Arch. Biol. Sci., Belgrade, 62 (3), 789-797, 2010
DOI:10.2298/ABS1003789L

MORPHOLOGICAL VARIATION OF THE COMMON LIZARD (*ZOOTOCA VIVIPARA* JACQUIN, 1787) IN THE CENTRAL BALKANS

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Abstract – The variation in some of the morphometric, meristic and qualitative characters of the Common Lizard (*Zootoca vivipara*) in five population samples from mountains of the Central Balkans was analyzed using univariate and multivariate statistics. The morphological differentiation was greater in males than in females and is more expressed in the morphometric than in the pholidosis and qualitative characters. The largest differences in morphometric traits appeared between the populations from the Šara and Stara Planina mountains. The "median" pileus pattern generally prevailed, with the appearance of other states in a certain proportion in some of the populations. Further analyses of the possible morphological and ecological distinctions of the population from Mt Tara are proposed.

Keywords: Viviparous lizard, morphology, scalation, geographic variation, The Balkan Peninsula

UDC 598.112(497-191.2)

INTRODUCTION

The Common Lizard, *Zootoca vivipara* (Jacquin, 1787), has the largest distribution of all lizard species, occurring throughout Eurasia from NW Spain to Sakhalin island in the China Sea (Böhme, 1997). The Balkan Peninsula is an area of the southern edge of its range. There the Common Lizard is restricted to humid habitats at higher altitudes in mountains, reaching 2400 m (Dely and Böhme, 1984; Böhme, 1997). The patchy distribution is particularly character for the central part of the peninsula where the occurrence of the Common Lizard has been reported on several mountains (Radovanović, 1951; Džukić, 1974) that are dissimilar in their geological origin and history (Dimitrijević, 1995), as well as in biotopes of different floristic provinces of the Balkan boreal subregion (Stevanović, 1995).

Owing to its large areal presence and its specificity in reproductive bimodality, the Common Lizard has been the subject of many studies into the morphological variability, life-history traits, karyological and molecular affinities of its populations, especially in recent years (e.g. Guillaume et al., 2006; Kupriyanova et al., 2006; Surget-Groba et al., 2006; Arribas, 2008). To date, some of the Balkan populations have been included in genetic studies (Guillaume et al., 1997; Heulin et al., 2000; Surget-Groba et al., 2006), while integrative morphological analyses of the species included restrictive samples of some eastern (Bulgaria) and western (Slovenia, Croatia) Balkan populations (see Arribas, 2001, 2008; Guillaume et al., 2006). To the best of our knowledge, comparative morphological analyses of populations from the Central Balkans have not been carried out.

This paper deals with morphological characters of samples of *Z. vivipara* collected from the central part of the Balkan Peninsula (Serbia and Montenegro) in order to characterize the pattern of morphological variation in this part of the southern periphery of the species' range.

MATERIAL AND METHODS

Samples and measurements

A total of 96 adult specimens from five localities in Serbia and Montenegro were used (Fig. 1). 1 – Mt.Tara, Serbia (Baturski Rzav: Jezero, 960m above

Figure 1. Location of *Zootoca vivipara* populations used in this study. 1-Tara Mt. (Serbia); 2- Mt. Kopaonik (Serbia); 3- Mt. Stara (Serbia); 4- Mt. Bjelasica (Montenegro); 5- Mt. Šara (Serbia).

sea level, 43° 54'N, 19° 22'E; 0 females, 3 males); 2 – Mt.Kopaonik, Serbia (Pančićev vrh, 2000m, 43° 16'N, 20° 49'E, Mali Karaman, 1904 m, 43° 17'N, 20° 50'E, Mala Gobelja, 1800m, 43° 19'N, 20° 49'E, Velika Gobelja, 1870m, 43° 18'N, 20° 49'E; 9 females, 5 males); 3 – Mt. Stara, Serbia (Stražna čuka, 1740m, 43° 16'N, 22° 48'E, Kopren: Šošina vunija, 1840m, 43° 17'N, 22° 48'E, Ponor, 1434m, 43° 15'N, 22° 48'E; 13 females, 7 males); 4 – Mt. Bjelasica, Montenegro (Pešića jezero, 1840m, 42° 51'N, 19° 41'E; 3 females, 0 males); 5. Mt. Šara, Serbia (Piribeg: Crvena karpa, 42° 10'N, 21° 02'E, 1800-1900m, Štrbački jalovarnik, 42° 11'N, 21° 02'E, 1700m and Čemerište, 2050-2100m, 41° 54'N, 20° 42'E; 32 females, 24 males).

