THE VENTRAL CRANIAL SIZE AND SHAPE VARIATION BETWEEN MALES AND FEMALES OF EUROPEAN BROWN FROGS: *RANA DALMATINA*, *R. GRAECA* AND *R. TEMPORARIA* (ANURA, AMPHIBIA)

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ABSTRACT

Sexual size and shape dimorphism is a very common phenomenon widely studied in the field of evolutionary biology. The differences between sexes are related to their life strategies and driven by the two evolutionary processes, sexual and natural selection. In amphibians, females are larger sex due to high correlation with fecundity, while dimorphism in body shape is often related to intrasexual competition for opposite sex during the breeding season. The main aim of this study is to describe patterns of ventral cranial size and shape variation between males and females of three species of European brown frogs, *Rana dalmatina*, *R. graeca*, *R. temporaria*, from the Balkan Peninsula. Our results showed that species *R. dalmatina* and *R. graeca* are sexually dimorphic for the ventral cranial size while species *R. graeca* and *R. temporaria* for the ventral cranial shape. Sexual dimorphism in cranial size is most probably an indirect consequence of natural selection favoring larger body for higher fecundity in explosive breeders like brown frogs. Cranial shape variation is under a strong influence of size variation but without allometric scaling between sexes. Sexual shape differences of analyzed brown frog species were most pronounced in the level of connection between cranium and jaw which indicate that differential diet of males and females can be a factor affecting observed sexual dimorphism patterns. More detailed studies of males and females microhabitats are necessary to conclude if differences in intersexual ecology affect intersexual size and shape differences in the cranium.

Keywords: Cranium, Sexual dimorphism, Geometric morphometrics.

INTRODUCTION

Size and shape variations are the principal sources of biological diversity (Hallgrímsson & Hall, 2005) and their origin and maintenance are in the focus of many biological disciplines. Sexual size and shape dimorphism, the morphological difference between sexually mature males and females, is a very common phenomenon widely studied in the field of evolutionary biology (Fairbairn, 2013). The differences between sexes are related to their life strategies and driven by the two evolutionary processes, sexual and natural selection (Shine, 1989; Monnet & Cherry, 2002).

Sexual dimorphism in size (*sexual size dimorphism*, SSD) is the most prominent and most obvious aspect of sexual dimorphism (Shine, 1989; Fairbairn, 1997; Fairbairn et al., 2007). SSD is the topic of a large number of studies for a long time (e.g. Snyder et al., 1976, Shine, 1979; Price, 1984; Cox & Calsbeek, 2010; McPherson & Chenoweth, 2012; Plavcan, 2012; Friedman & Remeš, 2016; Cooper, 2018; Ng et al., 2019), while sexual shape dimorphism (*sexual shape dimorphism*, ShD) is more extensively explored in the last two decades (e.g. Herrel et al., 1999; Bonduriansky, 2006; Berns, 2013; Vladić et al., 2019).

Amphibians are an appropriate group for the sexual dimorphism studies as the difference between males and females is evident for number of morphological features such as body size and shape and body coloration (Monnet & Cherry, 2002; Hoffman & Blouin, 2000; Bell & Zamudio, 2012; Zhang & Lu, 2013; Petrović et al., 2017; Vukov et al., 2018). For example, females are larger sex in 90% of anuran species as the larger body is correlated with higher fecundity (Shine, 1979). Dimorphism in body shape is often related to intrasexual competition through contests for dominance or attracting the opposite sex during the breeding season (Shine, 1979).

Even though the sexual dimorphism of the skull is widely studied in animal groups such as mammals (Gittleman & Valkenburgh, 1997; Morris & Carrier, 2016; Porobić et al., 2016) and lizards (Kuo et al., 2009; Ljubisavljević et al., 2010; Borczyk et al., 2014), it is understudied in amphibians (Ivanović et al., 2007; Ivanović et al., 2008; Ivanović & Kalezić, 2012), especially in anurans (but see Vukov et al., 2018; Krstić Račkovic et al., 2019). Therefore, the aim of this study is to describe patterns of ventral cranial size and shape variation between males and females of three species of European brown frogs: *Rana temporaria*, *R. dalmatina* that are widely distributed throughout Europe (Sillero et al., 2014), and *R. graeca*, species endemic in the Balkan Peninsula (Džukić & Kalezić, 2004).

