

## SEXUAL SHAPE DIMORPHISM IN *BUFO VERRUCOSISSIMUS* (PALLAS, 1814) FROM LAKE BORÇKA KARAGÖL, TÜRKİYE

CANTEKIN DURSUN\*, NURHAYAT ÖZDEMİR and SERKAN GÜL

*Department of Biology, Faculty of Arts and Sciences, Recep Tayyip Erdogan University,  
Rize, 53100, Türkiye*

*E-mail: cantekin.dursun@erdogan.edu.tr; <https://orcid.org/0000-0001-7766-1470>  
(corresponding author)*

*E-mail: nurhayat.ozdemir@erdogan.edu.tr; <https://orcid.org/0000-0002-3880-5846>*

*E-mail: serkan.gul@erdogan.edu.tr; <https://orcid.org/0000-0002-0372-7462>*

Sexual dimorphism, i.e., differences in morphology between sexes of a species caused by intersexual differences in the strength or direction of sexual or natural selection, is prevalent throughout the animal kingdom. Contrary to differences in body size, little is known about sexual shape dimorphism in anurans. Here we investigated intersexual shape differences in *Bufo verrucosissimus* (Pallas, 1814) by taking 27 linear measurements to characterize body morphology of 23 males and 23 females. Relative to their overall body size, males had longer limbs and larger eyes, while females displayed larger parotoid glands and had wider heads. We speculate that these intersexual differences may have arisen due to differences in natural and sexual selection regimes acting on males and females, especially during foraging, mate searching, or intrasexual competition.

Key words: Anura, Bufonidae, PCA, morphometry.

### INTRODUCTION

Sexual dimorphism is a widespread phenomenon in the animal kingdom and refers to significant morphological differences between males and females of the same species such as in body size, shape, and colour (SHINE 1989, ANDERSSON 1994, MORI *et al.* 2017). The occurrence of sexual dimorphism can be caused by different selection pressures due to sexual or natural selection. It is generally explained by one of three main hypotheses (PINCHEIRA-DONOSO *et al.* 2017, SHUKER *et al.* 2021). The intrasexual competition hypothesis states that sexual selection favours males exhibiting larger body sizes or higher character values in specific morphologies that provide them with advantages in male-male competition and in territorial defence. In contrast, the fecundity selection hypothesis suggests that there is selection for larger females or females exhibiting enlarged reproductive organs because they can produce more offspring as they have more volume for eggs or developing embryos and can invest more energy into reproduction. Finally, the niche segregation hypothesis suggests that niche divergence between individuals of different sexes can result in shape differences via natural selection.

© Authors

Published by the Hungarian Natural History Museum  
and the Biological Section of the Hungarian Academy of Sciences.  
The journal uses a CC BY-NC license.

The difference in body size or mass between the sexes is referred to as sexual size dimorphism “SSD”, while intersexual differences in other quantitative morphological traits such as colour represent sexual shape dimorphism “SShD” (FAIRBAIRN 1997, FAIRBAIRN *et al.* 2007, BERNS 2013). SSD arising due to sexual or natural selection is a widely studied pattern in sexually reproducing animals (PARKER 1992, ISAAC 2005, COX *et al.* 2007, PINCHEIRA-DONOSO *et al.* 2021). It is noted that the analysis of shape dimorphism can contribute to the understanding of reproductive traits, enhanced fitness, and adaptation to ecological conditions (OLSSON *et al.* 2002, BUTLER & LOSOS 2002, ENGLMAIER *et al.* 2022, LI *et al.* 2022).

In anurans, sexual dimorphism is prevalent in size and shape and is affected by intersexual differences in life-history traits such as age at sexual maturity and growth rate. In anurans, most species studied (~90%) are known to have a female-biased SSD (SHINE 1979). Ultimately, this general trend is interpreted as a consequence of selection acting to increase female fecundity (WOOLBRIGHT 1983, MONNET & CHERRY 2002, SILVA *et al.* 2020), but, proximately, it can be attributed to intersexual differences in life-history traits such as growth rate, lifespan, or age at maturity (HAN & FU 2013; ALAEI *et al.* 2021, MARANGONI *et al.* 2021). Conversely, selective forces may favour larger body sizes in males by increasing reproductive success if larger males are preferred by females or if larger male have an advantage in male-male competition (YU *et al.* 2010, HUDSON & FU 2013). In contrast to numerous SSD studies, less attention has been paid to SShD in anurans. Recently, however, the number of studies investigating body shape dimorphism has increased to describe intersexual differences (DI CERBO & BLANCARDI 2012, MAGALHÃES *et al.* 2016, DE OLIVERA-LÓPEZ *et al.* 2021, SANCHES *et al.* 2022). Morphological traits exhibiting SShD may result from differences in selection pressures acting on females and males, and they may be useful in the identification of sex. On the other hand, studying SShD is useful for revealing hidden variation and phenotypic responses to environmental changes. Finally, testing for body shape differences between sexes can provide starting points for the study of reproductive traits, ecological roles, and adaptive mechanisms.

The Caucasian toad *Bufo verrucosissimus* (Pallas, 1814) is distributed throughout Caucasia including Azerbaijan, Georgia, south-western Russia, and north-eastern Türkiye, as well as in Lebanon and the southern shores of Türkiye (RECUERO *et al.* 2012, JABLONSKI & SADEK 2019, ÖZDEMİR *et al.* 2020). The species has become the subject of numerous morphological studies, and some of them have indicated the presence of female-biased SSD (ORLOVA & TUNIYEV 1989, TARKHNISHVILI 1994, KIDOV *et al.* 2015; KIDOV *et al.* 2017, DURSUN & ÖZDEMİR 2022) as reported in other *Bufo* toads (CVETKOVIĆ *et al.* 2005, ARNTZEN *et al.* 2013, JAVANBAKHT *et al.* 2022). However, little is known about SShD

in *B. verrucosissimus* (ÜZÜM *et al.* 2021). Here we comprehensively investigated SShD in Caucasian toads by taking linear measurements of a large number of different body parts and controlling for overall body size.

