



Fluctuating asymmetry and individual variation in the skull shape of the common wall lizard (*Podarcis muralis* Laurenti, 1768) estimated by geometric morphometrics

Aleksandar Urošević¹, Katarina Ljubisavljević¹ & Ana Ivanović²

¹Department of Evolutionary Biology, Institute for Biological Research "Siniša Stanković", University of Belgrade, Bulevar Despota Stefana 142, 11060, Belgrade, Serbia

²Institute of Zoology, Faculty of Biology, University of Belgrade, Studentski trg 16, 11000 Belgrade, Serbia

We explored individual variation and asymmetry in the skull shape of the common wall lizard (*Podarcis muralis* Laurenti, 1768) across four populations representing different habitats, by employing geometric morphometrics. We found directional and fluctuating asymmetry across the analysed populations, without differences in fluctuating asymmetry among populations. Patterns of individual variation and fluctuating asymmetry were highly correlated within and among populations. Asymmetric skull shape variation was similar in all populations, and was mostly related to the jaw adductor muscle chamber. Our results imply that the uniform pattern of skull fluctuating asymmetry results from a high level of canalisation. Directional asymmetry can be related to anatomical and behavioural lateralisation.

Key words: fluctuating asymmetry, island, *Podarcis muralis*, skull, variation

INTRODUCTION

Fluctuating asymmetry (FA) is a pattern of small deviations from the bilateral symmetry, and can be used as a measure of developmental instability (Palmer & Strobeck, 1986; Debat & David, 2001; Leamy & Klingenberg, 2005). It can be induced by intrinsic or extrinsic stress factors (Parsons, 1990; Møller & Swaddle, 1997; Klingenberg & McIntyre, 1998). Intrinsic stress factors can be genetic (Soulé, 1986; Caughley, 1994; Klingenberg & Nijhout, 1999), such as inbreeding depression or genetic drift (Frankham, 1997, 1998; Eldridge et al., 1999). Potentially important extrinsic factors may include habitat fragmentation (Sarre, 1996; Lens et al., 1999; Ljubisavljević et al., 2005), insularity (Sarre & Dearn, 1991; Crnobrnja-Isailović et al., 2005; Băncilă et al., 2010), undernourishment (Swaddle & Witter, 1994), thermal stress (Savage & Hogarth, 1999), high population densities (Møller et al., 1995), parasites (Møller, 1992), increased intraspecific competition (Witter & Swaddle, 1994; Rettig et al., 1997), pollution (Kozlov et al., 1996; Eeva et al., 2000; Amaral et al., 2012) and other anthropogenic disturbances in urban and sub-urban environments (Doyle et al., 1977; Ditchkoff et al., 2006; Tull & Brussard, 2007; Lucas & French, 2012; Lazić et al., 2013). Some studies further showed that increased interspecific competition actually decreases the intraspecific level of FA. Such patterns could be explained by higher mortality and lower adaptive value of asymmetric individuals, as they suffer more from

interspecific competition for resources and have less reproductive success (Thornhill, 1992; Møller et al., 1998; Tomkins & Kotiaho, 2001).

The common wall lizard (*Podarcis muralis* Laurenti, 1768) is a suitable model-system for testing different extrinsic factors which influence FA. *Podarcis* lizards can be abundant, and inhabit places with varying levels of potential stressors (Crnobrnja-Isailović et al., 2005; Amaral et al., 2012; Lazić et al., 2013). For example, in *Podarcis* species from the Iberian Peninsula, the degree of isolation (distance of the island from the shore) and history of colonisation can be related to FA levels (Băncilă et al., 2010). Also, the FA levels of various meristic traits of *P. muralis* are higher in urban than in rural populations, highlighting the significance of anthropogenic stress in urban environments (Lazić et al., 2013).

To explore individual variation and asymmetry in *P. muralis* we analysed skull shape. The skull is a complex morphological structure with high adaptive significance often used in studies of developmental stability (Debat et al., 2000; Willmore et al., 2005; Breno et al., 2011; Jojić et al., 2011). Previous research on skull shape variation among *P. muralis* populations (Urošević et al., 2014) showed that insularity significantly affects skull shape of *P. muralis*, in addition to habitat sharing with another lacertid species (*P. melisellensis* Braun, 1877). Here, we deepened the study on morphological variation by exploring the level and the pattern of FA, and its correlation with individual shape variation. *Podarcis muralis* is widely distributed in the Central Balkans,

including small islands of the Skadar Lake archipelago (Montenegro) which are of relatively recent, postglacial origin (Stanković, 1976). Since the level of genetic differentiation between the populations studied is minimal (Crnobrnja et al., 1994) and the islands in Skadar Lake are of recent origin (Stanković, 1976) we can rule out the effects of the “genetic stress” and island age and focus on the extrinsic factors which can affect the level of FA – environmental stress.

The main aims of our study were to (i) examine all populations for the presence of fluctuating and directional asymmetry, (ii) test whether populations differ in the extent of FA, and (iii) explore correlations between individual variation and FA, describing patterns of both symmetric and asymmetric shape variation.

MATERIAL AND METHODS

Sample collection

The common wall lizard is a small, insectivorous lacertid lizard common to central, eastern and southern Europe. The nominotypical subspecies *P. muralis muralis* inhabits the central Balkan Peninsula (Crnobrnja et al., 1994). In this study, we used samples from two mainland populations (city of Belgrade, Serbia: 25 females and 23 males; Zeta, the bank of the Skadar Lake: 15 females and 14 males) and two Skadar Lake insular populations (Malo Starčevo island, Montenegro: 20 females and 17 males; Malo Beško island: 15 females and 15 males). The Belgrade population is exposed to anthropogenic influence, while the other populations are not considered to be exposed to any major anthropogenic stressors. The detailed descriptions of populations from Skadar Lake area are provided elsewhere (Džukić, 1977; Crnobrnja et al., 1994; Aleksić et al., 2009). In Belgrade and Malo Starčevo island, *P. muralis* is the single *Podarcis* species in the habitat. However, *P. muralis* from the Zeta mainland and Malo Beško island share their habitat with the *P. melisellensis*, an ecologically and morphologically similar species (Džukić, 1977; Arnold, 1987).

All specimens were initially collected for use in other studies and deposited in the Herpetological Collection of the Institute for Biological Research ‘Siniša Stanković’, University of Belgrade, Serbia. Details about the sample were published previously (Aleksić, 1997; Aleksić et al., 2009; Urošević et al., 2012, 2014).