*BIOLOGY*
MATERIAL AND METHODS

In this study, we included 112 adult skulls (R. dalmatina: 20 males, 19 females; R. graeca: 19 males, 17 females; R. temporaria: 27 males, 10 females) obtained from the Batrachological Collections of the Institute for Biological Research “Siniša Stanković,” Belgrade (Đukić et al., 2015). The sample size in this geometric morphometric study was large enough for the appropriate estimation of different parameters (Cardini & Elton, 2007).

Skulls were taken from adult specimens (determined by gonad examination) and they were cleared with trypsin and potassium hydroxide (KOH), stained with Alizarin Red S to distinguish cranial elements and their articulations better and then preserved in glycerol. Images of the ventral cranium were obtained with a Sony DSC-F828 digital camera (resolution 8.0 MP; Sony Corp., Tokyo, Japan). Eighteen specific two-dimensional landmarks for the ventral cranium were digitized using TpsDig2 software (Rohlf, 2008). The chosen configuration of landmarks provides an adequate summary of specific aspects of the ventral cranium morphology. The specific positions of the selected landmarks are shown in Figure 1.

![Figure 1. Location of the selected landmarks on the ventral cranium of a brown frog (R. temporaria).](image)

We applied Generalized Procrustes analysis (GPA) (Dryden & Mardia, 1998) to analyse interspecific, sexual, and body size-related variation in the ventral cranium of brown frogs. A GPA was used to obtain a matrix of shape co-ordinates from which differences due to the position, scale, and orientation were removed (Dryden & Mardia, 1998). Variation in ventral cranial size (centroid size - CS) was evaluated by ANOVA with sex as factors. Determining the level of sexual dimorphism in size, the standard index is calculated by CS values: \( I_{SSD} = \text{size of the larger sex}/\text{size of the smaller sex} \). The differences in shape between sexes are described as Procrustes distances. Procrustes distance, a linear measure of shape differences between landmark configurations, was used as an index of sexual dimorphism in shape (\( I_{SSD} \)).

To access the optimal estimate of the impact of allometry on shape changes, we employed two-way permutational MANCOVA with species and sex as factors and CS as covariable. Factor × CS interaction would indicate that size-dependent shape changes differ between the species or sexes. The percentage of predicted allometry with the statistical significance of the allometric regressions was tested with permutation tests against the null hypothesis of allometry independence. Residuals from the multivariate regression of shape variables on CS were used to visualize non-allometric shape changes in the ventral cranium between sexes by discriminant analysis, and to calculate size corrected index of sexual dimorphism in shape (\( I_{SSDcorr} \)).

Analyses were performed in MorphoJ (Klingenberg, 2011), Statistica (StatSoft Inc., 2011), and in R 3.2.0 (R Core Team, 2015).

RESULTS

Cranial size differs significantly between sexes for R. dalmatina and R. graeca with larger cranium in females, but not for R. temporaria (Table 1). Females had larger body size in our sample (R. dalmatina: females L = 63.9 mm, males L = 57.2 mm; R. graeca: females L = 68.1 mm, males L = 61.9 mm; R. temporaria: females L = 85.1 mm, males L = 75.7 mm). The calculated SSD indexes (Table 1) and mean CS values for R. dalmatina and R. graeca showed that females had 12% larger cranium than males.

<table>
<thead>
<tr>
<th>species</th>
<th>CS means</th>
<th>CS SD</th>
<th>( I_{SSD} )</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>R. dalmatina</td>
<td>m 33.47</td>
<td>2.73</td>
<td>1.13</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td>f 37.72</td>
<td>5.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R. graeca</td>
<td>m 45.71</td>
<td>3.95</td>
<td>1.13</td>
<td>0.010</td>
</tr>
<tr>
<td></td>
<td>f 48.06</td>
<td>5.33</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R. temporaria</td>
<td>m 39.07</td>
<td>4.54</td>
<td>1.05</td>
<td>0.156</td>
</tr>
<tr>
<td></td>
<td>f 44.01</td>
<td>5.48</td>
<td></td>
<td></td>
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</tbody>
</table>

Regression analyses showed that the ventral cranium of males and females for all three analysed brown frog species share the same allometric trajectory (\( F = 0.6744, P = 0.6438 \)), with 16.91% of shape variation explained by the size variation (\( P \))
Sexual shape differences were significant for *R. graeca* and *R. temporaria*, and not significant for *R. dalmatina*, before and after removing the allometric component of the ventral cranium variation (Table 2). The calculated SShD indexes were similar for *R. graeca* and *R. temporaria* (Table 2).