## MATERIAL AND METHODS

We used the morphological dataset recorded in our previous studies (ÖZDEMİR *et al.* 2020, DURSUN & ÖZDEMİR 2022), including 46 adult *B. verrucosissimus* individuals (23 males and 23 females) sampled from Lake Borçka Karagöl, Artvin, Türkiye. Fieldwork was done during the breeding season. We followed previous studies to design the analytic framework (XIONG *et al.* 2016a,b, DURSUN *et al.* 2022). The sex determination of adults was done following the presence of nuptial pads in males, and their absence in females. We obtained measurements of 27 different variables from right side (for a representative figure see CASTELLANO & GIACOMA 1998) using a digital calliper to the nearest 0.01 mm: snout-vent length (SVL), length of the head (LHEAD), width of the head (WHEAD), minimum distance between the nostrils (INTNOS), distance between the nostril and the tip of the snout (NOSTIP), minimum distance from the nostril to the anterior corner of the eye (NOSEYE), eye-tympanum distance (EYETYM), horizontal diameter of the eye (DEYE), diameter of the tympanum (DTYM), length of the parotoid gland (LPAR), distance between the elbows with humerus kept perpendicular to the body axis (WGRASP), radioulna length (RADUL), length of the hand (LHAND), length of the first finger (L1FING), length of the femur (LFEM), length of the tibia (LTIB), length of the tarsus (LTARS), length of the foot (LFOOT), minimum distance from the distal extreme of the inner metatarsal tubercle and the web between the third and fourth digit (WEB), length of the metatarsal tubercle (LMET), interorbital distance (IOD), distance between the anterior parts of parotoids (PDA), distance between the posterior parts of parotoids (PDP), left parotoid width (LPW), right parotoid width (RPW), length of the inner metatarsal tubercle (LIMT), and width of the inner metatarsal tubercle (WIMT).

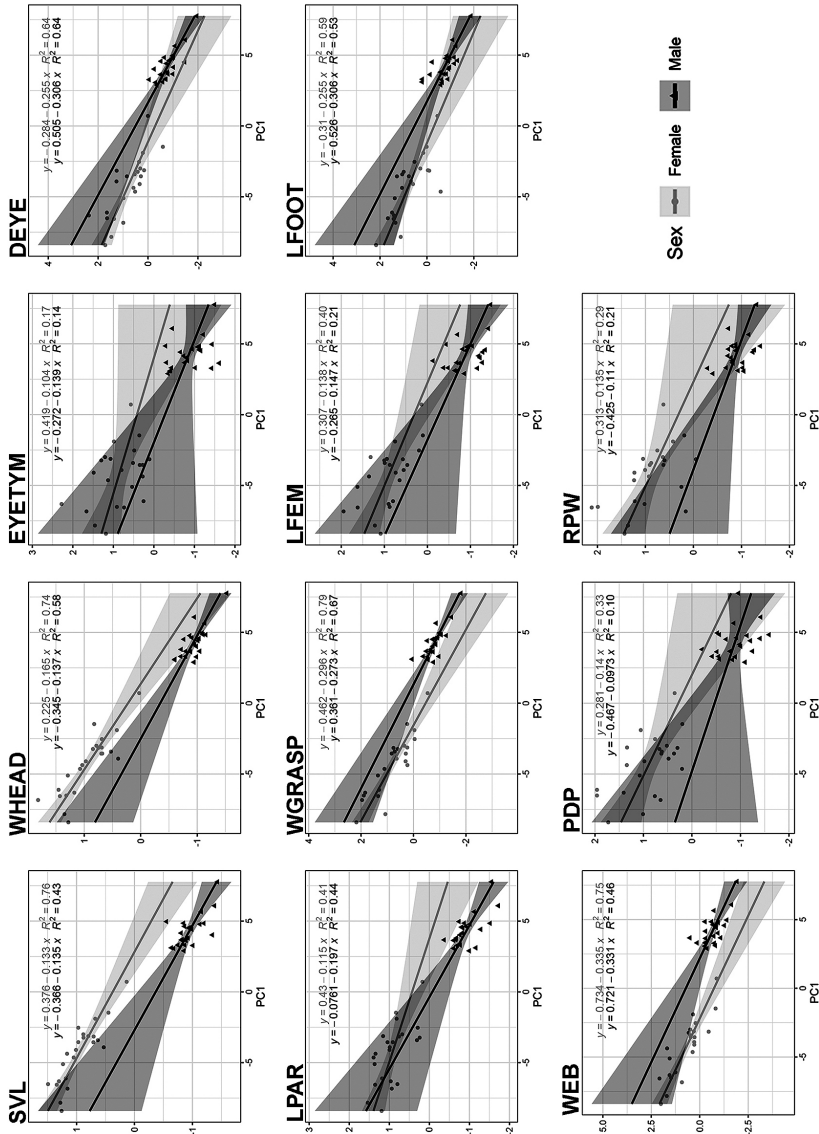
All statistical analyses were executed in R Programming Language (R CORE TEAM 2022). Descriptive statistics were calculated using the package *psych* (REVELLE 2021). The original dataset was z-transformed to minimize distortion effects using the package *clusterSim* (WALESIK & DUDEK 2020). We assessed adherence to the normality assumption using the Kolmogorov-Smirnov test in the package *olsrr* (HEBBALI 2020). The homogeneity of variances among z-transformed variables was verified using Levene's test in the package *car* (FOX & WEISBERG 2019). We performed a Principal component analysis on size measures and saved PC1 scores as measures of overall body size. The PCA returned two principal components with Eigenvalues >1. Subsequently, we ran ANCOVA analyses using the "lm" and "Anova" functions and a type III error. PCA and ANCOVA analyses were carried out using *stats* package (R CORE TEAM 2021). We entered PC1 scores as a covariate, sex as a factor, and z-transformed values as dependent variables. We also entered the interaction between sex and PC1 scores to test for homogeneity of regression slopes. The homogeneity of variances was supported by Levene's test in all variables ( $P > 0.05$ ). To assess the direction of sexual shape dimorphism we used post-hoc tests while applying Bonferroni correction for multiple testing in the package *rstatix* (KASSAMBARA 2020). We visualized results using the package *ggplot2* (WICKHAM 2016).