Skull preparation and landmarks

The skulls of *P. muralis* were cleared by dermestid beetles (Malo Starčevo population) or trypsin and potassium hydroxide (Belgrade, Zeta and Malo Beško population, Dingerkus & Uhler, 1977), stained with Alizarin Red S to better distinguish between cranial elements and their articulations, and preserved in glycerol. Digital images of the ventral side of the skull submerged in glycerol, with palatal view oriented parallel to the image plane, were taken with a Sony DSC F828 (resolution 8.0 MP; Sony Corp., Tokyo, Japan). The skulls were placed in the centre of the optical field and the camera setup and placement of the lens relative to each specimen (3 cm from each cranium) were kept constant to minimise image error

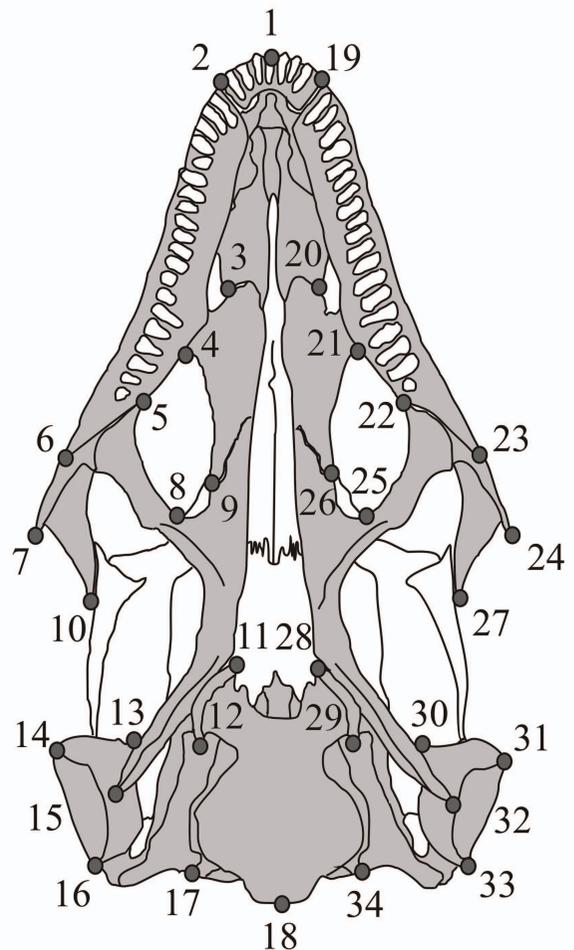


Fig. 1. Landmarks digitised on the ventral side of the cranium: 1 - Tip of premaxilla (tip of the snout); 2, 19 - Suture between premaxilla and maxilla; 3, 20 - Suture between vomer and palatine; 4, 21 - Anteriormost point of subocular foramen; 5, 22 - Anteriormost point of ectopterygoid; 6, 23 - Posterior tip of maxilla; 7, 24 - Lateralmost point of cranium; 8, 25 - Posteriormost point of subocular foramen; 9, 26 - Suture between pterygoid and palatine; 10, 27 - Posterior tip of jugal; 11, 28 - Anterior tip of basipterygoid process; 12, 29 - Posterior tip of basipterygoid process; 13, 30 - Anteriormost point of quadrate; 14, 31 - Lateralmost point of quadrate; 15, 32 - Posterior tip of pterygoid process; 16, 33 - Posterior point of quadrate; 17, 34 - Posterior tip of otooccipital; 18 - Posteriormost point on the curve of the occipital condyle.

related to distortion (Mullin & Taylor, 2002). Thirty-four landmarks (16 symmetric and 2 median) were digitized (by A.U.) on the ventral cranium using TpsDig 2 software (Rohlf, 2008). We used a landmark configuration which was the same as in the previous studies of the lacertid lizards cranial morphology (Ljubisavljević et al., 2010; 2011; Urošević et al., 2012; 2013; 2014, Fig. 1). The chosen landmarks were present on all specimens in the sample and they sufficiently summarise the morphology of the ventral cranium symmetric structures, contact points between bones, tips of processes, or the point of maximum curvature of structures.

Preliminary analyses: size and shape variation and allometry

To assess the differences in skull size between sexes and populations, we employed an ANOVA with CS as the dependent variable and sex and population as factors. To assess variation in skull shape, we employed a MANOVA with Procrustes coordinates as the dependent variable and sex and population as factors.

In a previous study it was shown that these populations share same allometry based on separate-sex analyses (Urošević et al., 2014). To test if sexes share a common allometry, we performed a multivariate regression of symmetric and asymmetric shape components on log transformed CS for each sex, with populations pooled within each group, before comparing the directions of allometric vectors between sexes for both symmetric and asymmetric components of shape variation. The angle between the two allometric vectors was calculated as an arccosine of the signed inner products between the normalised regression vectors (Cheverud, 1982; Klingenberg & Zimmermann, 1992; Klingenberg & McIntyre, 1998; Drake & Klingenberg, 2008). This angle was assessed by comparisons of angles with pairs of random vectors drawn in the multivariate tangent space. *P* values were calculated according to the close-form probability formula (Li, 2011) against the null hypothesis of the dissimilarity of vectors (Klingenberg & Marugán-Lobón, 2013).

Fluctuating asymmetry

We employed a Procrustes method (Auffray et al., 1996; Smith et al., 1997; Klingenberg & McIntyre, 1998) modified for landmark configurations with object symmetry as proposed by Klingenberg et al. (2002). First, the total shape variation of the symmetric structure was partitioned

into the symmetric and asymmetric component through Procrustes superimposition of the original landmark configurations and their mirror images. The asymmetry was quantified through the landmark deviations of the original configuration from the symmetric consensus of the original and mirror image (Mardia et al., 2000; Kent & Mardia, 2001). To estimate error, each configuration was digitised twice.

The sample sizes per population varied between 29 and 48 individuals (average 36), which is close to the recommended size for the correct estimate of FA ($n > 40$, Palmer, 1994). Before pooling sexes for the main analyses, we ran preliminary Levene tests on FA scores between sexes, for each population (Jojić et al., 2011). Deviations in the landmark configuration from the symmetric consensus was assessed via Procrustes ANOVA (Palmer & Strobeck, 1986; Palmer, 1994; Klingenberg & McIntyre, 1998) with the among-specimen main effect as a measure of individual variation, the main effect of side as a measure of the directional asymmetry, the side \times specimen interaction as a measure of fluctuating asymmetry (the deviation of each individual's asymmetry from the overall average asymmetry in shape), and the residual term representing measurement error. When assessing the FA via Procrustes ANOVA, we assumed that there is an equal amount of variation around each landmark ("Isotropic model", Goodall, 1991; Klingenberg & McIntyre, 1998). The level of FA (in units of Procrustes distance) for each group was estimated by the *FA10a* index of Palmer and Strobeck (1986) from the asymmetric component of shape variation. To estimate the statistical significance in FA among groups, the Levene's test was performed on the shape FA scores (Jojić et al., 2011). Tukey's HSD (honestly significant difference) post hoc test was performed to test for differences in FA scores between pairs of populations.

Table 1. Procrustes ANOVA for fluctuating asymmetry of the ventral cranium. Sum of squares (SS) and mean squares (MS) values are in the units of Procrustes distance. This analysis assumes that all landmarks have the same amount of isotropic variation. Pop. – Population. Ind. – Individual variation. Ref. – Reflection. Error – Measurement error.

