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On New Populations of *Rana holtzi* and *Rana macrocnemis* (Ranidae: Anura)

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Abstract: *Rana holtzi*, which is only known from Karagöl at altitude 2500 m and Çinigöl at altitude 2600 m, was determined from Eğrigöl for the first time at altitude 3000 m. Thus, the distribution range of *R. holtzi* in the Bolkar Mountains was extended. In addition, specimens of *Rana macrocnemis* were recorded from Seviçova (2500 m a.s.l.), located in the Bolkar Mountains near Ereğli (Konya), for the first time. Furthermore, the village of Örtülü (Maden, Elazığ), where specimens of *Rana macrocnemis* were captured, is determined to be southernmost distribution point of *R. macrocnemis*.

Mountain frog specimens captured from new localities were compared in terms of significant taxonomical features with regard to the literature. In addition, ecological observations of localities where specimens were captured were determined.

Key Words: *Rana holtzi*, *Rana macrocnemis*, new locality

Rana holtzi ve *Rana macrocnemis*'in Yeni Populasyonları Hakkında

Özet: Bu çalışmada, şimdiye kadar Bolkar Dağı'nda yalnız Karagöl ve Çinigöl'den bilinen *Rana holtzi* türünün bu dağın yaklaşık 3000 m yüksekliğinde Eğrigöl mevkiinde de yaşamakta olduğu ilk kez saptanmıştır. Böylece türün bu dağda daha geniş bir bölgeye yayılmış olduğu meydana çıkarılmıştır. Ayrıca Bolkar Dağı'nın Konya Ereğli'si tarafına yakın kısmında yaklaşık 2500 m yükseklikte *Rana macrocnemis* türü de Seviçova mevkiinden ilk olarak tespit edilmiştir. Ayrıca *Rana macrocnemis* örneklerinin yakalandığı Örtülü Köyü (Maden-Elazığ) *Rana macrocnemis* türünün yayılış alanının en güney sınırını teşkil etmektedir.

Çalışmamızda yeni lokalitelerden temin edilen materyalin önemli taksonomik özellikleri literatür bilgileriyle karşılaştırmalı olarak değerlendirilmiştir. Ayrıca örneklerimizin toplandıkları lokalitelerde saptanan ekolojik gözlemlere de yer verilmiştir.

Anahtar Sözcükler: *Rana holtzi*, *Rana macrocnemis*, yeni lokalite

Introduction

Mountain frogs specimens collected from certain regions of Turkey have been included in *Rana macrocnemis*, *Rana camerani*, and *Rana holtzi* since 1969 (Boulenger, 1885; Werner, 1898, 1902, 1914; Bird, 1936; Bodenheimer, 1944; Mertens, 1952; Başoğlu and Hellmich, 1958; Eiselt, 1965). Baran (1969) examined the systematic position of the Anatolian mountain frogs by collecting specimens from new localities, in addition to previously known localities. As a result of comparative biometrical analyses, he stated that *R. macrocnemis*, *R. camerani*, and *R. holtzi* are separate species and the population of Uludağ is included in *R. macrocnemis*, the population of Erciyes in *R. camerani*, and the population of Karagöl (in the Taurus Mountains) in *R. holtzi*. Baran (1969) also stated that populations from some mountains in Western Anatolia are included in *R. macrocnemis* and specimens from localities in Central Anatolia in *R. camerani*. Baran and Atatür (1986) described a new subspecies as *R. macrocnemis tavasensis* from Akdağ (Tavas, Denizli).

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This paper provides detailed information on the taxonomic status of mountain frogs specimens collected from new localities. In addition, observations on habitats of the specimens were determined.

Materials and Methods

The materials were collected from 3 different localities (*R. holtzi* – Eğrigöl, Çamlıyayla, Mersin; *Rana macrocnemis* – Seviçova, Ereğli, Konya; *R. camerani* – Örtülü village, Maden, Elazığ) and deposited in the ZDEU (Zool. Dept. Ege Univ.) collection. The pattern and coloration characteristics were recorded from live specimens; later the alcohol-formaldehyde fixed specimens (3 parts 40% formaldehyde + 7 parts 70% alcohol) were kept in 70% ethanol. The morphometric measurements were obtained with a digital caliper of 0.01 mm sensitivity. The measurements and ratios were obtained according to Baran (1969) and Terentjev and Chernov (1965). Morphometric indices and ratios were used to test for similarities and differences between the sexes. Ratios and indices were used due to uncertainty regarding age groups and because it was not known whether growth was isometric or not. The data were examined for conformation to assumption of normality (the Kolmogorov-Smirnov test) and homogeneity (Fmax). To compare the sexes morphometrically, an independent-samples t test was used. P values of less than 0.05 were considered statistically significant.