## RESULTS

Medians and interquartile ranges of raw morphometric measurements are presented in Table 1. The first principal component (PC1) explained 80.44%, the second component (PC2) explained 5.00% of the variance. The interaction terms between sex and PC1 were non-significant (all  $P > 0.05$ ; Fig. 1),

**Table 1.** Descriptive statistics of raw morphometric measurements (mm). For explanations of abbreviations see Material and methods.

Variable	Females		Males	
	Median	Interquartile range	Median	Interquartile range
SVL	104.23	99.98–108.45	74.43	70.61–75.72
LHEAD	24.59	23.63–26.88	17.16	16.90–17.97
WHEAD	33.22	32.60–25.60	23.80	22.86–24.22
INTNOS	6.13	5.90–6.48	4.84	4.59–4.96
NOSTIP	3.64	3.34–4.01	3.25	2.68–3.64
NOSEYE	5.24	5.04–5.77	3.73	3.39–3.92
EYETYM	4.31	3.89–4.70	2.75	2.44–3.13
DEYE	9.00	8.66–9.97	7.30	7.01–7.63
DTYM	3.51	2.99–3.86	2.81	2.15–3.22
LPAR	22.97	22.16–23.87	15.92	14.76–16.40
WGRASP	76.91	72.24–82.90	62.60	60.36–64.52
RADUL	30.19	28.64–31.77	23.88	22.08–25.28
LHAND	28.50	25.66–30.10	20.28	19.17–20.78
L1FING	12.15	11.31–12.99	7.98	7.22–8.62
LFEM	41.99	40.66–44.14	30.85	28.83–32.24
LTIB	28.84	26.06–32.98	20.44	19.48–25.80
LTARS	24.64	23.07–26.38	18.60	17.45–19.60
LFOOT	41.43	37.90–43.03	33.72	32.70–34.82
WEB	23.70	23.11–26.70	20.24	19.63–21.84
LMET	4.31	3.75–5.09	3.07	2.65–3.41
IOD	12.79	12.35–13.40	8.49	8.00–9.27
PDA	23.70	22.25–25.34	16.57	15.77–17.43
PDP	34.20	32.65–38.09	23.94	22.39–26.00
RPW	8.29	7.78–8.82	5.33	5.05–5.85
LPW	8.17	7.20–7.82	5.15	4.89–5.46
LIMT	6.30	6.10–6.78	4.65	4.42–4.89
WIMT	3.53	3.24–3.75	2.65	2.44–2.74



**Fig. 1.** Relationships between body size and shape characters in males (triangles) and females (dots). Z-transformed character values are plotted against PC1 component scores used as estimates of overall body size. Only characters exhibiting significant sexual dimorphism are shown. Please note the similarity between sexes in regression slopes within characters and the varying direction of sexual shape dimorphisms. Shaded areas represent 95% confidence intervals

indicating no difference in regression slopes. Therefore, the interaction term was removed from the models. Results of ANCOVAs indicated that 11 out of 26 characters differed between sexes (all  $P < 0.05$  after Bonferroni-correction) (Table 2). Compared to females and relative to their overall body size, males

**Table 2.** The result of ANCOVA test is presented below. Significant sex-biased body shape differences were noted based on post-hoc test results. Abbreviations: F = female, M = male; n.s. = non-significant differences. (\*):  $P < 0.05$ ; (\*\*):  $P < 0.01$ ; (\*\*\*):  $P < 0.001$ .

Variable	ANCOVA		
	F-value	Significance level	Sex bias
SVL	$F_{1,43} = 29.305$	***	F
LHEAD	$F_{1,43} = 1.351$	n.s.	n.s.
WHEAD	$F_{1,43} = 12.077$	**	F
INTNOS	$F_{1,43} = 0.301$	n.s.	n.s.
NOSTIP	$F_{1,43} = 1.846$	n.s.	n.s.
NOSEYE	$F_{1,43} = 0.474$	n.s.	n.s.
EYETYM	$F_{1,43} = 4.558$	*	F
DEYE	$F_{1,43} = 5.391$	*	M
DTYM	$F_{1,43} = 3.631$	n.s.	n.s.
LPAR	$F_{1,43} = 9.626$	**	F
WGRASP	$F_{1,43} = 13.678$	**	M
RADUL	$F_{1,43} = 1.881$	n.s.	n.s.
LHAND	$F_{1,43} = 2.399$	n.s.	n.s.
L1FING	$F_{1,43} = 0.341$	n.s.	n.s.
LFEM	$F_{1,43} = 4.520$	*	F
LTIB	$F_{1,43} = 2.731$	n.s.	n.s.
LTARS	$F_{1,43} = 1.403$	n.s.	n.s.
LFOOT	$F_{1,43} = 4.474$	*	M
WEB	$F_{1,43} = 19.017$	***	M
LMET	$F_{1,43} = 0.635$	n.s.	n.s.
IOD	$F_{1,43} = 0.800$	n.s.	n.s.
PDA	$F_{1,43} = 0.322$	n.s.	n.s.
PDP	$F_{1,43} = 4.110$	*	F
RPW	$F_{1,43} = 5.145$	*	F
LPW	$F_{1,43} = 0.329$	n.s.	n.s.
LIMT	$F_{1,43} = 0.405$	n.s.	n.s.
WIMT	$F_{1,43} = 1.074$	n.s.	n.s.

had longer back foot and webbings, larger eyes, and a larger distance between fore elbows, while females have a larger body, wider head proportion, and parotoid characteristics.

## DISCUSSION

This study documents that male and female *B. verrucosissimus* differ in their body shape. Our results demonstrated that the sexes differed in head width. In anurans, the dimension of head parts can be related to body size (VITT & COOPER 1985) as we observed in the eye-tympanum distance. Previous studies suggested that intersexual differences in size and shape may be associated with variation in dietary preferences in bufonids (CRNOBRNJA-ISAILOVIĆ *et al.* 2012, DURSUN *et al.* 2022). CRNOBRNJA-ISAILOVIĆ *et al.* (2012) also emphasized that *B. bufo* males have a higher proportion of small prey items in their dietary preferences than females. Therefore, we propose that variation in dietary composition may be a reason for the observed intersexual divergence in head width, as reported in several Anuran species (ARANTES *et al.* 2015, VUKOV *et al.* 2018; RAČKOVIĆ *et al.* 2019; ZHANG *et al.* 2020b). It may also influence mating success, i.e., an elongated snout shape may help males dislodge rivals from the backs of females (MOZAFFARI & MOGHARI 2012).

