lack of both patterns would result in the Plain pattern, whereas the occurrence of both would provide a Bare pattern. Such hypotheses need however confirmation.

Two main drivers of dorsal pattern variation in *Montivipera latifii* may be hypothesised: a genetic and/or an ecological basis. The present study, based on random observations of *M. latifii* in nature, was not sufficient to allow us to speculate about the genetic basis of the different dorsal patterns in this species. However, we can propose a scenario whereby the two distinct dorsal patterns of *M. latifii*, the incomplete zigzag pattern (Spotted) and stripy pattern (Stripy), are controlled by two genes. The complete zigzag pattern, which is combination of these two basic patterns, is observed when both genes are expressed. If we considering that the population in Lar Valley is in Hardy-Weinberg equilibrium, gene A for the Spotted characteristic (A is dominant over a) and gene B for the Stripy characteristic (again, B is dominant over b), the gene combinations AABB, AABb, AaBB and AaBb would provide a Bare pattern, aaBB and aaBb a Stripy pattern, and AAbb and Aabb a Spotted pattern, whereas the combination aabb would result in the lack of a dorsal pattern (Plain). Using this hypothesis, the proportions of each pattern observed in Lar Valley can allow a rough estimation of the frequency of both genes (A=0.40 and B=0.58).

In snakes, the dorsal pattern is often related to a defence strategy. For instance, the zigzag pattern in European vipers has been shown to act as an aposematic signal for birds (Wüster et al., 2004; Niskanen and Mappes, 2005). In addition, stripy patterns are thought to confuse predators over the direction and speed of movement of the snake by making it difficult to visually focus on any specific point along the animal (Wolf and Werner, 1994).

Comparing the environment of the different species of the genus, *M. latifii* can be found at altitudes higher than 2400 m in central Alborz, which is among the highest of the elevated habitats (Nilson and Andrén, 1986). *Montivipera latifii* is also found in one of the most sparsely vegetated habitats within the genus. Consequently, the lack of vegetation may have selected for different dorsal patterns. However, this hypothesis should be investigated, for instance, by studying the predation rates of the different patterns by analysing the survival rate of snakes with both patterns (*via* capture-mark-recapture methods) or by experiments using Plasticine snakes.

ACKNOWLEDGEMENTS

We are grateful to Abbas Zare, head of the Poisonous Animals Unit, Razi Vaccine and Serum Institute, for his cooperation. Also, we thank our colleagues H. Gudarzi, Y. Werner, R. Behruz, H. Parsa, H. Salehi, N. Kian and H. Rostami for their kind cooperation.

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