Population	Effect	SS	MS	df	<i>F</i>	<i>p</i> (param.)	% total
Belgrade	Ind.	0.086882	0.000058	1504	10.47	<.0001	87.83
	Ref.	0.000480	0.000015	32	2.72	<.0001	0.49
	Ind. \times Ref.	0.008298	0.000006	1504	5.20	<.0001	8.39
	Error	0.003257	0.000001	3072			
Zeta	Ind.	0.058185	0.000065	896	11.39	<.0001	87.43
	Ref.	0.000715	0.000022	32	3.92	<.0001	1.07
	Ind. \times Ref.	0.005107	0.000006	896	4.16	<.0001	7.67
	Error	0.002540	0.000001	1856			
Malo Starčevo	Ind.	0.075001	0.000063	1184	9.88	<.0001	87.69
	Ref.	0.000657	0.000021	32	3.20	<.0001	0.77
	Ind. \times Ref.	0.007593	0.000006	1184	6.49	<.0001	8.89
	Error	0.002277	0.000001	2304			
Malo Beško	Ind.	0.056123	0.000061	928	9.89	<.0001	84.27
	Ref.	0.001249	0.000039	32	6.38	<.0001	1.88
	Ind. \times Ref.	0.005674	0.000006	928	3.30	<.0001	8.52
	Error	0.003555	0.000002	1920			

Table 2. Tukey's HSD post hoc test for differences in FA scores between populations. Differences are significant at $p < 0.05$.

	Belgrade	Zeta	Malo Starčevo	Malo Beško
Belgrade		0.9951	0.2172	0.8097
Zeta	0.9951		0.4581	0.9386
Malo Starčevo	0.2172	0.4581		0.8237
Malo Beško	0.8097	0.9386	0.8237	

We also employed a non-isotropic model, proposed by Mardia et al. (2000) for structures with object symmetry. It avoids the assumption of equal, independent and isotropic variation of all landmarks. The model is based on a Lawley–Hotteling trace T_g^2 (Seber, 1984) extended to the two-factor MANOVA (Mardia et al., 2000). The mean squares of the individual \times reflection effect were used as the error covariance matrix. For the parametric test, significance levels were computed by an approximation via the F distribution (Klingenberg et al., 2002). This test takes variation of each landmark into account and therefore has higher statistical power, especially for testing directional asymmetry (Klingenberg et al., 2002). Since the number of individuals in datasets from Zeta and Malo Beško was lower than the number of landmarks, we performed the two-factor MANOVA test on the reduced number of landmarks. We excluded four pairs of landmarks: 6 and 23, 10 and 27, 12 and 29, 13 and 30, which were already represented by at least one other landmark (quadrate, basiptyergoid, maxillar and jugal bones).

To test if the developmental basis of FA is distinct from individual variation in each population, we compared covariance matrices of individual variation and individual \times reflection interaction within populations, including diagonal entries of the matrices. If the developmental processes that lead to individual variation also generate fluctuating asymmetry, then the covariance matrices should be similar (Klingenberg & McIntyre, 1998; Klingenberg et al., 2002). Partial covariance matrices were compared using the Mantel matrix correlation test (Mantel, 1967; Cheverud et al., 1989; Sokal & Rohlf, 1995) adapted for geometric morphometrics of structures with object symmetry (Klingenberg & McIntyre, 1998; Klingenberg et al., 2002). The significance of matrix correlations was assessed by the matrix permutation test with 10000 permutations against the null hypothesis of complete independence of the matrices. Only paired landmarks were used for the comparison of symmetric and asymmetric components. To test if both the individual variation and FA are correlated among populations, we did pairwise comparisons of both individual variation and FA covariance matrices among populations (Jojić et al., 2011), using the same matrix comparison method as above.

Landmark covariation patterns were further investigated via principal component analysis (PCA). PCAs display patterns of variation so they can be interpreted biologically, since coefficients of principal components

(PCs) can be displayed directly as joint displacement of landmarks (Klingenberg & McIntyre, 1998). To refine comparison of covariance matrices among populations, we compared individual PCs for each individual variation and FA by calculating an arccosine of the signed inner products between their respective eigenvectors (Cheverud, 1982; Klingenberg & Zimmermann, 1992; Klingenberg & McIntyre, 1998; Debat et al., 2000). A statistical assessment of this angle was achieved by a comparison with angles between pairs of random vectors drawn in the multivariate tangent space. P values were calculated according to the close-form probability formula (Li, 2011) against the null hypothesis of the dissimilarity of vectors (Klingenberg & Marugán-Lobón, 2013). Since we had six pairwise comparisons for each PC eigenvector, we applied a Bonferroni correction.

Procrustes ANOVA, Mantel tests, PCA, multivariate regressions and vector comparisons were performed using the MorphoJ program (Klingenberg, 2011). Standard statistical procedures were performed via the Statistica software package (Statistica for Windows; StatSoft, Inc., Tulsa, OK, USA).

RESULTS

The populations significantly differed in skull size (ANOVA $F=161.41$; $p < 0.0001$), and sexual size dimorphism (ANOVA $F=437.18$; $p < 0.0001$). Differences in skull shape were also significant among populations (ANOVA, Wilks' $\lambda=0.0006$; $p < 0.0001$) and sexes (ANOVA, Wilks' $\lambda=0.0872$; $p < 0.0001$). The allometry of the symmetric shape component was pronounced for both sexes (11% of total variance explained, $p < 0.0001$ for females; 10.9% of total variance explained, $p < 0.0001$ for males). Allometric trajectories of females and males were similar (22.9°, $p < 0.0001$). The allometry of the asymmetric shape component was not statistically different between populations (0.9% explained, $p=0.8265$ for females; 0.9% explained, $p=0.3574$ for males) so we did not make further assessments. Preliminary Levene's test on FA scores showed that, for each population, there is no difference

Table 3. MANOVA test for directional asymmetry and fluctuating asymmetry of the ventral cranium, done on the reduced set of landmarks. The error effect used to test the main effect of reflection is the individual \times reflection interaction. Pop. - Population. Ind. - Individual variation. Ref. - Reflection.

