

A Geometric Morphometric Analysis for Ranid Taxonomy: Chondrocranial Shape Variation of Five Ranid Species (Anura: Ranidae)

Ranid Taksonomisinde Geometrik Morfometrik Analiz: Beş Ranid Türünün Kondrokranyal Şekil Varyasyonu (Anura: Ranidae)

Research Article

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ABSTRACT

A landmark-based geometric morphometrics of the dorsal view of the chondrocranium was analysed for 188 specimens to investigate the hypothesis of species shape and size differences among *Rana tavasensis*, *R. macrocnemis*, *Pelophylax ridibundus*, *P. bedriagae* and *P. caralitanus*. Canonical variate analysis (CVA) is applied to determine whether the shape and size differences in the chondrocranium of five ranids can be used to classify those at a taxonomic level. The result of CVA analysis shows that five ranid species examined here discriminate as mountain (*Rana* sp.) and water (*Pelophylax* sp.) frogs. In discriminant function analysis (DFA), each pair of species was analyzed and the results show that there is no interspecific shape difference among *Pelophylax* species. However, the interspecific shape variations in the cornua trabeculae, articular process of the palatoquadrate and processus muscularis quadrati, were present between *Rana* species (*R. macrocnemis* and *R. tavasensis*).

Key Words

Geometric morphometrics, *Rana*, *Pelophylax*, chondrocranium.

ÖZ

Kondrokraniumun dorsal görüntüsünün landmark işaretlemelerine dayalı geometrik morfometrik analizi, *Rana tavasensis*, *R. macrocnemis*, *Pelophylax ridibundus*, *P. bedriagae* ve *P. caralitanus* türlerinin arasındaki şekil ve boyut farklılıklarının hipotezini araştırmak için 188 örnekte analiz edildi. Kanonik varyans analizi beş ranid türünün şekil ve boyut farklılıklarının taksonomik seviyede sınıflandırmak için kullanılıp kullanılmayacağını belirlemek için uygulandı. Analiz sonucu gösterdi ki araştırılan beş ranid türü dağ (*Rana* sp.) ve su (*Pelophylax* sp.) kurbağaları olarak ayrıldı. Diskriminant analizinde, her tür çifti analizi edildi ve *Pelophylax* türleri arasında türlerarası bir farklılık gözlenmedi. Ancak, processus muscularis quadrati, palatokuadratin artikular process'i ve cornua trabeculae'daki türler arası şekilsel varyasyonlar *Rana* türleri (*R. macrocnemis* and *R. tavasensis*) arasında bulunmuştur.

Anahtar Kelimeler

Geometrik morfometrik, *Rana*, *Pelophylax*, kondrokranium.

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INTRODUCTION

The family Ranidae is one of the most diverse amphibian groups and contains 389 recognized species [1]. The taxonomy of this family has been investigated by several researchers [2-13]. However, there is still a gap in the literature about the shape change in the larval anuran chondrocranium. Although the use of geometric morphometrics in amphibian species has increased in recent years, detailed studies of geometric morphometrics in the larval anuran chondrocranium are received relatively limited attention [14-17]. Larson [15] described the interspecific variation of larval chondrocranial shape and allometry among six ranid species (*Rana sylvatica*, *R. catesbeiana*, *R. calamitans*, *R. palustris*, *R. pipiens* and *R. sphenoccephala*).

Morphological studies have attracted much attention because of their use in definition of species [15, 18-20]. Moreover, understanding the function of the morphological structures provides important data about the taxonomy, ecology and development of species. Taxonomy discriminate groups by using color patterns of species despite variations of the color patterns of species and bases greatly on morphology. Therefore, it may be sometimes problematic to describe species by relying on morphological features. Geometric morphometrics (GMM) is a technique that plays an important role in many biological studies. The most important advantage of GMM is to have comprehensive data about shape that is obtained from a set of landmarks [21]. Variations in body shape of species result in the ability that occupy habitats or reproduce successfully.

The genera *Rana* and *Pelophylax* are still unclear. Some researchers suggest that morphometric data

cannot be used to identify *P. ridibundus*. They also suggest that the taxonomic status of *P. bedriagae* needs more studies [22]. Moreover, morphologies of five ranids are highly similar in their genus and generally, there are no differences on coloration and morphological patterns in these morphs. Ranids examined in this study mostly live in sympatry. It makes these genera as an ideal model for investigating morphometric differences.

