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A Preliminary Study on Morphology and Serology of *Pelodytes caucasicus* Boulenger 1896 Populations From North-Eastern Turkey

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Abstract: The present study compares the blood serum proteins of two populations of *Pelodytes caucasicus* from the north-eastern Black Sea region of Turkey (Rize and Trabzon) by polyacrylamide-disc electrophoresis. A set of two characters differed males of Trabzon from Rize's ones. No discernible difference was found between the electropherograms of blood serum proteins of the two populations. The densitometric curves of blood serum proteins obtained here for *Pelodytes caucasicus* differ from those of other anuran species, *Rana ridibunda*, *Bufo viridis*, *Pelobates fuscus* and *Bombina bombina*.

Key words: *Pelodytes caucasicus*, morphology, serology, Turkey

INTRODUCTION

The Caucasian Parsley Frog, *Pelodytes caucasicus* Boulenger 1896, can be found from western Caucasus, northern Azerbaijan, Mountainous Karabagh and southern Tiflis to the coast of Georgia and eastern Black Sea region of Anatolia^[1-6]. Various studies^[2,4,6] on ecology and zoogeography of *Pelodytes caucasicus* in the Caucasian region had also increased the distribution range of the species.

Although the species was only previously known to inhabit the eastern Black Sea region of Turkey^[3,4,7] more recently fourteen different localities were given for the species' distribution in north-eastern Turkey^[8].

Available literature has revealed the lack of detailed taxonomic studies on the *Pelodytes caucasicus* populations in Turkey. Former studies were mostly on the distribution and ecology of the species^[3,7,8].

This report includes both morphologic (colour and pattern, morphometric) and serologic (distribution of blood serum proteins) features of *Pelodytes caucasicus* from north-eastern Turkey. This is also the first electrophoretic separation of the blood serum proteins from the *Pelodytes caucasicus* specimens collected from NE Anatolia.

MATERIALS AND METHODS

For morphometric examination electrophoresis, a total of 39 (36♂♂, 3♀♀) specimens was collected from two

different localities (Fig. 1) and deposited in the museum of the Zoology Department of Ege University (ZDEU). The material used in this study was as follows: From Rize (n = 27) ZDEU 206/1996; 1-27 (24♂, 3♀), Çamlıhemşin, Rize-Turkey, 31.08.1996, leg U. Kaya and M. Tosunoğlu. From Trabzon (N = 12) ZDEU 60/1997; 1-12 (12♂), Uzungöl, Trabzon-Turkey, 20.05.1997, leg. M. Tosunoğlu and U. Kaya.

The collected material was delivered alive to the laboratory. Colour and pattern characteristics and colour slides were taken from each examined specimen. Blood samples for serological analysis were obtained from the ventricle and then the etherised specimens were properly fixed and stored in 70% ethanol. Morphometric measurements were done with dial calipers sensitive to 0.02 mm.

The specimens were evaluated on the basis of the works of Terentjev and Chernov^[9], Baran^[10]. The ten different morphometric measurements used in this study are: Snout-vent length (SVL), head length (HL), head width (HW), femur length (FL), tibia length (TL), inner metatarsal length (IML), nostril length (NL), nostril width (NW), eyelid width (EW), interorbital distance (IO).

Blood serum proteins were separated according to the polyacrylamide-disc-electrophoresis method of Davis^[11], slightly modified by Özeti and Atatür^[12], Arıkan^[13]. The qualitative evaluation of the separations were done on densitometric tracing curves, obtained from a Gelman ACD-15 Model 39430 densitometer scanning at 500 nm. The electropherograms were also visually compared.

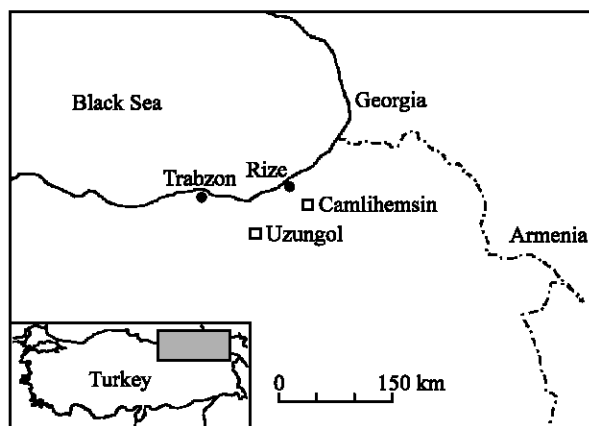


Fig. 1: The localities where the *Pelodytes caucasicus* specimens were captured

Morphometric analysis was carried out using the Minitab (Minitab Inc. Manuel, 1991: P.C. Version release 8. Quickest Inc., Rosemont, Pennsylvania). Sexual dimorphism was tested according to formulas given by Sokal and Rohlf^[4]:

