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Morphological and Serological Investigations on the Mountain Frogs of the Mid-Taurus Range Between East Longitudes 33° and 36° *

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Abstract: In the present study, a total of 115 (65 ♂♂, 50 ♀♀) specimens of mountain frogs from the mid-Taurus Range between east longitudes 33° and 36° were investigated from the morphological and serological points of view. From the data obtained, no differences were found at species level among the 3 populations from the region.

Key Words : Mountain frogs, *Rana macrocnemis*, Morphology, Blood serum proteins, Electrophoresis

33°-36° Doğu Boylamlar Arası Orta Torosların Dağ Kurbağaları Üzerinde Morfolojik ve Serolojik Araştırmalar

Özet: Mevcut çalışmada 33°. - 36°. Doğu Boylamlar Arası Orta Toroslar'dan toplam 115 (65 ♂♂, 50 ♀♀) dağ kurbağası örneği morfolojik ve serolojik yönden incelenmiştir. Elde edilen bulgulara göre, söz konusu bölgeden incelenen üç populasyon arasında tür seviyesinde bir farklılık saptanamamıştır.

Anahtar Sözcükler : Dağ Kurbağası, *Rana macrocnemis*, Morfoloji, Kan-serum proteinleri, Elektroforez

Introduction

The first species described among Anatolian mountain frogs was *Rana macrocnemis* found at Uludağ (Bursa) by Boulenger (1). Another species (*R. camerani*) was first described from Caucasica (2), and later recorded from Mount Erciyes in Turkey (3). A third species was recorded from Anatolia and described as *R. holtzi* from Lake Maden in the Tauruses by Werner (4). After the first description, Werner (3) and Boulenger (5) suggested that *R. holtzi* and *R. macrocnemis* were synonymous. On the other hand, Lantz and Cyren (6) and Bodenheimer (7) were of the opinion that *R. camerani* and *R. macrocnemis* were identical and as a result, the systematics of these 3 species have become a center of debate. In subsequent studies, Mertens (8), Başoğlu and Hellmich (9) and Eiselt (10) mentioned the presence of 3 species but suggested that more samples were needed to clarify the situation. According to Baran (11), who studied the morphology of a large number of live samples, and Özeti (12), who carried out osteological studies the same material, there are 3 distinct species.

In this study, samples collected from Çamlıyayla, the Bolkar Mountains and Aladağlar of Mid-Tauruses between east longitudes 33° and 36° were evaluated morphologically and serologically.

Materials and Methods

The samples used in this study were collected from 3 different regions of the Mid-Tauruses (Çamlıyayla and environs, Karagöl-Çinigöl in the Bolkar Mountains and Körmenlik Plateau in Aladağlar) between east longitudes

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33° and 36° and were deposited in the museum of ZDEU (Zoology Department, Ege University). The region in question and the localities where samples were collected are shown in Figure 1 and information about the samples studied is given in Table 1.

The study was carried out in terms of: a) Morphology and biometry and b) Analysis of blood sera proteins via polyacrylamide disc electrophoresis.

Color-pattern analysis of the samples collected from the field was carried out and color photographs were taken if needed. The various biometric measurements that were used in descriptions of the samples were taken using a Mauser brand dial caliper with 0.02 mm divisions. The measurements and ratios were taken according to Baran (11) and Terentjev and Chernov (13).

The blood required for the polyacrylamide disc elec-

Table 1. Data on mountain frogs collected from the Mid-Taurus Range, between east longitudes 33° and 36°.