Pop.	Effect.	Pillai tr.	p (param.)
Belgrade	Ref.	0.85	0.0001
	Ind \times Ref	17.3	0.0001
Zeta	Ref.	0.97	0.0173
	Ind \times Ref	16.81	0.0001
Malo Starčevo	Ref.	0.87	0.0044
	Ind \times Ref	17.93	0.0001
Malo Beško	Ref.	0.98	0.0022
	Ind \times Ref	15.45	0.0001

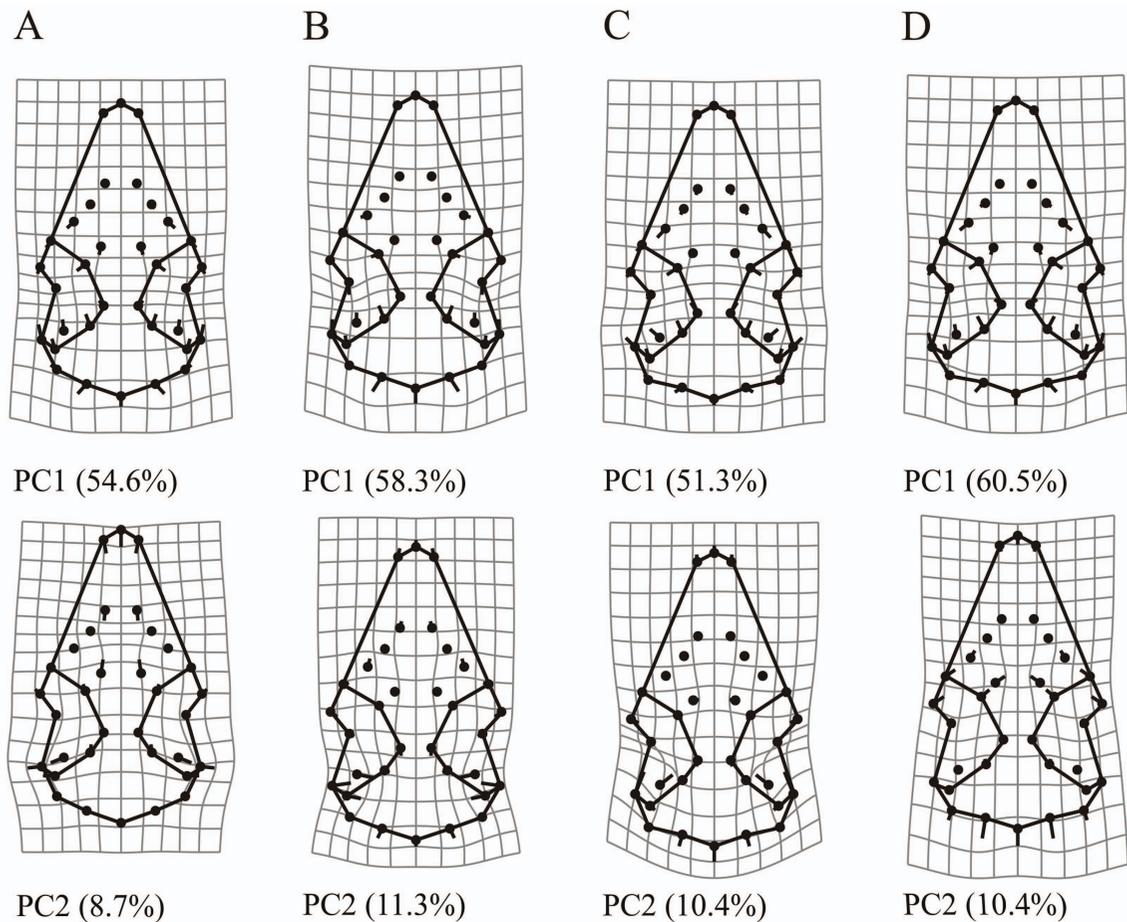


Fig. 2. First two PCs of the symmetric component of shape variation for all of the populations. The lines between landmarks 6, 7, 8, 10, 11, 12, 14 and 15 as well as 23, 24, 25, 27, 28, 29, 31 and 32 define the jaw adductor muscle chambers.

in FA between sexes (ANOVA, $F_{1,46}=0.31$; $p=0.5791$ for Belgrade; $F_{1,27}=0.05$; $p=0.8190$ for Zeta; $F_{1,36}=0.37$, $p=0.5466$ for Malo Starčevo and $F_{1,28}=0.50$; $p=0.4842$ for Malo Beško). The remainder of the analyses were therefore conducted on pooled sexes.

Procrustes ANOVA showed that the main effects of individual variation, directional asymmetry, and FA were highly significant (Table 1). Variation among individuals

accounted for the largest portion of shape variation in all populations. Both directional (Reflection) and fluctuating asymmetry (Individual \times Reflection) were significant in all populations, and FA values exceeded measurement error in all cases. The percentage of shape variation explained by directional asymmetry was smaller than shape variation explained by both individual variation and FA (Table 1).

Table 4. Matrix correlations and the results of the matrix correlation test for the individual variation (Ind) and fluctuating asymmetry (FA), within and among populations.

Comparison	Correlation	p
Ind. vs. FA in Belgrade	0.317	0.0017
Ind. vs. FA in Zeta	0.324	0.0027
Ind. vs. FA in Malo Starčevo	0.317	0.0017
Ind. vs. FA in Malo Beško	0.224	0.0382
Ind. vs. Ind. between Belgrade and Zeta	0.840	<.0001
Ind. vs. Ind. between Belgrade and Malo Starčevo	0.847	<.0001
Ind. vs. Ind. between Belgrade and Malo Beško	0.948	<.0001
Ind. vs. Ind. between Zeta and Malo Starčevo	0.728	<.0001
Ind. vs. Ind. between Zeta and Malo Beško	0.856	<.0001
Ind. vs. Ind. between Malo Starčevo and Malo Beško	0.854	<.0001
FA vs. FA between Belgrade and Zeta	0.639	<.0001
FA vs. FA between Belgrade and Malo Starčevo	0.579	<.0001
FA vs. FA between Belgrade and Malo Beško	0.604	<.0001
FA vs. FA between Zeta and Malo Starčevo	0.545	<.0001
FA vs. FA between Zeta and Malo Beško	0.588	<.0001
FA vs. FA between Malo Starčevo and Malo Beško	0.530	<.0001

FA values were similar in all populations (Belgrade: $FA10a=1.68 \times 10^{-3}$; Zeta: $FA10a=1.66 \times 10^{-3}$; Malo Starčevo: $FA10a=1.86 \times 10^{-3}$; Malo Beško: $FA10a=1.65 \times 10^{-3}$). Levene's test on FA scores showed that there is no statistical difference in FA levels among populations (ANOVA, $F_{3,141}=0.56$; $p=0.646$). The lack of statistically significant differences in FA among populations was

further confirmed by pairwise comparisons using the Tukey HSD test (Table 2). The two-way MANOVA confirmed significant DA (Side) and FA (Individual \times Reflection) in all populations (Table 3).