$$t = \frac{\bar{X}_1 + \bar{X}_2}{\sqrt{\frac{S_1^2}{n_1} + \frac{S_2^2}{n_2}}} \quad \text{and} \quad t_T = \frac{(S_1^2 / n_1) + (S_2^2 / n_2)}{\frac{S_1^2}{n_1} + \frac{S_2^2}{n_2}} t_2$$

where, \bar{X} = is mean, S = variance, n = samples and t = table values (n-1). Thus, if the calculated t is greater than theoretical t(t_T), difference between two groups

is discernible. In order to compare populations, the MAYR's^[15] formula for Coefficient of Difference was considered: $CD = (X_1 - X_2) / (SD_1 + SD_2)$, where, X_1 is the mean for the first group, X_2 is the mean for second group, SD_1 is the standard deviation for the first group and SD_2 is the standard deviation for the second group. When the value of CD is 1.28 or higher, it is accepted that there is a disparity between the groups in terms of characters being compared.

RESULTS AND DISCUSSION

Pattern and coloration: The predominant coloration of the back is brown. This background is covered with rather symmetrical warts or lumps that provide the thickly dotted appearance on the rear side and striped aspect from middle of the body to the head. The ventral side is yellowish white and the underside of the legs is pinkish red. The front part of the bottom of body and head features lightly coloured greyish brown stains. These stains are not found on the rear part of the abdomen or lower sides of the legs. In addition to the stains, there are also small granular protrusions on the abdomen. These granules are also visible on both sides of the body. On the inner side of femur and tibia of the forelimbs, male specimens have four large black protuberances. Of these, two large protuberances are on inner forelimb digits while two smaller ones are on the inner surface of the forearms and shoulders. Dorsal and ventral colour and pattern characteristic of *P. caucasicus* from the eastern Black Sea region of Turkey correspond to those given by Nikosky^[1],

Table 1: Statistical analyses of various parameters of the specimens from Rize and Trabzon

| Characters (mm) | Rize specimens | | | | Females | | | |
|-------------------|----------------|-------------|------------|------|---------|-------------|------------|------|
| | Males | | | | | | | |
| | N | Range | Mean±SE | SD | N | Range | Mean±SE | SD |
| SVL | 24 | 43.48-52.08 | 48.04±0.42 | 2.10 | 3 | 46.70-50.28 | 48.87±1.10 | 1.91 |
| HL | 24 | 13.62-15.84 | 14.53±0.12 | 0.61 | 3 | 14.22-15.30 | 14.70±0.31 | 0.55 |
| HW | 24 | 16.26-19.90 | 17.49±0.16 | 0.82 | 3 | 16.10-17.34 | 16.68±0.36 | 0.62 |
| NL | 24 | 6.04-7.30 | 6.62±0.07 | 0.36 | 3 | 5.68-6.40 | 5.98±0.21 | 0.37 |
| NW | 24 | 5.24-7.24 | 6.28±0.09 | 0.44 | 3 | 6.20-6.86 | 6.52±0.19 | 0.33 |
| IO | 24 | 3.98-5.32 | 4.85±0.06 | 0.33 | 3 | 4.12-4.46 | 4.23±0.11 | 0.19 |
| EW | 24 | 2.70-3.94 | 3.35±0.07 | 0.36 | 3 | 3.44-3.96 | 3.72±0.15 | 0.26 |
| FL | 24 | 21.66-25.86 | 23.77±0.24 | 1.21 | 3 | 21.46-24.20 | 22.65±0.81 | 1.40 |
| TL | 24 | 23.46-26.58 | 24.84±0.18 | 0.91 | 3 | 21.96-24.22 | 23.05±0.65 | 1.13 |
| IMT | 24 | 1.70-3.80 | 2.37±0.09 | 0.44 | 3 | 1.88-3.20 | 2.56±0.38 | 0.66 |
| Trabzon specimens | | | | | | | | |
| SVL | 12 | 40.28-48.26 | 43.67±0.71 | 2.47 | - | - | - | - |
| HL | 12 | 11.26-14.74 | 13.46±0.27 | 0.96 | - | - | - | - |
| HW | 12 | 13.86-16.16 | 15.32±0.19 | 0.65 | - | - | - | - |
| NL | 12 | 5.24-6.78 | 6.12±0.13 | 0.45 | - | - | - | - |
| NW | 12 | 4.50-5.42 | 4.91±0.08 | 0.29 | - | - | - | - |
| IO | 12 | 4.40-5.40 | 4.88±0.10 | 0.37 | - | - | - | - |
| EW | 12 | 2.80-3.68 | 3.24±0.08 | 0.28 | - | - | - | - |
| FL | 12 | 20.38-23.90 | 22.28±0.31 | 1.07 | - | - | - | - |
| TL | 12 | 21.40-24.88 | 23.33±0.29 | 1.02 | - | - | - | - |
| IMT | 12 | 2.02-3.20 | 2.62±0.08 | 0.28 | - | - | - | - |

