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ARAŞTIRMA MAKALESİ **RESEARCH PAPER**

Morphological Variation of *Pelodytes caucasicus* Boulenger, 1896 (Anura: Pelodytidae) from Turkey [*]

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Abstract: The Parsley frog is represented by a single species from Anatolia which is called *Pelodytes caucasicus*. This species is categorized as a near threatened in the conservation list and has very limited distribution along the North East Black Sea and its populations have been poorly studied. In this study, we analyzed morphological variation of 59 specimens from different localities from Anatolia (Artvin; Karagöl, Rize; Çat and Trabzon; Hıdırnebi) unraveling the morphological differences among *P. caucasicus* populations. For this purpose, we used multivariate analysis such as principal component analysis (PCA) and linear discriminant analysis (LDA). Although there was considerable an overlap among morphological variation of localities according to PCA analysis, slight differences were observed in LDA analyses. In PCA analysis, the Head Width (HW), Head length (HL), and Snout vent length (SVL) are primarily discriminating characters for *P. caucasicus*. In LDA analysis, the NL, HW, and HL are primarily discriminating characters. This study will contribute to the limited data of *P. caucasicus* and enlarge the knowledge of geographic variation for *P. caucasicus* in Anatolia.

Keywords: Caucasian, morphometric differences, *P. caucasicus*, Turkey.

Türkiye'deki *Pelodytes caucasicus* Boulenger, 1896 (Anura: Pelodytidae) Türünün Morfolojik Çeşitliliği

***Sorumlu yazar:**
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Öz: Anadolu'da Pelodytidae familyasının Parsley kurbağası *Pelodytes caucasicus* adı ile bilinen tek türü dağılış göstermektedir. Bu tür koruma listelerine tehdite açık olarak kategorize edilir.Doğu Karadeniz boyunca kısıtlı yayılıma sahiptir ve populasyonları az çalışılmıştır. Bu çalışmada Anadolu'daki (Artvin; Karagöl, Rize; Çat ve Trabzon; Hıdırnebi) *P. caucasicus* populasyonları arasındaki morfolojik farklılıkları ortaya çıkarmak için 59 bireyin morfolojik çeşitliliği analiz edilmiştir. Bu amaç doğrultusunda temel bileşenler analizi (PCA) ve doğrusal ayırım analizi (LDA) gibi çoklu analizler kullanılmıştır. Temel bileşenler analizinde lokaliteler arası çakışmalar gözüksede doğrusal ayırım analizinde az da olsa farklılık gözlenmiştir. Temel bileşenler analizine göre Baş Genişliği (HW), Baş Uzunluğu (HL) ve Burun Ucu-Kloak Arası Mesafe (SVL) öncül ayırıcı karakterlerdir. Doğrusal ayırım analizine göre NL, HW ve HL öncül ayırıcı karakterlerdir. Bu çalışma *P. caucasicus*'un kısıtlı verisine katkı sağlamış ve türün coğrafik varyasyonu ile ilgili bilgiyi genişletmiştir.

Anahtar kelimeler: Kafkasya, morfolojik farklılıklar, *P. caucasicus*, Türkiye.

INTRODUCTION

The Pelodytidae family is represented by one genus (*Pelodytes*) and four species overall its distribution areas. Three of species of *Pelodytes* (*P. atlanticus*, *P. ibericus*, *P. punctatus*) inhabited in Iberian Peninsula and around France and one of them (*P. caucasicus*) inhabited to Caucasian region.

Pelodytes caucasicus, is an endemic species of the Caucasian Isthmus, the region delimiting Europe and Asia, and lying between the Black Sea in the west and the

[*] *This study was produced from the master thesis.*

Caspian Sea in the east (Litvinchuk & Kidov, 2018). This species is distributed in the western Republic of Georgia, Krasnodar Region of Russia, and extreme northeastern Turkey, along with isolated populations in the central part of Turkey's northern coast and Georgia-Azerbaijan border area (Kaya et al., 2009). Caucasian Parsley frog (*P. caucasicus*) is characterized by fragmented populations, and little information about its population's features exists. Above all, *P. caucasicus* is cataloged as Near Threatened (NT) in the International Union for Conservation of Nature (IUCN) Red List of Threatened Species.

