

Patterns of cranial sexual dimorphism in the yellow-bellied toad (*Bombina variegata*, Bombinatoridae)

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Abstract. This study provides first insight into patterns of adult cranial size and shape sexual dimorphism in the yellow-bellied toad (*Bombina variegata*). Our results revealed significant cranial sexual size and shape differences in this species, with a very small impact of allometry on the shape diversity. The pattern of cranial sexual dimorphism indicates early differentiation of the sexes followed by parallel growth trajectories. Males have a larger cranium than females. Shape differences between the sexes are pronounced in the trophic part of the cranium. In comparison to females males have the lateral part of the nasal displaced posteriorly, a shorter anterior pterygoid process and the posterior part of the quadratojugal and pterygoid displaced toward the snout. Therefore, males have a wider but shorter posterior part of the cranium. Adaptation to divergent trophic niches driven by natural selection rather than sexual selection could have led to sexual size and shape differences in the yellow-bellied toad. However, further analysis of cranial variation patterns including ontogenetic aspects of cranial variation and ecological niche analyses are crucial to elucidate how different developmental and evolutionary mechanisms act on the cranium and result in size and shape sexual dimorphism.

Key words: cranium, size, shape, allometry, anurans.

Introduction

Sexual dimorphism, defined as phenotypic differences between conspecific males and females, is a widespread phenomenon in animals (Shine 1989). The impact of sexual dimorphism on evolution and ecology of organisms is usually profound and crucial for better understanding the patterns and processes at both micro and macroevolutionary levels (Butler et al. 2007, Berns & Adams 2013, De Lisle & Rowe 2015). At least two adaptive processes and their interplay can lead to the evolution of sexual dimorphism in phenotypic traits: sexual selection and natural selection. Sexual selection arises from variance in mating success, while natural selection arises from variance in other components of fitness (e.g. viability, fecundity, fertility) (Arnold & Wade 1984). For example, sexual selection is accepted in general as an explanation for sexual size differences in anurans, where competition between males (intrasexual selection) and female choice (intersexual selection) are the main drivers (Shine 1989).

Sexual dimorphism in anuran amphibians exists for many traits, such as coloration (Bell & Zamudio 2012), skin texture and skin glands (Brunetti 2015), vocalization and breeding behavior (Shine 1979, Leininger & Kelley 2015), body size (Han & Fu 2013) and body shape (Herrel et al. 2012).

Regarding body size, most of the studied amphibian species have larger females than males due to the advantage of a larger body for increased fecundity (Shine 1989, Liao & Chen 2012, Han & Fu 2013). Nevertheless, competition among males, female choice, parental care and vocalization are the main drivers for larger individuals among males (Shine 1989, Kupfer 2007). Although sexual shape dimorphism for different morphological traits, such as whole body shape (Malmgren & Thollesson 1999, Ivanović et al. 2008, Hasumi 2010, Reinhard & Kupfer 2015), limb shape (Balogová et al. 2015) and cranial shape (Ivanović & Kalezić

2012), has been widely studied for tailed amphibians, few analyses of sexual shape dimorphism have been made in anurans. They showed that head and forelimb shapes were sexually dimorphic, with males having narrower heads (Di Cerbo & Biancardi 2012, Arantes et al. 2015) and longer forelimbs (Lee 2001, Arantes et al. 2015).

The importance of examining cranial morphological variation in evolutionary studies and therefore in studies of sexual dimorphism, is reflected through the lack of in-depth knowledge about the causes of its morphological complexity. The cranium comprises different regions, such as the auditory region, braincase, orbits and nasal regions that develop semi-independently and thus can be recognized as different modules (Moss & Young 1960, Emerson & Bramble 1993). In addition, the interplay of different developmental and evolutionary mechanisms that affect different parts of the cranium emphasizes cranial complexity. In order to understand proximal processes and evolutionary pressures that act in each sex and lead to the absence or presence of morphological differentiation in the cranium of adult males and females, the first step is a description of the patterns of cranial sexual dimorphism.

The cranium of anurans has been widely analyzed in the light of taxon determination, phylogenetic relationships and evolutionary studies (Mendelson et al. 2000, Fabrezi 2006, Smirnov 2011, Acevedo et al. 2016, Yildirim & Kaya 2017), development and integration (Hanken & Hall 1987, 1988, Roček 1999, Larson 2000, Vera & Ponsa 2004, Schoch 2006, Yildirim & Kaya 2016), ossification sequences (Weisbecker & Mitgutsch 2010, Harrington et al. 2013) and diet (Emerson 1985). Cranial sexual dimorphism has been evaluated mostly in mammals (Gittleman & Valkenburgh 1997, Morris & Carrier 2016, Porobić et al. 2016) and lizards (Kaliontzopoulou et al. 2008, Kuo et al. 2009, Ljubisavljević et al. 2010) with, to the best of our knowledge, just one paper regarding tailed amphibians (Ivanović & Kalezić 2012), and no studies on an-

urans.

Patterns of sexual dimorphism in the yellow-bellied toad (*Bombina variegata*, Bombinatoridae) have rarely been investigated (Radojičić et al. 2002, Di Cebro et al. 2011), so shape variation in complex morphological structures such as the cranium, has not yet been assessed. Therefore, the goal of this study was to describe patterns of cranial size and shape sexual dimorphism in adult *B. variegata*. We used geometric morphometrics as a powerful tool for an in-depth investigation of morphological structures as this allows an integrated study of morphological variation (Bookstein et al. 1999, 2003).