ZDEU	Collecting Localities	ALTITUDE E(M)	Number of Specimens	Collecting Dates	Collected by
49/96	Atlılar Mevki/Çamlıyayla-MERSıN	1360	8 (5 ♂♂, 3♀♀)	12.05.1996	TBAG-1385
50/96	Kuyualanı/Çamlıyayla-MERSıN	1360	6 (4 ♂♂, 2♂♂)	12.05.1996	TBAG-1385
59/96	AlanyalıKöyü/Çamlıyayla-MERSiN	1200	2♀♀	12.05.1996	TBAG-1385
110/96	Karagöl/Bolkar Dağları-Nı–DE	2460	12 (10 ♂♂, 2♀♀)	08.07.1996	TBAG-1385
143/96	Karagöl/Bolkar Dağları-Nı–DE	2460	48 (25 ♂♂, 23♀♀)	09.07.1996	TBAG-1385
145/96	Çinigöl/Bolkar Dağları-Nı–DE	2500	3♀♀	09.07.1996	TBAG-1385
74/97	Karagöl/Bolkar Dağları-Nı–DE	2460	15 (11 ♂♂, 4♀♀)	01.06.1997	TBAG-1385
71/97	Körmenlik Yaylası/Aladağlar-Nı–DE	2910	21 (10 ♂♂, 11♀♀)	02.06.1997	TBAG-1385

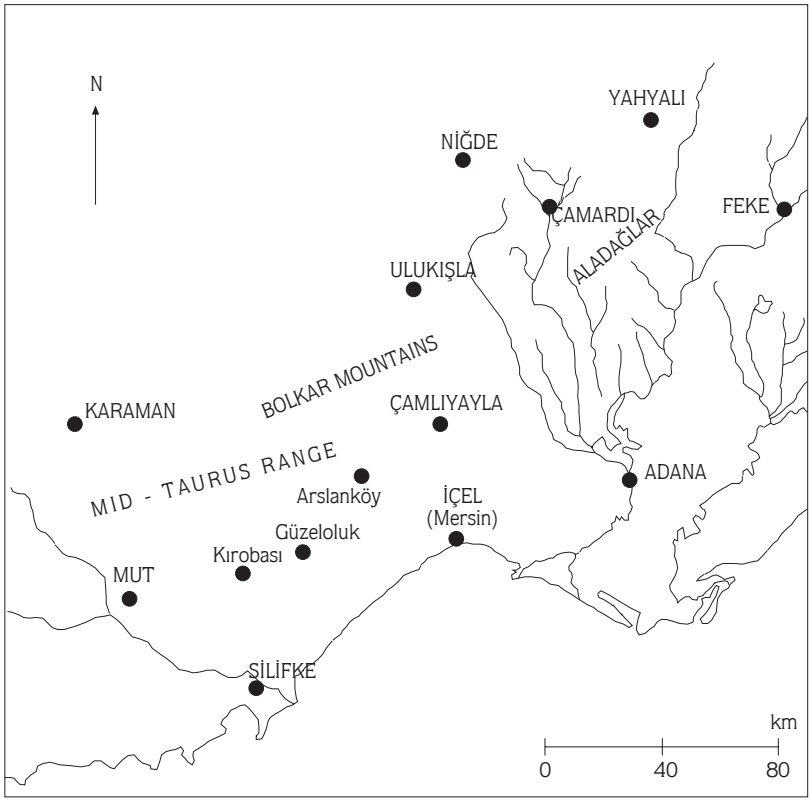

Figure 1. The study area.

trophoresis of serum proteins was taken via heparinized hematocrit capillary tubes. The tubes were centrifuged at 600 g for 5 minutes and serum samples separated from blood cells were kept at -20ºC until used for electrophoretic separation, which was accomplished according to Arıkan (14), who had slightly modified Davis' (15) protocol using a polyacrylamide disc electrophoresis apparatus (Canalco Model 1200). For the electrophoretic separation of serum proteins, an aliquot of 5 µl of serum sample was used. Following the separation, gels were stained with 0.5% Amido black (Naphtol Blue Black 10-13), destained passively and preserved in 7% acetic acid. Separated and stained proteins were directly evaluated qualitatively.

Results

Morphological analysis and Biometry

Samples obtained from the Bolkar Mountains and Çamlıyayla were sexually mature. Because no difference was found between the sexes on the basis of the characteristics studied, male and female individuals were evaluated together.