Body size is a fundamental morphological trait and important in a physiological, ecological, and behavioral context of a species (Schäuble, 2004). The population of species that inhabit a wide range of environments frequently displays divergent morphologies that correlate with differences in ecological parameters (Rivera, 2008). Despite that, in some situations morphological differentiation of amphibians can be very small and involves mainly differences in body proportions (Babik & Rafinski, 2000).

Comparative studies are pivotal for expanding awareness about the current situation of populations and tender differences among populations and for explaining the reason for the effects of different factors on populations. Previously, some works have been carried out on distribution, ecology, breeding, age structure, helminth parasites, hematology, morphology, and serology of *P. caucasicus* in Turkey (Steiner, 1968; Franzen, 1999; Arıkan et al., 2003; Tosunoğlu & Taşkavak 2004; Arıkan et al., 2007; Erişmiş et al., 2009, Yıldırımhan et al., 2009, Litvinchuk & Kidov, 2018). Limited studies are existing about morphological characters of *P. caucasicus* through its distribution area, especially in Turkey. In addition to that, newly introduced areas are involved in literature in Turkey. Thus far, possible morphological separation during species evolution remains completely unknown.

In this study, we determined whether morphological characters differ significantly from one locality to another and characterized these differences for *P. caucasicus*. Body differences were analyzed using multivariate methods such as principal component analysis (PCA) and linear discriminant analysis (LDA). Specimens of three different populations from Artvin, Rize, and Trabzon were evaluated according to morphological variation according to PCA and LDA for the first time in literature

MATERIAL AND METHOD

Morphological data were obtained from 59 specimens from 3 different localities Karagöl; Artvin (latitude/longitude: 41.38; 41.85) (16♂, 2♀), Çat; Rize (latitude/longitude: 40.86; 40.94) (21♂, 1♀) and Hıdırnebi; Trabzon (latitude/longitude: 40.96; 39.43) (17♂, 2♀) throughout the Eastern Black Sea region of Turkey (Figure 1). The morphological measurement was taken with the permission of the local ethics committee of Çanakkale Onsekiz Mart University for animal experiments (approval reference number: 2019/09). No specimens were taken to the laboratory. All measurements were taken in the field.

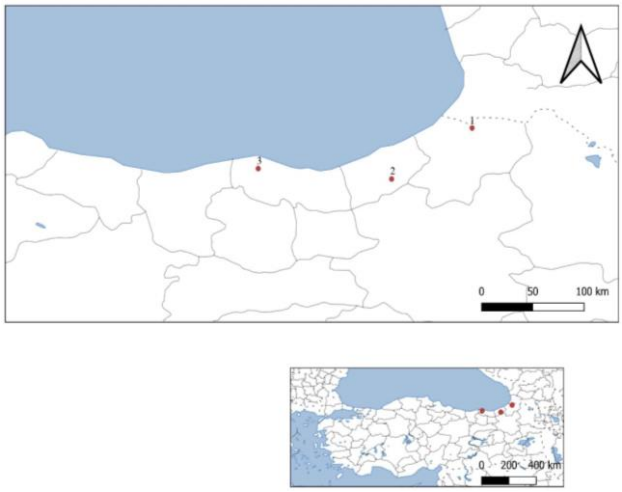

Figure 1. Map of sampling localities (1: Karagöl; Artvin, 2: Çat; Rize and 3: Hıdırnebi; Trabzon.

We sampled specimens in July 2020 and July 2021 in their activation time. Sex determination was performed by the absence or presence of the appearance of black tubercles, especially on the venter during the breeding season in males. In advance of measurement, all specimens were anesthetized and all individuals were measured at one time. Using a digital caliper to the nearest 0.1 mm, we measured the following six morphological characters: snout-vent length (SVL), head length (HL), head width (HW), Nostril length (NL), femur length (FL), tibia length (TL). To minimize bias introduced by measurements and avoid extraneous error quantification of body dimensions, all frogs were collected by a single observer (T. Ergül Kalaycı).

Because we had insufficient data for female individuals, we analyzed pool data for each location (females and males were not separately analyzed). We used principal components analysis (PCA) to find the best low-dimensional representation of variation in the data to determine whether morphological variation could form the basis of detectable group structure. We used Linear Discriminant analysis for specified morphological clustering. The function "prcomp" with "scale = TRUE" was used for the principal component analyses, clustering individuals in the multivariate space of the first two principal components (PC1 and PC2); and the function "lda" in package "MASS" (Venables & Ripley, 2002) was used for the linear discriminant analysis. Statistical

analysis was performed using the R (R Development Core Team, 2021).