Matrix correlations between individual variation and FA were significant for all populations (Table 4). Correlations between all pairs of populations were highly

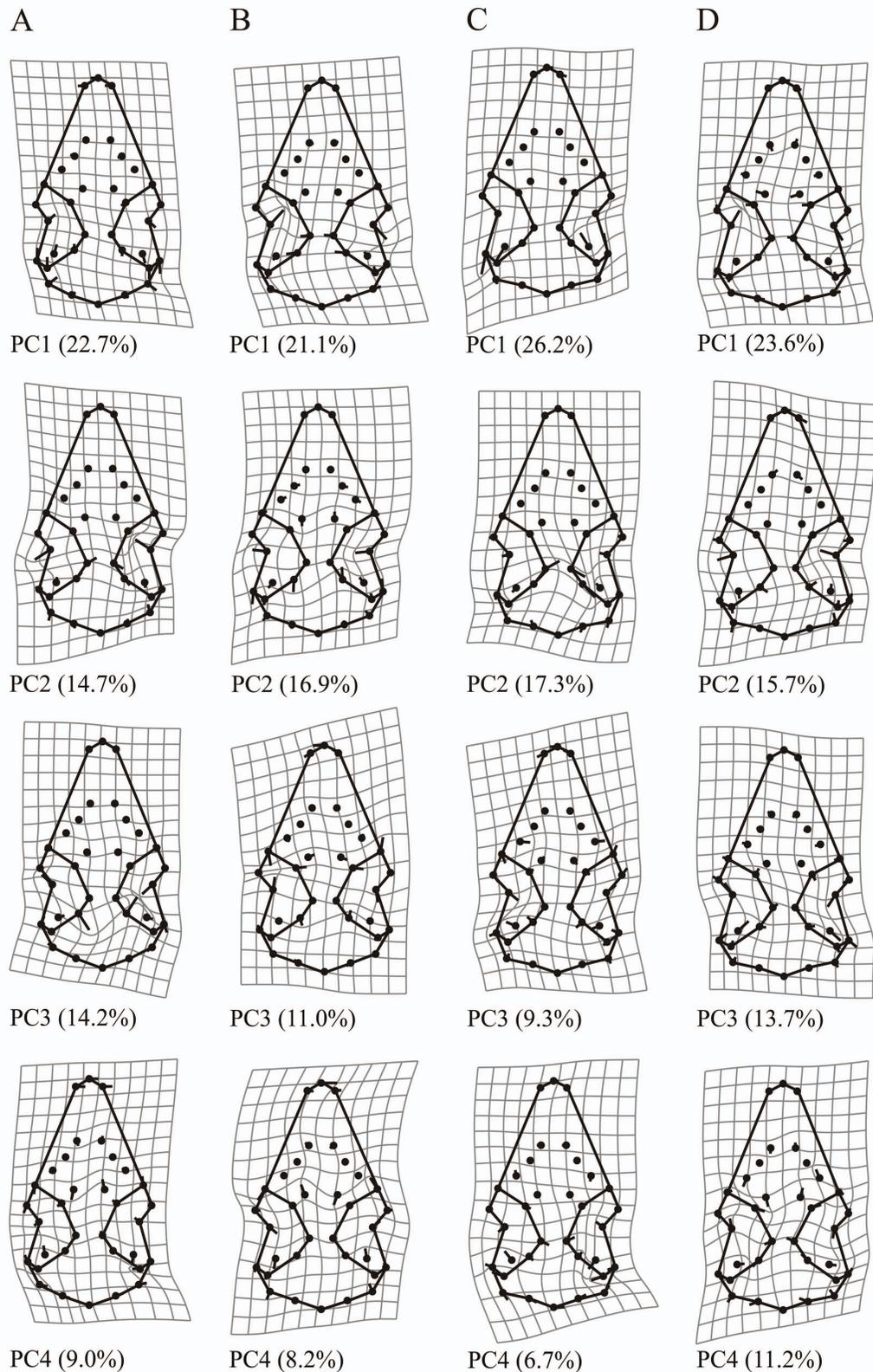


Fig. 3. Patterns of the FA illustrated by the first four PCs, for all of the populations. The lines between landmarks 6, 7, 8, 10, 11, 12, 14 and 15 as well as 23, 24, 25, 27, 28, 29, 31, and 32 define the jaw adductor muscle chambers. A – Belgrade, B – Zeta, C – Malo Starčevo, D – Malo Beško

Table 5. The angles (in degrees) between eigenvectors of PC1 and PC2 for individual variation. *P* values are given after the Bonferroni correction.

Comparison	PC1	<i>p</i>	PC2	<i>p</i>
Belgrade vs. Zeta	25.3	<.00001	52.3	>0.0001
Belgrade vs. Malo Starčevo	25.6	<.00001	58.7	0.0003
Belgrade vs. Malo Beško	11.2	<.00001	77	0.0348
Zeta vs. Malo Starčevo	37.5	<.00001	50.4	>0.0001
Zeta vs. Malo Beško	23.6	<.00001	71.6	0.0123
Malo Starčevo vs. Malo Beško	23.5	<.00001	69.2	0.0072

significant for both individual variation and FA (Table 4). For symmetric shape variation, principal component analyses showed that PC1 alone accounted for more than 50% of the individual variation in all populations (Fig. 2). Variation in FA was more evenly spread across PCs, and the first four PCs explained the highest portion of variation (60.62% in Belgrade, 57.14% in Zeta, 59.34% in Malo Starčevo and 64.16% in Malo Beško). For measurement error, PC1 explained the low level of variation (11.25% for Belgrade, 15.39 for Zeta, 16.92% for Malo Starčevo and 15.87% for Malo Beško), and the variation slowly tapered in subsequent PCs.

For the symmetric shape variation, more positive scores on the PC1 were associated with a larger skull base, and smaller jaw adductor muscle chambers (Fig. 2). The shape differences associated with PC2 included variation in the elongation of the rostrum and general cranium width and length, as well as differences in cranium base and jaw adductor muscle chambers. For the lizards from Belgrade, specimens with positive PC scores have a wider ventral cranium with shorter rostrum and enlarged jaw adductor muscle chambers. In the Zeta and Malo Starčevo populations, the trend was general elongation and narrowing of the ventral cranium, with enlargement of the cranium base, and Malo Beško lizards showed a tendency toward a narrowing of the cranium and shortening of the rostrum, with pronounced enlargement of the cranium base (Fig. 2).

The first PCs were highly correlated among all groups, and the angles between eigenvectors were relatively small. The angles between second PCs had higher values. After Bonferroni correction, the second PCs were also correlated among all groups, albeit only marginally significantly between Malo Beško and Belgrade as well as Malo Beško and Zeta (Table 5).

Differences in fluctuating asymmetry were mostly related to the jaw adductor muscle chamber, especially to the placement of the tip of the jugal, quadrate bones and basiptergoid processes. These patterns were similar for all populations, especially on the PC1 and PC 2 (Fig. 3). However, the PCs were not correlated among all populations – angles of PC1 eigenvectors significantly differed between Belgrade and Zeta and Belgrade and Malo Beško. Angles of PC2 differed between Belgrade and Zeta and Belgrade and Malo Starčevo. PC3 eigenvectors were not correlated between Zeta and Malo Beško. The PC4 eigenvectors were only correlated between Belgrade and Malo Starčevo and Malo Beško and Malo Starčevo (Table 6).