Material Lists

Rana holtzi

ZDEU 14/1999. 1-14♂♂, 15-25♀♀, 26-28 juv., Eğrigöl, Çamlıyayla, Mersin, 09.07.1999, leg. İ. Baran, S. Balık.

Rana macrocnemis

ZDEU 15/1999. 1-29♂♂, 30-38♀♀, 39-42 juv., Seviçova, Ereğli, Konya, 09.07.1999, leg. İ. Baran, S. Balık; ZDEU 186/2005. 1-3♂♂, 4-6♀♀, 7-10 subad., 11-14 juv., Örtülü village, Maden, Elazığ, 11.06.2005, leg. İ. Baran, Y. Kumlutaş, Ç. Ilgaz, A. Avcı.

Results and Discussion

Eğrigöl Population

Morphological characters: Specimens examined from Eğrigöl had few warts and thin skin. This characteristic feature distinguishes *R. holtzi* from *R. macrocnemis* (Baran, 1969; Baran and Atatür, 1986). Coloration and pattern features of the dorsum of the specimens from Eğrigöl were typical to those of *R. holtzi* in that the dorsal stripe was not present. In addition, the color and pattern features not mentioned in this paper were similar to those of typical *R. holtzi* stated in the literature (Werner, 1898; Baran, 1969; Baran and Atatür, 1986).

According to the results of the independent-samples t test, the differences found between the sexes were not significant ($P > 0.05$) and so male and female specimens were pooled. SVL of our specimens ranged from 40.24 to 56.94, with a mean of 45.20 mm (Table 1).

An important point that needs to be emphasized concerning the body measurements of mountain frogs is that *R. holtzi* specimens are shorter than the other 2 mountain frog (*R. macrocnemis* and *R. camerani*) species (Werner, 1898; Baran, 1969; Baran and Atatür, 1986). In fact, the total body length average (45.20 mm) of the Eğrigöl material is shorter than that of the populations obtained from other regions. This emanates from the information that the active period of specimens in the Eğrigöl region is quite short and the creek side that they live on is not very rich in terms of food. There is not sufficient time or food for the growth of frog specimens, which spend at least 7 months of the year under the grass roots, which form rather thick pads on the creek sides. This also causes the individuals that create the population to remain small and thin. Accordingly, mountain frogs were rarely seen on snow-water creek sides in Eğrigöl.

According to results published by Picariello et al. (1999), including the morphology and S1 satellite DNA of brown frogs, 3 distinct mountain frog species in Turkey should be included in 1 species, that is, for the priority law of nomenclature, *R. macrocnemis*. Veith et al. (2003) considered that 3 mountain frog species do not necessarily define monophyletic lineages according to DNA sequencing analyses. Çevik et al. (2006) evaluated mountain frog specimens from Uludağ, Erciyes Mountain, and Karagöl using morphological features and electrophoresis. They stated that *R. holtzi* is a distinct mountain species.

All the morphological and the biotope features at roughly 3000 m a.s.l. for our mountain frog material, which was analyzed from Eğrigöl, are not different from those of *R. holtzi* (Werner, 1898; Baran, 1969; Baran and Atatür, 1986; Çevik et al., 2006).

Table 1. Morphological measurements (in millimeters) and ratios of the Eğrigöl specimens.