RESULTS

The mean values for SVL, HL, HW, NL, FL and TL were found as 49.60, 15.69, 15.09, 3.38, 23.12 and 25.73 mm for Artvin, respectively. For Rize, we found 43.38, 15.11, 13.83, 4.26, 19.38, and 21.58 mm for SVL, HL, HW, NL, FL and TL, respectively. The mean values for SVL, HL, HW, NL, FL and TL were found as 49.76, 16.12, 16.94, 3.43, 19.83, and 21.97 mm for Trabzon, respectively.

There is no clear separation for *P. caucasicus* individual from three localities (Artvin, Rize, and Trabzon). We retained the first two components for the principal component analysis (PCA). These two components explained 73.51% of the morphological variation. The factor loadings for the first PC were all positive, with the heaviest loadings on SVL and HL. The loadings of the second PC were weighted heavily positive for SVL, HL, and HW, and negatively for NL, FL, and TL (Table 1).

Table 1. PCA loadings for *P. caucasicus.* snout-vent length (SVL), head length (HL), head width (HW), Nostril length (NL) femur length (FL), tibia length (TL).

	PC1	PC2	PC3	PC4	PC5	PC6
SVL	0.444	0.123	0.332	-0.811	-0.139	-0.026
HL	0.463	0.402	-0.118	0.371	-0.654	0.213
HW	0.431	0.511	0.068	0.233	0.673	-0.205
NL	0.187	-0.038	-0.929	-0.299	0.096	-0.035
FL	0.422	-0.543	0.049	0.214	-0.143	-0.677
TL	0.437	-0.516	0.071	0.123	0.267	0.672

The continuous variables presented high correlation values for the first component except for NL (Table 2).

Table 2. Correlation of variables with PC1 and PC2 explained for *P. caucasicus* (geographic location as a factor).

Variable	PC1	PC2
SVL	0.786	0.139
HL	0.819	0.455
HW	0.763	0.577
NL	0.330	-0.043
FL	0.747	-0.613
TL	0.773	-0.583

The most important character contributing to PC1 was the snout-vent length (SVL). PC2 was positively correlated with SVL, HL, and HW and negatively correlated with NL, FL, and TL. The graphical representation of PC1/PC2 showed an overlap for three distribution area members (Figure 2). Although, Trabzon and Artvin showed no clear separation from Rize, specimens from Rize could be separated by their smaller size (Figure 2).

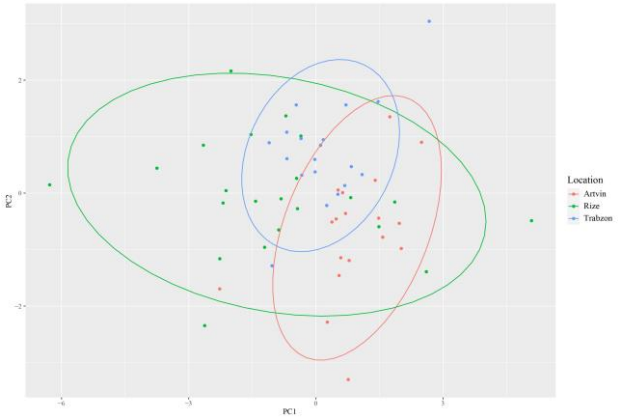

Figure 2. Ordination of the first and second principal components for *P. caucasicus* (Red dots represent Artvin individuals and green dots represent Rize individuals, while blue dots represent Trabzon individuals).

The results from PCA suggested that we could find the best separation of groups by employing LDA. The prior probabilities of the linear discriminant analysis (LDA) among localities were as follows: Artvin (0.28), Trabzon (0.28) and Rize (0.43). LDA analysis showed the function 1 explained 76.2% of the variance whereas function 2 explained 23.8% of the variance (Figure 3).

