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Source: Folia Zoologica, 66(1) : 11-21

Published By: Institute of Vertebrate Biology, Czech Academy of Sciences

URL: https://doi.org/10.25225/fozo.v66.i1.a4.2017

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Folia Zool. – 66 (1): 11–21 (2017)

Sexual dimorphism in size and shape of traits related to locomotion in nine anuran species from Serbia and Montenegro

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Received 19 October 2016; Accepted 20 February 2017

Abstract. Investigation of sexual dimorphism patterns give us insight in how organisms adapt to fulfill their roles (reproductive, ecological, social) and enhance fitness. We examined sexual size and shape dimorphism in traits related to locomotion of nine anuran species from Serbia and Montenegro (*Hyla arborea*, *Bombina variegata*, *Bufotes viridis*, *Rana temporaria*, *R. graeca*, *R. dalmatina*, *Pelophylax* kl. *esculentus*, *Pelobates fuscus* and *P. syriacus*). Sexual size dimorphism in studied species ranges from female and male-biased, to absence of dimorphism. Sexual shape dimorphism was most pronounced in the forelimbs while the differences were small or did not exist in the hindlimbs and sacral traits. Males of most species were characterized by longer proximal parts (humerus and radioulna) and shorter distal forelimb parts, while the opposite pattern was observed in females. Also, some cases of significant intersexual shape differences are size-dependent. This indicates that these male traits are under stronger selective pressures to increase relative to body size, which may allow them to outcompete other males. Different types of behaviour can indirectly shape the limb morphology and lead to differences between the sexes. More data on the ecology and natural history of these animals are needed to explore the factors underlying the observed sex differences.

Key words: Amphibia, intersexual difference, forelimb, hindlimb, sacral traits

Introduction

Sexual dimorphism as a differentiation of adult males and females has been explained mainly as a consequence of sexual and natural selection (Shine 1989, Andersson 1994, Fairbairn 1997). Under these selective pressures, males and females often diverge in a variety of traits, such as body size and shape, colour, ornaments, and physiological characteristics (Shine 1979, Halliday & Verrell 1986, Kupfer 2007). Sexual selection leads to secondary sexual characteristics in anuran males such as bright colour, larger limbs, oral tusks and spines, while natural selection reduces competition between sexes through ecological adaptations such as niche segregation, difference in ontogeny and predator-prey interactions (Carothers 1984, Bell & Zamudio 2012).

Examination of sexual dimorphism patterns allowed us to increase knowledge of how organisms adapt to fulfill their reproductive, ecological and social roles and enhance their fitness (Fairbairn 1997). Most studies that investigate sexual dimorphism in frogs are dedicated to differences in overall body size (Shine 1979, Halliday & Verrell 1986, Monnet & Cherry 2002, Nali et al. 2014, Liao et al. 2015). In 90 % of anuran species females are the larger sex (Shine 1979). In species with male combat, selection favour larger males due to success in intrasexual struggles (Shine 1979, Wells & Schwartz 2007). Even though females are substantially larger in body size, males may often exceed them in other body dimensions (Lee 2001).

Little is known about possible intersexual differences in body parts related to locomotion. Nevertheless, a few literature data noted sex differences for limb's traits. In some species males have longer humerus, radioulna or total forelimbs (Lee 2001, Di Cerbo & Biancardi 2012, Arantes et al. 2015). Longer and robust forelimbs allow males to retain a firm grip on the female in amplexus (Howard & Kluge 1985). Males can also have longer hindlimbs (Zug 1978, Herrel et al. 2012), and/or their elements, such as femur, tibiofibula and foot (Radojčić et al. 2002, Kraus 2008, Streicher et al. 2012, Hudson et al. 2016).

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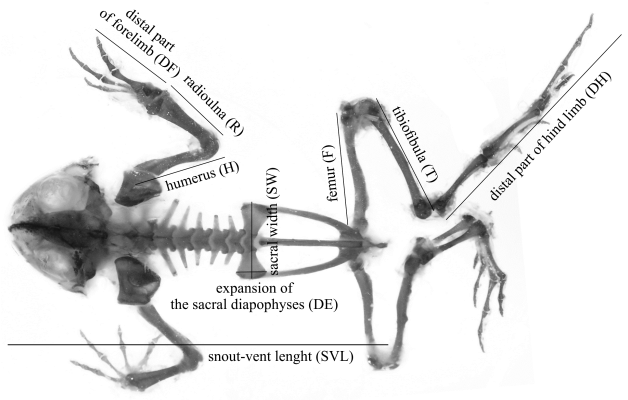


Fig. 1. Limb and sacral measurements used in this study; forelimb elements: humerus (H), radioulna (R), distal part of forelimb (DF), hindlimb elements: femur (F), tibiofibula (T), distal part of hindlimb (DH) and snout-vent-length (SVL). Forelimb length (FL) and hindlimb length (HL) were calculated as sum of their segments.