	♂♂ + ♀♀					♂♂					♀♀				
	N	M	Range	SD	SE	N	M	Range	SD	SE	N	M	Range	SD	SE
SVL	25	45.20	40.24-56.94	5.37	1.12	14	46.03	40.70-56.94	5.59	1.49	11	43.90	40.24-56.38	5.04	1.68
FL	25	23.44	20.20-29.62	2.80	0.58	14	23.91	20.42-29.62	2.74	0.73	11	22.70	20.20-29.14	2.88	0.96
TL	25	24.04	21.36-30.08	2.55	0.53	14	24.59	21.80-30.08	2.60	0.69	11	23.20	21.36-28.62	2.36	0.79
HL	25	16.45	12.20-21.32	2.14	0.45	14	16.95	14.12-20.62	1.77	0.47	11	15.69	12.20-21.32	2.52	0.84
HW	25	17.64	15.00-22.36	2.01	.4191	14	17.72	15.70-20.50	1.79	0.48	11	17.51	15.00-22.36	2.42	0.81
MTL	25	2.46	1.96-3.14	0.36	0.08	14	2.55	2.00-3.14	0.35	0.09	11	2.32	1.96-3.04	0.36	0.12
FTL	25	4.93	4.00-5.82	0.53	0.11	14	5.10	4.28-5.82	0.51	0.14	11	4.66	4.00-5.30	0.47	0.16
SVL / FL+TL	25	0.95	0.90-1.00	0.03	0.07	14	0.95	0.90-1.00	0.03	0.01	11	0.96	0.91-1.00	0.03	0.01
SVL / FL	25	1.94	1.74-2.16	0.11	0.02	14	1.93	1.74-2.15	0.11	0.03	11	1.96	1.81-2.16	0.11	0.04
SVL / TL	25	1.88	1.76-1.97	0.06	0.01	14	1.87	1.76-1.97	0.06	0.02	11	1.89	1.83-1.97	0.05	0.02
HL/HW	25	0.93	0.81-1.05	0.06	0.01	14	0.96	0.87-1.05	0.06	0.02	11	0.89	0.81-0.96	0.05	0.02
TL / FL	25	1.03	0.93-1.18	0.06	0.01	14	1.03	0.93-1.18	0.06	0.02	11	1.02	0.97-1.11	0.05	0.02
MTL / FTL	25	0.50	0.37-0.59	0.06	0.01	14	0.50	0.37-0.59	0.06	0.02	11	.50	0.43-0.57	0.05	0.02

(N: Number of specimens, M: Mean, Range: Extreme values, SD: Standard deviation, SE: Standard errors of the means, SVL: Snout-vent length, FL: Femur length, TL: Tibia length, HL: Head Length, HW: Head width, MTL: Metatarsal tubercle length, FTL: First toe length).

Rana holtzi, which is endemic, is known from Karagöl at altitude 2500 m and from Çinigöl at altitude 2600 m (Werner, 1898, 1902; Baran, 1969; Baran and Atatür, 1986). Karagöl, which is the type locality of *R. holtzi*, is covered with meadows on the north, west and east sides (Baran, 1969). Baran (1969) noted that his study population was large, without giving any quantification. *R. holtzi* has been on the IUCN Red List of Threatened Species and categorized as an Endangered (EN) species since 1996. Baran et al. (2001) stated that the population size of *R. holtzi* declined approximately 60%-70% in Karagöl because of the introduction of the common carp, *Cyprinus carpio*. Kaya et al. (2005) estimated the population size of *Rana holtzi* in Karagöl to be between 725 and 1432 based on a mark-recapture study. They also stated that this species is facing a very high risk of decline in its natural habitat. Eğrigöl, where the new population was detected, is approximately 16 km southeast of Karagöl and is 3000 m a.s.l. In this flat region, there are relatively slow flowing creeks that are created by snow water. The creek sides are covered with prairie where sheep herds are pastured. At the same time, pads formed by grass roots on the creek sides provide a suitable environment for the mountain frogs to winter under. While the specimens that belong to *Rana holtzi* were acquired from the prairies on 3 different creek sides, no specimens were collected from other creeks with similar properties. Later, Çinili Lake, which is slightly higher, was observed but it was later determined that the lake side was not suitable for mountain frog habitation. Therefore, it was discovered that *R. holtzi*, which was thought to be living only around Karagöl and Çinigöl, has in fact spread to a wider area of the Bolkar Mountains.