To the best of our knowledge, this is the first study that addresses the chondrocranial shape variations of the five ranids. In this study we aimed to: 1) compare dorsal chondrocranium of three ranid species from the genus *Pelophylax* and two ranid species from the genus *Rana* using geometric morphometrics to determine interspecific shape variation; 2) to evaluate chondrocranial shape pattern for intra- and interspecific taxonomy; 3) to consider the phylogenetic signal with the geometric morphometric analysis.

MATERIALS and METHODS

Study Areas and Specimens Examined

A total of 188 larvae of the five ranid species: *Pelophylax bedriagae*, *P. ridibundus*, *P. caralitanus*, *Rana macrocnemis* and *R. tavaresensis* were obtained during fieldwork between 2013 and 2016. Information on Ranid species examined here is given in Table 1. Larvae were staged according to Gosner [23]. Clearing and double staining technique follows that of Wassersug [24]. All specimens measured in this study were deposited in ZDEU (Zoology Department Dokuz Eylül University) at Dokuz Eylül University.

Landmark Selection

A total of 10 landmarks based on Larson [14] were used for this study. Samples between

Table 1. List of specimens used in analysis.

Species	Location	Elevation (m)	Date
<i>Pelophylax ridibundus</i>	Efteni Lake, Düzce, Turkey	118	15.04.2016
<i>Pelophylax bedriagae</i>	Saklı Lake, Denizli, Turkey	960	10.04.2015
<i>Pelophylax caralitanus</i>	Işıklı Lake, Denizli, Turkey	818	31.05.2015
<i>Rana tavaresensis</i>	Tavas, Denizli, Turke	1646	16.04.2015
<i>Rana macrocnemis</i>	Uludağ, Bursa, Turkey	2221	24.04.2013

Gosner Stages 26-40 were used for geometric morphometric analysis because of occurrence the remodeling of chondrocranium after Stage 40. Landmarks are described as follows:

1. Point of maximum curvature between the confluences of the cornua trabeculae.
2. Anteromedial corner of the cornua trabeculae.
3. Anterolateral corner of the cornua trabeculae.
4. Anteromedial corner of the articular process of the palatoquadrate.
5. Anterolateral corner of the articular process of the palatoquadrate.
6. Anteroventral margin of the processus muscularis quadrati.
7. Posteroventral margin of the processus muscularis quadrati.
8. Maximum posterior curvature of the otic capsule.
9. Maximum anterior curvature of the otic capsule.
10. Anterior attachment of the commissura quadratocranialis anterior to the braincase.

Morphometric Analyses

Dorsal view of chondrocranium of five ranids was photographed with Leica DFC295 with a digital camera. A total of 10 landmarks were digitized in 188 specimens in total. I used 33 specimens from *Pelophylax bedriagae*, 42 specimens from

P. caralitanus, 32 specimens from *P. ridibundus*, 45 specimens from *Rana macronemis* and 36 specimens from *R. tavasensis* for geometric morphometric analyses. These landmarks were digitized on right side of each dorsal chondrocranium in tpsDig (v.2.16; Rolf, 2010). MorphoJ (version 1.05d) software was used for characterizing the primary variations in dorsal chondrocranial shape among ranid species. We analyzed the shape differences of ranid species by using multivariate statistics (Canonical variance analysis (CVA), Principle component analysis (PCA) and Discriminant function analysis (DFA).

RESULTS

Principle component analysis displays variation among samples. The first four principle components (PC) explained 80.9% of the total variance in the data. PC 1 accounts for 34.37% of the variation and PC 2 for 31.39% of the variation. A plot of principle component 1 (PC1) against principle component 2 (PC2) explain 65.7% of the total variance. However, there were no clusters (Figure 1).

Canonical variance analysis (CVA) is a method used to display the shape variation that best differentiate among specimens. Procrustes distances were calculated by using a canonical

