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SEXUAL SIZE AND SHAPE DIMORPHISM IN *SALAMANDRA SALAMANDRA* (AMPHIBIA, CAUDATA, SALAMANDRIDAE) FROM THE CENTRAL BALKANS

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Abstract - Sexual size dimorphism is one of the key evolutionary features that has been studied in many organisms. On the other hand, sexual shape dimorphism has not been examined as well despite being as important as size dimorphism. Therefore, we analyzed the sexual size and shape dimorphism (SSSD) of *Salamandra salamandra* from the territory of the central Balkans. In addition, we wanted to reconsider if there is some regularity in the geographical distribution of SSSD in the investigated area. Significant differences in size and shape between the sexes were found for the whole sample and among the analyzed groups. Females were larger than males and had bigger heads, interlimb distances and a parotid gland, while males had bigger tails, forelimbs, hindlimbs, and forefoot and hindfoot length. Our results reveal a strong effect of locality on trait variation. This variation from the general pattern of SSSD is not substantial but still has to be considered.

Key words: SSSD, morphology, fire salamander, Serbia, Montenegro

INTRODUCTION

Sexual dimorphism, i.e. the existence of phenotypic differences between the sexes, is widespread in animals (Andersson, 1994; Fairbairn, 1997; Kupfer, 2007). Sexual size dimorphism, as an aspect of sexual dimorphism, comprises the presence of differences in the mean values of morphometric characters in sexually mature individuals of both sexes. Among amphibians, sexual dimorphism is most apparent in differences in body size. Females are generally larger than males as is the case in about 90% of frogs and 61% of tailed amphibians (Shine, 1979). Differences in body size between males and females were found in many tailed amphibians (Rebelo and Caetano, 1995; Cvetković et al., 1996; Kalezić et al., 2000; Luiseli et al., 2001; Miaud et al., 2001; Olgun et al., 2001; Ivanović et al., 2008; Üzüm, 2009). Generally, females are larger than males (Cvetković et al., 1996; Dandova et al., 1998; Luiseli et al., 2001; Olgun et al., 2001; Miaud et al., 2001; Romano et al., 2009; Sharrifi et al., 2012). However, there are some cases, where males are larger than females (Kalezić et al., 2000; Fontenot and Seigel, 2008).

Little attention is given to the differences in body shape between the sexes in comparative studies of Urodelas (but see Ivanović et al., 2008; Romano et al., 2009; Hasumi, 2010), though there is no reason to believe that shape dimorphism is any less important than size dimorphism.

The central Balkans are inhabited by only one species of salamander, the fire salamander *Salamandra salamandra* (Linnaeus, 1758). Although this is the most common type of salamander, not only in the Balkans but also in Europe, differences in body size and shape between males and females have not been explored much. Male fire salamanders do not have specific secondary sexual characteristics that would enable them to be easily distinguished from females. They do not have the crest that characterizes other representatives of tailed amphibians during the period of breeding. In addition, there are no differences in the qualitative traits between males and females; therefore, it is impossible to determine the sex based on the type and arrangement of spots on the dorsal or ventral side of the body (Džukić, 1993).

However, although the differences between the sexes are small and hardly noticeable, they do exist. Females are generally larger than males, and this is particularly evident in the period of gestation. On the other hand, males have a more slender body, long tail and long limbs. In addition, during the period of breeding, the males have a more swollen cloaca (Degani, 1986; Rebelo and Caetano, 1995; Griffiths, 1996; Kuzmin, 2000).

Since the differences in body size between the males and females of only two populations of fire salamanders in the central Balkans were found (Kalezić et al., 2000), and there are no studies comparing shape between the sexes, we believe that there are good reasons to study the sexual size and shape dimorphism (SSSD) in the same species, but over a much larger area, including much larger populations. We analyzed the SSSD of the entire sample from the territory of the central Balkans, and within the individual, geographically well-defined groups. Our aim was to test the potential presence of gender differences and try to understand the nature of these differences. We also wanted to reconsider if there is some regularity in the geographical distribution of SSSD in the investigated area.