DISCUSSION

Our study on four *P. muralis* populations showed that, despite differences in skull shape among populations and sexes, they are characterised by conserved allometry (Urošević et al., 2014) and FA (present study). Such a result can be explained by adaptive significance and the high level of canalisation of skull shape, which would prevent large fluctuations in FA levels among populations exposed to different types and intensities of stressors (Debat & David, 2001; Jojić et al., 2011). We also found significant DA within each population. DA is common in the animal kingdom (Auffray et al., 1996; Klingenberg & McIntyre, 1998; Tamura et al., 1999; Leamy et al., 2001; Klingenberg et al., 2002; Willmore et al., 2005; Jojić et al., 2011). One of the most important causes of directional asymmetry in skull shape could be cerebral lateralisation associated with behavioural and anatomical asymmetries, and has been previously found in *P. muralis* (Bonati et al., 2008). The strong covariance between

Table 6. The angles (in degrees) between eigenvectors of PC1, PC2, PC3 and PC4 for FA. *p* values are given after the Bonferroni correction. Insignificant *p* values are given in *italic*.

Comparison	PC1	<i>p</i>	PC2	<i>p</i>	PC3	<i>p</i>	PC4	<i>p</i>
Belgrade vs. Zeta	81.254	0.0664	82.852	0.0817	65.674	0.0029	88.473	0.1472
Belgrade vs. Malo Starčevo	51.142	>0.0001	69.565	0.0077	64.354	0.0020	64.267	0.0019
Belgrade vs. Malo Beško	84.228	0.0963	68.501	0.0060	54.855	0.0001	88.527	0.1479
Zeta vs. Malo Starčevo	73.575	0.0185	83.749	0.0911	68.831	0.0065	85.27	0.10809
Zeta vs. Malo Beško	46.33	>0.0001	56.525	0.0002	82.27	0.0759	84.778	0.10249
Malo Starčevo vs. Malo Beško	69.531	0.0077	76.353	0.0310	60.153	0.0005	74.336	0.02149

individual variation and FA in all populations suggests the absence of distinctive developmental mechanisms that lead to FA. Such a pattern can be explained by the interaction between developmental homeostasis and individual fitness (Clarke, 1998; Klingenberg & McIntyre, 1998; Debat et al., 2000). Some dissimilarities in morphological patterns of FA emerged. For instance, the Belgrade population differs from Zeta and Malo Beško on PC1 and from Zeta on PC2, and Malo Starčevo differs from Zeta on PC2. Differences in the morphological pattern of FA between the Belgrade population and Zeta and Malo Beško could be explained by the effect of urban environment on developmental stability (Debat et al., 2000; Lazić et al., 2013), or phenotypic plasticity and differential effects of functional and behavioural traits (habitual asymmetries in the prey handling, territorial or copulatory bites) on symmetric and asymmetric components of morphological variation (Klingenberg et al., 2002).

Shape differences associated with individual variation appear similar to the shape differences associated with sexual dimorphism and allometry - proportional enlargement of the jaw adductor muscle chambers and reduction of the cranium base in larger animals (Ljubisavljević et al., 2010, 2011). The remainder of the observed shape variation could be attributed to the changes in cranium width and length, which is analogous to the shape changes described previously (Urošević et al., 2014). Shape differences related to FA mainly involved aspects of the jaw adductor muscle chamber. Such differences are likely due to differences in adductor muscle size and differences in strain on the surrounding skeletal elements (Herring & Teng, 2000; Curtis et al., 2011). It is possible that skeletal elements subjected to relatively large strains show greater variability than skeletal elements associated with weaker muscles (Wood & Lieberman, 2001). The increased FA in cranial elements associated with jaw musculature could be related to bite side preference (lateralisation) or simply the initial FA in the muscle development (Willmore et al., 2005; Bonati et al., 2008). Our results do not coincide with Lazić et al. (2013), who showed higher FA levels in urban populations of *P. muralis*. However, differences in FA level were detected on univariate meristic traits with low functional relevance, which are expected to have less developmental stability compared to traits with high functional relevance, such as in the skull (Palmer & Strobeck, 1986; Clarke, 1998).

In conclusion, the observed pattern of cranial FA in *P. muralis* from the Central Balkans suggests a high level of canalisation in skull shape. Including more populations from various habitats in different parts of the species range, such as more isolated, old insular populations or those from more heavily polluted habitats (close vicinity of industrial facilities, for instance) could reveal further trends in individual variation and FA of the *P. muralis* skull.

ACKNOWLEDGEMENTS

We would like to thank Antigoni Kaliontzopoulou and

Vincent Debat for constructive reviews. We would also like to thank Vida Jojić, Tanja Vukov, Nataša Tomašević-Kolarov and Miloš Kalezić for helpful suggestions. This study was funded by the Serbian Ministry of Education, Science and Technological Development, grant no. 173043.

REFERENCES

- Aleksić, I. (1997). Age-related variability of morphological and anatomical traits in lizard populations *Podarcis muralis* (Laurenti, 1768) and *Lacerta oxycephala* (Dumeril and Bibron, 1839). Ph.D. Thesis. University of Belgrade: Faculty of Biology. [In Serbian with English summary].
- Aleksić, I., Ivanović, A., Crnobrnja - Isailović, J. & Kalezić, M.L. (2009). Species and sex size/shape differences in the lacertid community (*Podarcis* ssp. and *Archeolacerta* sp.) from the Skadar Lake region (Montenegro). *Italian Journal of Zoology* 76, 43–52.
- Amaral, M.J., Carretero, M.A., Bicho, R.C., Soares, A.M. & Mann, R.M. (2012). The use of a lacertid lizard as a model for reptile ecotoxicology studies: Part 1 – Field demographics and morphology. *Chemosphere* 87, 757–764.
- Arnold, E.N. (1987). Resource partitioning among lacertid lizards in southern Europe. *Journal of Zoology* 1, 739–782.
- Auffray, J.-C., Alibert, P., Renaud, S., Orth, A. & Bonhomme, F. (1996). Fluctuating asymmetry in *Mus musculus* subspecific hybridization: traditional and Procrustes comparative approach. In *Advances in morphometrics*, 275–283. Marcus, L.F., Corti, M., Loy, A., Naylor, G.J.P. & Slice, D.E. (eds). New York: Plenum Press.
- Băncilă, R., van Gelder, I., Rotteveel, E., Loman, J. & Arntzen, J.W. (2010). Fluctuating asymmetry is a function of population isolation in island lizards. *Journal of Zoology* 282, 266–275.
- Bonati, B., Csermely, D. & Romani, R. (2008). Lateralization in the predatory behaviour of the common wall lizard (*Podarcis muralis*). *Behavioural Processes* 79, 171–174.
- Breno, M., Leirs, H. & Van Dongen, S. (2011). No relationship between canalization and developmental stability of the skull in a natural population of *Mastomys natalensis* (Rodentia: Muridae). *Biological Journal of the Linnean Society* 104, 207–216.
- Caughley, G. (1994). Directions in conservation biology. *Journal of Animal Ecology* 63, 215–244.
- Cheverud J.M. (1982). Relationships among ontogenetic, static, and evolutionary allometry. *American Journal of Physical Anthropology* 59, 139–149.
- Cheverud, J.M., Wagner, G.P. & Dow, M.M. (1989). Methods for the comparative analysis of variation patterns. *Systematic Zoology* 38, 201–213.
- Clarke, G.M. (1998). The genetic basis of developmental stability. V. Inter- and intra-individual character variation. *Heredity* 80, 562–567.
- Crnobrnja, J., Džukić, G., Aleksić, I., Vujičić, L. & Avramov, S. (1994). Distribution and genetic variation of the common wall lizard (*Podarcis muralis*, Lacertidae) populations from lake Skadar: Preliminary results. *Bios (Macedonia, Greece)* 2, 275–286.
- Crnobrnja – Isailovic, J., Aleksić, I. & Bejakovic, D. (2005). Fluctuating asymmetry in *Podarcis muralis* populations from Southern Montenegro: detection of environmental