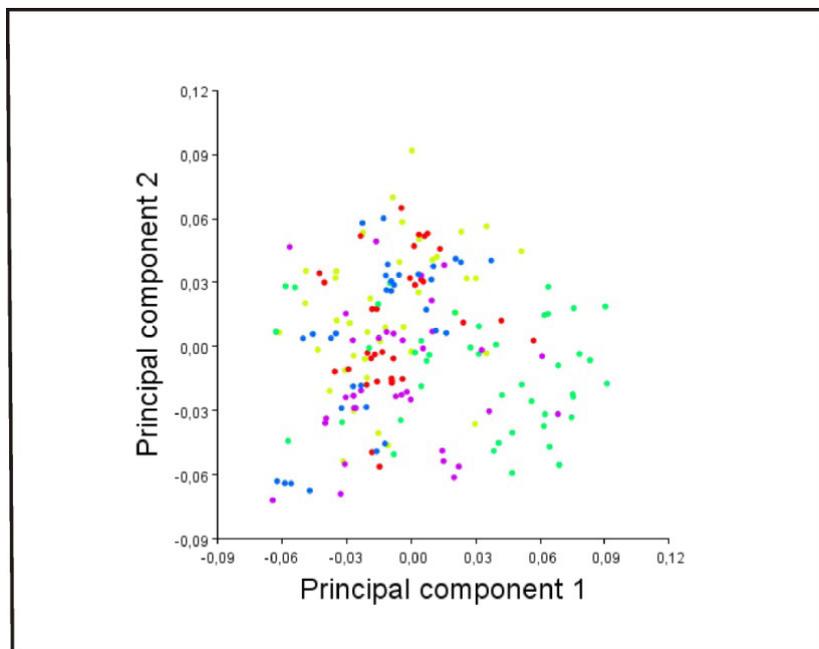


Figure 1. Scatter plot of PCA in five ranids.

variate analysis and contain a permutation test with 10000 iterations for testing shape variation of each species. Figure 2 displays deformation grids of ranids correlated with the canonical variate 1 (CV 1) and CV 2 and shows shape differences in the chondrocranium of five ranids. In CVA, the first two canonical variate (CV1) explain 85.8% of the total variance. The first four components explained 100% of the variance. The scores of the two CVs for ranid species showed that specimens grouped into two distinct clusters while there was a high degree of overlap in *Pelophylax* species.

The first axis separates *Rana* sp. (*Rana macrocnemis* and *R. tavasensis*) from *Pelophylax* sp. (*Pelophylax bedriagae*, *P. ridibundus* and *P. caralitanus*) (Figure 2). *Rana tavasensis* and *R. macrocnemis* with a negative score on CV1 whereas

Pelophylax bedriagae, *P. ridibundus* and *P. caralitanus* have a positive score on the same CV.

Rana tavasensis with positive score on CV2, has laterally orientated the cornua trabeculae (Landmarks 1, 2 and 3), a larger the articular process (Landmarks 4 and 5), a smaller the processus muscularis quadrati (Landmarks 6 and 7) and a larger and more lateral oriented the otic capsule (Landmarks 8 and 9) (Figure 2A). *Rana macrocnemis* has negative score on CV2, possess a smaller the cornua trabeculae (Landmarks 1, 2 and 3), a longer the articular process (Landmarks 4 and 5), a larger the processus muscularis quadrati (Landmarks 6 and 7) and more medial oriented and smaller the otic capsule (Landmarks 8 and 9) (Figure 2B). *Rana* species with negative scores on CV1, has more lateral orientated and