We found that males had larger eyes relative to their body size than females. HUANG *et al.* (2019) did a comparative study where they studied eye size in anurans and found that it was highly correlated with body mass, while other factors had only limited effects. However, BURROWES (2000) studied sexual selection in *Eleutherodactylus cooki* and showed that the larger eye of males enhances their visual capability and can provide advantages in the form of enhanced mating success. ANDERSSON (1994) observed that males of species with mating systems ruled by scramble competition have larger eyes to increase mobility and locate females. Therefore, the divergence in eye size may be a result of sexual selection.

We revealed male-biased SShD in the hindlimb-relevant variables. LEHTINEN (2003) suggested that larger male hindlimbs in Mantellid frogs contribute to male-male competition abilities and faster locomotion. PETROVIĆ *et al.* (2017) emphasized that Anuran males with relatively longer hindlimbs have an advantage during migration. ZHIPING (2013) stated that the mass of hindlimb muscles was larger in males than in females of *Bufo gargarizans*, and that this trait positively affected male mating success. Our results also indicated a significant difference in distance between the elbows with humerus kept perpendicular to the body axis. This dimorphic trait has been reported for several bufonid toads and is thought to increase amplexus success and dispersal distance during the breeding season (OKA *et al.* 1984, LEE 2001, CLARKE *et al.* 2019, DURSUN *et al.* 2022).



Females exhibited relatively longer femurs than males. Similarly, LO VALVO & GIACALONE (2008) biometrically analysed Sicilian green toad and they associated larger body parts with body size. QUIROGA *et al.* (2015) observed larger femurs in females of *Odontophrynus cf. barrioi* and suggested that this may have evolved as it is beneficial for females when moving eggs in ponds during amplexus. Therefore, the larger femur in *B. verrucosissimus* may contribute to moving egg chain in water.

The results of our study confirmed sexual dimorphism in parotoid size as revealed by the morphological characters parotoid length, right parotoid width and posterior distance between parotoids. This accords with results of HUDSON *et al.* (2021) who studied 3779 adult *Rhinella marina* individuals and showed that females had larger and more elongated parotoids. Similar results regarding female biased parotoid size dimorphism have also been documented in *Rhinella arenarum* (REGUEIRA *et al.* 2017) and *Bufo bufo* (BÓKONY *et al.* 2019, ZAMORA-CAMACHO 2022). CHEN *et al.* (2017) suggested that female *Rhinella marina* exhibiting larger bodies may be more visible to predators, hence their need for larger parotoids as a defensive adaptation. The observation that the relative width of the right parotoid differed between sexes, but the width of the left parotoid did not is in line with results of previous studies which reported size and width differences between right and left parotoids both between sexes and between genetic lineages of *B. verrucosissimus* (SINSCH *et al.* 2009, ÖZDEMİR *et al.* 2020). In addition, ARNTZEN *et al.* (2013) recorded highly variable parotoid size and shape characteristics between the sexes of *B. bufo* and *B. spinosus* distributed in Western Europe. Therefore, we think that the shape dimorphism of parotoids in *B. verrucosissimus* is similar to other *Bufo* taxa.

In this study, we found that females are larger than males. The generally accepted explanation for female-biased SSD in anurans is provided by the fecundity selection hypothesis: The large body size of females provides an advantage in terms of enhanced fecundity. This sex difference is mainly associated with reproductive traits such as clutch and egg sizes, number of offspring produced, and fertility rate as was previously reported for the genus *Bufo* (READING 1986, TARKHNISHVILI 1993, LIAO *et al.* 2015). Nonetheless, females may also be larger than males due to higher growth rates and delayed reproduction (MIAUD *et al.* 1999, VALDEZ & MANEYRO 2016). Although larger size usually benefits males competing for females (DAVIES & HALLIDAY 1978, VÁGI & HETTYEY 2016), being smaller can also be advantageous for males by resulting in higher mobilization capacity and agility (HUDSON *et al.* 2020, ZHANG *et al.* 2020a).

In summary, we document sexual shape dimorphism in *B. verrucosissimus* regarding the head, parotoid glands, hindlimbs, and forelimbs. To fur-



ther expand our knowledge of shape differences between sexes, the use of geometric morphometric applications will be of great help and may provide new insights also related to the variation found in this study.

\*

Acknowledgement – The data in this study was collected with the permission of the local ethics committee (Republic of Türkiye Recep Tayyip Erdogan University Local Ethics Committee for Animal Experiments, approval reference numbers: 2014/4 and 2019/9).