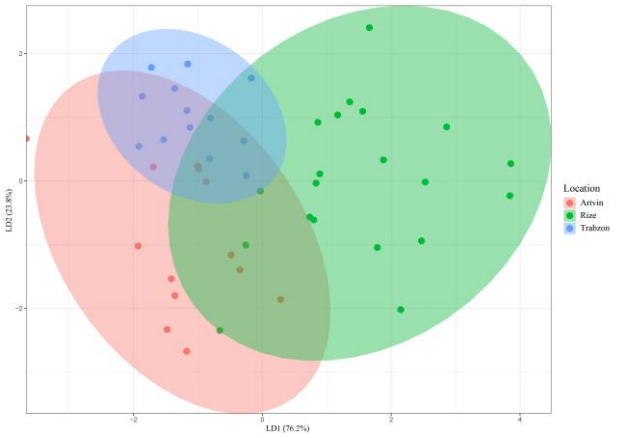

Figure 3. Plot of linear discriminant axis 1 versus linear discriminant axis 2 from morphometric characters of *P. caucasicus* specimens.

Artvin had the higher error rate in specimen classification (4 of 18 individuals, error = 22.22 %) followed by Rize (2 of 22 individuals, error= 9.09 %) and Trabzon (1 of 19 individuals, error= 5.26 %). A total of % 89.83 correct classification was achieved in LDA analysis. Differentiation along the LD1 is driven by the number of NL, HL, and HW (Table 3). The LD2 is driven primarily by HW and HL (Table 3).

Table 3. Coefficients of linear discriminant.

Variables	LD1	LD2
SVL	-0.187	0.009
HL	0.281	-0.271
HW	-0.274	0.498
NL	0.927	0.188
FL	-0.187	-0.026
TL	0.025	-0.286

DISCUSSION

This is the first study to compare the morphological features of *P. caucasicus* from Artvin (Karagöl), Rize (Çat village), and Trabzon (Hıdırnebi plateau) localities. Though *P. caucasicus* is distributed to restricted areas along with Caucasian and categorized as threated species in different conservation lists, there is limited data available for morphological comparison of Caucasian Parsley frog from Anatolia. Although morphometric for female individuals is missing, due to the lack of production of advertisement signals and the difficulty of sampling, this study gathers new information about *P. caucasicus*, which is highly required for this under-studied and near threated species.

Tosunoğlu and Taşkavak (2004) previously compared the specimen from Trabzon (Uzungöl) and Rize (Çamlıhemşin) and found that two populations are similar according to the coefficient of difference values. Their results also resembled the populations from Georgia and Caucasus (Tarkhnishvili & Gökhelashvili, 1999). As Franzen (1999) specified the eastern Black Sea populations of Turkey do not differ morphologically from the neighbor populations. The study of Tosunoğlu & Taşkavak (2004) was the only study existing in the literature about the morphological comparison of *P. caucasicus* from Anatolia. According to our study, Rize specimen slightly differed from Trabzon in particular LDA analysis.

Comparison between two analyses showed that LDA provided higher resolution than PCA. In PCA analysis, three populations were completely intermixed with each other. But in LDA analysis, Rize specimens proneness to Artvin and Trabzon specimens disappeared.

It is essential to recognize characters that differentiate populations from morphologically distinguished populations. In this study, we found that SVL, HL, and HW are primarily distinguishable characters for *P. caucasicus* in PCA analysis. In terms of LDA analysis, NL, HW, and HL are primarily discriminated values for *P. caucasicus*.

The specimens from Rize differed from their narrow head size from Trabzon and Artvin. Former studies have indicated anurans co-occurring with predators to exhibit a narrower head compared to inhabiting predator-free environments (Tejedo et al., 2010). Rize specimens that experience greater predation pressure should exhibit a narrower head. Also, the habitat of Rize specimens tends to dry compared to Artvin and Trabzon. Destruction of water bodies will limit the food resources and amphibians will not be able to find appropriate places for reproduction. Eventually, this will restrain the body proportions.

Geographic variation in body size of anurans could result from either phenotypic plasticity or adaptation to particular environments (Rivas et al., 2018). It is vital to the understanding interplay of morphological variation to understand how species survive across different landscapes (Shaffer et al., 2015). *P. caucasicus* is near threated species and its population is seriously threatened by habitat changes. The conclusions of this study give us a chance to look through the *P. caucasicus*'s life history attributes and see whether morphology is changing in different habitats in the current state. Further research for *P. caucasicus* inclusive more variables will contribute to the literature.

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