- stress in insular populations. *Amphibia-Reptilia* 26, 149–158.
- Curtis, N., Jones, M.E.H., Shi, J., O'Higgins, P., et al. (2011). Functional relationship between skull form and feeding mechanics in *Sphenodon*, and implications for diapsid skull development. *PLoS ONE* 6, e29804.
- Debat, V. & David, P. (2001). Mapping phenotypes: canalization, plasticity and developmental stability. *Trends in Ecology and Evolution* 16, 555–561.
- Debat, V., Alibert, P., David, P., Paradis, E. & Auffray, J.C. (2000). Independence between developmental stability and canalization in the skull of the house mouse. *Proceedings of the Royal Society of London series B Biological Sciences* 267, 423–430.
- Dingerkus, G. & Uhler, L.D. (1977). Enzyme clearing of alcian blue stained whole small vertebrates for demonstration of cartilage. *Stain Technology* 52, 229–232.
- Ditchkoff, S.S., Saalfeld, S.T. & Gibson, C.J. (2006). Animal behavior in urban ecosystems: Modifications due to human-induced stress. *Urban Ecosystems* 9, 5–12.
- Doyle, W.J., Kelley, C. & Siegel, M.I. (1977). The effects of audiogenic stress on the growth of long bones in the laboratory rat (*Rattus norvegicus*). *Growth* 41, 183–189.
- Drake A.G. & Klingenberg C.P. (2008). The pace of morphological change: historical transformation of skull shape in St. Bernard dogs. *Proceedings of the Royal Society of London Series B Biological Sciences* 275, 71–76.
- Džukić, G. (1977). Medjusobno kompetitivno isključivanje *Lacerta oxycephala* i *Lacerta muralis* na ostrvima Skadarskog jezera. [Mutual competitive exclusion of *Lacerta oxycephala* and *Lacerta muralis* on Skadar Lake islands]. Sadržaji referata, 75–76. Novi Sad: III simpozijum biosistematičara Jugoslavije. [In Serbian].
- Eeva, T., Tanhuanpää, S., Råbergh, C., Airaksinen, S., et al. (2000). Biomarkers and fluctuating asymmetry as indicators of pollution-induced stress in two hole-nesting passerines. *Functional Ecology* 14, 235–243.
- Eldridge, M.D.B., King, J.M., Loupis, A.K., Spencer, P.B.S., et al. (1999). Unprecedented low levels of genetic variation and inbreeding depression in an island population of the black footed rock wallaby. *Conservation Biology* 13, 531–541.
- Frankham, R. (1997). Do island populations have less genetic variation than mainland populations? *Heredity* 78, 311–327.
- Frankham, R. (1998). Inbreeding and extinction: Island populations. *Conservation Biology* 12, 665–675.
- Goodall, C.R. (1991). Procrustes methods in the statistical analysis of shape. *Journal of the Royal Statistical Society B* 53, 285–339.
- Herring, S.W. & Teng, S. (2000). Strain in the braincase and its sutures during function. *American Journal of Physical Anthropology* 112, 575–593.
- Jojić, V., Blagojević, J. & Vujošević, M. (2011). B chromosomes and cranial variability in yellow-necked field mice (*Apodemus flavicollis*). *Journal of Mammalogy* 92, 396–406.
- Kent, J.T. & Mardia, K.V. (2001). Shape, Procrustes tangent projections and bilateral symmetry. *Biometrika* 88, 469–485.
- Klingenberg, C.P. (2011). MorphoJ: an integrated software package for geometric morphometrics. *Molecular Ecology Resources* 11, 353–357.
- Klingenberg C.P. & Zimmermann M. (1992). Static, ontogenetic, and evolutionary allometry: a multivariate comparison in nine species of water striders. *American Naturalist* 140, 601–620.
- Klingenberg, C.P. & McIntyre, G.S. (1998). Geometric morphometrics of developmental instability: analyzing patterns of fluctuating asymmetry with Procrustes methods. *Evolution* 52, 1363–1375.
- Klingenberg, C.P. & Nijhout, H.F. (1999). Genetics of fluctuating asymmetry: a developmental model of developmental instability. *Evolution* 53, 358–375.
- Klingenberg, C.P., & Marugán-Lobón, J. (2013). Evolutionary covariation in geometric morphometric data: analyzing integration, modularity and allometry in a phylogenetic context. *Systematic Biology* 62, 591–610.
- Klingenberg, C.P., Barluenga, M. & Meyer, A. (2002). Shape analysis of symmetric structures: quantifying variation among individuals and asymmetry. *Evolution* 56, 1909–1920.
- Kozlov, M.V., Wilsey, B.J., Koricheva, J. & Haukioja, E. (1996). Fluctuating asymmetry of birch leaves increases under pollution impact. *Journal of Applied Ecology* 33, 1489–1495.
- Lazić, M.M., Kaliontzopoulou, A., Carretero, M.A. & Crnobrnja-Isailović, J. (2013). Lizards from urban areas are more asymmetric: Using fluctuating asymmetry to evaluate environmental disturbance. *PLoS One* 8, e84190.
- Leamy, L.J. & Klingenberg, C.P. (2005). The genetics and evolution of fluctuating asymmetry. *Annual Reviews of Ecology, Evolution and Systematics* 36, 1–21.
- Leamy, L.J., Meagher, S., Taylor, S., Carroll, L. & Potts, W.K. (2001). Size and fluctuating asymmetry of morphometric characters in mice: their associations with inbreeding and the *t*-haplotype. *Evolution* 55, 2333–2341.
- Lens, L., Van Dongen, S., Wilder, C.M., Brooks, T.M. & Matthysen, E. (1999). Fluctuating asymmetry increases with habitat disturbance in seven bird species of a fragmented Afrotropical forest. *Proceedings of the Royal Society B: Biological Sciences* 266, 1241–1246.
- Li, S. (2011). Concise formulas for the area and volume of a hyperspherical cap. *Asian Journal of Mathematics and Statistics* 4, 66–70.
- Ljubisavljević, K., Tome, S., Džukić, G. & Kalezić, M.L. (2005). Morphological differentiation of isolated population of the Italian Wall Lizard (*Podarcis sicula*) of the southeastern Adriatic coast. *Biologia* 60, 189–195.
- Ljubisavljević, K., Urošević, A., Aleksić, I. & Ivanović, A. (2010). Sexual dimorphism of skull shape in lacertid lizard species (*Podarcis* spp., *Dalmatolacerta* sp., *Dinarolacerta* sp.) revealed by geometric morphometrics. *Zoology* 113, 168–174.
- Ljubisavljević, K., Polović, L., Urošević, A. & Ivanović, A. (2011). Patterns of morphological variation in the skull and cephalic scales of the lacertid lizard *Algyroides nigropunctatus*. *Herpetological Journal* 21, 65–72.
- Lucas, L.D. & French, S.S. (2012). Stress-induced tradeoffs in a free-living lizard across a variable landscape: consequences for individuals and populations. *PLoS ONE* 7, e49895.
- Mantel, N. (1967). The detection of disease clustering and a generalized regression approach. *Cancer Research* 27, 209–220.
- Mardia, K.V., Bookstein, F.L. & Moreton, I.J. (2000). Statistical