Material and methods

Yellow-bellied toad (*B. variegata*, Serbia, Prohor Pčinjski: 34 males, 26 females) specimens were obtained from the Batrachological Collections of the Institute for Biological Research "Siniša Stanković," Belgrade. The sample size in this geometric morphometric study was large enough for appropriate estimation of different parameters (Cardini & Elton 2007). The body size (SVL - snout-vent length) of each specimen was measured before the skulls were cleaned.

Skulls were taken only from adult specimens (determined by gonad examination) and were cleared with trypsin and potassium hydroxide (KOH), stained with Alizarin Red 5 to better distinguish cranial elements and their articulations (Dingerkus & Uhler 1977) and then preserved in glycerol. Images of each dorsal and ventral cranium were obtained with a Sony DSC-F828 digital camera (resolution 8.0 MP; Sony Corp., Tokyo, Japan). Ten two-dimensional landmarks for the dorsal cranium and thirteen for the ventral cranium were digitized using TpsDig2 software (Rohlf 2008). The chosen configuration of landmarks provides an adequate summary of specific aspects of dorsal and ventral cranium morphology. The positions and definitions of the chosen landmarks are shown in Fig. 1.

First, we examined how absolute head size (log CS, see below) differences between the sexes were related to total body size (log SVL). This allows questions about the direct target of evolutionary mechanisms, overall body size or head size to be answered.

We applied Generalized Procrustes analysis (GPA) (Dryden & Mardia 1998) in MorphoJ 1.02b (Klingenberg 2011) to analyze sexual dimorphism in dorsal and ventral cranial size and shape. A GPA was used to obtain a matrix of shape co-ordinates from which differences due to position, scale and orientation were removed (Dryden & Mardia 1998). Variation in dorsal and ventral cranium size was evaluated by Student's t-test on centroid size (CS). Variations in dorsal and ventral cranial shape between the sexes were analyzed by multivariate analysis of variance (MANOVA) on cranial shape variables (partial warps and uniform components), which can be used as shape variables in any conventional statistical analysis (Zelditch et al. 2004). The differences in shape between females and males were quantified as Procrustes distances. The Procrustes distance, a linear measure of shape differences between landmark configurations, was used as an index of sexual dimorphism in shape (ISShD). To quantify the level of sexual dimorphism in size (SSD), the standard index of SSD was calculated using CS values: $ISSD = \text{size of the larger sex (males)} / \text{size of the smaller sex (females)}$.

In order to access the optimal estimate of the impact of allometry on shape changes, pooled within-group allometric regression using centroid size (Size) was performed on the Procrustes coordinates (Shape). Our assumption for using this method was that groups shared the same allometry (Klingenberg 2016). The homogeneity of allometric slopes between the sexes was tested using the homogeneity of slope test, with shape variables as dependent variables, sex as factors and CS as an independent variable. Factor \times CS interaction would indicate that size-dependent shape changes differ between the sexes. The percentages of predicted allometry in each sex were

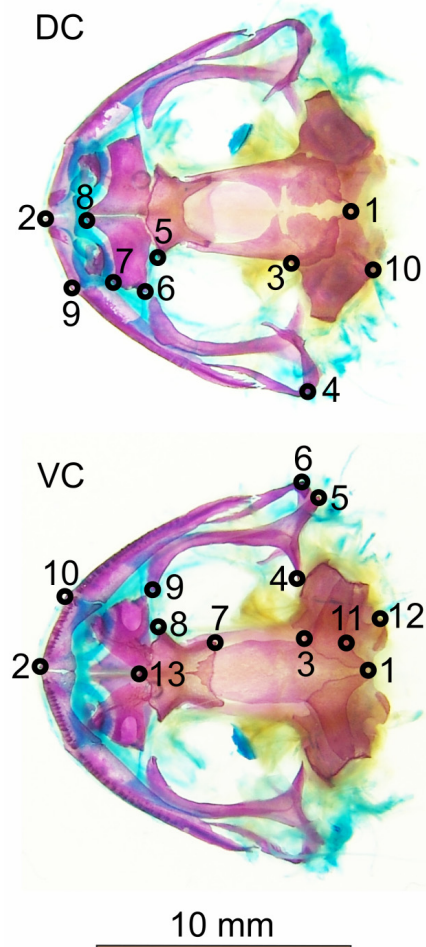


Figure 1. Two-dimensional landmarks digitized on the dorsal and ventral cranium of an individual of *Bombina variegata*.

Dorsal cranium (DC): 1. Medial point of exoccipital; 2. Anterior-most point of premaxilla; 3. Suture between parietal and prootic; 4. Lateralmost point of squamosal; 5. Lateralmost point of sphenethmoid; 6. Lateralmost point of nasal (posterior); 7. Lateralmost point of nasal (anterior); 8. Medial point of nasal; 9. Most anterior point of suture between premaxilla and maxilla; 10. Posteriormost point of exoccipital.

Ventral cranium (VC): 1. Posteriormost point of parasphenoid; 2. Anteriormost point of premaxilla; 3. Anteriormost base of lateral process of parasphenoid; 4. Tip of medial pterygoid process (towards the auditory capsule); 5. Tip of lateral pterygoid process (towards the quadrate); 6. Most lateral point of quadratum; 7. Lateralmost point of sphenethmoid (posterior); 8. Lateralmost point of sphenethmoid (anterior); 9. Tip of anterior pterygoid process (towards the maxilla); 10. Anteriormost point of maxilla; 11. Posteriormost base of lateral process of parasphenoid; 12. Tip of occipital condyle; 13. Anteriormost point of sphenethmoid.

calculated with the statistical significance of the allometric regressions (tested with permutation tests against the null hypothesis of allometry independence).