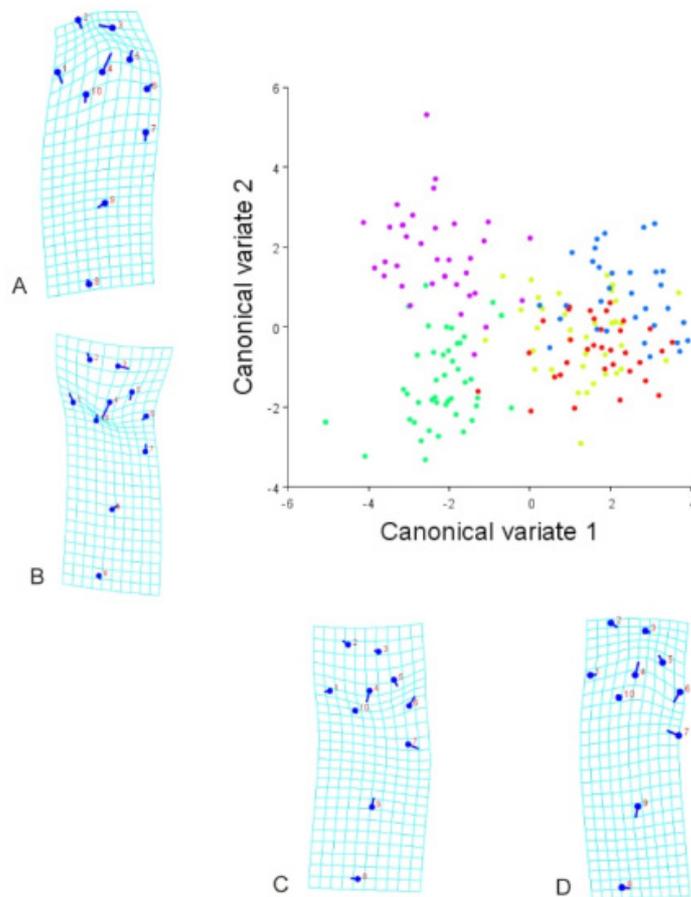


Figure 2. Scatter plot of CVA in five ranids (red: *P. bedriagae*, blue: *P. ridibundus*, yellow: *P. caralitanus*, green: *R. macrocnemis* and purple: *R. tavasensis*). A. positive curvature of landmarks on CV 2, B. negative curvature of landmarks on CV 2, C. negative curvature of landmarks on CV 1, D. positive curvature of landmarks on CV 1.

smaller the cornua trabeculae (Landmarks 1, 2 and 3), more anterior orientation of the articular process (Landmarks 4 and 5), a smaller the processus muscularis quadrati (Landmarks 6 and 7) and more lateral oriented and smaller the otic capsule (Landmarks 8 and 9) (Figure 2C). *Pelophylax bedriagae*, *P. ridibundus* and *P. caralitanus* that have positive scores on CV1 possess more medial orientation of the cornua trabeculae (Landmarks 1, 2 and 3), more posterior orientated the articular process (Landmarks 4 and 5), a larger the processus muscularis quadrati (Landmarks 6 and 7) and more medial oriented and larger the otic capsule (Landmarks 8 and 9) (Figure 2D). Procrustes distances calculated for five ranid species. *Pelophylax* species have lower distances among themselves. First, *P. caralitanus* and *P. bedriagae* have a lower distance (0.019)

than others do. Second, *P. ridibundus* and *P. bedriagae* show a lower distance (0.026). Finally, *P. ridibundus* and *P. caralitanus* exhibit a lower distance (≈ 0.027) than the remaining species. Also, Mahalanobis distances calculated among species is similar with the results of Procrustes distances (The lowest distance belongs to first *P. caralitanus* and *P. bedriagae* (2.31) and then *P. ridibundus* and *P. bedriagae* (2.48).

Discriminant function analysis (DFA) was used to perform distinct analyses for each pair of species. According to DFA, there were no interspecific shape differences among *Pelophylax* species (Figures 3A, B and C). However, the interspecific shape variations, which were caused by the cornua trabeculae, articular process of the palatoquadrate and processus muscularis

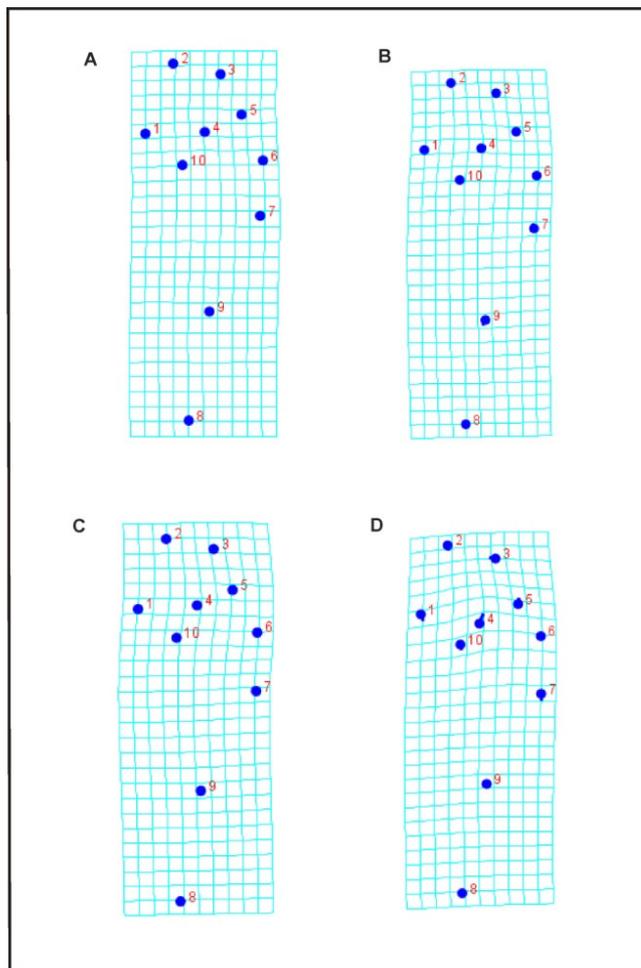


Figure 3. The results of DFA analysis. A. *Pelophylax bedriagae*-*P. caralitanus*, B. *P. bedriagae*-*P. ridibundus*, C. *P. caralitanus*-*P. ridibundus*, D. *Rana macrocnemis*-*R. tavasensis*.