- assessment of bilateral symmetry of shapes. *Biometrika* 87, 285–300.
- Møller, A.P. (1992). Parasites differentially increase the degree of fluctuating asymmetry in secondary sexual characters. *Journal of Evolutionary Biology* 5, 691–699.
- Møller, A.P. & Swaddle, J.P. (1997). *Asymmetry, developmental stability, and evolution*. Oxford: Oxford University Press.
- Møller, A.P., Sanotra, G.S. & Vestergaard, K.S. (1995). Developmental stability in relation to population density and breed of chickens *Gallus gallus*. *Poultry Science* 74, 1761–1771.
- Møller, A.P., Milinski, M. & Slater, P.J.B. (1998). *Advances in the Study of Behavior: Stress and Behavior*. London: Academic Press.
- Mullin, S.K. & Taylor, P.J. (2002). The effects of parallax on geometric morphometric data. *Computers in Biology and Medicine* 32, 455–464.
- Palmer, A.R. (1994). Fluctuating Asymmetry Analysis: A primer. In *Developmental Instability: Its Origins and Evolutionary Implications*, 335–364. Markow, T.A. (ed). Dordrecht: Kluwer.
- Palmer, A.R. & Strobeck, C. (1986). Fluctuating asymmetry: measurement, analysis, patterns. *Annual Review of Ecology and Systematics* 17, 391–421.
- Parsons, P.A. (1990). Fluctuating asymmetry: an epigenetic measure of stress. *Biological Reviews* 65, 131–145.
- Rettig, J.E., Fuller, R.C., Corbett, A.L. & Getty, T. (1997). Fluctuating asymmetry indicates levels of competition in an even-aged poplar clone. *Oikos* 80, 123–127.
- Rohlf, F.J. (2008). *TpsDig2. Version 2.12*. Stony Brook: Department of Ecology and Evolution. State University of New York. Available from: <<http://life.bio.sunysb.edu/morph/>>.
- Sarre, S. (1996). Habitat fragmentation promotes fluctuating asymmetry but not morphological divergence in two geckos. *Researches on Population Ecology* 38, 57–64.
- Sarre, S., Dearn, J.M. (1991). Morphological variation and fluctuating asymmetry among insular populations of the sleepy lizard, *Trachydosaurus rugosus* Gray (Squamata: Scincidae). *Australian Journal of Zoology* 39, 91–104.
- Savage, A. & Hogarth, P.J. (1999). An analysis of temperature-induced fluctuating asymmetry in *Asellus aquaticus* (Linn.). *Hydrobiologia* 411, 139–143.
- Seber, G.A.F. (1984). *Multivariate observations*. New York: John Wiley and Sons.
- Smith, D.R., Crespi, B.J. & Bookstein, F.L. (1997). Fluctuating asymmetry in the honey bee, *Apis mellifera*: effects of ploidy and hybridization. *Journal of Evolutionary Biology* 10, 551–574.
- Sokal, R.R. & Rohlf, F.J. (1995). *Biometry: the principles and practice of statistics in biological research*. New York: W. H. Freeman.
- Soulé, M.E. (1986). *Conservation biology: The science of scarcity and diversity*. Sunderland: Sinauer Associates.
- Stanković, S. (1976). *Jezero sveta*. [Lakes of the World]. Beograd: Mala biblioteka Srpskog geografskog društva. [In Serbian].
- Swaddle, J.P. & Witter, M.S. (1994). Food, feathers and fluctuating asymmetries. *Proceedings of the Royal Society of London B* 255, 147–152.
- Thornhill, R. (1992). Fluctuating asymmetry, interspecific aggression and male mating tactics in two species of Japanese scorpionflies. *Behavioral ecology and sociobiology* 30, 357–363.
- Tamura, K., Yonei-Tamura, S. & Izpisua Belmonte, J.C. (1999). Molecular basis of left-right asymmetry. *Development Growth & Differentiation* 41, 645–656.
- Tomkins, J.L. & Kotiaho, J.S. (2001). *Fluctuating Asymmetry. Encyclopedia of life sciences*. London: Macmillan Publishers Ltd, Nature Publishing Group. / www.els.net
- Tull, J.C. & Brussard, P.F. (2007). Fluctuating Asymmetry as an Indicator of Environmental Stress from Off-Highway Vehicles. *Journal of Wildlife Management* 71, 1944–1948.
- Urošević, A., Ljubisavljević, K., Jelić, D. & Ivanović, A. (2012). Variation in the cranium shape of wall lizards (*Podarcis* spp.): effects of phylogenetic constraints, allometric constraints and ecology. *Zoology* 115, 207–216.
- Urošević, A., Ljubisavljević, K. & Ivanović, A. (2013). Patterns of cranial ontogeny in lacertid lizards: morphological and allometric disparity. *Journal of Evolutionary Biology* 26, 399–415.
- Urošević, A., Ljubisavljević, K. & Ivanović, A. (2014). Variation in skull size and shape of the Common wall lizard (*Podarcis muralis*): allometric and non-allometric shape changes. *Contributions to Zoology* 83, 67–77.
- Willmore, K.E., Klingenberg, C.P. & Hallgrímsson, B. (2005). The relationship between fluctuating asymmetry and environmental variance in rhesus macaque skulls. *Evolution* 59, 898–909.
- Witter, M.S. & Swaddle, J.P. (1994). Fluctuating asymmetry, competition and dominance. *Proceedings of the Royal Society of London B* 256, 299–303.
- Wood, B. & Lieberman, D.E. (2001). Craniodental variation in *Paranthropus boisei*: a developmental and functional perspective. *American Journal of Physical Anthropology* 116, 13–25.

Accepted: 24 November 2014