Specimens from Bolkar Mountain (Karagöl-Çinigöl) Population

A total of 78 individuals (46 ♂♂ and 32 ♀♀) from Karagöl-Çinigöl populations in the Bolkar Mountains were studied. The morphometric measurements and the ratios derived from these measurements for this population are given in Table 2.

In the specimens from the Karagöl-Çinigöl population, the ground color of the dorsum, including the legs, was yellowish green to grayish green, often with darkish green and sometimes darkish brown or dark brown spots. While in 32% of the specimens studied, spots were more abundant and homogenous (Figure 2A), in 53.85%, spots were in different shapes and sizes and less abundant (Figure 2B), and only in 14% spots were few and larger (Figure 2C). The dorsal spots were surrounded by a lighter colored circle. Spots on the hind legs were in longitudinal bands. There was no vertebral stripe in any of the specimens studied from the Karagöl-Çinigöl population.

The venter was generally pinkish and sometimes yellowish or grayish. There was a large number of disordered spots under the head and neck. There were no spots on the abdominal region. The dorsal skin was usually soft and smooth.

Specimens from the Aladağlar Population

A total of 21 specimens (10 ♂♂, 11 ♀♀) were studied from Körmenlik Plateau (Aladağlar). The morphometric measurements concerning this population and the ratios obtained from them are given in Table 3.

The ground color of the dorsum was grayish brown or light brick red. Over this, there were spots of different hues from brown to greenish brown. A vertebral stripe lighter than the dorsum was present in 47% of the specimens and various-sized spots were arranged in a row on both sides of the vertebral stripe (Figure 3A); there was no vertebral stripe in 42.86% of the specimens and spots were dispersed in a disordered manner (Figure 3B). There were a few spots in 9.52% and the smaller spots were distributed on each side of the vertebral stripe (Figure 3C). There were no lighter colored circles around the spots. There were large dark spots in transverse bands on the hind legs. There were no spots on the abdomen but there were very small spots under the head.

Table 2. Morphometric measurements and ratios obtained from Karagöl-Çinigöl population in Bolkar Mountains.

CHARACTERS	EXT.	M	SD	SE
Snout-Vent Length (L.)	33.16-59.70	47.15	6.13	0.69
Snout-Vent L./Femur +Tibia L.	0.83-0.99	00.93	0.04	0.00
Snout-Vent.L./Tibia L.	1.58-1.94	01.78	0.07	0.01
Snout-Vent.L./Head L.	2.79-3.46	03.06	0.13	0.02
Snout-Vent.L./Head Width (W.)	2.27-3.05	02.56	0.14	0.02
Snout-Vent L./ First Toe L.	6.91-10.52	08.36	0.62	0.07
Snout-Vent.L./Metatarsal Tubercle L.	14.54-21.19	17.23	1.38	0.16
Snout-Vent.L./Femur L.	1.02-1.21	01.09	0.04	0.00
Tibia L./ Metatarsal Tubercle L.	7.97-11.81	09.68	0.84	0.10
First Toe L./ Metatarsal Tubercle L.	1.57-2.56	02.07	0.20	0.02
Head L./ Head W.	0.71-0.97	00.84	0.05	0.01