This has been often linked with the ability of males to eliminate competition because robust hindlimbs allow amplectant males to expel rivals through kicking (Wells 1979).

On the other hand, in rare cases females can exceed males in some traits, proximal and distal parts of hindlimbs (Ponssa et al. 2011, Quiroga et al. 2015), pelvic traits (Blain et al. 2015) and trunk length (Kamath & Sreekar 2016). There are several causes for this form of sexual dimorphism and main of those are related to fecundity or/and reproductive behaviour of females (Nali et al. 2014, Quiroga et al. 2015).

Significant amount of literature on sexual dimorphism in anuran limbs is dealing with the investigation of muscles mass and size, wherein males have more robust muscles of fore- and hindlimbs in comparison to females (Kirby 1983, Yekta & Blackburn 1992, Lee 2001, Lee & Corrales 2002, Clark & Peters 2006, Navas & James 2007, Liao et al. 2012, Liao & Chen 2012).

In anurans, limbs are primary involved in locomotion, hindlimbs directly in generating jumping force, while forelimbs in controlling landing and decreasing impact force (Nauwelaerts & Aerts 2006). Limbs also have a variety of other important functions during mating, prey capture and manipulation during feeding and skin shedding (Anderson 1994, Duellman & Trueb 1994, Valdez & Nishikawa 1997). All these functions provide opportunity for the action of selective forces on limb size and shape that could be operating on the sexes in the same or opposite directions, and generate different patterns of limb sexual dimorphism. In this context, we aim to determine intersexual differences in body size and shape of traits related to locomotion and existence of common pattern of sexual dimorphism in nine anuran species: *Hyla arborea*, *Bombina variegata*, *Bufotes viridis*, *Rana temporaria*, *R. graeca*, *R. dalmatina*, *Pelophylax* kl. *esculentus*, *Pelobates fuscus* and *P. syriacus* from Serbia and Montenegro. Sexual dimorphism regarding differences in body parts related to locomotion has not been particularly investigated, hence the present paper aims to contribute to better understanding of sexual variation in anurans.

Material and Methods

We studied a total of 127 adult males and 91 adult females belonging to five families and nine anuran species (Table 1). All analyzed species originate from Serbia and Montenegro to minimize the impact of local environmental conditions on phenotypic change through changes in life-history traits (Enriquez-Urzelai et al. 2015 and references there in). The sexes of individuals were determined by inspection of gonads. All individuals were from batrachological collection of the Institute for Biological Research "Siniša Stanković", Belgrade, Serbia (Table S3).

Data

All the samples were preserved in 75 % ethanol; skeleton was cleared with trypsin and potassium hydroxide (KOH), and differentially stained, using Alizarin Red S for bone, and Alcyan Blue for cartilage, and preserved in glycerol (Dingerkus & Uhler 1977). All measurements were taken on the right side of the body with a digital caliper (precision 0.01mm). We analyzed body size and all three skeletal limbs segments: the stylopod (humerus, femur), zeugopod (radioulna, tibiofibula) and autopod (distal part of fore- and hindlimb, comprises of carpal, tarsals, metapodials and phalanges). Measures were scored as: snout-vent-length (SVL, distance from top of the head to the posterior edge of the cloaca basis); forelimb traits: humerus (H), radioulna (R), distal part of forelimb (DF, distance from radiocarpal joint to the tip of third finger); hindlimb traits: femur (F), tibiofibula (T), distal part of hindlimb (DH, distance from tibiotarsal joint to the tip of fourth finger); sacral traits: sacral width (SW), and sacral diapophyseal expansion (DE). Total forelimb (FL) and hindlimb length (HL) were calculated as sum of their segments (Fig. 1). The mean values of the raw data are given in the supplementary (Tables S1, S2).

Statistical analyses

All variables were log-transformed before analyses to ensure normality (Kolmogorov-Smirnov test, $p < 0.01$). Data from all nine anuran species were always treated separately. One-way analysis of variance

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Table 1. Family, locality, number of sexes and total number for each species; M – males, F – females, N – number of individuals, RS – Republic of Serbia, MN – Montenegro.