quadrati, were present between *R. macrocnemis* and *R. tavasensis* (Figure 3D). These differences were as follows: (1) the cornua trabeculae are shorter and located laterally, (2) the articular process of the palatoquadrate tends to be located more posteriorly and (3) the processus muscularis quadrati is smaller.

DFA shows that the shape variations existed between the genera *Rana* and *Pelophylax*. The shape differences between *P. bedriagae* and

R. macrocnemis were as follows: (1) more anteriorly located the articular process of the palatoquadrate, (2) more medial orientated and smaller the processus muscularis quadrati. Moreover, the shape variations were available between *P. bedriagae* and *R. tavasensis*. These were: (1) more laterally located the cornua trabeculae, (2) more medial orientated and smaller the processus muscularis quadrati and (3) more laterally orientated the otic capsule (Figures 4A and D).

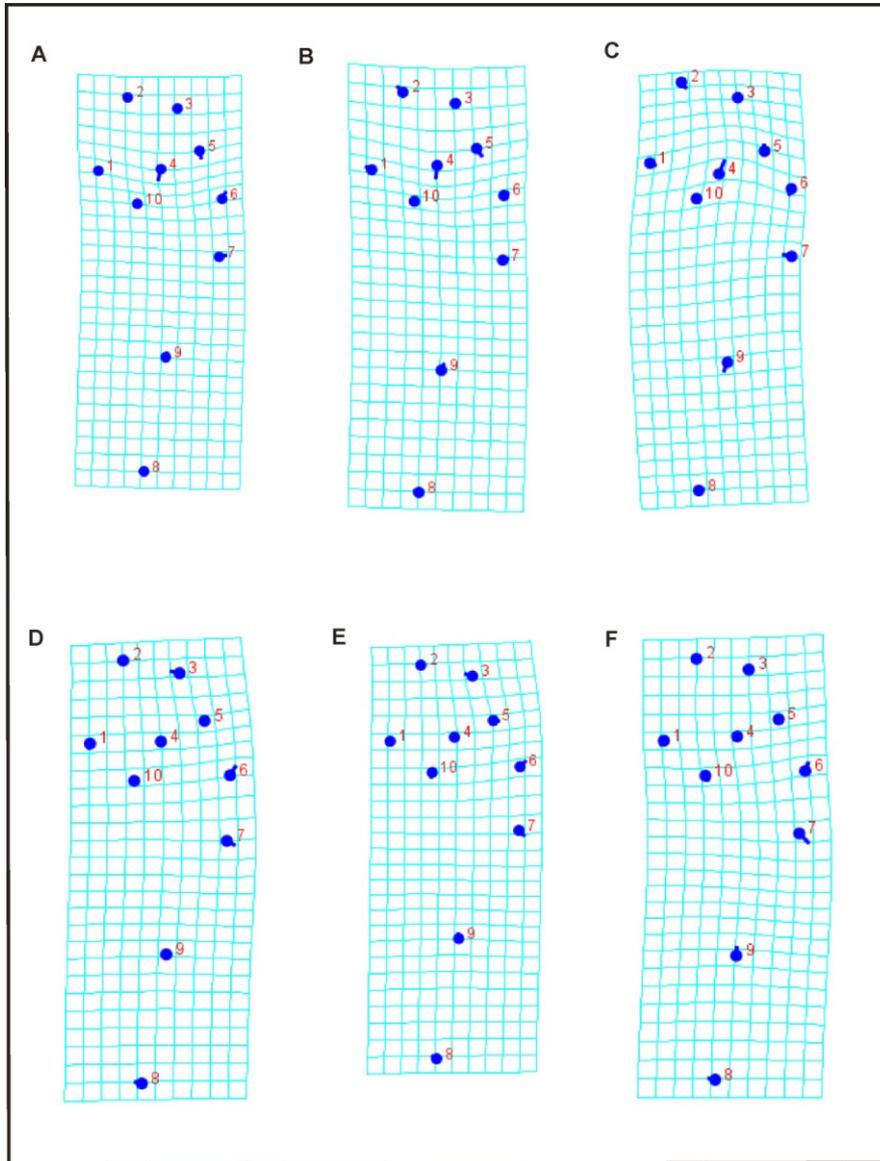


Figure 4. The result of DFA analysis in five ranids. A. *Pelophylax bedriagae*-*R. macrocnemis*, B. *P. caralitanus*-*R. macrocnemis*, C. *P. ridibundus*-*R. macrocnemis*, D. *Pelophylax bedriagae*-*R. tavasensis*, E. *P. caralitanus*-*R. tavasensis*, F. *P. ridibundus*-*R. tavasensis*.

The shape differences between *P. caralitanus* and *R. macrocnemis* were as follows: (1) more laterally located and smaller the cornua trabeculae, (2) more anteriorly oriented the articular process of the palatoquadrate, (3) smaller processus muscularis quadrati, (4) smaller the otic capsule. However, the shape differences between *P. caralitanus* and *R. tavasensis* were caused by the more medially oriented and smaller the processus muscularis quadrati, more laterally oriented the cornua trabeculae, more medially located the articular process of the palatoquadrate and more laterally located and smaller the otic capsule (Figures 4B and E).

The shape differences between *P. ridibundus* and *R. macrocnemis* were as follows: (1) more posteromedially oriented the articular process of the palatoquadrate, (2) more laterally located and larger the processus muscularis quadrati, (3) more laterally orientated and larger the otic capsule and (4) more medially oriented the cornu trabeculae. However, the shape differences between *P. ridibundus* and *R. tavasensis* were caused by the more medially oriented and smaller the processus muscularis quadrati, and more laterally located and smaller the otic capsule (Figures 4C and F).

DISCUSSION