Seviçova Population

Morphological characters: In 38 adult specimens the SVL ranged from 41.34 to 57.26, with a mean of 49.00 mm. The Seviçova population was slightly larger than the *R. holtzi* populations. In addition, the skin of the specimens from Seviçova had slightly more warts than the *R. holtzi* population. The color and pattern features of the Seviçova populations were typical of *Rana macrocnemis* (Baran, 1969; Baran and Atatür, 1986). While in 32.0% of the specimens examined a vertebral stripe lighter than the dorsum was clear, in 48.0% of the specimens a stripe on the dorsum was absent. The remaining (20.0%) specimens had a barely perceptible vertebral stripe. It is stated that one of the diagnostic features of *R. macrocnemis* is having barely perceptible vertebral stripe (Boulenger, 1885; Werner, 1902; Baran, 1969; Baran and Atatür, 1986).

According to the results of the independent-samples t test, differences found between the sexes were not significant ($P > 0.05$), and so male and female specimens were pooled. The morphometric data of the specimens are given in Table 2.

Our specimens, which were analyzed from the Seviçova region for the first time, do not differ from *R. macrocnemis* in terms of color and pattern condition and body measurements. This is also justified by the explanations given above. Thus, the existence of *R. macrocnemis* was confirmed in the area towards Ereğli of the Bolkar Mountains.

Seviçova is located on the road ascending from Ereğli (Konya) to Bolkar Mountain plateau. It is a small plain area with a slight slope that stands between 2 mountains, running in a north-south direction. The spring water on the north side of the plain forms small creeks. These creeks generate ponds with plenty of vegetation on open plains. On other sections, they produce moors. In Seviçova's prairies and ponds, which are approximately 24 km southeast of Karagöl as the crow flies, considerably dense and well-developed *R. macrocnemis* specimens live, since insect samples are very frequently encountered due to the large plant variety in or near the waters here.

Recording *R. macrocnemis* species in Seviçova, which is located approximately 500 m below Eğrigöl where *R. holtzi* lives, shows similarity to Meydan, which is located 450 m below Karagöl. It was explained by Baran (1969) that *R. macrocnemis* species live in the wetlands that are on the way to the village of Maden just down from Karagöl. The Seviçova population is also completely isolated from the Eğrigöl population. There is a similar isolation between Karagöl and Meydan.

Örtülü Village Population

Morphological characters: The SVL of adult specimens varied from 42.60 to 61.18, with a mean of 47.93 mm. SVL of the Örtülü population was longer than that of *R. holtzi*. A vertebral stripe lighter than the dorsum was usually present (93.0%) without a single specimen having barely perceptible stripe. In addition, the venter of the largest female specimen (SVL 61.18 mm)

Table 2. Morphological measurements (in millimeters) and ratios of Seviçova specimens (for abbreviations see Table 1).

	♂♂ + ♀♀					♂♂					♀♀				
	N	M	Range	SD	SE	N	M	Range	SD	SE	N	M	Range	SD	SE
SVL	38	49.00	41.34-57.26	4.94	0.80	29	48.83	41.34-57.26	4.99	0.93	9	49.52	42.38-55.66	5.02	1.67
FL	38	25.45	20.26-30.70	2.97	0.48	29	25.50	20.26-30.70	3.14	0.58	9	25.27	21.66-28.94	2.48	0.83
TL	38	26.58	22.00-31.92	2.91	0.47	29	26.90	22.12-31.92	2.87	0.53	9	25.54	22.00-30.04	2.95	0.98
HL	38	18.64	15.46-23.44	2.04	0.33	29	18.72	15.46-23.44	2.08	0.39	9	18.36	15.66-21.00	2.01	0.67
HW	38	18.24	14.08-21.50	2.18	0.35	29	18.30	14.68-21.50	2.14	0.40	9	18.05	14.08-21.00	2.44	0.81
MTL	38	2.73	2.04-4.66	0.50	0.08	29	2.74	2.04-4.66	0.54	0.10	9	2.71	2.22-3.20	0.39	0.13
FTL	38	5.04	4.00-7.14	0.74	0.12	29	4.84	4.00-5.98	0.54	0.10	9	5.68	4.56-7.14	0.95	0.32
SVL / FL+TL	38	0.94	0.88-1.07	0.04	0.01	29	0.93	0.88-0.99	0.03	0.01	9	0.97	0.92-1.07	0.05	0.02
SVL / FL	38	1.93	1.78-2.15	0.08	0.01	29	1.92	1.78-2.06	0.08	0.01	9	1.96	1.87-2.15	0.08	0.03
SVL / TL	38	1.85	1.65-2.44	0.12	0.02	29	1.82	1.65-1.91	0.06	0.01	9	1.95	1.78-2.44	0.19	0.06
HL/HW	38	1.02	0.90-1.15	0.05	0.01	29	1.03	0.90-1.15	0.06	0.01	9	1.02	0.95-1.11	0.05	0.02
TL / FL	38	1.05	0.78-1.18	0.06	0.01	29	1.06	0.98-1.18	0.04	0.01	9	1.01	0.78-1.14	0.10	0.03
MTL / FTL	38	0.55	0.31-0.87	0.10	0.02	29	0.56	0.42-0.87	0.08	0.02	9	0.50	0.31-0.64	0.14	0.05