Species	Locality	Sex	n
Pelobatidae			
Pelobates fuscus	Deliblatska peščara, Hrastovača, RS	M	16
		F	12
Pelobates syriacus	Deliblatska peščara, Đurica, RS	M	7
		F	14
Hylidae			
Hyla arborea	Vir Pazar, MN	M	19
		F	11
Bombinatoridae			
Bombina variegata	Prohor pčinjski, RS	M	18
		F	12
Bufonidae			
Bufotes viridis	Fruška Gora, RS	M	10
		F	8
Ranidae			
Pelophylax kl. *esculentus*	Zaječar, RS	M	17
		F	8
Rana graeca	River Gornja Trešnjica, RS	M	12
		F	7
Rana temporaria	Šara Mountain, RS	M	13
		F	9
Rana dalmatina	Vražja pond, RS	M	15
		F	10

(ANOVA) was applied to investigate sex differences in traits related to locomotion and to establish absolute size patterns.

Additionally, in order to separate size from shape we followed methodology of Kaliontzopoulou et al. (2010) and calculated an isometric size (SIZE) of each individual by projecting all log-transformed linear measurements on an isometric vector. We then regressed each linear trait on size and obtained residuals, which were used as size-corrected traits to represent shape variation (Kaliontzopoulou et al. 2010).

To investigate whether the significant shape differences between the sexes are size-dependent, we regressed shape variables on SIZE using the homogeneity of slope test, with shape variables as dependent variables, sex as factor and SIZE as a covariate. The null hypothesis states that shape is isometric, i.e. that it does not change in relation to size. A significant sex × SIZE interaction would indicate that changes in size-dependent shape differ between sexes. In cases of multiple groups comparisons Bonferroni correction was done. The level for statistical significance was defined as $p < 0.05$.

The statistical package STATISTICA 10.0 and R language and environment for statistical computing were used for data analysis (StatSoft Inc. 2010, R Development Core Team 2013). We also employed modified versions of the R-scripts for size-corrected variables provided by Baur & Leuenberger (2011, see "Supplementary material").

Results

Size

The results of ANOVA test showed significant sex differences in SIZE for five species (Table 2). For, *H. arborea*, *R. dalmatina* and *P. fuscus* female-biased sexual size dimorphism was found, while in *B. variegata*, and *P.* kl. *esculentus* males were larger. No significant differences in SIZE were observed for *B. viridis*, *R. temporaria*, *R. graeca* and *P. syriacus* (Table

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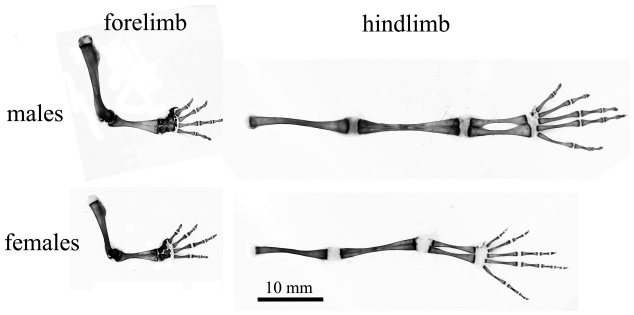


Fig. 2. Fore- and hindlimb of male and female of *Bombina variegata*.

2). As we recognized three size-related groups: species with larger females, species with larger males and species without sexual SIZE dimorphism, the results of absolute and size corrected (shape) variation in traits related to locomotion were presented according to this grouping. Descriptive statistics for absolute and size-corrected traits can be seen in Tables 2-5.

Species with larger females

1) Absolute size variation – *Hyla arborea* females had higher values for forelimb traits H, R, FL, hindlimb

Table 2. Descriptive statistics of isometric size (SIZE) and absolute forelimb traits (log – transformed) for males (M) and females (F) of examined anuran species. Values are the mean ± standard error with Bonferroni corrected *p* – significance value of ANOVA results (bold – statistically significant). H – humerus, R – radioulna, DF – distal forelimb part, FL – forelimb length.