MATERIALS AND METHODS

Samples

We examined the specimens of fire salamander deposited in the herpetological collections of the University of Priština (temporarily in Kosovska Mitrovica), Faculty of Science and Mathematics, Biology Department and from the Institute for Biological Research, University of Belgrade. In total, 370 ethanol-preserved specimens were analyzed: 140 males and 230 females. The specimens were from 47 localities and pooled into 11 groups according to their geographical origin (see Appendix). The geographical groups generally correspond to phyto- and zoogeographic characterization of the former Yugoslavia (Stevanović, 1992). The location of the fire salamander population samples and populations' grouping is represented in Fig. 1.

Studied characters

Morphometric analysis was done on 17 traits that determine the size and shape of the body and head of the fire salamanders. Measurements were taken only on sexually mature individuals. Reproductively mature males and females were identified on dissection and gonad survey basis. The measured traits were: L – total length, Lsd – length from the snout to the anterior edge of cloaca basis, Lsv – snout-vent length (from the snout to the posterior edge of the cloaca basis), Tl - tail length, Lc – head length, Ltc – head width, Ac – head height, D – interlimb distance, Lpa – forelimb length, Lpp – hindlimb length, Dn – distance between exterior nostrils, Do – eye diameter, Spp – minimal distance between orbits, Lpr – parotid gland length, A – forefoot length (measured from the base of foot to the end of 3rd toe), P – hindfoot length (measured from the base of foot to the end of the 4th toe), Lm - jaw length (measured from the snout to the corner of the mouth). All measurements were taken with a digital caliper to a precision of 0.01 mm by the same person (N.L.).

Statistical analyses

To reduce the impact of overall size, Mosimann's approach of obtaining shape variables was used (Darroch and Mosimann, 1985). This adjustment removed isometric size but not size-related (allometric) shape. The geometric mean (GM) of all traits per individual was used as a measure of size for every specimen. In

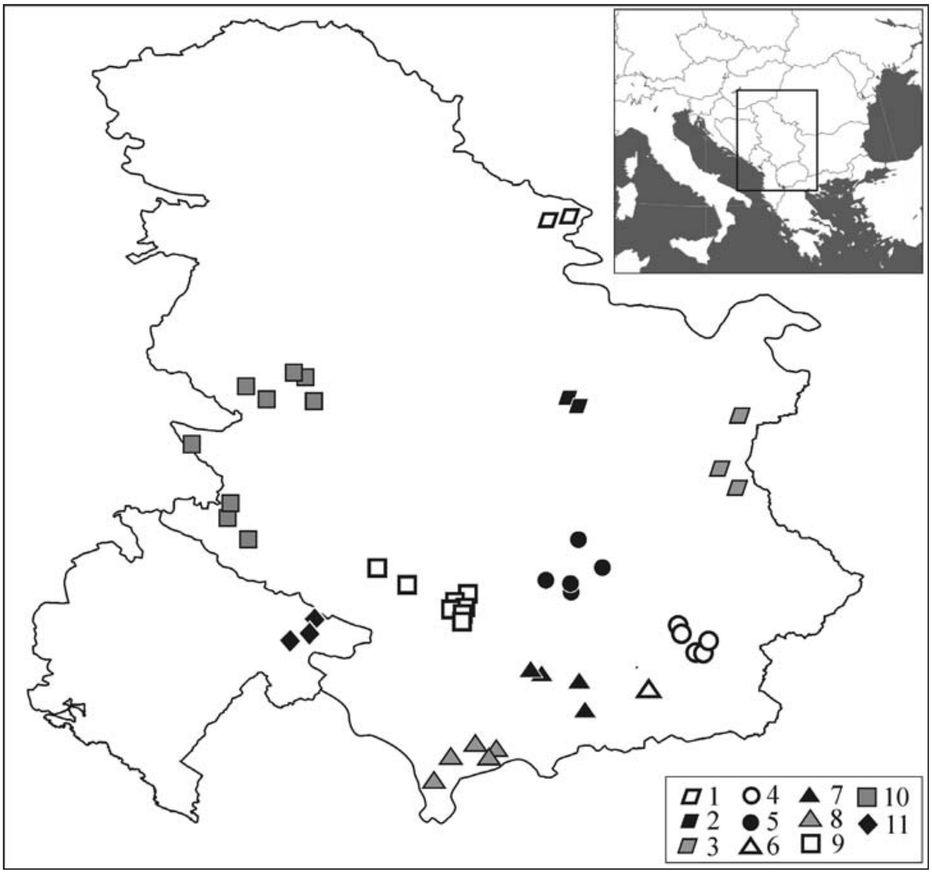

Fig. 1. Location of *S. salamandra* population samples and population grouping (see Appendix).

order to estimate the differences in size and shape between the sexes as well as among the groups, we performed two-way ANOVA and MANOVA tests with sex and group as sources of variability. A more detailed analysis of sexual dimorphism was performed by ANOVA on the total sample as well as for every analyzed group in order to obtain a possible correlation between SSSD and geographical area. All statistical analyses were performed using the computer package Statistica® (STATISTICA for Windows. StatSoft, Inc., Tulsa, OK), considering P <0.05 as the level for significance.