Specimens preserved in 70% ethanol were from the herpetological collection of the Institute for Biological Research, Belgrade.

The specimens were examined for the following characters:

Morphometric characters: snout-vent length (L.cor), head length (L.cap), head width (Lt.cap), head height (Alt.cap), mouth length (L.fo), mouth width (Lt.fo), forelimb length (L.pa), hindlimb length (L.pp), length of fourth toe on hindlimb (L.dg), pileus length (L.pil), pileus width (Lt.pil), distance between fore and hind limbs (Pap).

Scalation characters: numbers of supraciliary scales (CIL), postocular scales (POC), temporal scales (TMP), supratemporal scales (STM), supralabial scales anterior to subocular (SLB), sublabial scales (SUB), submaxilar scales (SMX), gular scales (GUL), collar scales (COL), ventral scales (VNT), dorsal scales (DOR), praeanal scales (PRA), femoral pores (FPO), femural scales (FEM), subdigital scales (SDG). Qualitative characters: (I) pileus type: a – normal, b – with additional scales and anomalies. (II) masseteric plate: a – single, b – indistinct, c – divided. (III) contact between the supranasal and first loreal scales: a – wide contact, b – contact at one point, c – lack of contact. (IV) disposition of supranasals: a – wide contact ("median" type), b – contact at one point ("cross" type), c – lack of contact ("transversal" type), d – supernumerary scale among supranasals ("rectangular" type). (V) disposition of prefrontals: a - wide contact ("median" type), b – contact at one point ("cross" type), c – lack of contact ("transversal" type), d – supernumerary scale among prefrontals ("rectangular" type). (VI) row of supraciliary granules: a – absent; b – present. (VII) number of postnasal scales: a – one, b - two superposed, c – absent (fused with the first loreal scale). (VIII) number of first loreals: a – one, b – two, c – absent, fused with the postnasal scale, d – absent, fused with the second loreal scale. (IX) number of the second loreals: a – one, b – two, c – absent, fused with the first loreal scale. (X) number of preocular scales: a – one; b - two and more. (XI) presence of black spots on the ventral scales: a – on all scales, b – on majority of scales, c – on some scales, d – only in the lower part of the trunk, e – absent. (XII) dorsal pattern: a – broad continuous or discontinuous vertebral band, dark dorsolateral stripes of distinct large spots, b – broad continuous or discontinuous vertebral band, dark dorsolateral stripes of small or indistinct spots, c – narrow continuous or discontinuous vertebral band, dark dorsolateral stripes of distinct large spots, d – narrow continuous or discontinuous vertebral band, dark dorsolateral stripes of small or indistinct spots, e – vertebral band of irregular scattered points, dark dorsolateral stripes of small or indistinct spots, f – vertebral band indistinct, dark dorsolateral stripes of small or indistinct spots. Some of the qualitative characters were recorded following Voipio (1992) and Arribas (2008).

Counts were taken under a stereoscopic microscope, while the body and head dimensions were taken with digital calipers to the nearest 0.01 mm. Symmetrical characters were checked on both sides. Data processing for morphometric characters concerned the mean of the right and left values. Analyses of bilateral counts concerned the sum of scales in both sides in order to increase the number of phenotypic classes represented. Meristic characters with a small number of states were treated as qualitative characters, and a combination of states for both the right and left side was used.

Statistical analyses

All the statistical procedures used in this study are fully described in our previous studies of morphological variation in the lacertid species (e.g. Ljubisavljević et al., 2006, 2007) and included ANOVA (for scalation and snout-vent length), ANCOVA (with L.cor as covariate for other morphometric characters), the post-hoc Tukey HSD test for quantitative characters and the Williams' corrected G test for qualitative characters. Only characters showing significant geographical variation were subjected to multivariate analysis (Canonical Discriminant Analysis- CDA). The analyses were carried out using the computer package STATISTICA (StatSoft, Inc. 1997).