In order to access size-independent shape variation, residuals of pooled within-group allometric regression were used for statistical analysis and investigation of shape variation of the dorsal and ventral cranium. Canonical variate analysis (CVA) was used to verify which shape variations could distinguish the sexes regarding dorsal and ventral cranium. The differences in size-independent shape between females and males were quantified as Procrustes distances. The index of sexual dimorphism in shape - standardized (ISShD(stand)) was calculated as the Procrustes distance between

sexes using the size-independent shape variation.

Results

Differences in body and cranial size

The t-test showed non-significant difference in body size between the sexes ($P > 0.05$). The interaction $\log\text{SVL} \times \text{sex}$ was also non-significant ($F = 0.00094$, $P > 0.05$), indicating no sex specific cranial size/body size trajectories. However, males and females of the yellow-bellied toad had significantly different cranial size (t-test: dorsal cranium $P < 0.001$; ventral cranium $P < 0.01$), with male crania being larger at a given body size (Table 1). Although significant cranial SSD was not pronounced, males had a 6% larger dorsal cranium and a 4% larger ventral cranium than females (Table 1).

Differences in cranial shape

Multivariate analysis of variance with sex as a factor indicated significant variation in shape between the sexes for the dorsal but not for the ventral cranium (dorsal cranium Wilks' Lambda = 0.5379, $F_{17,42} = 2.1227$, $P < 0.05$; ventral cranium Wilks' Lambda = 0.5396, $F_{23,34} = 1.26134$, $P > 0.05$). The permutation test (10000 iterations) of pairwise Procrustes distances showed statistical significance for the differences between mean dorsal and ventral cranial shape of males and females (dorsal $P < 0.01$; ventral $P < 0.05$). As a quantitative index of sexual dimorphism in cranial shape, Procrustes distances between mean dorsal and ventral cranial shapes for males and females are given in Table 1 (see I_{SShD}).

Analysis of cranial allometry

The homogeneity of slope test revealed that allometric slopes for males and females were the same for both dorsal and ventral cranium (dorsal cranium Wilks' Lambda = 0.7312, $F_{17,40} = 0.8648$, $P > 0.05$; ventral cranium Wilks' Lambda = 0.6036, $F_{23,32} = 0.9135$, $P > 0.05$). Therefore, analysis of allometric shape changes was conducted on the whole sample (sexes pooled).

Multivariate regression of shape on log-transformed CS showed that shape changes were significantly correlated with changes in size for both dorsal and ventral cranium, but that allometric changes were not that pronounced (dorsal cranium 6.38 %, $P < 0.001$; ventral cranium 6.83 %, $P < 0.001$).

Dorsal cranial shape changes from smaller to larger CS included coordinated displacement of landmarks on the nasal (landmarks 6, 7, 8), sphenethmoid (landmark 5), maxilla (landmark 9), frontoparietal (landmark 3) and prootic (landmark 10). Individuals with larger CS had bigger but narrower nasals, more elongated maxillas, narrower frontoparietals and shortened posterior part of the cranium (Fig. 2). Ventral cranial shape changes from smaller to larger CS included coordinated displacement of landmarks on the sphenethmoid (landmarks 7, 8, 13), pterygoid (landmarks 5, 6, 9) and occipital condyle (landmark 12). Individuals with larger CS had wider and longer sphenethmoid, more elongated pterygoid processes, and shortened posterior part of the cranium (Fig. 2).

Non-allometric cranial sexual shape dimorphism

As intersex differences in shape related to size exist, com-

Table 1. Means and standard deviations of dorsal and ventral cranium size, indices of sexual size dimorphism (ISSD) and sexual shape dimorphism, unstandardized and standardized values (I_{SShD} , $I_{\text{SShD}}(\text{stand.})$). SD, standard deviation.

	N	CS Mean	CS SD	ISSD	I_{SShD}	$I_{\text{SShD}}(\text{stand.})$
Dorsal cranium						
males	34	15.70	0.88	1.0615	0.0166	0.0170
females	26	14.79	0.92			
Ventral cranium						
males	34	17.52	0.97	1.0453	0.0168	0.0194
females	24	16.76	0.88			

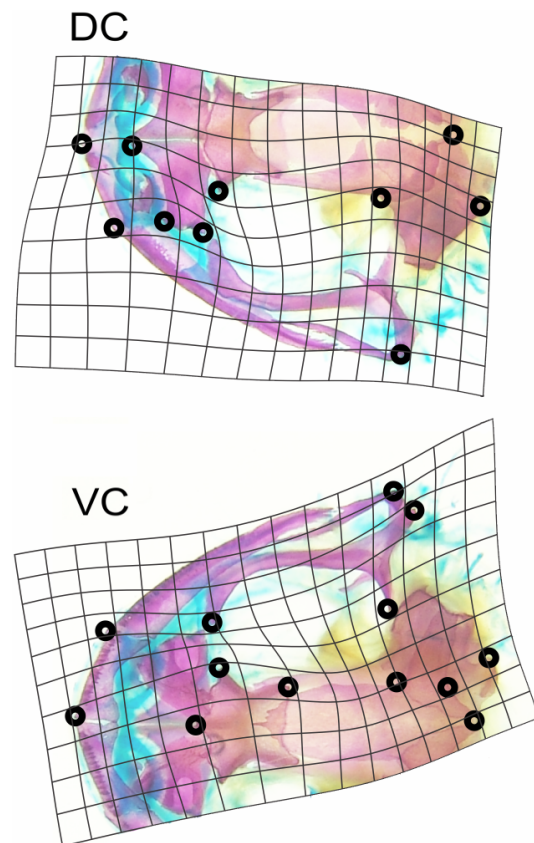


Figure 2. Cranium shape changes associated with static allometry in the yellow-bellied toad from smaller to larger centroid size.