## REFERENCES

- ALAEI, R., PESARAKLOO, A., NAJIBZADEH, M. & MIRKAMALI, S. J. (2021): Life-history traits and the first demographic data of Iranian population of the West Asian Lemon-Yellow Tree Frog, *Hyla savignyi* (Audouin, 1827). – *Acta Zoologica Academiae Scientiarum Hungaricae* 67(3): 247–261. <https://doi.org/10.17109/AZH.67.3.247.2021>
- ANDERSSON, M. (1994): *Sexual selection*. – Princeton University Press, Princeton, New Jersey, 624 pp.
- 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(2): 329–342. <https://doi.org/10.1643/CH-14-092>
- ARNTZEN, J. W., McATEAR, J., RECUERO, E., ZIERMANN, J. M., OHLER, A., VAN ALPHEN, J. & MARTINEZ-SOLANO, I. (2013): Morphological and genetic differentiation of *Bufo* toads: two cryptic species in Western Europe (Anura, Bufonidae). – *Contributions to Zoology* 82(4): 147–169. <https://doi.org/10.1163/18759866-08204001>
- BERNS, C. M. (2013): The evolution of sexual dimorphism: understanding mechanisms of sexual shape differences. Pp. 1–16. In: MORIYAMA, H. (ed.): *Sexual dimorphism*. – In-Tech, Rijeka.
- BÓKONY, V., ÜVEGES, B., VEREBÉLYI, V., UJHEGYI, N. & MÓRICZ, Á. M. (2019): Toads phenotypically adjust their chemical defences to anthropogenic habitat change. – *Scientific Reports* 9(1): 1–8. <https://doi.org/10.1038/s41598-019-39587-3>
- BURROWES, P. A. (2000): Parental care and sexual selection in the Puerto Rican cave-dwelling frog, *Eleutherodactylus cooki*. – *Herpetologica* 56(3): 375–386.
- BUTLER, M. A. & LOSOS, J. B. (2002): Multivariate sexual dimorphism, sexual selection, and adaptation in Greater Antillean Anolis lizards. – *Ecological Monographs* 72(4): 541–559. [https://doi.org/10.1890/0012-9615\(2002\)072\[0541:MSDSSA\]2.0.CO;2](https://doi.org/10.1890/0012-9615(2002)072[0541:MSDSSA]2.0.CO;2)
- CASTELLANO, S. & GIACOMA, C. (1998): Morphological variation of the green toad, *Bufo viridis*, in Italy: a test of causation. – *Journal of Herpetology* 32(4): 540–550.
- CHEN, W., HUDSON, C. M., DEVORE, J. L. & SHINE, R. (2017): Sex and weaponry: The distribution of toxin storage glands on the bodies of male and female cane toads (*Rhinella marina*). – *Ecology and Evolution* 7(21): 8950–8957. <https://doi.org/10.1002/ece3.2914>
- CLARKE, G. S., SHINE, R. & PHILLIPS, B. L. (2019): May the (selective) force be with you: Spatial sorting and natural selection exert opposing forces on limb length in an invasive amphibian. – *Journal of Evolutionary Biology* 32: 994–1001. <https://doi.org/10.1111/jeb.13504>

- COX, R. M., BUTLER, M. A. & JOHN-ALDER, H. B. (2007): The evolution of sexual size dimorphism in reptiles. Pp. 38–49. *In*: FAIRBAIN, D., BLANCKENHORN, W. U. & SZÉKELY, T. (eds): *Sex, size and gender roles: evolutionary studies of sexual size dimorphism*. – Oxford University Press, Oxford.
- CRNOBRNJA-ISAILOVIĆ, J., ČURČIĆ, S., STOJADINOVIĆ, D., TOMAŠEVIĆ-KOLAROV, N., ALEKSIĆ, I. & TOMANOVIĆ, Ž. (2012): Diet composition and food preferences in adult common toads (*Bufo bufo*) (Amphibia: Anura: Bufonidae). – *Journal of Herpetology* **46**(4): 562–567. <https://doi.org/10.1670/10-264>
- CVETKOVIĆ, D. D., TOMAŠEVIĆ, N., ALEKSIĆ, I. D. & CRNOBRNJA-ISAILOVIĆ, J. M. (2005): Assessment of age and intersexual size differences in *Bufo bufo*. – *Archives of Biological Sciences* **57**(2): 157–162. <https://doi.org/10.2298/ABS0502157C>
- DAVIES, N. B. & HALLIDAY, T. R. (1978): Deep croaks and fighting assessment in toads *Bufo bufo*. – *Nature* **274**: 683–685. <https://doi.org/10.1038/274683a0>
- DE OLIVERA-LÓPEZ, V., CAMARGO, A. & MANEYRO, R. (2021): Morphometric differentiation and sexual dimorphism in *Limnomedusa macroglossa* (Duméril & Bibron, 1841) (Anura: Alsodidae) from Uruguay. *Acta Herpetologica* **16**(1): 11–25. [https://doi.org/10.36253/a\\_h-8955](https://doi.org/10.36253/a_h-8955)
- DI CERBO, A. R. & BIANCARDI, C. M. (2012): Are there real sexual morphometric differences in yellow-bellied toads (*Bombina* spp.; Bombinatoridae)? – *Amphibia-Reptilia* **33**(2): 171–183. <https://doi.org/10.1163/156853812X634062>
- DURSUN, C. & ÖZDEMİR, N. (2022): Morphological variability and age structure in a population of *Bufo verrucosissimus* (Anura: Bufonidae) from Artvin, Turkey. – *Phyllomedusa* **21**(1): 31–49. <https://doi.org/10.11606/issn.2316-9079.v21i1p31-49>
- DURSUN, C., GÜL, S. & ÖZDEMİR, N. (2022): Sexual size and shape dimorphism in Turkish common toads (*Bufo bufo* Linnaeus 1758). – *The Anatomical Record* **305**(6): 1548–1558. <https://doi.org/10.1002/ar.24801>
- ENGLMAIER, G. K., ANTONOV, A. & WEISS, S. J. (2022): General patterns of sexual dimorphism in graylings (*Thymallus*), with a comparison to other salmonid species. – *Reviews in Fish Biology and Fisheries* **32**(2): 645–667. <https://doi.org/10.1007/s11160-021-09694-4>
- FAIRBAIRN, D. J. (1997): Allometry for sexual size dimorphism: pattern and process in the coevolution of body size in males and females. – *Annual Review of Ecology and Systematics* **28**: 659–687. <https://doi.org/10.1146/annurev.ecolsys.28.1.659>
- FAIRBAIRN, D. J., BLANCKENHORN, W. U., & SZÉKELY, T. (2007): *Sex, size and gender roles: evolutionary studies of sexual size dimorphism*. – Oxford University Press, Oxford, 280 pp.
- FOX, J. & WEISBERG, S. (2019): *An R companion to applied regression*. – Thousand Oaks, Sage, CA, 608 pp.
- HAN, X. & FU, J. (2013): Does life history shape sexual size dimorphism in anurans? A comparative analysis. – *BMC Evolutionary Biology* **13**(1): 1–11. <https://doi.org/10.1186/1471-2148-13-27>
- HEBBALL, A. (2020): *olsrr*: tools for building OLS regression models. R package version 0.5.3. <https://CRAN.R-project.org/package=olsrr>
- HUANG, C. H., ZHONG, M. J., LIAO, W. B. & KOTRSCHAL, A. (2019): Investigating the role of body size, ecology, and behavior in anuran eye size evolution. – *Evolutionary Ecology* **33**(4): 585–598. <https://doi.org/10.1007/s10682-019-09993-0>
- HUDSON, C. M. & FU, J. (2013): Male-biased sexual size dimorphism, resource defense polygyny, and multiple paternity in the Emei moustache toad (*Leptobranchium borin-gii*). – *PloS One* **8**(6): e67502. <https://doi.org/10.1371/journal.pone.0067502>