		isosize		H		R		DF		FL	
Species	Sex	mean (SE)	*p*	mean (SE)	*p*	mean (SE)	*p*	mean (SE)	*p*	mean (SE)	*p*
Pelobates fuscus	M	–0.080 (0.008)	**0.026**	1.125 (0.006)	0.342	0.882 (0.005)	1.000	1.126 (0.005)	**0.006**	1.536 (0.004)	**0.037**
	F	–0.005 (–0.024)		1.149 (0.011)		0.896 (0.017)		1.170 (0.010)		1.566 (0.010)	
Pelobates syriacus	M	0.019 (0.054)	0.923	1.183 (0.028)	1.000	0.918 (0.027)	1.000	1.177 (0.026)	0.910	1.586 (0.027)	0.879
	F	0.103 (0.040)		1.215 (0.019)		0.943 (0.017)		1.205 (0.018)		1.615 (0.018)	
Hyla arborea	M	–0.284 (0.016)	**0.013**	1.029 (0.007)	**0.018**	0.807 (0.008)	**0.002**	1.113 (0.008)	0.063	1.478 (0.007)	**0.009**
	F	–0.177 (0.024)		1.073 (0.010)		0.868 (0.009)		1.156 (0.012)		1.526 (0.010)	
Bombina variegata	M	–0.291 (0.016)	**0.009**	1.046 (0.013)	**0.001**	0.816 (0.008)	**0.003**	0.989 (0.008)	0.702	1.439 (0.008)	**0.003**
	F	–0.402 (0.023)		0.940 (0.014)		0.755 (0.009)		0.984 (0.009)		1.381 (0.010)	
Bufotes viridis	M	0.248 (0.016)	0.158	1.348 (0.006)	1.000	1.106 (0.005)	1.000	1.277 (0.007)	0.217	1.732 (0.005)	0.908
	F	0.298 (0.013)		1.357 (0.007)		1.109 (0.008)		1.296 (0.006)		1.743 (0.006)	
Pelophylax kl. *esculentus*	M	0.215 (0.022)	**0.000**	1.291 (0.011)	**0.020**	1.048 (0.008)	**0.009**	1.265 (0.011)	0.160	1.692 (0.010)	**0.026**
	F	–0.041 (0.093)		1.168 (0.041)		0.906 (0.046)		1.191 (0.032)		1.584 (0.038)	
Rana graeca	M	0.134 (0.022)	0.815	1.280 (0.011)	1.000	1.031 (0.006)	1.000	1.221 (0.010)	0.254	1.667 (0.009)	0.500
	F	0.178 (0.049)		1.276 (0.020)		1.030 (0.019)		1.265 (0.019)		1.681 (0.019)	
Rana temporaria	M	0.245 (0.030)	0.531	1.318 (0.014)	0.950	1.070 (0.012)	0.969	1.263 (0.018)	0.256	1.707 (0.014)	1.000
	F	0.282 (0.046)		1.319 (0.019)		1.069 (0.021)		1.322 (0.018)		1.729 (0.018)	
Rana dalmatina	M	0.023 (0.020)	**0.002**	1.205 (0.010)	**0.001**	1.034 (0.007)	0.087	1.169 (0.009)	**0.027**	1.619 (0.008)	**0.002**
	F	0.157 (0.010)		1.251 (0.005)		1.062 (0.008)		1.217 (0.003)		1.662 (0.004)	

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Table 3. Descriptive statistics of absolute hindlimb and pelvic traits (log – transformed) for males (M) and females (F) of examined anuran species. Values are the mean ± standard error with Bonferroni corrected *p* – significance value of ANOVA results (bold – statistically significant). F – femur, T – tibia, DH – distal hindlimb part, HL – hindlimb length, SW – sacral width, DE – diapophyseal expansion.

		F		T		DH		HL		SW		DE	
Species	Sex	mean (SE)	*p*	mean (SE)	*p*	mean (SE)	*p*	mean (SE)	*p*	mean (SE)	*p*	mean (SE)	*p*
Pelobates fuscus	M	1.289 (0.005)	**0.029**	1.215 (0.005)	0.059	1.502 (0.004)	0.194	1.830 (0.004)	0.062	0.912 (0.005)	**0.002**	1.025 (0.009)	0.437
	F	1.324 (0.010)		1.247 (0.010)		1.526 (0.011)		1.859 (0.010)		0.968 (0.011)		1.059 (0.016)	
Pelobates syriacus	M	1.348 (0.023)	0.878	1.292 (0.024)	0.794	1.528 (0.024)	1.000	1.878 (0.024)	1.000	0.981 (0.019)	0.887	0.983 (0.021)	0.700
	F	1.388 (0.019)		1.333 (0.017)		1.562 (0.015)		1.916 (0.017)		1.016 (0.017)		1.045 (0.021)	
Hyla arborea	M	1.271 (0.007)	**0.010**	1.280 (0.007)	**0.014**	1.442 (0.006)	**0.014**	1.816 (0.006)	**0.010**	0.864 (0.008)	**0.029**	0.437 (0.011)	0.777
	F	1.321 (0.011)		1.329 (0.012)		1.482 (0.009)		1.861 (0.010)		0.921 (0.016)		0.470 (0.021)	
Bombina variegata	M	1.195 (0.006)	**0.002**	1.168 (0.008)	**0.003**	1.426 (0.006)	**0.002**	1.756 (0.006)	**0.002**	0.896 (0.006)	0.557	0.743 (0.016)	1.000
	F	1.131 (0.012)		1.109 (0.010)		1.373 (0.010)		1.699 (0.010)		0.873 (0.015)		0.727 (0.018)	
Bufotes viridis	M	1.409 (0.007)	0.177	1.406 (0.007)	0.770	1.656 (0.007)	0.977	1.984 (0.007)	0.915	1.174 (0.010)	**0.038**	0.771 (0.015)	**0.023**
	F	1.432 (0.006)		1.415 (0.007)		1.666 (0.005)		1.995 (0.007)		1.214 (0.005)		0.837 (0.006)	
Pelophylax kl.*esculentus*	M	1.519 (0.010)	**0.047**	1.559 (0.010)	0.054	1.734 (0.009)	0.109	2.092 (0.009)	0.072	1.109 (0.011)	**0.037**	0.367 (0.014)	**0.008**
	F	1.417 (0.039)		1.459 (0.041)		1.645 (0.040)		1.996 (0.040)		0.982 (0.050)		0.198 (0.047)	
Rana graeca	M	1.513 (0.008)	0.589	1.580 (0.007)	0.975	1.698 (0.009)	0.541	2.081 (0.008)	0.733	1.018 (0.010)	0.620	0.288 (0.022)	0.612
	F	1.534 (0.018)		1.601 (0.017)		1.712 (0.020)		2.099 (0.018)		1.048 (0.029)		0.312 (0.042)	
Rana temporaria	M	1.530 (0.014)	0.458	1.572 (0.014)	0.412	1.750 (0.015)	0.971	2.105 (0.014)	0.451	1.112 (0.013)	0.864	0.400 (0.013)	1.000
	F	1.549 (0.019)		1.593 (0.019)		1.768 (0.019)		2.125 (0.019)		1.107 (0.027)		0.368 (0.031)	
Rana dalmatina	M	1.453 (0.009)	0.165	1.516 (0.009)	**0.005**	1.658 (0.009)	**0.009**	2.028 (0.009)	**0.002**	0.961 (0.010)	**0.031**	0.238 (0.018)	**0.001**
	F	1.496 (0.004)		1.582 (0.005)		1.717 (0.002)		2.085 (0.003)		1.059 (0.004)		0.308 (0.010)	