parison of mean shapes after removing the effect of size on shape showed statistically significant differences in the dorsal and ventral cranium between the sexes (permutation test of pairwise Procrustes distances: dorsal cranium $P < 0.01$, ventral cranium $P < 0.01$). The quantitative index of non-allometric sexual dimorphism in cranial shape, Procrustes distances between mean dorsal and ventral cranial shape for males and females are given in Table 1 (see $I_{\text{SShD}}(\text{Stand.})$).

Non-allometric shape changes in the dorsal cranium between males and females were caused by displacement of landmarks on the squamosal (landmark 4), prootic (landmarks 1, 10), frontoparietal (landmark 3) and nasal (landmarks 6, 7). Males had a wider and shorter posterior part of the cranium with the lateral nasal displaced posteriorly (Fig. 3).

Non-allometric shape changes in the ventral cranium between males and females were caused by displacement of landmarks on the quadratojugal (landmark 6), posterior and anterior pterygoid processes (landmarks 5, 9), occipital

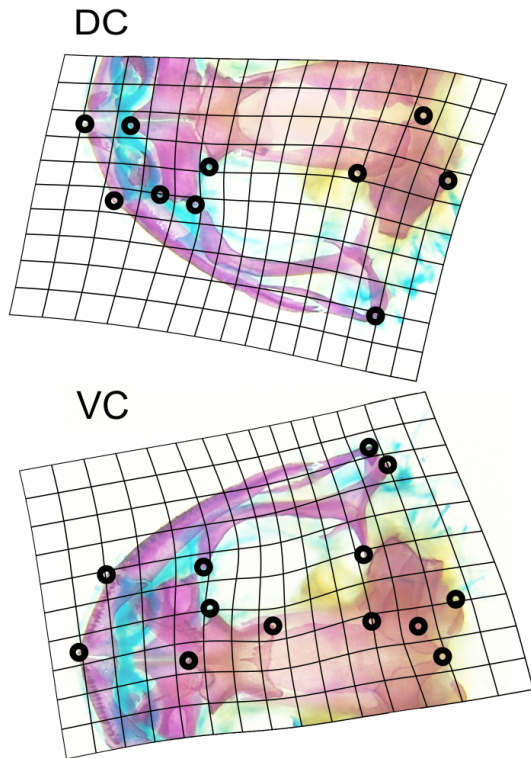


Figure 3. Cranium shape changes associated with non-allometric SShD between the sexes in the yellow-bellied toad (from females to males).

condyle (landmark 12), posterior part of the parasphenoid (landmark 1). Males had a wider posterior part of the cranium, shorter anterior pterygoid processes and displaced posterior part of the quadratojugal and pterygoid towards the snout (Fig. 3).

Discussion

Our results provide first insight into patterns of sexual dimorphism for adult cranial size and shape in the yellow-bellied toad (*B. variegata*). While no significant sex differences were observed for body size, cranial size and shape differences were statistically significant with a very small impact of allometry on shape differences. Thus, male toads have a slightly larger cranium than females at a given body size. More pronounced sexual divergence is present in the trophic part of the cranium where males have a wider but shorter posterior part.

Our analysis shows that differences in cranial size between the sexes in the yellow-bellied toad are consistent with other studies on head sexual size dimorphism in the same and closely related groups (e.g. anurans - Katsikaros & Shine 1997, lizards - Carpenter 1995, Herrel et al. 2001; snakes - Shine 1991, Andjelković et al. 2016). The most commonly observed dimorphism could be explained by adaptive processes and therefore by the influence of natural and/or sexual selection. Selection can act on overall body size, or a particular body part with specific features that might exhibit over-development in one sex independently of total size. Since male yellow-bellied toads had a larger cranium than females with no significant body size difference

between the sexes, the adaptive processes could be related directly to head dimensions and not overall body size.

A larger cranium in one sex is a result of sexual selection if head enlargement (relative to body size) has some advantage in social interaction, intraspecific combat or courtship. Moreover, natural selection can affect cranial size if head enlargement provides some advantage in consuming large prey, antipredator display, and sensory organ ability. Many studies in lizards and anurans have confirmed that increased male head size increases their bite force, which is advantageous in male-male combats and/or copulation with females (Carpenter 1995, Herrel et al. 2001, Katsikaros & Shine 1997). Studies of snakes connected divergence in head size between the sexes with divergence in feeding behavior, diet and differential prey selection between the sexes (Shine 1993, Vincent & Herrel 2007).