RESULTS

Significant variations between the sexes in shape dimorphism (MANOVA, Wilks' lambda = 0.56, df1 = 17, df2 = 332, P < 0.0001) and the groups (MANOVA, Wilks' lambda = 0.21, df1 = 170, df2 = 2900, P < 0.0001) were found. In addition, a difference in size between the sexes was found on the whole sample (2-way ANOVA, F_1= 7.83, P = 0.005) as well as among the groups (2-way ANOVA, F_{10} = 10.50, P <0.0001), but interaction between sex and group was not found (sex*group: F_{10} = 0.92, P = 0.514).

The analysis of variance on the whole sample showed that sexes differed in 10 of 17 traits: length from the snout to the anterior edge of cloaca basis (Lsd), tail length (Tl), head width (Ltc), head height (Ac), interlimb distance (D), forelimb length (Lpa), hindlimb length (Lpp), parotid gland length (Lpr), forefoot length (A) and hind foot length (P) (Table 1). For Lsd, Ltc, Ac, D and Lpr, females were statis-

Table 1. Size (GM) and shape differences (all other morphometric traits) between the sexes of *S. salamandra*. Abbreviations of traits are given in "Materials and Methods". Bold – statistically significant *P* values ($P < 0.05$).

Traits	F	*P*
GM	20.56	**0.0001**
L	1.03	0.3097
Lsd	9.67	**0.0020**
Lsv	0.73	0.3937
Tl	21.55	**0.0001**
Lc	0.26	0.6071
Ltc	9.36	**0.0024**
Ac	27.03	**0.0001**
D	19.45	**0.0001**
Lpa	60.22	**0.0001**
Lpp	34.15	**0.0001**
Dn	0.08	0.7735
Do	1.67	0.1975
Spp	0.48	0.4891
Lpr	7.31	**0.0072**
A	18.74	**0.0001**
P	11.60	**0.0007**
Lm	0.29	0.5905

Table 2. Descriptive statistics of morphometric traits examined for *S. salamandra*. Std.- Standard Deviation. Abbreviations of traits are given in "Materials and Methods".

	Females (N=230)			Males (N=140)		
Traits	Mean	±	Std.	Mean	±	Std.
GM	25.86		1.53	25.05		1.88
L	179.31		11.38	174.70		12.94
Lsd	98.68		6.93	93.29		7.12
Lsv	109.00		7.38	104.82		8.07
Tl	80.51		7.22	81.28		7.51
Lc	23.47		2.09	22.82		2.11
Ltc	19.75		1.27	18.72		1.25
Ac	12.84		1.86	11.52		1.81
D	53.91		6.56	49.29		6.08
Lpa	30.28		2.35	31.23		2.89
Lpp	34.50		2.15	34.83		3.05
Dn	6.44		0.49	6.21		0.60
Do	6.89		0.62	6.59		0.73
Spp	9.72		6.62	9.03		0.83
Lpr	13.78		1.16	13.02		1.17
A	13.31		1.31	13.45		1.53
P	16.41		1.39	16.37		1.69
Lm	16.47		1.26	16.01		1.30

tically larger than males, while in other traits males were bigger. Means and standard deviations of analyzed traits on GM and non-transformed measures are presented in Table 2.