traits F, T, DH, HL and sacral trait SW (Tables 2, 3). In *R. dalmatina* females had larger forelimb traits H, DF and FL, also hindlimb traits T, DH, HL, and sacral traits SW and DE (Tables 2, 3). Females of *P. fuscus* had higher values for forelimb DF, FL, while only F in hindlimbs and sacral SW (Tables 2, 3). 2) Size-corrected variation (shape) – in *H. arborea* sex differences were observed only for humerus, where males had higher values (Table 4). *R. dalmatina* males had longer radioulna, and total forelimb and femur in hindlimbs. The homogeneity of slope showed significant allometry for radioulna. Females had only

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Table 4. Descriptive statistics of size-corrected (shape) forelimb traits for males (M) and females (F) of examined anuran species. Values are the mean ± standard error with Bonferroni corrected *p* – significance value of ANOVA results (bold – statistically significant). H – humerus, R – radioulna, DF – distal forelimb part, FL – forelimb length.

		H		R		DF		FL	
Species	Sex	mean (SE)	*p*	mean (SE)	*p*	mean (SE)	*p*	mean (SE)	*p*
Bombina variegata	M	–0.036 (0.017)	**0.001**	–0.046 (0.017)	0.291	–0.128 (0.017)	**0.010**	–0.073 (0.006)	0.228
	F	–0.169 (0.011)		–0.075 (0.016)		–0.029 (0.014)		–0.095 (0.006)	
Bufotes viridis	M	0.121 (0.004)	**0.005**	0.083 (0.008)	**0.042**	–0.004 (0.011)	1.000	0.063 (0.005)	**0.027**
	F	0.091 (0.005)		0.041 (0.009)		–0.011 (0.011)		0.039 (0.005)	
Pelobates fuscus	M	–0.066 (0.010)	0.449	–0.105 (0.008)	0.368	–0.025 (0.013)	1.000	–0.062 (0.007)	1.000
	F	–0.086 (0.010)		–0.148 (0.025)		0.001 (0.018)		–0.068 (0.004)	
Pelobates syriacus	M	–0.032 (0.011)	0.510	–0.121 (0.012)	0.466	–0.007 (0.015)	1.000	–0.046 (0.010)	0.870
	F	–0.041 (0.007)		–0.147 (0.011)		–0.026 (0.010)		–0.062 (0.005)	
Hyla arborea	M	–0.082 (0.006)	**0.004**	–0.073 (0.007)	0.188	0.149 (0.008)	0.747	0.010 (0.003)	0.723
	F	–0.087 (0.008)		–0.041 (0.012)		0.142 (0.018)		0.013 (0.008)	
Pelophylax kl. *esculentus*	M	0.022 (0.008)	0.430	–0.017 (0.011)	0.071	0.002 (0.008)	**0.027**	0.003 (0.004)	1.000
	F	–0.005 (0.012)		–0.088 (0.022)		0.087 (0.028)		0.010 (0.009)	
Rana graeca	M	0.078 (0.006)	**0.002**	0.024 (0.009)	**0.044**	–0.019 (0.008)	**0.025**	0.027 (0.003)	0.875
	F	0.024 (0.007)		–0.023 (0.010)		0.038 (0.014)		0.015 (0.007)	
Rana temporaria	M	0.053 (0.010)	0.218	0.003 (0.016)	0.523	–0.034 (0.020)	**0.035**	0.008 (0.005)	0.712
	F	0.021 (0.007)		–0.035 (0.020)		0.064 (0.020)		0.022 (0.009)	
Rana dalmatina	M	0.016 (0.011)	0.353	0.142 (0.007)	**0.002**	–0.029 (0.012)	0.990	0.028 (0.005)	**0.002**
	F	–0.011 (0.008)		0.072 (0.013)		–0.051 (0.007)		–0.009 (0.004)	