One factor most commonly involved in cranial size enlargement is male territorial behavior (e.g. Ljubisavljević et al. 2010). Breeding males of the yellow-bellied toad defend their territory during short-term spawning aggregations. Territorial males try to climb on the back of the intruder and eventually obtain a hold on the others back or around its legs. The loser emits release calls and rapidly swims away after being set free without direct male to male combat (Seidel, 1999). As they do not use just their head in some specific aggressive territorial behavior, the whole body size and not just relative head size could be an advantage in these intraspecific combats. Therefore, the larger cranium in *B. variegata* males found here probably cannot be explained only by male territorial behavior. Further, more detailed studies are needed to prove/disprove this.

On the other hand, the hypothesis of dietary divergence of the sexes is most often used to explain how an increase in the maximum size of prey might be reflected in an evolutionary increase in head size, which in the most cases is accompanied with head shape changes (Shine 1977). If dietary divergence in prey size is the main driver for cranium sexual dimorphism, the most pronounced differences should be in parts directly associated with feeding. And indeed our results revealed the most pronounced shape changes in the skull of the yellow bellied toad were in the part associated with feeding. The dorsal and ventral cranium show differences between the sexes mainly in trophic structures (associated with feeding) such as squamosal, quadratojugal, pterygoid, with small differences in non-trophic structures (making up the roof of the brain case and cranium) such as the prootic, frontoparietal, nasal, occipital and posterior part of the parasphenoid. In comparison to females males had the lateral part of the nasal displaced posteriorly, shorter anterior pterygoid processes and the posterior part of quadratojugal and pterygoid displaced towards the snout. That means that males have a wider but shorter posterior part of the cranium. Therefore, our study indicates that divergence in feeding behavior could be the driver for cranial sexual dimorphism in the yellow bellied toad. Indeed, males and females of this species differ in diet and feeding techniques. Males mostly use the so called "active foraging" feeding method, while females use both the "active foraging" and "sit and wait" techniques (Perry & Pianka 1997). However, diet analyses for the yellow-bellied toad are scarce (Ghiurcă & Zaharia 2005, Bisa et al. 2007), particularly those dealing

with niche divergence between the sexes (Sas et al. 2005). Although females consumed larger amounts of prey with greater mobility (Sas et al. 2005), prey size was not quantified. Therefore analysis of diet composition in parallel with cranial shape analysis should be performed to elucidate if prey size plays an important role in shaping the cranium of this species. However, additional aspects of interaction between individual and habitat could promote evolution of sexual dimorphism, for example ecological niche segregation, differential sensitivity of the sexes to ecological factors, phenotypic plasticity (Temeles et al. 2000, Jones et al. 2009, Stillwell & Fox 2007, but see Delph & Bell 2008). Unfortunately, more data are needed to elucidate the importance of these factors for variation in sexual size and shape, especially regarding the cranium.

Allometric analysis for *B. variegata* showed that only 7 % of cranial shape changes for both sexes were due to changes in size, with no difference in allometric slopes between males and females. Adult cranial shape differences followed with parallel allometric trajectories indicate that the development strategy for this species could be through early differentiation in cranial shape between the sexes (Sanger et al. 2013). Parallel growth trajectories point to the possible existence of a common developmental mechanism for each sex. During growth, yellow-bellied toads have larger but narrower nasals, more elongated maxillas, narrower frontoparietals, wider and longer sphenethmoid, elongated pterygoid processes and shortened posterior part of the cranium. The most pronounced changes are in the middle part of the skull (changes in nasal and sphenethmoid). Studies of allometric changes in anurans are important because this group goes through profound morphological changes during metamorphosis, and their skull is structurally repatterned from a larval skull, well suited for aquatic feeding and respiration, to a skull adapted to terrestrial life (Hanken & Summers 1988). Therefore, early and late cranial growth periods probably are not correlated and shapes could be stage specific. As allometric growth is one of the dominant but not deeply explored factors of morphological variation and an important source of diversification (Gould 1966, Webster & Zelditch 2005), more ontogenetic sampling and analyses of yellow-bellied toads would be a crucial step towards understanding the developmental origins of their cranial morphological variation. As cranial shapes differ between the sexes in the adult stage, it would be interesting to determine when during ontogeny males and females start to diverge. Different studies have shown that adult sexual size dimorphism in amphibians could be influenced by size at hatching, rate of growth, duration of the growth period, age at maturation, size-dependent survival (Monnet & Cherry 2002). Yellow-bellied toad adult males have a higher survival rate and a lower body size at maturation than females, but a similar age at maturation and longevity (Barandun et al. 1997). These life history traits could influence the observed cranial sexual dimorphism, but further analyses of complex relationships between life histories and cranial sexual dimorphism are necessary.

Although many studies describe and analyze different aspects of anuran morphology, data about cranial sexual size and shape dimorphism are scarce. That still keeps us far from large comparative studies and an ultimate ecological

understanding of sex-specific cranium evolution.