Table 3. Morphological measurements (in millimeters) and ratios of Örtülü specimens (for abbreviations see Table 1).

	1♂	2♂	3♂	4♀	5♀	6♀
SVL	47.74	46.88	43.80	61.18	42.60	45.40
FL	24.10	23.22	22.66	31.70	20.08	22.22
TL	28.26	26.58	26.24	33.16	20.28	26.34
HL	21.04	15.90	19.76	23.84	15.66	16.54
HW	17.70	16.00	16.32	21.60	14.78	17.26
MTL	2.74	2.42	2.20	3.44	2.06	2.40
FTL	4.02	4.46	4.98	4.80	3.16	4.36
SVL / FL+TL	0.91	0.94	0.90	0.94	1.06	0.93
SVL / FL	1.98	2.02	1.93	1.93	2.12	2.04
SVL / TL	1.69	1.76	1.67	1.84	2.10	1.72
HL/HW	1.18	0.99	1.21	1.10	1.06	0.96
TL / FL	1.17	1.14	1.16	1.05	1.01	1.19
MTL / FTL	0.68	0.54	0.44	0.72	0.65	0.55

was pink. The morphometric data of the adult specimens are given in Table 3. It was discovered for the first time that the distribution area of mountain frog species, which was previously thought to be from West, East and Central Anatolia (Baran, 1969; Baran and Atatür, 1986), extends to Örtülü in South Anatolia.

The specimens were collected from a meadow along a small river situated north of Örtülü between 16.00 and 18.00. The temperature was 27 ºC when the specimens were captured. The sympatric amphibian species was *R. ridibunda* Pallas, 1771. The altitude at which the sampling was carried out was 1460 m a.s.l.

R. macrocnemis was first described from Uludağ by Boulenger (1885). Later, another mountain frog species, *R. camerani*, was described from Tabizhuri Lake and Akhalkalaki in the Caucasus by Boulenger (1886), and reported from Erciyes Mountain by Werner (1902). Lantz and Cyren (1913) and Bodenheimer (1944) stated that *Rana camerani* was similar to *R. macrocnemis*. According to results on the morphological and osteological characteristics of large sample of mountain frogs, 3 distinct species were determined to inhabit Anatolia (Baran, 1969; Özeti, 1970). Tarkhnishvili et al. (1999) mentioned the presence of 2 subspecies (*R. m. macrocnemis* and *R. m. camerani*) in the Caucasus. Picariello et al. (1999) included 3 supposed mountain frog species in 1 species (*R. macrocnemis*) according to the results of S1 satellite DNA and morphology of mountain frog specimens. No significant morphological differences without some pattern and coloration of specimens from Çamlıyayla, Bolkar Mountain and Aladağ Mountain were established (Arıkan et al., 2001). Veith et al. (2003) considered that 3 mountain frog species do not necessarily define monophyletic lineages according to DNA sequencing analyses. Çevik et al. (2006) evaluated mountain frog specimens from Uludağ, Erciyes Mountain, and Karagöl using morphological features and electrophoresis. They stated that *R. camerani* from Erciyes Mountain should be recognized as a synonym of *R. macrocnemis*.

In light of the previous studies mentioned above, mountain frog specimens from Örtülü should be included in *Rana macrocnemis*.

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