- HUDSON, C. M., VIDAL-GARCÍA, M., MURRAY, T. G. & SHINE, R. (2020): The accelerating anuran: evolution of locomotor performance in cane toads (*Rhinella marina*, Bufonidae) at an invasion front. – *Proceedings of the Royal Society B* **287**(1938): 20201964. <https://doi.org/10.1098/rspb.2020.1964>
- HUDSON, C. M., BROWN, G. P., BLENNERHASSETT, R. A. & SHINE, R. (2021): Variation in size and shape of toxin glands among cane toads from native-range and invasive populations. – *Scientific Reports* **11**(1): 1–11. <https://doi.org/10.1038/s41598-020-80191-7>
- ISAAC, J. L. (2005): Potential causes and life-history consequences of sexual size dimorphism in mammals. – *Mammal Review* **35**(1): 101–115. <https://doi.org/10.1111/j.1365-2907.2005.00045.x>
- JABLONSKI, D. & SADEK, R. A. (2019): The Caucasian Toad, *Bufo verrucosissimus* (Pallas, 1814) in the Levant: evidence from mitochondrial DNA. – *Herpetozoa* **32**: 255–258. <https://doi.org/10.3897/herpetozoa.32.e37560>
- JAVANBAKHT, H., HEIDARI, N. & ALINEZHADI, I. (2022): Evaluation of sexual dimorphism on the basis of morphometric traits in Talysh toad, *Bufo eichwaldi* (Litvinchuk, Borkin, Skorinov & Rosanov, 2008), along with the evaluation of the conservation status of the species. – *Nova Biologica Reperta* **9**(1): 17–28. <https://doi.org/10.29252/nbr.9.1.17>
- KASSAMBARA, A. (2020): rstatix: pipe-friendly framework for basic statistical tests. R package version 0.6.0. <https://CRAN.R-project.org/package=rstatix>.
- KIDOV, A. A. & MATUSHKINA, K. A. (2015): Fecundity of female *Bufo caucasicus* *Bufo verrucosissimus* (Pallas, 1814) in vitro. – *Vestnik Buryatskogo gosudarstvennogo universiteta – Bulletin of Buryat State University* **4**: 75–80.
- KIDOV, A. A., MATUSHKINA, K. A. & AFRIN, K. A. (2017): To research expansion and variability of *Bufo caucasicus* *Bufo verrucosissimus* (Pallas, 1814) in Karachay-Cherkessia. – *Vestnik Tambovskogo universiteta – Bulletin of Tambov University. Natural and Engineering Sciences Series* **22**(5–1): 917–920.
- LEE, J. C. (2001): Evolution of a secondary sexual dimorphism in the toad, *Bufo marinus*. – *Copeia* **2001**(4): 928–935. [https://doi.org/10.1643/0045-8511\(2001\)001\[0928:EOASSD\]2.0.CO;2](https://doi.org/10.1643/0045-8511(2001)001[0928:EOASSD]2.0.CO;2)
- LEHTINEN, R. M. (2003): Parental care and reproduction in two species of *Mantidactylus* (Anura: Mantellidae). – *Journal of Herpetology* **37**(4): 766–768.
- LI, H., CHEN, S., JIANG, J., HE, B. & ZHANG, M. (2022): Exploring sexual differences in external morphology and limb muscles of *Hylarana guentheri* (Anura: Ranidae) during non-breeding season. – *Acta Zoologica* <https://doi.org/10.1111/azo.12448>
- LIAO, W. B., LIU, W. C. & MERILÄ, J. (2015): Andrew meets Rensch: sexual size dimorphism and the inverse of Rensch's rule in Andrew's toad (*Bufo andrewsi*). – *Oecologia* **177**(2): 389–399. <https://doi.org/10.1007/s00442-014-3147-8>
- LO VALVO, M. & GIACALONE, G. (2013): Biometrical analyses of a Sicilian green toad, *Bufo siculus* (Stöck et al. 2008), population living in Sicily. – *International Journal of Morphology* **31**: 681–686. <https://doi.org/10.4067/S0717-95022013000200055>
- MAGALHÃES, R. F., GARDA, A. A., MARQUES, N. C. S. & BRANDAO, R. A. (2016): Sexual dimorphism and resource utilisation by the Veadeiros waterfall frog *Bokermannohyla pseudopseudis* (Anura: Hylidae). – *Salamandra* **52**(2): 171–177.
- MARANGONI, F., TEJEDO, M. & COGĂLNICEANU, D. (2021): Can age and growth patterns explain the geographical variation in the body size of two toad species? – *Anais da Academia Brasileira de Ciências* **93**(2): e20190470. <https://doi.org/10.1590/0001-376520210190470>