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References

- Acevedo, A.A., Lampo, M., Cipriani, R. (2016): The cane or marine toad, *Rhinella marina* (Anura, Bufonidae): two genetically and morphologically distinct species. *Zootaxa* 4103: 574-586.
- Andjelković, M., Tomović, L., Ivanović, A. (2016): Variation in skull size and shape of two snake species (*Natrix natrix* and *Natrix tessellata*). *Zoomorphology* 135: 243-253.
- Arantes, Í.D.C., Vasconcellos, M.M., Boas, T.C., Veludo, L.B., Colli, G.R. (2015): Sexual dimorphism, growth, and longevity of two toad species (Anura, Bufonidae) in a Neotropical Savanna. *Copeia* 103: 329-342.
- Arnold, S.J., Wade, M.J. (1984): On the measurement of natural and sexual selection: applications. *Evolution* 38: 720-734.
- Balogová, M., Nelson, E., Uhrin, M., Figurová, M., Ledecký, V., Zysk, B. (2015): No sexual dimorphism detected in digit ratios of the fire salamander (*Salamandra salamandra*). *Anatomical Record* 298: 1786-1795.
- Barandun, J., Reyer, H.U., Anholt, B. (1997): Reproductive ecology of *Bombina variegata*: aspects of life history. *Amphibia-Reptilia* 18: 347-355.
- Bell, R.C., Zamudio, K.R. (2012): Sexual dichromatism in frogs: natural selection, sexual selection and unexpected diversity. *Proceedings of the Royal Society B, Biological Sciences*: rspb20121609.
- Berns, C.M., Adams, D.C. (2013): Becoming different but staying alike: patterns of sexual size and shape dimorphism in bills of hummingbirds. *Evolutionary Biology* 40: 246-260.
- Bisa, R., Sfenthourakis, S., Fragedakis-Tsolis, S., Chondropoulos, B. (2007): Population density and food analysis of *Bombina variegata* and *Rana graeca* in mountainous riverine ecosystems of northern Pindos (Greece). *Journal of Biological Research-Thessaloniki* 8: 129-137.
- Bookstein, F., Schaefer, K., Prossinger, H., Seidler, H., Lieder, M., Stringer, C., Weber, G.W., Arsuaga, J.L., Slice, D.E., Rohlf, F.J., Recheis, W., Mariam, A.J., Marcus, L.F. (1999): Comparing frontal cranial profiles in archaic and modern homo by morphometric analysis. *Anatomical Record* 257: 217-224.
- Bookstein, F.L., Gunz, P., Mitteroecker, P., Prossinger, H., Schaefer, K., Seidler, H. (2003): Cranial integration in *Homo*: singular warps analysis of the midsagittal plane in ontogeny and evolution. *Journal of Human Evolution* 44: 167-187.
- Brunetti, A.E., Hermida, G.N., Luna, M.C., Barsotti, A.M., Jared, C., Antoniazzi, M.M., Rivera-Correa, M., Berneck, B.V., Faivovich, J. (2015): Diversity and evolution of sexually dimorphic mental and lateral glands in *Cophomantini treefrogs* (Anura: Hylidae: Hylinae). *Biological Journal of Linnean Society* 114: 12-34.
- Butler, M.A., Sawyer, S.A., Losos, J.B. (2007): Sexual dimorphism and adaptive radiation in *Anolis* lizards. *Nature* 447: 202-205.
- Cardini, A., Elton, S. (2007): Sample size and sampling error in geometric morphometric studies of size and shape. *Zoomorphology* 126: 121-134.
- Carpenter, G.C. (1995): Modeling dominance: the influence of size, coloration, and experience on dominance relations in tree lizards (*Urosaurus ornatus*). *Herpetological Monographs* 9: 88-101.
- Stillwell, R., Fox, C.W. (2009): Geographic variation in body size, sexual size dimorphism and fitness components of a seed beetle: local adaptation versus phenotypic plasticity. *Oikos* 118: 703-712.
- De Lisle, S.P., Rowe, L. (2015): Independent evolution of the sexes promotes amphibian diversification. *Proceedings of the Royal Society B, Biological Sciences*: rspb.2014.2213.
- Delph, L.F., Bell, D.L. (2008): A test of the differential-plasticity hypothesis for variation in the degree of sexual dimorphism in *Silene latifolia*. *Evolutionary Ecology Research* 10: 61-75.
- Di Cerbo, A.R., Biancardi, C.M. (2012): Are there real sexual morphometric differences in yellow-bellied toads (*Bombina* spp.; Bombinatoridae)? *Amphibia-Reptilia* 33: 171-183.
- Di Cerbo, A.R., Dino, M., Milesi, S., Biancardi, C.M. (2011): Long term monitoring of yellow-bellied toad populations in Italy. pp. 34-35. In: *Societas Europaea Herpetologica and Deutscher Herpetologentag (DGHT)* (eds.), Abstracts Book and Programme, 16th European Congress of Herpetology (SEH) (Luxembourg and Trier, 25-29 September 2011).
- Dingerkus, G., Uhler, L.D. (1977): Enzyme clearing of alcian blue stained whole small vertebrates for demonstration of cartilage. *Stain Technology* 52: 229-232.