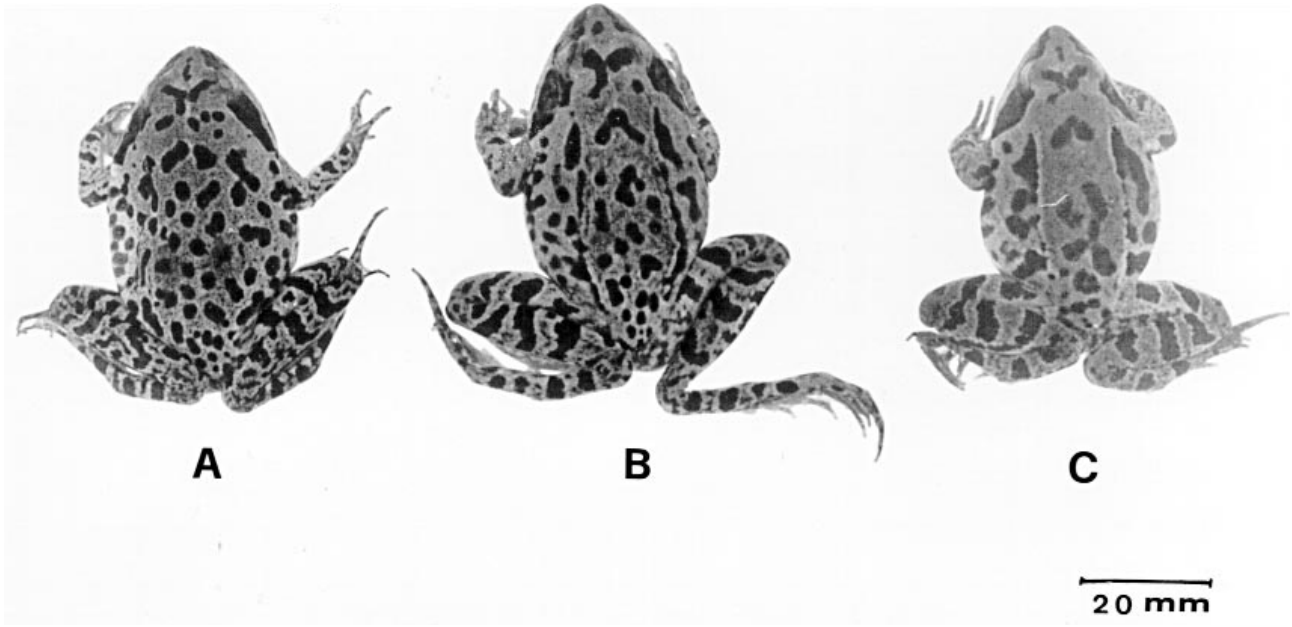

Figure 2. Dorsal pattern types of Karagöl-Çinigöl specimens from Bolkar Mountains.

Table 3. Morphometric measurements and ratios obtained from Aladağlar (Körmenlik Plateau) population.

CHARACTERS	EXT.	M	SD	SE
Snout-Vent Length (L.)	36.08-50.12	45.16	3.83	0.84
Snout-Vent L./Femur +Tibia L.	0.90-0.98	00.93	0.02	0.01
Snout-Vent.L./Tibia L.	1.71-1.88	01.77	0.05	0.01
Snout-Vent.L./Head L.	2.57-2.93	03.05	0.11	0.02
Snout-Vent.L./Head Width (W.)	2.57-2.93	02.74	0.09	0.02
Snout-Vent L./ First Toe L.	7.91-10.54	09.26	0.77	0.17
Snout-Vent.L./Metatarsal Tubercle L.	12.82-17.16	14.85	1.11	0.24
Snout-Vent.L./Femur L.	1.04-1.16	01.09	0.03	0.01
Tibia L./ Metatarsal Tubercle L.	6.32-9.90	08.30	0.08	0.18
First Toe L./ Metatarsal Tubercle L.	1.35-1.93	01.61	0.18	0.04
Head L./ Head W.	0.830.98	00.90	0.03	0.01

A vertebral stripe was present in 57.14% of the specimens from the Körmenlik Plateau population.

Specimens from the Çamlıyayla (Mersin) Population

A total of 16 specimens (9 ♂♂, 7 ♀♀) were studied from Çamlıyayla (Mersin). The morphometric measurements concerning this population and their estimated ratios are given in Table 4.

