

## A Serological Investigation of *Pelodytes caucasicus* and *Pelobates syriacus* (Amphibia, Anura) Populations in Turkey

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**Abstract:** Blood serum proteins of specimens belonging to *Pelodytes caucasicus* and *Pelobates syriacus* populations were compared qualitatively and quantitatively by means of polyacrylamide disc electrophoresis and densitometry methods. It was determined that the blood serum proteins of both populations demonstrated qualitative and quantitative differences. This result supports the morphological and osteological studies emphasizing the fact that the genus *Pelodytes* needs to be treated as a separate family (Pelodytidae).

**Key Words:** Anura, *Pelodytes caucasicus*, *Pelobates syriacus*, blood-serum proteins, polyacrylamide disc electrophoresis

### *Pelodytes caucasicus* ve *Pelobates syriacus* (Amphibia, Anura) Populasyonlarının Serolojik Yönden İncelenmesi

**Özet:** Bu çalışmada, *Pelodytes caucasicus* ve *Pelobates syriacus* populasyonlarına ait örneklerin kan serum proteinleri poliakrilamid disk elektroforezi ve densitometri yöntemleri ile kalitatif ve kantitatif açıdan değerlendirilerek karşılaştırılmıştır. İki tür populasyonlarında kan-serum proteinlerinin hem kalitatif hem de kantitatif farklılıklar gösterdiği saptanmıştır. Bu sonuç *Pelodytes* genusunun ayrı bir familya (Pelodytidae) olarak değerlendirilmesi gereğine dikkat çeken morfolojik ve osteolojik çalışmalarını destekler durumdadır.

**Anahtar Sözcükler:** Anura, *Pelodytes caucasicus*, *Pelobates syriacus*, kan-serum proteinleri, poliakrilamid disk elektroforezi

### Introduction

The Caucasian parsley frog, *Pelodytes caucasicus* (Boulenger, 1896), is an endemic species distributed in the western Caucasus. It is known to inhabit only the eastern Black Sea region in Turkey. The land frog, *Pelobates syriacus* (Boettger, 1889), on the other hand, has a distribution range including Israel, Syria, Anatolia, and Trans-Caucasia. Although there are a number of studies on the taxonomy, distribution, ecology, and biology of these species (Nikolsky, 1962; Zaloğlu, 1964; Steiner, 1968; Darevsky et al., 1971; Golubev, 1985; Engelman and Günther, 1986; Atatür and Yılmaz, 1986; Chubinishvili et al., 1995; Uğurtaş 1995; Tarkhnishvili, 1996; Baran et al., 1997; Baran and Atatür, 1998; Franzen, 1999; Tarkhnishvili and Gökheleshvili, 1999),

the literature scan we conducted did not reveal any study related to their serology.

Until recently, some researchers (Nikolsky, 1962; Başoğlu et al., 1994) placed the genus *Pelodytes* under the subfamily Pelobatinae within the family Pelobatidae, while others, such as Engelman and Günther (1986), Baran and Atatür (1998), Franzen (1999), Tarkhnishvili and Gökheleshvili (1999), and Maglia (1993), emphasized that it should be accepted as a different family (Pelodytidae), based on morphological and osteological studies.

In the present study, blood serum proteins of specimens belonging to the 2 frog species were examined electrophoretically and we attempted to establish their taxonomical features.

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## Materials and Methods

The sexually mature specimens used in this study were collected in 1994 and 2001. Records pertaining to the material, which are deposited at the Ege University Department of Zoology Museum (ZDEU), are as follows:

### *Pelobates syriacus*

ZDEU 21/1994. 1-12 (6 male, 6 female), Bursa, Turkey, 09.06.1994, leg. İ. H. Uğurtaş.

### *Pelodytes caucasicus*

ZDEU 34/2001. 1-12 (6 male, 6 female), Uzungöl (1100 m), Trabzon-Turkey, 20. 07. 2001, leg. M. Tosunoğlu and H. Arıkan.

Blood samples were taken from the ventriculus with heparinized hematocrit tubes, and the blood sera were centrifuged at 600 g and then kept at  $-20^{\circ}\text{C}$ . The separation of blood serum proteins were conducted with a Canalco 1200 electrophoresis apparatus using Davis's (1964) polyacrylamide disc electrophoresis method as modified by Özeti and Atatür (1979) and Arıkan (1983). Blood serum samples of  $5\ \mu\text{l}$  were used in the electrophoretic separation. Then, the separation gels were stained with 0.5% Amido black (Naphthol blue black 10-B) prepared with 7% acetic acid. Removal of extra stain was conducted passively in 7% acetic acid baths. Densitometric curves of the separation gels were obtained by means of a Gelman ACD 15 Model 39430 densitometer.