significantly higher SW (Tables 4, 5). Sex related shape differences in *P. fuscus* were absent.

Species with larger males

1) Absolute size variation – in *B. variegata* males had higher values for forelimb traits H, R, FL and for hindlimb traits F, T, DH, HL (Tables 2, 3). For *P.* kl. *esculentus* males had significantly higher values for forelimb traits H, R, FL, hindlimb trait F, and sacral traits SW and DE (Tables 2, 3). 2) Size-corrected variation (shape) – ANOVA test confirmed sex differences in *B. variegata* for forelimb traits H, DF and hindlimb trait F (Tables 4, 5, Fig. 2). Males had higher values for femur and humerus, while females had higher values for distal part of forelimbs. A significant sex × SIZE interaction was recorded for femur. In *P.* kl. *esculentus* females had longer distal fore- and hindlimb elements (DF and DH) (Tables 4, 5). The homogeneity of slope showed allometry for distal forelimb element.

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Table 5. Descriptive statistics of size-corrected hindlimbs and pelvic traits for males (M) and females (F) of examined anuran species. Values are the mean ± standard error with Bonferroni corrected *p* – significance value of ANOVA results (bold – statistically significant). F– femur, T– tibia, DH – distal hindlimb part, HL – hindlimb length, SW – sacral width, DE – diapophyseal expansion.

		F		T		DH		HL		SW		DE	
Species	Sex	mean (SE)	*p*	mean (SE)	*p*	mean (SE)	*p*	mean (SE)	*p*	mean (SE)	*p*	mean (SE)	*p*
Bombina variegata	M	–0.119 (0.007)	**0.014**	–0.183 (0.008)	0.396	–0.066 (0.009)	1.000	–0.113 (0.005)	0.165	0.053 (0.011)	0.078	0.653 (0.024)	0.565
	F	–0.157 (0.007)		–0.209 (0.008)		–0.077 (0.014)		–0.135 (0.008)		0.109 (0.017)		0.725 (0.031)	
Bufotes viridis	M	–0.165 (0.007)	0.717	–0.175 (0.007)	0.283	–0.075 (0.006)	0.162	–0.128 (0.004)	0.078	0.152 (0.014)	0.137	0.176 (0.021)	**0.020**
	F	–0.161 (0.005)		–0.202 (0.009)		–0.101 (0.007)		–0.152 (0.006)		0.196 (0.006)		0.278 (0.012)	
Pelobates fuscus	M	–0.114 (0.006)	1.000	–0.287 (0.006)	1.000	–0.102 (0.005)	0.168	–0.155 (0.004)	0.843	–0.122 (0.012)	0.066	1.089 (0.016)	0.889
	F	–0.109 (0.005)		–0.289 (0.004)		–0.122 (0.005)		–0.163 (0.003)		–0.068 (0.013)		1.093 (0.019)	
Pelobates syriacus	M	–0.077 (0.006)	1.000	–0.209 (0.011)	1.000	–0.142 (0.008)	1.000	–0.143 (0.006)	1.000	–0.062 (0.027)	0.900	0.894 (0.018)	0.593
	F	–0.069 (0.008)		–0.199 (0.007)		–0.147 (0.006)		–0.140 (0.003)		–0.066 (0.011)		0.951 (0.019)	
Hyla arborea	M	0.050 (0.004)	1.000	0.068 (0.005)	1.000	–0.037 (0.006)	1.000	0.015 (0.004)	0.891	–0.030 (0.009)	0.793	–0.059 (0.017)	1.000
	F	0.058 (0.009)		0.073 (0.009)		–0.050 (0.009)		0.014 (0.008)		–0.005 (0.016)		–0.091 (0.030)	
Pelophylax kl. *esculentus*	M	0.122 (0.006)	1.000	0.210 (0.007)	0.389	0.138 (0.009)	**0.047**	0.153 (0.006)	0.073	0.037 (0.012)	0.653	–0.720 (0.022)	0.055
	F	0.142 (0.017)		0.237 (0.009)		0.188 (0.009)		0.188 (0.008)		–0.001 (0.028)		–0.854 (0.032)	
Rana graeca	M	0.188 (0.005)	1.000	0.341 (0.008)	0.801	0.136 (0.007)	1.000	0.210 (0.005)	1.000	–0.092 (0.008)	0.747	–0.821 (0.032)	1.000
	F	0.192 (0.009)		0.346 (0.015)		0.123 (0.013)		0.207 (0.010)		–0.067 (0.030)		–0.809 (0.049)	
Rana temporaria	M	0.115 (0.005)	1.000	0.210 (0.008)	1.000	0.143 (0.010)	1.000	0.153 (0.006)	1.000	0.013 (0.012)	0.486	–0.676 (0.026)	0.183
	F	0.123 (0.009)		0.223 (0.009)		0.149 (0.010)		0.161 (0.007)		–0.035 (0.025)		–0.785 (0.034)	
Rana dalmatina	M	0.162 (0.005)	**0.003**	0.304 0.004)	0.272	0.154 (0.004)	0.867	0.198 (0.003)	1.000	–0.113 (0.013)	**0.002**	–0.826 (0.025)	1.000
	F	0.125 (0.005)		0.322 (0.007)		0.155 (0.007)		0.195 (0.004)		–0.021 (0.006)		–0.799 (0.014)	