RESULTS

Quantitative characters

Preliminary analyses detected significant differences between the sexes (results not shown) and therefore the results are presented for males and females separately.

Table 1. Standardized coefficients of variation of the first three canonical variates of male *Zootoca vivipara*. Only characters showing significant geographical variation were subjected to CDA. Abbreviations of characters are given in "Materials and methods".

Characters	CV 1	CV 2	CV 3
L.cap	-0,482	-0,142	-0,354
Lt.cap	0,100	-1,747	1,967
Alt.cap	0,827	0,033	1,162
Lt.fo	0,621	0,729	-0,939
L.pa	-0,618	0,264	-0,259
L.pp	0,188	-0,431	-0,343
L.dg	1,476	-0,056	-0,178
L.pil	0,358	-0,005	-0,859
Lt.pil	-1,980	2,022	-0,630
CIL	0,116	0,591	0,347
SAN	0,387	-0,471	0,323
GUL	-0,654	-0,870	0,025
COL	-0,181	0,118	0,784
VNT	-0,893	-0,026	-0,101
DOR	0,505	-0,288	0,062
FPO	0,661	0,002	0,012
SDG	0,142	0,150	-0,259
Eigenvalue	14,476	4,643	2,704
% of variance	66,33	87,61	100,00

The results of an ANOVA test for L. cor. and an ANCOVA for other morphometric traits indicated that nine and five out of twelve examined characters presented significant geographical variation for males and females, respectively (ANCOVA/ANOVA, $P < 0.05$ for L.cap, Lt. cap, Alt. cap, Lt.fo, L.pa, L.pp, L.dg, L.pil, Lt. pil in males; Alt. cap, L.pp, L.pil, Lt. pil, Pap in females). Paired testing revealed that the greatest differences were between Mt. Stara and Mt. Šara in both sexes (Tukey HSD test, $P< 0.05$ in 3 variables in females and 10 variables in males, respectively). The limb

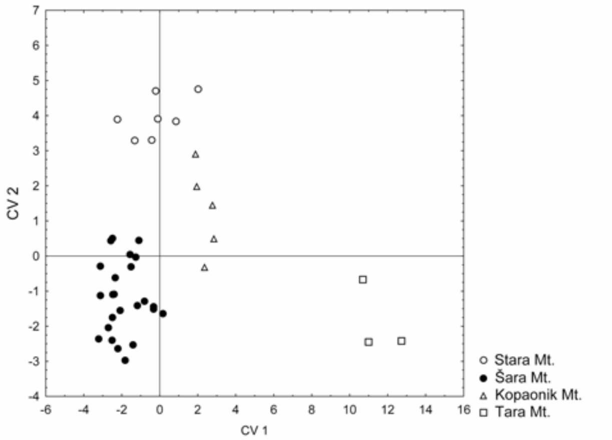

Figure 2. Scatterplot of the first and second canonical variates (CV) for quantitative characters in male *Zootoca vivipara*.

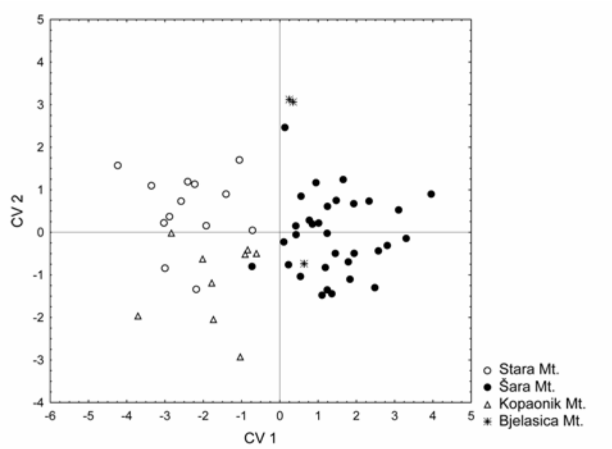

Figure 3. Scatterplot of the first and second canonical variates (CV) for quantitative characters in female *Zootoca vivipara*.

dimensions and pileus length showed the greatest differences across the paired comparisons in males, while the head height was the most variable character in females (in 4 and 2 out of 6 pairwise comparisons, respectively).