Due to the fact that there were no differences in the electropherograms of blood serum samples based on sex, males and females of each population were evaluated together. In the qualitative evaluation, electrophoretic curves of the serum proteins were compared. In the quantitative evaluation, on the other hand, individual protein fractions or proportional protein percentages of close fraction groups were obtained by means of densitometry, and range diagrams were prepared by computing the albumin/globulin (A/G) proportions using these values.

## Results

The gel photographs and the densitometric curves of the 2 species are shown in Figure 1. Protein fractions of *Pelobates syriacus* could only be divided into 8 fractions or fraction groups: 1 in the albumin and 7 in the globulin region. In *P. caucasicus* there were 9 fractions or fraction groups: 1 in the albumin and 8 in the globulin region (Figure 1).

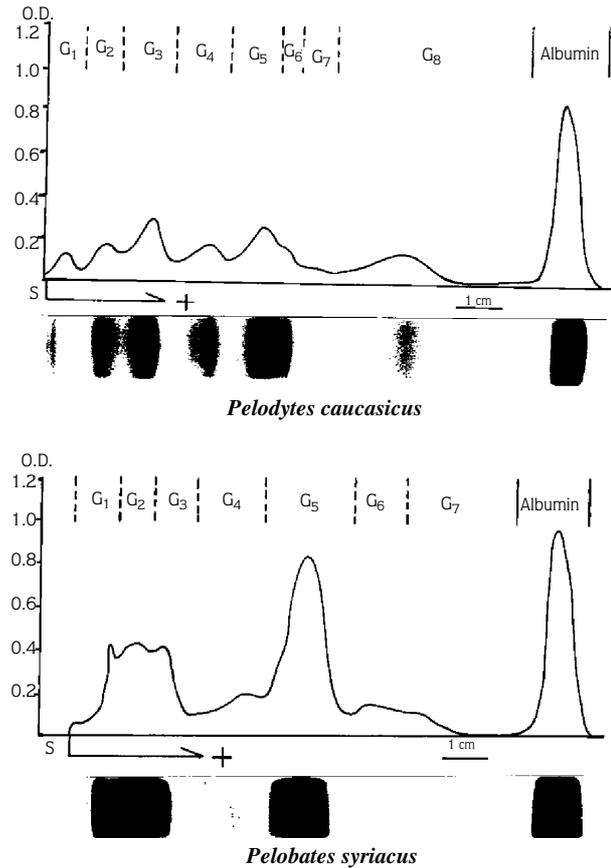


Figure 1. Electropherograms (gel photographs) showing the electrophoretic separation of the blood-serum proteins of the adult male *Pelodytes caucasicus* and *Pelobates syriacus*, together with their densitometric tracing curves. G<sub>1</sub> to G<sub>8</sub> designate the globulin zone protein bands, or band-groups (O.D.: optical density; S: start (junction between spacer gel and separated gel)).

The position of proportional protein percentages within the population, with respect to protein fractions or fraction groups that have been separated electrophoretically, are shown in the distribution given in Figure 2A and B. In the *P. syriacus* population, there are significant variations in the G<sub>1</sub>, G<sub>2</sub>, G<sub>3</sub>, and G<sub>5</sub> protein fractions and in the albumin fraction.

The average amount of albumin was  $25.0 \pm 0.952\%$  and the A/G proportion was  $0.336 \pm 0.018$  for the *P. syriacus* population, while the average amount of albumin was  $28.84 \pm 0.802\%$  and the A/G proportion was  $0.407 \pm 0.016$  for the *P. caucasicus* population (Table).