Species with no sexual SIZE dimorphism

1) Absolute size variation – *Bufotes viridis* females had higher values for sacral traits SW and DE (Table 3). No significant sex differences in absolute size traits were observed for *R. temporaria*, *R. graeca* and *P. syriacus*. 2) Size-corrected variation (shape) – *B. viridis* males had longer forelimb traits H and R, while females had larger sacral DE (Tables 4, 5). Based on homogeneity of slopes, allometric changes were related to H and DE. In *Rana temporaria* only sex differences were reported for forelimb trait DF, where females showed significantly higher values (Table 4). Results for *R. graeca* showed that males had longer forelimb traits H and R, while females had higher values for distal forelimb part DF (Table 4). The homogeneity of slopes showed significant allometry for radioulna. No significant sex differences in size-corrected (shape) traits were observed for *P. syriacus*.

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Discussion

Sexual differences in limb and sacral traits in anurans can provide important information of the underlying evolutionary mechanisms, due to their role in connection of the organism and the environment, through locomotion, reproduction and feeding. Obtained results indicate variable pattern in size and shape of examined traits between sexes across different anuran species (species-specific sexual dimorphism), which is consistent with data from the literature (Shine 1979, 1989, Duellman & Trueb 1994, Lee 2001, Monnet & Cherry 2002, Nali et al. 2014). Important part of this variation is related to sex differences in forelimbs, and only small proportion is referred to differences in hindlimbs and sacral traits.

Absolute size variation

Sexual size dimorphism has been a topic of intense study for years, and well documented in a number of anuran species (Shine 1979). In this study, sexual size dimorphism ranged from female and male-biased, to absence of sexual dimorphism across species. As previous studies noted, *Pelobates fuscus*, *Hyla arborea* and *Rana dalmatina* follow female-biased sexual size dimorphism seen in majority of anurans (Monnet & Cherry 2002, Székely & Nemes 2002). Females of *Pelobates syriacus*, *Rana graeca* and *R. temporaria* were larger, but not significantly. The well accepted explanation for female-biased sexual dimorphism is fecundity selection, which is based on a positive correlation between fecundity and body size of females, and is followed by higher growth rate and delaying maturity (Crump 1974, Andersson 1994, Monnet & Cherry 2002, Liao & Lu 2009a, b, Liao & Lu 2011, Liao et al. 2012, Liao & Chen 2012). Males of *Bombina variegata* and *Pelophylax* kl. *esculentus* are larger than females, which is a condition seen only in 10 % of anuran species (Shine 1979). Other studies documented the same pattern in *B. variegata* (Radojčić et al. 2002, Di Cerbo & Biancardi 2012), while females of *P.* kl. *esculentus* were larger than males (Krizmanić 2008). The main reason for lager body in males is the higher reproductive success (advantage in male-male direct competitions, and in mating calls that can be preferred by females) (Woolbright 1983, Katsikaros & Shine 1997, Lodé & Le Jacques 2003). Besides intrasexual competition, female choice for larger males can affect size of male body (Lips 2005).