N: Number of samples, Min: Minimum data, Max: Maximum data, M: Mean, SD: Standard deviation, SE: Standard error

Table 2: Probability values to show the statistical differences between males and females of the Rize specimens, and between two sub-populations of *Pelodytes caucasicus*

| Characters | Males and females of Rize | | Males of Rize and Trabzon | | Rize -Trabzon |
|------------|---------------------------|---------------|---------------------------|---------------|---------------|
| | Calculated t | Theoretical t | Calculated t | Theoretical t | CD |
| SVL | 0.362 | 5.787 | 2.209 | 2.350 | 0.956 |
| HL | 0.893 | 5.768 | 3.867 | 2.382* | 0.682 |
| HW | 3.104 | 5.263 | 11.818 | 2.266* | 1.476** |
| NL | 7.679 | 6.018* | 7.793 | 2.359* | 0.617 |
| NW | 3.232 | 5.235 | 29.539 | 2.226* | 1.877** |
| IO | 20.347 | 4.167* | 0.662 | 2.342 | 0.043 |
| EW | 7.847 | 5.099* | 3.160 | 2.261* | 0.172 |
| FL | 0.957 | 6.170 | 3.344 | 2.292* | 0.654 |
| TL | 2.367 | 6.236 | 4.381 | 2.342* | 0.782 |
| IMT | 0.746 | 6.347 | 5.490 | 2.219* | 0.347 |

* Indicated differences and ** Indicated probable subspecific separation

Engelmann and Günther^[2], Tarkhnishvili and Gökhelashvili^[6] for the Caucasian populations.

Morphometry: Morphometric data on both populations is summarized in Table 1. In three characters, nostril length (NL), interorbital distance (IO) and eyelid width (EW), males of *P. caucasicus* from Rize were found to be different from females (Table 2). Similar analyses^[4] separated males of the Rize specimens from those from Trabzon according to the eight characters considered (Table 2).

Regarding MAYR's^[5] CD (coefficient of difference), according to eight morphometric characters compared, the two populations were found quite similar (<1.28). However, the computed CD values of 1.47 and 1.87, respectively, differed head width (HW) and nostril width (NW) of Trabzon specimens from those of Rize.

Results found in morphometric examinations coincide with those given for populations from Georgia and Caucasus^[6,9]. As stated by Franzen^[3] the eastern Black Sea populations of Turkey do not differ morphologically from the neighbour populations.

Serological evaluation: All specimens examined were sexually mature. Since no discernible qualitative differences were apparent between the serum protein pherograms (in the densitometric tracing curves) of the males and the females within a sample, the data from the two sexes were pooled for further analyses. In qualitative comparisons between the specimens from Trabzon (Uzungöl) and Rize (Çamlıhemşin), no differences were found regarding the albumin and globulin regions. In both regions, blood serum proteins could be separated into 10 fractions or fraction groups (Fig. 2A and B).

There have been no previous studies on the electrophoretic separation of the blood serum proteins of *Pelodytes caucasicus* from Turkey. When we examined the blood serum proteins of two populations with the method of polyacrylamide-disc electrophoresis, it was determined that the number of protein fractions comprise