The ground color of the dorsum and legs was slightly pinkish brown or gray brown. In 68.75% of the specimens (Figure 4A) blackish or dark brownish spots were present on the dorsum and head and were distributed on both sides of the vertebral stripe in a straight line; small spots were present on both sides of the vertebral stripe in 18.75% of the specimens (Figure 4B); and in the remaining 12.5% various sized spots were irregularly dispersed (Figure 4C).

The ground color of the venter was pinkish or pinkish yellow. No spots were present. However, many small spots were present on the ventral side of the head. A vertebral stripe was present in 87.50% of the specimens.

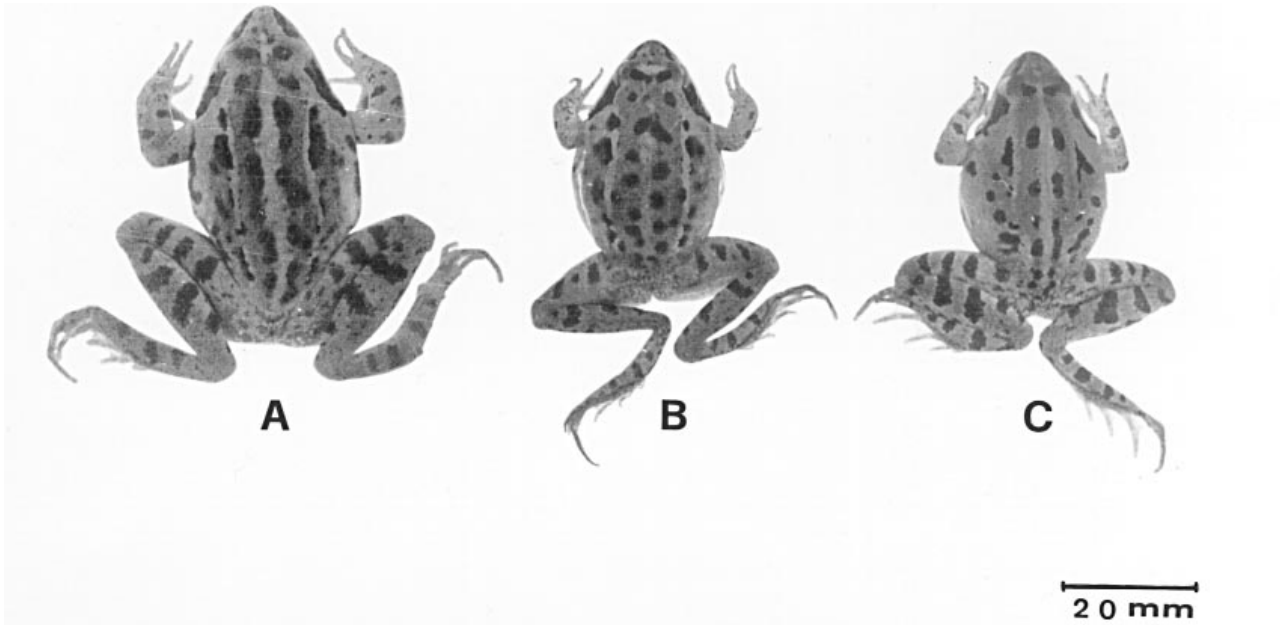

Figure 3. Dorsal pattern types of Aladağlar (Körmenlik Plateau) specimens..

Table 4. Morphometric measurements and ratios obtained from Çamlıyayla (Mersin) population.

CHARACTERS	EXT.	M	SD	SE
Snout-Vent Length (L.)	33.44-64.44	44.20	10.56	2.64
Snout-Vent L./Femur +Tibia L.	0.87-0.97	00.92	0.03	0.006
Snout-Vent.L./Tibia L.	1.64-1.88	01.76	0.05	0.01
Snout-Vent.L./Head L.	2.73-3.33	02.99	0.17	0.04
Snout-Vent.L./Head Width (W.)	2.42-2.80	02.63	0.13	0.03
Snout-Vent L./ First Toe L.	8.08-10.43	09.57	0.52	0.13
Snout-Vent.L./Metatarsal Tubercle L.	13.56-18.47	15.82	1.26	0.32
Snout-Vent.L./Femur L.	0.94-1.20	01.09	0.05	0.01
Tibia L./ Metatarsal Tubercle L.	7.66-9.91	08.99	0.63	0.16
First Toe L./ Metatarsal Tubercle L.	1.37-1.99	01.66	0.15	0.04
Head L./Head W.	0.78-0.95	00.88	0.05	0.01