- MIAUD, C., GUYÉTANT, R. & ELMBERG, J. (1999): Variations in life-history traits in the common frog *Rana temporaria* (Amphibia: Anura): a literature review and new data from the French Alps. – *Journal of Zoology* **249**(1): 61–73. <https://doi.org/10.1111/j.1469-7998.1999.tb01060.x>
- MONNET, J. M. & CHERRY, M. I. (2002): Sexual size dimorphism in anurans. – *Proceedings of the Royal Society of London. Series B: Biological Sciences* **269**(1507): 2301–2307. <https://doi.org/10.1098/rspb.2002.2170>
- MORI, E., MAZZA, G. & LOVARI, S. (2017): Sexual dimorphism. Pp. 1–7. In: VONK, J. & SHAKELFORD, T. (eds): *Encyclopedia of animal cognition and behavior*. – Springer International Publishing, Switzerland.
- MOZAFFARI, O. & MOGHARI, E. S. (2012): Sexual dimorphism in *Bufo eichwaldi*'s snout shape with description of its usage in male-male competition. – *Russian Journal of Herpetology* **19**(4): 349–351. <https://doi.org/10.30906/1026-2296-2012-19-4-349-351>
- OKA, Y., OHTANI, R., SATOU, M. & UEDA, K. (1984): Sexually dimorphic muscles in the forelimb of the Japanese toad, *Bufo japonicus*. – *Journal of Morphology* **308**: 297–308. <https://doi.org/10.1002/jmor.1051800310>
- OLSSON, M., SHINE, R., WAPSTRA, E., UJVARI, B. & MADSEN, T. (2002): Sexual dimorphism in lizard body shape: the roles of sexual selection and fecundity selection. – *Evolution* **56**(7): 1538–1542. <https://doi.org/10.1111/j.0014-3820.2002.tb01464.x>
- ORLOVA, V. F. & TUNIYEV, B. S. (1989). On the taxonomy of the Caucasian common toads belonging to the group *Bufo bufo verrucosissimus* (Pallas) (Amphibia, Anura, Bufonidae). – *Bjulleten' Moskovskogo Obscestva Ispytatelej Prirody Otdel biologiceskij* **94**(3): 13–24.
- ÖZDEMİR, N., DURSUN, C., ÜZÜM, N., KUTRUP, B. & GÜL, S. (2020): Taxonomic assessment and distribution of common toads (*Bufo bufo* and *B. verrucosissimus*) in Turkey based on morphological and molecular data. – *Amphibia-Reptilia* **41**(3): 399–411. <https://doi.org/10.1163/15685381-bja10009>
- PARKER, G. A. (1992): The evolution of sexual size dimorphism in fish. – *Journal of Fish Biology* **41**: 1–20. <https://doi.org/10.1111/j.1095-8649.1992.tb03864.x>
- PETROVIĆ, T. G., VUKOV, T. D. & KOLAROV, N. T. (2017): Sexual dimorphism in size and shape of traits related to locomotion in nine anuran species from Serbia and Montenegro. – *Folia Zoologica* **66**(1): 11–21. <https://doi.org/10.25225/fozo.v66.i1.a4.2017>
- PINCHEIRA-DONOSO, D. & HUNT, J. (2017): Fecundity selection theory: concepts and evidence. – *Biological Reviews* **92**(1): 341–356. <https://doi.org/10.1093/beheco/arab055>
- PINCHEIRA-DONOSO, D., HARVEY, L. P., GRATTAROLA, F., JARA, M., COTTER, S. C., TREGENZA, T. & HODGSON, D. J. (2021): The multiple origins of sexual size dimorphism in global amphibians. – *Global Ecology and Biogeography* **30**(2): 443–458. <https://doi.org/10.1111/geb.13230>
- QUIROGA, L. B., SANABRIA, E. A. & MARANGONI, F. (2015): Sexual size dimorphism and age in *Odontophrynus cf. barrioi* (Anura: Odontophrynidae) from the Monte Desert, Argentina. – *Journal of Herpetology* **49**(4): 627–632. <https://doi.org/10.1670/13-216>
- R CORE TEAM. (2022): R: A language and environment for statistical computing. R foundation for statistical computing, Vienna, Austria. <https://www.R-project.org/>.
- RAČKOVIĆ, J. K., KOLAROV, N. T. & VUKOV, T. (2019): The ventral cranial size and shape variation between males and females of European brown frogs: *Rana dalmatina*, *R. graeca* and *R. temporaria* (Anura, Amphibia). – *Bulletin of Natural Sciences Research* **9**(2): 6–11. <https://doi.org/10.5937/univtho9-21223>