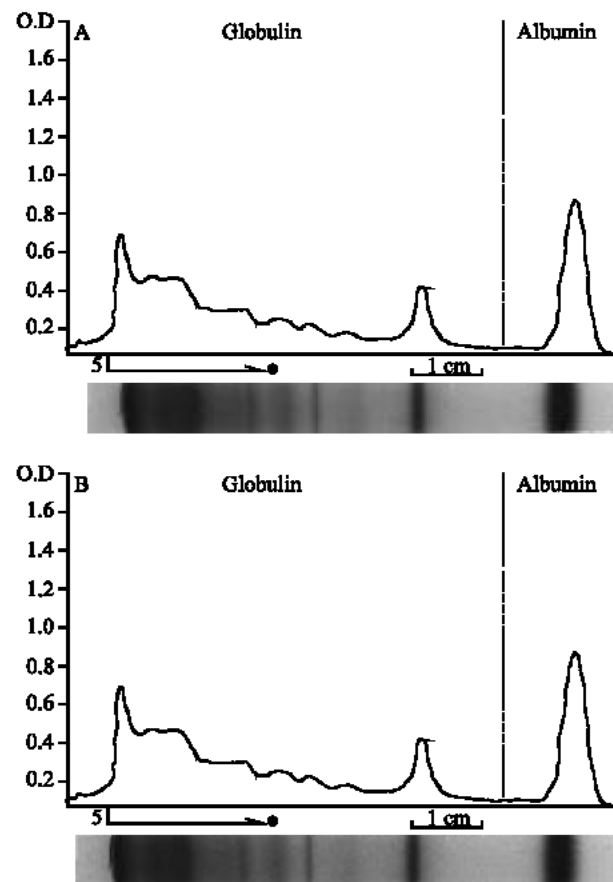


Fig. 2: Electropherograms (gel photograph) showing the electrophoretic separation of the blood-serum proteins of the adult male *Pelodytes caucasicus* specimens from Çamlıhemşin, Rize (A) and Uzungöl, Trabzon (B) together with their densitometric tracing curves. O.D.: Optical density

of 10 fractions or fraction groups in the Turkish specimens of *Pelodytes caucasicus*. When densitometric curves of two populations were considered, no discernible differences were found between both fraction

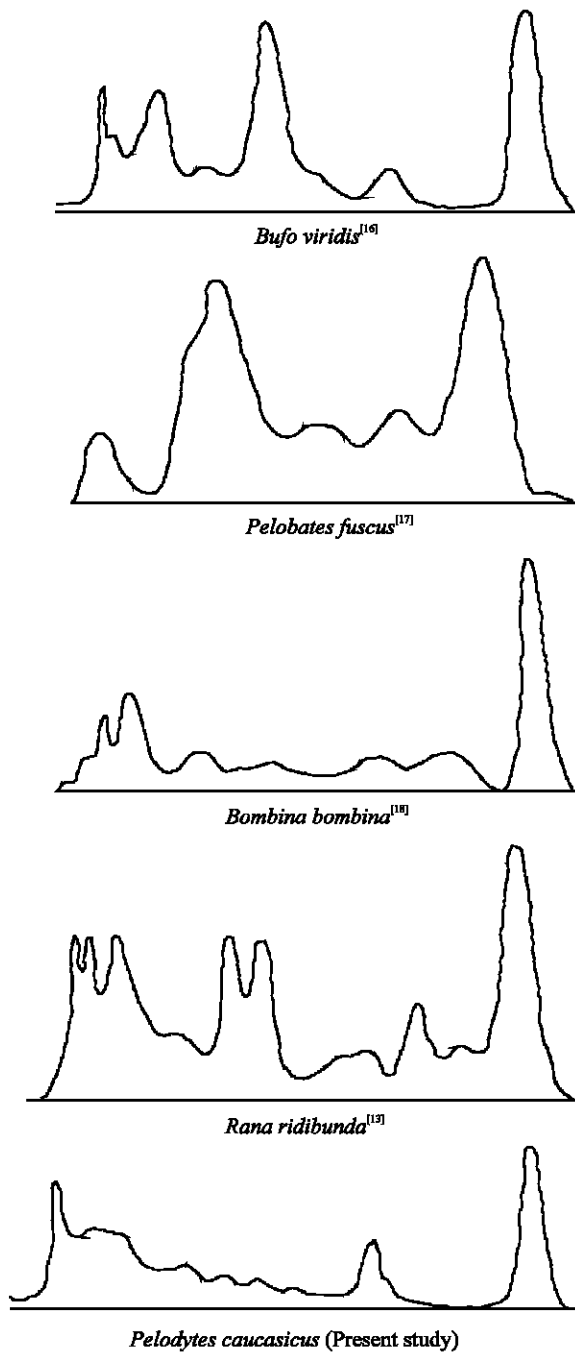


Fig. 3: A comparison of electrophoretic separation of the blood serum proteins of various anuran specimens [Blood serum proteins of the specimens considered here were separated according to the polyacrylamide disc-electrophoresis method of Davis^[11]]

numbers and dispersions of blood serum proteins. However, if the densitometric curves of serum proteins

obtained here are compared with those of other anuran species (*Rana ridibunda*^[13], *Bufo viridis*^[16], *Pelobates fuscus*^[17], *Bombina bombina*^[18]), it is clearly seen that the species *Pelodytes caucasicus* differs in its dispersion of albumin and globulin (Fig. 3).