Serum Proteins

Blood serum proteins of only the Karagöl-Çinigöl population from the Bolkar Mountains and the Körmenlik Plateau population from Aladağlar were separated and evaluated using polyacrylamide disc electrophoresis. Specimens of these populations were sexually mature. As our electrophoretic results on blood sera of the sexes did not show differences, males and females were evaluated together.

Each population was surveyed for intrapopulational variation and then comparisons between populations were conducted.

Electropherograms of 2 selected specimens representing the 2 populations in our research area are given in Figure 5. There was no apparent qualitative difference found between the 2 populations in terms of their blood sera proteins. However, there were some quantitative differences detected within and between populations, especially at the level of globulin fractions.

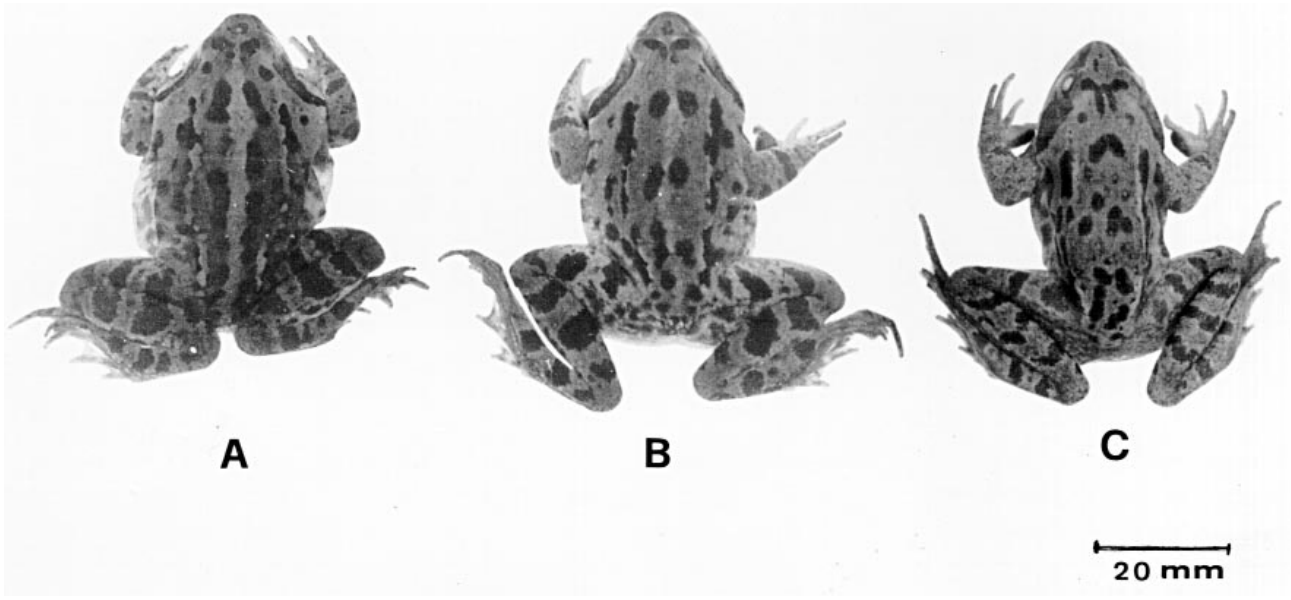

Figure 4. Dorsal pattern types of Çamlıyayla (Mersin) specimens.