Results from the analyses of variance of within-group differences between the sexes showed a similar pattern of SSD in the analyzed traits. In groups 5 and 7 no significant differences between females and males were found. In the other groups, there were some statistically significant differences in traits, but without any regularity among the groups. All traits followed the general pattern of differences between females and males as in the whole sample. In group 1 males were larger than females in Lpa ($F_{1,35} = 4.34$, $P = 0.044$). In group 2 males were larger in Tl ($F_{1,30} = 4.31$, $P = 0.046$), Lpa ($F_{1,30} = 13.22$, $P = 0.001$), Lpp ($F_{1,30} = 4.32$, $P = 0.046$) and A ($F_{1,30} = 5.09$, $P = 0.031$). Males from group 3 were larger in Do ($F_{1,17} = 4.51$, $P = 0.049$). In group 4 females were larger in Lsd ($F_{1,36} = 6.06$, $P = 0.019$), Lsv ($F_{1,36} = 4.25$, $P = 0.047$), Ac ($F_{1,36} = 5.54$, $P = 0.024$) and males were larger in Lpa ($F_{1,33} = 13.44$, $P < 0.001$), Lpp ($F_{1,36} = 5.88$, $P = 0.020$), A ($F_{1,36} = 5.79$, $P = 0.021$) and P ($F_{1,36} = 8.72$, $P = 0.005$). In group 6 females were larger in interlimb distance (D) ($F_{1,23} = 15.96$, $P < 0.001$), but males were larger in Tl ($F_{1,23} = 5.16$, $P = 0.032$), Lpa ($F_{1,23} = 6.70$, $P = 0.016$), Lpp ($F_{1,23} = 7.22$, $P = 0.013$) and A ($F_{1,23} = 4.52$, $P = 0.044$). In group 8 males were larger in Lpa ($F_{1,33} = 10.10$, $P = 0.003$), but females were larger in Dn ($F_{1,33} = 4.79$, $P = 0.036$). Group 9 had larger females in GM ($F_{1,49} = 8.23$, $P = 0.006$), Ac ($F_{1,49} =$

7.46, P = 0.009) and larger males in Lpa ($F_{1,49}$ = 5.83, P = 0.019), A ($F_{1,49}$ = 6.41, P = 0.014) and P ($F_{1,49}$ = 6.10, P = 0.017). In group 10 females were larger in GM ($F_{1,49}$ = 5.69, P = 0.022) and D ($F_{1,35}$ = 13.78, P < 0.001) while males were larger in Lpp ($F_{1,35}$ = 7.28, P = 0.011), Dn ($F_{1,35}$ = 4.42, P = 0.043), Do ($F_{1,35}$ = 5.36, P = 0.026) and Spp ($F_{1,35}$ = 9.26, P = 0.004). In group 11 females were larger in Lc ($F_{1,25}$ = 5.18, P = 0.032), while males were larger in P ($F_{1,25}$ = 5.03, P = 0.034).

DISCUSSION

Sexual size dimorphism is under a strong influence of genetic and environmental factors and shaped by natural and sexual selection. The degree and direction of sexual size dimorphism often is a consequence of different selective regimes acting separately on males and females. In urodeles, females could be favored to be the larger sex by fecundity selection, which is reflected in the positive correlation between reproductive output (such as clutch size and egg size) and female size (Wells, 2007). Males could be favored as the larger sex by sexual selection, which is reflected in the positive correlation between male-to-male combat successes and male size (Shine, 1979). Bigger males are also favored because of the female choice of larger males (Halliday and Verrell, 1986). Sexual size dimorphism is female biased in ectotherms, with some exceptions (e.g. Kupfer, 2009). Our results confirm the presence of sexual size dimorphism in the fire salamander and that females are statistically the larger sex. In addition, in groups with statistically significant sexual size dimorphism females were larger.

So far, comparative studies have mostly focused on size dimorphism then on sexual shape dimorphism although shape dimorphism is as important as size. Sexual shape dimorphism could be the consequence of different allometric patterns between the sexes (Butler and Losos, 2002). Our results confirm the presence of sexual shape dimorphism and therefore an allometric pattern in intersex variation in the fire salamander. The main results are that females have longer interlimb distance and head width, and males have longer legs. This is consistent with the assumption that larger the interlimb distance in females is an adaptation to provide room for eggs (e.g. Shine, 1979). Longer legs in males could be beneficial in courtship performance, where individuals with longer legs have greater reproductive success. The difference in head shape between fire salamander males and females could be a consequence of different feeding strategies along a niche divergence process (Andersson, 1994).

In the Balkans, the existing studies revealed that sexual dimorphism exists with males being bigger than females (e.g. Kalezić et al., 2000). Our results are not consistent with previous results for the fire salamander in this region, but are concordant with the general pattern of sexual size dimorphism for salamanders (Shine, 1979). For sexual shape dimorphism, the key factor in the differences between the sexes may be a consequence of differences in some life-history traits.