This is the first study on differentiations among five ranid species by using geometric morphometrics. We tested the hypothesis that if there are intra- and interspecific differences between *Pelophylax* sp. and *Rana* sp.. Although the result of PCA did not show any separation among species, other multivariate analysis showed the separations among species.. The results of multivariate analysis (CVA) showed that the two genera are correctly separated from one another. *Rana macrocnemis* and *R. tavasensis* are classified as one cluster and three *Pelophylax* species (*P. ridibundus*, *P. bedriagae* and *P. caralitanus*) form another cluster. *Rana* species are distinct from *Pelophylax* species on the basis of the more lateral orientated and smaller the cornua trabeculae, more anterior orientation of the articular process, a smaller the processus muscularis quadrati and more lateral oriented

and smaller the otic capsule. *Pelophylax* species have closer similarities whereas *Rana* species significantly differed from one another. Moreover, DFA is an important method to examine the separation between two species. The results of DFA indicated that a high differentiation between the genera *Pelophylax* and *Rana* and within the genus *Rana*.

Intra- and interspecific morphological differences among species are often caused by an interaction between genetic and environmental factors [25]. Species are influenced by these environmental factors during the early developmental stages. However, this variability may not effect species differences at the molecular level [26]. The genus *Pelophylax* is formerly included in genus *Rana* and the taxonomic status of the genus *Pelophylax* is changed by Frost et al. [11]. The genus *Pelophylax* is the most complicated genus to identified [27]. Different color and patterns of ranids, especially *Pelohylax* species, exist in the same environment. These morphological variations leave the classification of ranids unclear.

Recently, the number of GMM studies has increased. This method has been used in many taxonomic groups like fish [28], amphibians [15], reptiles [29], mammals [30]. GMM based studies provide more powerful comparison than traditional morphological analyses that based on linear measurement data to discriminate shape variation of species. In this study, we investigated the chondrocranial anatomy in *Rana* and *Pelophylax* species and the results indicate that there are three main differences which are related with the larval upper jaw, hyobranchial skeleton and ossification time and sequence between the water (*Pelophylax* sp.) and mountain (*Rana* sp.) frogs (Yıldırım., in prep). In this sense, the results of geometric morphometrics are consistent with our unpublished study.

Turkish water frogs are characterized by intraspecific polymorphism in terms of the body size and dorsal color patterns [31]. Thus, molecular and bioacoustic studies are the reason of changing taxonomic status of these frogs [6,9,32-34]. GMM

is important to understand shape variation in amphibians and it is still an open field to provide different perspectives for future studies. I believe our study has provided an important data to address morphometric variation answers of anuran species for further inquiry.

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