The meristic traits appeared to be less variable than the morphometric characters. Significant locality differences were found for eight and five out of fifteen traits in males and females, respectively (ANOVA, $P < 0.05$ for CIL, SLB, GUL, COL, VNT, DOR, FPO, SDG in males; CIL, SUB, GUL, COL, FPO in females). Pairwise comparisons

Table 2. Standardized coefficients of variation of the first three canonical variates of female *Zootoca vivipara*. Only characters showing significant geographical variation were subjected to CDA. Abbreviations of characters are given in "Materials and Methods".

Characters	CV 1	CV 2	CV 3
Alt.cap	-0,356	-0,427	0,854
L.pp	-0,362	-0,649	-0,625
L.pil	0,127	1,441	0,417
Lt.pil	-0,484	0,666	-0,096
Pap	0,821	-1,084	-0,349
CIL	-0,340	0,028	-0,585
SUB	-0,003	0,104	0,369
GUL	0,556	0,064	-0,391
COL	-0,272	0,173	0,054
FPO	-0,674	-0,157	-0,237
Eigenvalue	3,113	0,475	0,287
% of variance	80,33	92,90	100,00

revealed only slight and inconsistent differences among some samples. Thus, in males the greatest differences were found between samples from Mts. Tara and Kopaonik ($P < 0.05$ in three characters), while among females significant differences for two characters existed only between Mts. Šara vs. Stara and Kopaonik .

The first three canonical variate axes (CV1 -3) explain the whole interpopulation variation (100%) in both sexes (Tables 1 and 2). Discrimination was however more pronounced among the males than among the females (see Figs. 2 and 3), which is reflected in the higher percentage of correctly classified cases in the males (100% vs. 91%, for males and females, respectively).

In males, the sample from Mt. Tara (that could not be analysed in females) is differentiated from the others in the lower right quadrant, and is characterized by the high values for all traits that accounted for most of the variation on the first axis

(L.dg, Alt.cap, FPO, L.pa, see Table 1), except for the number of ventral scales (lowest values). The second axis separates the samples from Mt. Stara in the most positive part from the Mt. Šara sample in the negative part (Fig. 2). This is due to the lower values for head dimensions (Lt.cap, Lt.pil and Lt.fo) and the number of supraciliary scales. The higher values for the number of gular scales in the Mt. Šara sample highly loaded the second CV.

In the females, the greatest contributions to variance on the first CV were recorded for the meristic characters – number of femoral pores (FPO) as negative, and number of gular scales (GUL) as positive factors, respectively (Table 2). The second CV showed a high weighting for the pileus dimensions (L.pil, Lt.pil) as positive factors and some limb and interlimb dimensions (L.pp and Pap as negative factors). The first CV mainly separated Mts. Šara and Bjelasica from the others on the basis of a greater number of gular scales but smaller number of femoral pores (Fig. 3). The second CV mainly separated the Stara and Kopaonik samples according to greater pileus and hindlimb dimensions, but smaller interlimb distance in females from Mt. Stara in relation to those from Mt. Kopaonik.

Qualitative traits

All qualitative characters except for the presence of black spots on the ventral scales in males ($G = 22.42$, $P < 0.01$), showed an insignificant variation among the localities. The males from Mt. Šara were mostly characterized by the presence of black spots only on some ventral scales (54%) and differed significantly from those from Mt. Stara ($G = 8.82$, $P < 0.05$) and Kopaonik ($G = 13.84$, $P < 0.01$) which were characterized by the presence of black spots on all the scales (54% and 80%, respectively). All females were generally characterized by a complete lack of black spots or the presence of black spots only on lower part of the trunk (XId, XIe 67 – 100%).