- Dryden, I.L., Mardia K.V. (1998): Statistical Shape Analysis, Wiley, Chichester, UK.
- Emerson, S.B. (1985): Skull shape in frogs: correlations with diet. *Herpetologica* 41: 177-188.
- Emerson, S.B., Bramble, D.M. (1993): Scaling, allometry, and skull design. pp. 384-421. In: Hanken, J., Hall, B.K. (eds.), *The skull, Functional and Evolutionary Mechanisms*. The University of Chicago Press, Chicago.
- Fabrezi, M. (2006): Morphological evolution of Ceratophryinae (Anura, Neobatrachia). *Journal of Zoological Systematics and Evolutionary Research* 44: 153-166.
- Ghiurcă, D., Zaharia, L. (2005): Data regarding the trophic spectrum of some population of *Bombina variegata* from Bacău County. *North-Western Journal of Zoology* 1: 15-24.
- Gittleman, J.L., Valkenburgh, B.V. (1997): Sexual dimorphism in the canines and skulls of carnivores: effects of size, phylogeny, and behavioural ecology. *Journal of Zoology* 242: 97-117.
- Gould, S.J. (1966): Allometry and size in ontogeny and phylogeny. *Biological Review* 41: 587-640.
- Han, X., Fu J.Z. (2013): Does life history shape sexual size dimorphism in anurans? A comparative analysis. *BMC Evolutionary Biology* 13:1-11.
- Hanken, J., Hall, B.K. (1987): Skull development during anuran metamorphosis. II. Role of thyroid hormone in osteogenesis. *Anatomy and Embryology* 178: 219-227.
- Hanken, J., Hall, B.K. (1988): Skull development during anuran metamorphosis: I. Early development of the first three bones to form—the occipital, the parasphenoid, and the frontoparietal. *Journal of Morphology* 195: 247-256.
- Hanken, J., Summers, C.H. (1988): Skull development during anuran metamorphosis: III. Role of thyroid hormone in chondrogenesis. *Journal of Experimental Zoology* 246: 156-170.
- Harrington, S.M., Harrison, L.B., Sheil, C.A. (2013): Ossification sequence heterochrony among amphibians. *Evolution & Development* 15: 344-364.
- Hasumi, M. (2010): Age, body size, and sexual dimorphism in size and shape in *Salamandrella keyserlingii* (Caudata: Hynobiidae). *Evolutionary Biology* 37: 38-48.
- Herrel, A., de Grauw, E., Lemos-Espinal, J.A. (2001): Head shape and bite performance in xenosaurid lizards. *Journal of Experimental Zoology* 290: 101-107.
- Herrel, A., Gonwouo, L.N., Fokam, E.B., Ngundu, W.I., Bonneaud, C. (2012): Intersexual differences in body shape and locomotor performance in the aquatic frog, *Xenopus tropicalis*. *Journal of Zoology* 287: 311-316.
- Ivanović, A., Kalezić, M.L. (2012): Sexual dimorphism in the skull geometry of newt species of *Ichthyosaura*, *Triturus* and *Lissotriton* (Salamandridae, Caudata, Amphibia). *Zoomorphology* 131: 69-78.
- Ivanović, A., Sotiropoulos, K., Furtula, M., Džukić, G., Kalezić, M.L. (2008): Sexual size and shape evolution in European newts (Amphibia: Caudata: Salamandridae) on the Balkan Peninsula. *Journal of Zoological Systematics and Evolutionary Research* 46: 381-387.
- Jones, K.S., Nakagawa, S., Sheldon, B.C. (2009): Environmental sensitivity in relation to size and sex in birds: meta-regression analysis. *The American Naturalist* 174: 122-133.
- Kalontzopoulou, A., Carretero, M.A., Llorente, G.A. (2008): Head shape allometry and proximate causes of head sexual dimorphism in *Podarcis* lizards: joining linear and geometric morphometrics. *Biological Journal of Linnean Society* 93: 111-124.
- Katsikaros, K., Shine, R. (1997): Sexual dimorphism in the tusked frog, *Adelotus brevis* (Anura: Myobatrachidae): the roles of natural and sexual selection. *Biological Journal of Linnean Society* 60: 39-51.
- Klingenberg, C.P. (2011): MorphoJ: an integrated software package for geometric morphometrics. *Molecular Ecology Resources* 11: 353-357.
- Klingenberg, C.P. (2016): Size, shape, and form: concepts of allometry in geometric morphometrics. *Development Genes and Evolution* 226: 113-137.
- Kuo, C.Y., Lin, Y.T., Lin, Y.S. (2009): Sexual size and shape dimorphism in an Agamid lizard, *Japalura swinhonis* (Squamata: Lacertilia: Agamidae). *Zoological Studies* 48: 351-361.
- Kupfer, A. (2007): Sexual size dimorphism in amphibians: an overview. pp. 50-59. In: Fairbairn, D.J., Blanckenhorn, W.U., Székely, T. (eds.), *Sex, Size and Gender Roles: Evolutionary Studies of Sexual Size Dimorphism*. Oxford University Press, Oxford.
- Larson, P.M. (2000): Chondrocranial development in larval *Rana sylvatica* (Anura: Ranidae): morphometric analysis of cranial allometry and ontogenetic shape change. *Journal of Morphology* 252: 131-144.
- Lee, J.C. (2001): Evolution of a secondary sexual dimorphism in the toad, *Bufo marinus*. *Copeia* 2001: 928-935.
- Leininger, E.C., Kelley, D.B. (2015): Evolution of courtship songs in *Xenopus*: Vocal Pattern Generation and Sound Production. *Cytogenetic and Genome Research* 145: 302-314.
- Liao, W.B., Chen, W. (2012): Inverse Rensch's rule in a frog with female-biased sexual size dimorphism. *Naturwissenschaften* 99: 427-431.