- READING, C. J. (1986): Egg production in the common toad, *Bufo bufo*. – *Journal of Zoology* **208**(1): 99–107. <https://doi.org/10.1111/j.1469-7998.1986.tb04712.x>
- RECUERO, E., CANESTRELLI, D., VÖRÖS, J., SZABÓ, K., POYARKOV, N. A., ARNTZEN, J. W., W, CRNOBRNJA-ISAILOVIC, J., KIDOV, A. A., COGALNICEANU, D., CAPUTO, F. P., NASCETTI, G. & MARTÍNEZ-SOLANO, I. (2012): Multilocus species tree analyses resolve the radiation of the widespread *Bufo bufo* species group (Anura, Bufonidae). – *Molecular Phylogenetics and Evolution* **62**(1): 71–86. <https://doi.org/10.1016/j.ympev.2011.09.008>
- REGUEIRA, E., DÁVILA, C., SASSONE, A. G., O'DONOHUE, M. E. A. & HERMIDA, G. N. (2017): Post-metamorphic development of skin glands in a true toad: Parotoids versus dorsal skin. – *Journal of Morphology* **278**(5): 652–664. <https://doi.org/10.1002/jmor.20661>
- REVELLE, W. (2021): *psych: Procedures for Personality and Psychological Research*. – Northwestern University, Evanston, Illinois, USA.
- SANCHES, P. R., SANTANA, M. M., OLIVEIRA-SOUZA, A. E. & COSTA-CAMPOS, C. E. (2022): Sexual dimorphism in *Rhinella major* (Anura: Bufonidae) from eastern Brazilian Amazonia. – *North-Western Journal of Zoology* **18**(2): 214–217.
- SHINE, R. (1979). Sexual selection and sexual dimorphism in the Amphibia. – *Copeia* **1979**(2): 297–306. <https://doi.org/10.2307/1443418>
- SHINE, R. (1989): Ecological causes for the evolution of sexual dimorphism: a review of the evidence. – *The Quarterly Review of Biology* **64**(4): 419–461. <https://doi.org/10.1086/416458>
- SHUKER, D. M., & KVARNEMO, C. (2021). The definition of sexual selection. – *Behavioral Ecology* **32**(5): 781–794. <https://doi.org/10.1093/beheco/arab055>
- SINSCH, U., SCHNEIDER, H., TARKHNISHVILI, D. N. (2009): *Bufo bufo* Superspezies – Erdkröten-Artenkreis – taxon *bufo* (Linnaeus, 1758) – Erdkröte – taxon *gredosicola* L. Müller und Hellmich, 1935 – Gredoserdkröte – taxon *spinosus* Daudin, 1803 – Riesenerdkröte – taxon *verrucosissimus* (Pallas, 1811) – Kolchische Erdkröte. Pp. 191–335. In: GROSSENBACHER, K. (ed.). *Handbuch der Reptilien und Amphibien Europas. Froschlurche II*. – Aula Verlag, Wiebelsheim.
- SILVA, N. R., BERNECK, B. V., DA SILVA, H. R., HADDAD, C. F., ZAMUDIO, K. R., MOTT, T., NALI, R. C., & PRADO, C. P. (2020): Egg-laying site, fecundity and degree of sexual size dimorphism in frogs. – *Biological Journal of the Linnean Society* **131**(3): 600–610. <https://doi.org/10.1093/biolinnean/blaa126>
- TARKHNISHVILI, D. (1993): Anurans of Borjomi Canyon: clutch parameters and guild structure. – *Alytes* **11**(4): 140–154.
- TARKHNISHVILI, D. N. (1994): Breeding of the toad *Bufo verrucosissimus*: sexual dimorphism and shifting spawning sites. – *Amphibia-Reptilia* **15**(2): 191–198. <https://doi.org/10.1163/156853894X00290>
- ÜZÜM, N., ÖZDEMİR, N., DURSUN, C., KUTRUP, B. & GÜL, S. (2021): Interspecific and intraspecific size and shape variation in skull of two closely related species *Bufo bufo* (Linnaeus, 1758) and *Bufo verrucosissimus* (Pallas, 1814) from Turkey. – *Turkish Journal of Zoology* **45**(2): 91–101. <https://doi.org/10.3906/zoo-2009-8>
- VÁGI, B. & HETTYEY, A. (2016). Intraspecific and interspecific competition for mates: *Rana temporaria* males are effective satyrs of *Rana dalmatina* females. – *Behavioral Ecology and Sociobiology* **70**: 1477– 484.
- VALDEZ, V. & MANEYRO, R. (2016). Reproductive biology of *Odontophrynus americanus* females (Duméril & Bribon, 1841) (Anura, Cycloramphidae) from Uruguay. – *Pan-American Journal of Aquatic Sciences* **11**(3): 188–197.



- VITT, L. J. & COOPER JR, W. E. (1985): The evolution of sexual dimorphism in the skink *Eumeces laticeps*: an example of sexual selection. – *Canadian Journal of Zoology* **63**(5): 995–1002. <https://doi.org/10.1139/z85-148>
- VUKOV, T., KRSTIČIĆ, J., PETROVIĆ, T. & TOMAŠEVIĆ KOLAROV, N. (2018): Patterns of cranial sexual dimorphism in the yellow-bellied toad (*Bombina variegata*, Bombinatoridae). – *North-Western Journal of Zoology* **14**(1): 44–49.
- WALESIAK, M. & DUDEK, A. (2020). The choice of variable normalization method in cluster analysis. Pp. 325–340. In: SOLIMAN, K. S. (ed.): *Education excellence and innovation management: A 2025 vision to sustain economic development during global challenges*. – International Business Information Management Association, Sevilla.
- WICKHAM, H. (2016): *ggplot2: Elegant graphics for data analysis*. Springer-Verlag, New York, 276 pp.
- WOOLBRIGHT, L. L. (1983): Sexual selection and size dimorphism in anuran amphibia. – *The American Naturalist* **121**(1): 110–119. <https://doi.org/10.1086/284042>
- XIONG, J. L., LIU, X. Y., ZHANG, X. M., LI, M. Y. & MIN, Y. (2016a). Sexual dimorphism of the Jilin Clawed Salamander, *Onychodactylus zhangyapingi* (Urodela: Hynobiidae: Onychodactylinae) from Jilin Province, China. – *Asian Herpetological Research* **7**: 220–226. <https://doi.org/10.16373/j.cnki.ahr.150057>
- XIONG, J., LI, M., LIU, X. & ZHANG, X. (2016b). Sexual dimorphism in Yellow-Spotted Salamander, *Pseudohynobius flavomaculatus* (Urodela: Hynobiidae). – *Russian Journal of Herpetology* **23**(3): 235–238. <https://doi.org/1026-229620162303-0235>
- YU, B. G., ZHENG, R. Q., ZHANG, Y. & LIU, C. T. (2010): Geographic variation in body size and sexual size dimorphism in the giant spiny frog *Paa spinosa* (David, 1875) (Anura: Ranoidae). – *Journal of Natural History* **44**(27–28): 1729–1741. <https://doi.org/10.1080/00222931003632682>
- ZAMORA-CAMACHO, F. J. (2022): Sex and habitat differences in size and coloration of an amphibian's poison glands match differential predator pressures. – *Integrative Zoology* **17**(5): 764–776. <https://doi.org/10.1111/1749-4877.12597>
- ZHANG, F., YUAN, C. & FENG, A. S. (2020a): Female concave-eared torrent frogs prefer smaller males. – *Journal of Zoology* **311**(4): 239–245. <https://doi.org/10.1111/jzo.12775>
- ZHANG, L., SHENG, Y., YUAN, X., YU, F., ZHONG, X. & CHEN, X. (2020b): Sexual dimorphism in Scutigiger *boulengeri*, an endemic toad from the Tibetan Plateau. – *Animal Biology* **70**(4): 445–457. <https://doi.org/10.1163/15707563-bja10041>
- ZHIPING, M. I. (2013): Sexual dimorphism in the hindlimb muscles of the Asiatic toad (*Bufo gargarizans*) in relation to male reproductive success. – *Asian Herpetological Research* **4**(1): 56–61. <https://doi.org/10.3724/SP.J.1245.2013.00056>

Revised version submitted May 2, 2023; accepted June 1, 2023; published June 30, 2023