Generally, the analyzed populations from the Central Balkans were characterized by: wide contact of prefrontals (Va 67 – 100%) and single loreals on one or both head sides (VIIIaa, VIIIab 60 – 100%; IXaa 80 – 100%). Also, they predominantly had normal pileus type (Ia 67 – 92%) and one postnasal scale (VIIaa 67 – 92%) while lacking supraciliary granules (VIaa 78 – 100%), except for the males from Mt. Tara where the pileus with additional scales and anomalies, supraciliary granules and two or none postnasal scales appeared in a greater proportion (Ib, VIaa, VIab, VIIab, VIIcc all 67%). Furthermore, the analyzed samples were characterized by: wide contact between the supranasal and first loreal scales on both head sides (IIIaa, 59 – 92%), except in the Kopaonik sample where the lack of contact on both head sides appeared in somewhat greater percentage (IIIcc 44% and 40% in females and males, respectively); wide contact between supranasals (IVa 89 -100%), except for the Kopaonik males and Mt. Stara females where contact at one point or lack of contact predominated (IV b + IVc 80% and 54% for Kopaonik and Mt. Stara, respectively).

The states of the masseteric plate (III) and number of preocular scales (X) showed great intra- and interpopulation variability, and there was no general prevalence of certain states. The same could be true for the dorsal pattern, although the data gave some indication of a prevalence of the pattern XIId in most samples (narrow vertebral band with dorsolateral stripes of small or indistinct spots, 37 – 67%), except in males from Kopaonik which were characterized by broad vertebral band and dorsolateral stripes of distinct large spots (XIIa 60%) and Tara males where a vertebral band of irregular scattered points and dorsolateral stripes of small or indistinct spots occurred in a greater proportion (67%).

As can be seen, the distinctiveness of some of the samples, such as the Tara and Kopaonik male samples, was observable, but still not statistically significant, probably due to the small sample size.

DISCUSSION

The morphological variation that was observed among the studied high-altitude populations of the Common Lizard in the Central Balkans appeared to be more expressed in the morphometric traits than in the pholidosis and qualitative characters. Also, morphological differentiation was greater in males than in females, a result that was also reported for the Common Lizard by Arribas (2008). Why the females of this species tend to be less variable is difficult to assess on the basis of the current data. A plausible explanation could be that different selection pressures may affect males in different ways from females.

The high altitude populations of South European lacertids often tend to be morphologically (but also genetically) well differentiated even in neighboring massifs (e.g. Arribas and Carranza, 2004; Ljubisavljević et al., 2007). However, it is still to be investigated whether the well-defined positions of the analyzed populations in the morphospace will disappear after the inclusion of samples from other regions (e.g. see Bulgarian high altitude populations in Guillaume et al., 2006). Previous studies have revealed a rather mosaic variability of *Z. vivipara* with respect more to local/ecological variations than to consistent differences between regions (Sas et al., 2004; Guillaume et al., 2006). However, when populations with different reproductive modes were compared, both oviparous forms showed consistent morphological differences in regard to all viviparous forms of this species (Guillaume et al., 2006; Arribas, 2008). These studies, together with molecular and karyotypic data, confirmed the validity of two subspecies *Z. v. carniolica* (eastern oviparous form in southern Austria, northern Italy, Slovenia, partly reaching the north-west of the Balkan Peninsula in its type locality on Mt. Snežnik and northwestern Croatia) and *Z. v. louislantzi* (western oviparous form in the Pyrenees and Cantabrian mountain regions), although there was no single character which could serve for diagnostic purposes.

Three of the five populations analysed here have been included in the phylogenetic study of Surget-Groba et al., (2006), which revealed the position of the Bjelasica, Šara and Kopaonik populations within the "Western Viviparous" clade. However, this clade may be complex and needs further study to determine if it is a valid subspecies (Arribas, 2008).

Although there was no significant geographical variation in body size, the largest differences in other morphometric traits appeared between Mts. Šara and Stara in both genders. Some differences in life-history characters between these populations have been already found and were explained as the results of fine-grained differences in the environmental conditions between these two localities (Crnobrnja-Isailović and Aleksić, 2004). The morphological distinction of the Tara males in comparison to the other populations could be simply due to the small sample size, but it could also be caused by adaptation to local environmental conditions, given that this population is found at a much lower altitude (960 m) with regard to the other analyzed populations. Also, the Tara population which occurs in relic habitats of refugial type (Džukić, 1974) deserves more thorough study for other reasons. Firstly, further survey **work** is essential to confirm whether the Common Lizard still exists on this mountain, as the latest field investigations did not confirm the presence of this species in previously recorded localities (Džukić, unpublished data). If still present, its reproductive mode should be studied because some lower- and medium-altitude populations from the north-western Balkans (245 – 1250 m, Heulin et al., 2000; Mayer et al., 2000) appeared particularly distinctive due to their oviparous reproductive mode (see above). Other populations from this study, as well those from the eastern Balkans (Bulgaria) exist at lower latitudes but higher altitudes (1434 – 2100 m this study; 1300 – 1950 m Bulgarian populations, see Guillaume et al., 1997) than those of the western Balkans and are viviparous (Guillaume et al., 1997; Crnobrnja-Isailović and Aleksić, 2004; Surget-Groba et al., 2006).