- Ljubisavljević, K., Urošević, A., Aleksić, I., Ivanović, A. (2010): Sexual dimorphism of skull shape in a lacertid lizard species (*Podarcis* spp., *Dalmatolacerta* sp., *Dinarolacerta* sp.) revealed by geometric morphometrics. *Zoology* 113: 168-174.
- Malmgren, J.C., Thollesson, M. (1999): Sexual size and shape dimorphism in two species of newts, *Triturus cristatus* and *T. vulgaris* (Caudata: Salamandridae). *Journal of Zoology* 249: 127-136.
- Mendelson, J.R., Silva, H.R., Maglia, A.M. (2000): Phylogenetic relationships among marsupial frog genera (Anura: Hylidae: Hemiphractinae) based on evidence from morphology and natural history. *Zoological Journal of the Linnean Society* 128: 125-148.
- Monnet, J.M., Cherry, M.I. (2002): Sexual size dimorphism in anurans. *Proceedings of the Royal Society of London B: Biological Sciences* 269: 2301-2307.
- Morris, J.S., Carrier, D.R. (2016): Sexual selection on skeletal shape in Carnivora. *Evolution* 70: 767-780.
- Moss, M.L., Young, R.W. (1960): A function approach to craniology. *American Journal of Physical Anthropology* 18: 281-292.
- Perry, G., Pianka, E.R. (1997): Animal foraging: past, present and future. *Trends in Ecology and Evolution* 12: 360-364.
- Porobić, J., Čirović, D., Jojić, V. (2016): Cranial variability of the Serbian golden jackal: geographic variation, sexual dimorphism and allometry. *Zoologischer Anzeiger* 261: 38-47.
- Radojičić, J.M., Cvetković, D.D., Tomović, L.M., Džukić, G., Kalezić, M.L. (2002): Sexual dimorphism in fire-bellied toads *Bombina* spp. from the central Balkans. *Folia Zoologica* 51: 129-140.
- Reinhard, S., Kupfer, A. (2015): Sexual dimorphism in a French population of the marbled newt *Triturus marmoratus* (Urodela: Salamandridae). *Salamandra* 51: 121-128.
- Roček Z. (1999): Developmental story of the anuran skull: does it provide any phylogenetic information? pp.35-50. In: Mиаud, C., Guyétant, R. (eds.), *Current Studies in Herpetology*. SEH 1998, Le Bourget du Lac, France.
- Rohlf, F.L. (2008): tpsDig version 2.11. SUNY, Stony Brook, NY.
- Sanger, T.J., Sherratt, E., McGlothlin, J.W., Brodie, E.D., Losos, J.B., Abzhanov, A., 2013. Convergent evolution of sexual dimorphism in skull shape using distinct developmental strategies. *Evolution* 67: 2180-2193.
- Sas, I., Covaciu-Marcov, S.D., Cușța, D., Cicort-Lucaciu, A.Ș., Popa, L. (2005): Food analysis in adults (males/females) and juveniles of *Bombina variegata*. *Analele Științifice ale Universității „Al. I. Cuza” Iași, s. Biologie Animală* 51: 169-177.
- Schoch, R.R. (2006): Skull ontogeny: developmental patterns of fishes conserved across major tetrapod clades. *Evolution & Development* 8: 524-536.
- Seidel, B. (1999): Water-wave communication between territorial male *Bombina variegata*. *Journal of Herpetology* 33: 457-462.
- Shine, R. (1977): Habitats, diets, and sympatry in snakes: a study from Australia. *Canadian Journal of Zoology* 55: 1118-1128.
- Shine, R. (1979): Sexual selection and sexual dimorphism in the Amphibia. *Copeia* 1979: 297-306.
- Shine, R. (1989): Ecological causes for the evolution of sexual dimorphism: a review of the evidence. *Quarterly Review of Biology* 64: 419-461.
- Shine, R. (1991): Intersexual dietary divergence and the evolution of sexual dimorphism in snakes. *American Naturalist* 138: 103-122.
- Shine, R. (1993): Sexual dimorphism in snakes. pp.49-86. In: Seigel, R.A., Collins, J.T., (eds), *Snakes: Ecology and Behavior*. McGraw-Hill, New York.
- Smirnov, S.V. (2011): Additional dermal ossifications in the anuran skull: morphological novelties or archaic elements? *Russian Journal of Herpetology* 4: 17-27.
- Temeles, E.J., Pan, I.L., Brennan, J.L., Horwitt, J.N. (2000): Evidence for ecological causation of sexual dimorphism in a hummingbird. *Science* 289: 441-443.
- Vera, M.C., Ponssa, M.L. (2014): Skeletogenesis in anurans: cranial and postcranial development in metamorphic and postmetamorphic stages of *Leptodactylus bufonius* (Anura: Leptodactylidae). *Acta Zoologica* 95: 44-62.
- Vincent, S.E., Herrel, A. (2007): Functional and ecological correlates of ecologically-based dimorphisms in squamate reptiles. *Integrative and Comparative Biology* 47:172-188.
- Webster, M., Zelditch, M.L. (2005): Evolutionary modifications of ontogeny: heterochrony and beyond. *Paleobiology* 31: 354-372.
- Weisbecker, V., Mitgutsch, C. (2010): A large - scale survey of heterochrony in anuran cranial ossification patterns. *Journal of Zoological Systematics and Evolutionary Research* 48: 332-347.
- Yildirim, E., Kaya, U. (2016): Patterns of cranial development in larval *Rana macrocnemis*: chondrocranial size and shape relationship with *Pelophylax bedriagae* (Anura: Ranidae). *Anatomical Record* 299: 711-721.
- Yildirim, E., Kaya, U. (2017): Chondrocranial differences in *Bufo variabilis* (Anura: Bufonidae): geometric morphometric comparison with two anuran species. *Turkish Journal of Zoology* 41: 241-249.
- Zelditch, M.L., Swiderski, D., Sheets, D., Fink, W.L. (2004): *Geometric morphometrics for biologists: a primer*. Elsevier, London.