In addition, our results reveal the strong effect of group (localities) on trait variation. In other words, an estimation of which traits are sexually dimorphic can vary by group. This variation from the general pattern of SSSD is not that substantial but still has to be considered. However, the actual causes of sexual shape differences between fire salamander males and females still have to be elucidated.

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APPENDIX

Group 1 (Vršački breg): Vršački breg (45° 08' N, 21° 25' E, 28 males, 6 females), Vršac (45° 06' N, 21° 18' E, 0 + 3).

Group 2 (Despotovac - Plažane): Despotovac (44° 05' N, 21° 26' E, 5 + 13), Plažane (44° 08' N, 21° 24' E, 7 + 7).

Group 3 (East Serbia): Miroč (44° 38' N, 22° 19' E, 3 + 8), Gornja Bela Reka (43° 46' N, 22° 12' E, 4 + 3), Jakovac (43° 39' N, 22° 18' E, 0 + 1).

Group 4 (Vlasina - Kukavica): Predejanska reka (42° 50' N, 22° 10' E, 4 + 23), Džepska reka (42° 47' N, 22º 07’ E, 0 + 1), Ljutež (42º 47’ N, 22º 08’ E, 0 + 1), Lipova čuka (42º 55’ N, 22º 00’ E, 1 + 3), Bara (42º 52’ N, 22º 01’ E, 5 + 0).

Group 5 (Jastrebac Group): Veliki Jastrebac (43º 23’ N, 21º 27’ E, 8 + 12), Pljakovo (43º 10’ N, 21º 17’ E, 1 + 0), Prokuplje (43º 14’ N, 21º 35’ E, 1 + 1), Bresnik (43º 09’ N, 21º 25’ E, 1 + 0), Tovrljane (43º 07’ N, 21º 25’ E, 1 + 0).

Group 6 (Čestelin): Čestelin (42º 35’ N, 21º 49’ E, 10 + 15).

Group 7 (Central parts of Kosovo and Metochia): Priština (42º 40’ N, 21º 10’ E, 0 + 2), Grmija (42º 39’ N, 21º 14’ E, 0 + 5), Novo Brdo (42º 37’ N, 21º 26’ E, 8 + 23), Gnjilane (42º 27’ N, 21º 28’ E, 3 + 3).

Group 8 (Šar - planina Group): Sevce (42º 12’ N, 20º 57’ E, 7 + 5), Vrbeštica (42º 14’ N, 20º 58’ E, 4 + 15), Prizren (42º 13’ N, 20º 44’ E, 1 + 0), Sredska (42º 16’ N, 20º 52’ E, 1 + 0), Dragaš (42º 04’ N, 20º 39’ E, 0 + 2).

Group 9 (Banjska - Novi Pazar): Banjska (42º 58’ N, 20º 46’ E, 2 + 0), Banjski Suvi Do (42º 59’ N, 20º 46’ E, 4 + 2), Bresnica (42º 58’ N, 20º 48’ E, 4 + 5), Banjska Reka (42º 59’ N, 20º 45’ E, 0 + 8), Rudine (43º 02’ N, 20º 45’ E, 0 + 7), Novi Pazar (43º 08’ N, 20º 30’ E, 1 + 1), Lukare (43º 04’ N, 22º 27’ E, 6 + 9), Goševo (43º 13’ N, 20º 20’ E, 0 + 2).

Group 10 (Western Serbia): Priboj (43º 34’ N, 19º 32’ E, 2 + 1), Pribojska Goleša (43º 30’ N, 19º 31’ E, 0 + 4), Prijepolje (43º 22’ N, 19º 38’ E, 0 + 4), Petnica (44º 15’ N, 19º 56’ E, 2 + 1), Valjevo (44º 16’ N, 19º 53’ E, 1 + 0), Maljen (44º 07’ N, 20º 00’ E, 1 + 0), Povlen (44º 08’ N, 19º 45’ E, 1 + 0), Medvednik (44º 12’ N, 19º 38’ E, 0 + 1), Tara (43º 53’ N, 19º 20’ E, 1 + 17).

Group 11 (Montenegro): Berane (42º 50’ N, 19º 52’ E, 3 + 4), Petnjica (42º 54’ N, 19º 57’ E, 0 + 3), Dobrodole (42º 57’ N, 19º 59’ E, 8 + 9.