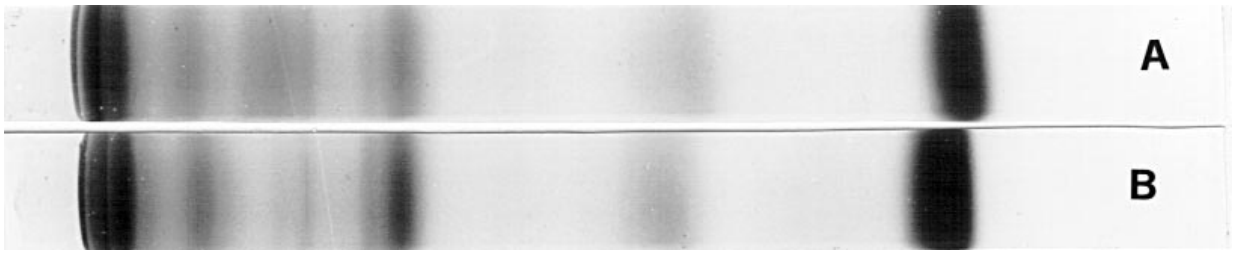

Figure 5. Blood sera protein electropherograms of specimens from 2 different populations; A. Karagöl- Bolkar Mountains, B. Körmenlik Plateau-Aladağlar.

Discussion and Conclusion

Although so far results from studies carried out on the taxonomy of mountain frogs based on morphology have indicated the presence of 3 species in Anatolia (*Rana macrocnemis*, *R. camerani* and *R. holtzi*) there is no consensus yet among scientists. In particular, the taxonomic positions of *R. camerani* and *R. macrocnemis* are not clear. Lantz and Cyren (6) and Bodenheimer (7) considered these 2 species to be identical and Delwig (16) described them as subspecies of the same species. Mertens (8), Baran (11), Özeti (12), Terentjev and Chernov (13), Boettger (17), and Başoğlu et al. (18), however, regard them as 2 different species. Baran and Atatür (19), who collected samples from Akdağ (Tavas, Denizli), reported a new subspecies (*R. m. tavasensis*). As shown by the literature, the taxonomy of Anatolian mountain frogs is still debatable.

According to our results, there is no difference between the 3 populations (Çamlıyayla, Karagöl-Çinigöl and Körmenlik Plateau) in terms of the morphometric measurements and related ratios (Table 2-4). There is a similarity between the Körmenlik Plateau and Çamlıyayla populations with respect to color-pattern characteristics. However, there are also some slight differences between the Karagöl-Çinigöl population and the Körmenlik Plateau and Çamlıyayla populations, i.e., the dorsal spots are surrounded by circles lighter than the ground coloration in the first population. This characteristic is not present in

the Çamlıyayla or Körmenlik populations. Although none of the specimens studied from the Karagöl-Çinigöl population has a vertebral stripe, there are vertebral stripes in 57.14% of the specimens from Körmenlik Plateau and in 87.50% of the specimens from Çamlıyayla. In other words, the occurrence of a vertebral stripe is over 50% in both the Körmenlik Plateau and Çamlıyayla populations.

Some authors (20-22) who have been working on the blood sera of amphibians have mentioned that genetic variations, age, physiology and environmental factors have an effect on the quality and quantity of protein fractions, but of these, only genetic variation causes qualitative differences. Therefore, qualitative differences have some importance in taxonomy.

Quantitative variations found at the level of globulins in this study are presumably because of age differences and physiological or environmental factors, and thus have no genetic basis and are of no use in taxonomy.

According to the morphological and serological results, it is impossible to say if there is a difference at species level between the mountain frogs dispersed in the Mid-Taurus region between east longitudes 33° and 36°. However, we believe that it is necessary to compare our results with similar results obtained from populations of *R. macrocnemis*, which were originally described from Uludağ, and those of *R. camerani,* which was originally described from Erciyes Mountain, in order to reveal the taxonomic status of the Anatolian mountain frogs.

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