As result, serograms demonstrated that *P. caucasicus* (Pelodytidae) is serologically different from *Bufo viridis* (Bufonidae) *Pelobates fuscus* (Pelobatidae) *Bombina bombina* (Bombinatoridae) and *Rana ridibunda* (Ranidae). This method, however, would probably be unsuitable to demonstrate differences between conspecific and geographically close population. On the other hand, the sampling size of Trabzon specimens was insufficient (12 males and no female). Thus, difference seen here in two morphometric characters of Rize and Trabzon specimens is insufficient to speculate on sub specific variation of both subpopulations. Further specimens from Trabzon could be obtained for further detailed statistical approach.

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REFERENCES

1. Nikolsky, A.M., 1962. Fauna of Russia and Adjacent Countries. Amphibians. Israel Prog. For Sci. Translations Ltd. Jerusalem, p: 119-120.
2. Engelmann, W.E. and R. Günther, 1986. Amphibians and Reptiles of Europe. Ferdinand Enke Verlag Stuttgart, pp: 146-147.
3. Başoğlu, M., N. Özeti and I. Yılmaz, 1994. Amphibians of Turkey. Fen Fak., Kitaplar Serisi. İzmir, 50: 30-58.
4. Tarkhnishvili, D.N., 1996. The distribution and ecology of the amphibians of Georgia and Caucasus: a biogeographical analysis. Z. Feldherpetol., Magdeburg, 3: 167-196.
5. Baran, I. and M.K. Atatür, 1998. Turkish Herpetofauna (Amphibians and Reptiles). Ankara (Ministry of Environment), pp: 214.
6. Tarkhnishvili, D.N. and R.K. Gökkelashvili, 1999. The Amphibians of the Caucasus. Advances in Amphibian research in the Former Soviet Union. Sofia-Moscow, 4: 94-106.
7. Baran, I., M. Tosunoğlu, U. Kaya and Y. Kumlutaş, 1997. On the herpetofauna of vicinity of Çamlıhemşin (Rize). Doğa Tr. J. Zool., 21: 409-416.
8. Franzen, M., 1999. Distribution and ecology of *Pelodytes caucasicus* Boulenger, 1896 in Turkey. Salamandra, Rheinbach, 35: 1-18.

9. Terentjev, P.V. and S.A. Chernov, 1949. Key to Amphibians and Reptiles. Israel Prog. For Sci. Transl. Jerusalem 1965, pp: 74.
10. Baran, I., 1969. An investigation on the mountain frogs of Anatolia. Ege Üniv. Fen Fak. İlmî Rap. Ser., İzmir, 80: 1-78.
11. Davis, B.J., 1964. Disc Electrophoresis II. Method and application to human serum proteins. Ann. N. Y. Acad. Sci., 121: 404-427.
12. Özeti, N. and M.K. Atatür, 1979. A preliminary survey of the serum proteins of a population of *Mertensiella luschani finikensis* Başoğlu and Atatür from Finike in South-western Anatolia. İstanbul Üni. Fen Fak. Mec., 44B: 23-29.
13. Arıkan, H., 1983. Serological investigations of *Rana ridibunda* in the Aegean region. Doğa Bilim Dergisi, Temel Bilimler C, Ankara, 7: 37-45.
14. Sokal, R.R. and F.J. Rohlf, 1981. Biometry. The Principles and Practice of Statistics in Biological Research. New York, W.H. Freeman and Company.
15. Mayr, E., 1969. Principles of Systematic Zoology. Mac. Graw-Hill Inc. New York, pp: 428.
16. Tosunoğlu, M., 1999. Morphological, osteological and serological investigations on *Bufo viridis* (Anura: Bufonidae) Populations in Turkey. Doğa Tr. J. Zool., 23/3: 849-871.
17. Flindt, R., H. Hemmer and R. Jaeger, 1968. The blood serum proteins of Central European Anurans. Zool. J. Physiol. Bd., 74: 155-163.
18. Özeti, N. and H. Arıkan, 1986. A preliminary survey on serum-proteins of the Turkish populations of *Bombina bombina* (Anura: Discoglossidae). İstanbul Üniv. Fen Fak. Seri B., 51: 13-18.