Finally, we have noted the variation in qualitative traits which has been widely discussed in previous studies (Voipio, 1968; Dely, 1978, 1981; Dely and Böhme, 1984; Heulin, 1986; Voipio, 1992; Arribas 2008). According to the scheme of dorsal pattern given by Arribas (2008), the analyzed populations (except the Kopaonik males) did not display the typical pattern of the "Western Viviparous" clade, but rather resembled those of other clades, even of the western oviparous form *Z.*

v. louislantzi (especially frequent in the males and females from Mt. Šara). However, part of the females from Mts. Stara and Šara resembled the “Central Viviparous I”, or “Eastern Viviparous” from Romania and Bulgaria.

Although it is proposed that the prefrontal pileus pattern of the Common Lizard is variable in the southern parts of Europe, which is explained as a result of the stenotopy of isolated populations in mountain areas (Dely and Böhme, 1984), we recorded that the “median” type, as the usual disposition for the species, prevailed in a high percentage in the Central Balkans both for supranasals and prefrontals (82% and 91% respectively, n = 96 for the whole sample). The “rectangular” type is believed to occur as a consequence of developmental instability experienced during embryonic development that is caused by various stresses (particularly under suboptimal environmental conditions) or to be produced by an extreme genotype (Voipio, 1992; Arribas, 2008). The “rectangular” disposition of supranasals was not recorded in the Central Balkan populations, while that of prefrontals was recorded in a smaller percentage in three populations (Mts. Šara, Stara and Kopaonik, 8 - 20%, respectively). Also we did not observe direct connections between the presence of pileus anomalies and rectangular pattern as proposed by Voipio (1992), or between the occurrence of rare pileus configuration and the absence of contact between the supranasals and first loreal scales, as recorded in the West Cantabrians (Arribas, 2008). Significant sexual differences in pileus constellation were found in the western France population (Heulin, 1986). We recorded significant but not consistent differences between the sexes in proportion to specimens with “median” disposition of supranasals in two populations (i.e. “median” type prevailed in the Mt. Stara males and in Kopaonik females: detailed results will be presented elsewhere). Although these results might be affected by the small sample size (especially in Kopaonik males), the appearance of “irregular” states also does not seem to be caused by environmental stress, at least on Mts. Šara and Stara where the first data on some life-history traits (Crnobrnja-Isailović and Aleksić, 2004; authors unpublished data) pointed to optimal environmental conditions at these particular localities.

Acknowledgment - This study was supported by the Serbian Ministry of Science and Technological Development (grant no. 143052, “Patterns of amphibian and reptile diversity on the Balkan Peninsula”).

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МОРФОЛОШКА ВАРИЈАБИЛНОСТ ПЛАНИНСКОГ ГУШТЕРА (*ZOOTOCA VIVIPARA* JACQUIN) У ЦЕНТРАЛНОМ ДЕЛУ БАЛКАНСКОГ ПОЛУОСТРВА

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У овом раду анализирана је морфолошка варијабилност популација планинског гуштера (*Zootoca vivipara*) из централног дела Балканског полуострва у погледу већег броја морфометријских, меристичких и квалитативних карактера помоћу униваријантих и мултиваријантних статистичких метода. Морфолошка диференцијација популација је више изражена код мужјака него код женки и то знатно више у морфометријским карактерима него у фолидози и квалитативним карактерима. Највеће разлике у погледу морфометријских карактера су утврђене између популација са Шар-планине и Старе планине. Доминира констелација пилеуса “медиан” типа, са појавом мањег процента осталих стања у појединим популацијама. Предлажу се даље анализе могуће морфолошке и еколошке различитости популације са планине Таре.