Table 2. Indices of sexual size dimorphism before (I_{SShD}) and after removing allometric component of variation (I_{SShDcorr}). Statistically significant values in bold.

<table>
<thead>
<tr>
<th>Species</th>
<th>I_{SShD}</th>
<th>P</th>
<th>I_{SShDcorr}</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>R. dalmatina</em></td>
<td>0.02</td>
<td>0.060</td>
<td>0.01</td>
<td>0.292</td>
</tr>
<tr>
<td><em>R. graeca</em></td>
<td>0.03</td>
<td><strong>0.001</strong></td>
<td>0.02</td>
<td><strong>0.048</strong></td>
</tr>
<tr>
<td><em>R. temporaria</em></td>
<td>0.03</td>
<td><strong>0.001</strong></td>
<td>0.03</td>
<td><strong>0.001</strong></td>
</tr>
</tbody>
</table>

Patterns of the intersexual shape variation were the same before and after removing the allometric component. Females of the *R. graeca* had wider skull than males in the posterior part of the cranium (landmarks 1, 2, 3, 4) but shorter snout (landmarks 14, 15, 16, 17), with anteriorly displaced contact between pterygoid and maxilla (landmarks 11, 12) (Figure 2A). In the *R. temporaria* females compared to males the tips of lateral pterygoid process and quadrate bones were displaced posteriorly (landmarks 1, 2, 3, 4), contacts between the palatine and maxilla and pterygoid and maxilla anteriorly (landmarks 9, 10, 11, 12), and snout was slightly longer (landmarks 16, 17) (Figure 2B).

DISCUSSION

Three species of the European brown frogs are sexually dimorphic for the ventral cranial size in *R. dalmatina* and *R. graeca* and for the ventral cranial shape in *R. graeca* and *R. temporaria*. Cranial shape variation is under a strong influence of size variation but without allometric scaling between sexes. The most pronounced shape dissimilarities between sexes are found in the posterior part of the ventral cranium.

Two evolutionary processes, sexual and natural selection, have the highest impact on the morphological variability of males and females (Shine, 1989; Andersson, 1994; Fairbairn, 1997; Hendry et al., 2014). Therefore, the difference between sexes is a consequence of differential interaction of each sex with environment and interaction between sexes (Herrel et al., 2001; Sacchi et al., 2009; Peiman & Robinson, 2010; Lailvaux et al., 2012). In the context of sexual selection, sexually dimorphic size and shape of the cranium could evolve through intrasexual competition where cranial traits provide advantages in breeding opportunities to some individuals through contests of dominance or attracting the opposite sex. For example, maximum bite force linked to features of cranial-jaw complex contributes to successful breeding in reptiles (Lappin & Husak, 2005; McBryer & Anderson, 2007). In addition, sexually dimorphic size and shape of the cranium could arise through natural selection and intersexual niche divergence in order to reduce intersexual competition for resources and habitat use. Indeed, many studies showed that skull dimorphism is mainly restricted to differences in the size and shape of the parts that can affect bite force and prey size (Vincent et al., 2004; Herrel et al., 2007; Kaliontzopoulou et al., 2007; Ljubisavljević et al., 2010).

Our results showed that females had larger ventral cranial and bodies than males, therefore sexual dimorphism in cranial size is most probably an indirect consequence of natural selection favoring larger body for higher fecundity in explosive breeders like brown frogs (Woolbright, 1983). In addition, brown frogs do not display male to male combats or territorial contests, or intersexual interactions like copulatory bites, so sexual dimorphism in cranial size is probably not a consequence of sexual selection. Sexual shape differences of analyzed brown frog species were most pronounced in the level of connection between cranium and jaw which indicate that differential diet of males and females can be a factor affecting observed sexual dimorphism patterns. However, studies about the diet of brown frogs males and females are almost not existing (Cicort et al., 2011) so the direct link between observed intersexual cranial shape variation and diet variation cannot be established. Study of the dorsal cranium variation in the European brown species showed that ecologically similar species (in habitat characteristics and type of locomotion) shared the cranial morphology (Krštić Račković et al., 2019). However, more detailed studies of males and females microhabitats are necessary.
to conclude if differences in intersexual ecology affect intersexual size and shape differences in the cranium.

Observed patterns of sexual dimorphism in cranial size and shape of three brown frog species indicate that further studies are necessary in order to find causes and consequences of intersexual variation of the cranium.

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REFERENCES