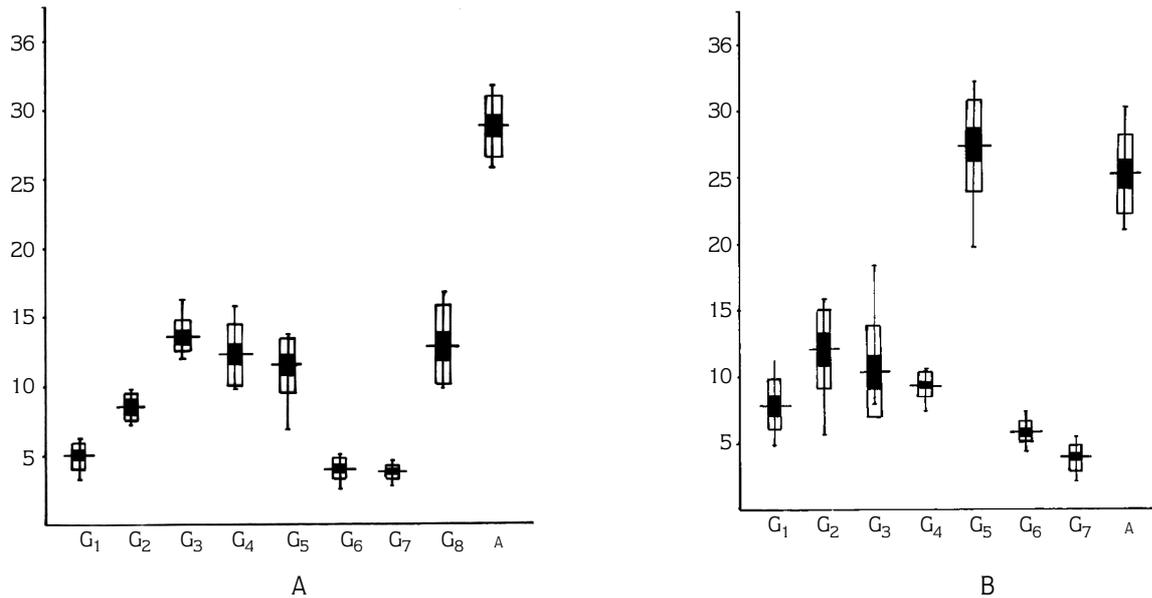


Figure 2. Variation of relative serum protein percentages of the sera obtained from *Pelodytes caucasicus* (A) and *Pelobates syriacus* (B) species (vertical line: range of observed variation; white rectangle: standard deviation; black rectangle: standard error).

Table. Average albumin (in percentage) and A/G rate values determined in *Pelobates syriacus* and *Pelodytes caucasicus* species (with standard deviation of the average).

Species	N	Albumin	A/G
<i>P. syriacus</i>	9	25.00 ± 0.952	0.336 ± 0.018
<i>P. caucasicus</i>	8	28.84 ± 0.802	0.407 ± 0.016

## Discussion and Conclusion

As stated in the introduction, the genus *Pelodytes* was considered under the subfamily Pelobatinae within the family Pelobatidae by Nikolsky (1962), Yılmaz (1994), and Baçoğlu et al. (1994). Later, based on both morphological and osteological studies conducted on *P. caucasicus* and *P. syriacus* species (Engelman and Günther 1986; Baran and Atatür, 1998; Franzen, 1999; Tarkhnisvili and Gökhelashvili, 1999), some researchers emphasized the need for considering the genus *Pelodytes* as a separate family (Pelodytidae).

Dessauer (1956), Chen (1967), Ferguson (1980), and Tosunoğlu and Taşkavak (2004), who carried out studies on the electrophoretic separation of the blood-serum proteins of amphibians, reported the taxonomical

significance of the number, rate, and density of protein fractions. Ferguson (1980) stressed the fact that genetic variation, age, sex, and physiological and environmental factors affected protein fractions, and stated that the genetic variation between these led to a qualitative difference in separation, and the others caused a quantitative difference, thus drawing attention to the taxonomical significance of qualitative differences.

According to results of electrophoretic and densitometric analyses, blood-serum proteins of the *P. caucasicus* and *P. syriacus* specimens demonstrated differences with respect to both quality and quantity (Figures 1 and 2, Table). Qualitatively, blood-serum proteins in *P. syriacus* were divided into 8 fractions (1 albumin, 7 globulin), whereas they were divided into 9

fractions (1 albumin, 8 globulin) in *P. caucasicus* (Figure 1). Quantitatively, the average amount of albumin in the *P. syriacus* population was 25% and the A/G proportion was 0.336, whereas the average amount of albumin in the *P. caucasicus* population was 28.84% and the A/G proportion was 0.407. Thus, there is a difference between the populations with respect to average amounts of albumin. Moreover, a significant variation was observed in the protein fractions G<sub>1</sub>, G<sub>2</sub>, G<sub>3</sub>, G<sub>4</sub>, and G<sub>5</sub>,

as well as in the albumin fraction of the *P. syriacus* population (Figure 2).

The present study's serological analysis results seem to confirm previous morphological and osteological studies conducted by various researchers. Consequently, we are of the opinion that it would be more proper to consider the genus *Pelodytes* within the family Pelodytidae.

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