Patterns of variation in absolute measures of fore- and hindlimbs and sacral vertebrae imply that variation of these characteristics is in concordance with variation of body size. In sex with larger isometric size, almost all traits related to locomotion were statistically larger in comparison to sex with smaller isometric size. Existing literature data confirmed similar pattern variation for *P. fuscus*, *P. syriacus* and *B. variegata.* Larger body was followed by longer femur and tibia in species from the genus *Pelobates* (Székely & Nemes 2002, Ugurtas et al. 2002). In *B. variegata* males exhibit lager body and higher values for humerus, tibia and distal part of hindlimbs (Radojčić et al. 2002, Di Cerbo & Biancardi 2012).

Size-corrected variation

Males of most species were characterized by longer proximal parts (humerus and radioulna) and shorter distal forelimb parts, while females had shorter proximal and longer distal forelimb elements. Also, some cases of significant intersex shape differences were size dependent. Allometric growth for humerus and radioulna in males showed that these traits are under stronger selective pressures for increase relative to body size in comparison to females. Since there is no difference in forelimb length, we assumed that longer humerus and radioulna in males were associated with shorter distal forelimb elements in order to maintain optimal length of forelimbs and their function in locomotion. Emerson (1991) suggested that total length of forelimbs may not be most appropriate measure for sexual selection in frogs, and emphasized functional aspect of their segments (humerus and radioulna) in amplexus. The increase of these elements allows stronger hold of males on females during amplexus and resistance of takeover by competing males (Howard & Kluge 1985). Previous studies confirmed that dimorphism is present also in muscle mass of forelimbs in anurans. Muscles involved in amplexus, such as *pectoralis*, *coracoradialis*, *coracobrachialis*, *flexor carpi radialis*, *extensor carpi radialis*, *sternoradialis* and *abductor indicus longus* were found to be larger in males than in females (Oka et al. 1984, Yekta & Blackburn 1992, Peters & Aulner 2000, Lee 2001, Clark & Peters 2006).

It is interesting that almost no differences between sexes were found for hindlimbs and sacral traits. Studies that examined relationship of morphology and locomotion in frogs emphasize biomechanical importance of hindlimbs and explanatory power of sacral traits in context of different locomotor performances (Emerson 1976, Jorgensen & Reilly 2013). Considering that locomotion is essential for animal survival, those traits in frogs can be under

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strong selective pressures in both sexes, and were not expected to differ considerably between sexes. Only in *B. variegata* and *R. dalmatina* femur was larger in males. Longer hindlimbs were present in species with male competition. Males with longer hindlimbs may have some advantage during amplexus or locomotor advantage in finding mates during migration (Wells 1979, Halliday 1980, Lee & Corrales 2002, Herrel et al. 2012). Additionally, *B. variegata* and *R. dalmatina* were dimorphic for sacral traits, with higher values in females. Observed allometry for sacral traits in females suggested that selection may be acting on trunk, because wider pelvic girdle would increase the space available for eggs. This could be expected as these anuran species are explosive breeders, where female frogs face stronger selective pressures for fecundity increase due to limited breeding period (Nali et al. 2014). Sexual dimorphism for sacral traits was not observed for other explosive breeders in our study (e.g. *R. temporaria*, *B. viridis*), which indicates that other factors could affect variation in pelvic girdle. Further studies are necessary to investigate the mechanisms of these findings.

This study points out the importance of adaptive mechanisms in disentangling dimorphism in shape and body size of examined anuran species. Different patterns of sexual size and shape dimorphism were established for absolute size variation and size-related shape variation but more data on the ecology and natural history of these animals are needed to be explored.

Acknowledgements

This research was financed by the Ministry of Education, Science and Technological Development of Republic of Serbia (Grant No. 173043). We thank Milena Cvijanović and Aleksandar Urošević for valuable comments on the first draft of the manuscript. We also would like to thank the two anonymous reviewers, who provided many constructive suggestions on earlier drafts of this article.

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Supplementary online material

Table S1. Descriptive statistics of snout-vent length (SVL) and forelimb traits for males (M) and females (F) of examined anuran species. Values are the mean ± standard error; H – humerus, R – radioulna, DF – distal forelimb part, FL – forelimb length.

Table S2. Descriptive statistics of hindlimb and pelvic traits for males (M) and females (F) of examined anuran species. Values are the mean ± standard error; F – femur, T – tibia, DH – distal hindlimb part, HL – hindlimb length, SW – sacral width, DE – diapophyseal expansion.

Table S3. Access numbers of batrachological collection – Institute for Biological Research "Siniša Stanković" (http://www.ivb.cz/folia_zoologica/supplemetarymaterials/petrovic_t_g_et_al_tables_s1_s3.doc).

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