

Classification of the mountain frogs of the Berçelan Plateau (Hakkari), east Anatolia (Turkey)

(Anura: Ranidae)

Systematische Zuordnung der Braunfrösche
des Berçelan Plateaus (Hakkari), Ost-Anatolien (Türkei)
(Anura: Ranidae)

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KURZFASSUNG

Die Braunfrösche des Berçelan Plateaus (Karadağ, Hakkari) in Ost-Anatolien wurden im Hinblick auf ihre äußere Morphologie (morphometrisch und nach Färbungsmerkmalen) sowie serologisch (Plasma-Proteinelektrophorese) untersucht. Danach und im Vergleich mit Material der typischen Fundorte von *Rana macrocnemis* BOULENGER, 1885 und *Rana holtzi* WERNER, 1898 zeigten die Frösche des Berçelan Plateaus große Übereinstimmung mit den Tieren des Uludağ (Bursa), die derzeit *R. macrocnemis* zugeordnet werden.

ABSTRACT

In the present study, the mountain frog population of the Berçelan Plateau (Karadağ, Hakkari) in east Anatolia was examined both in terms of their morphology (morphometric and color-pattern characteristics) and serology (blood plasma protein electrophoresis). According to these studies and when compared with frogs from the type localities of *Rana macrocnemis* BOULENGER, 1885, and *Rana holtzi* WERNER, 1898, the mountain frogs of the Berçelan Plateau strongly resembled the specimens from Uludağ (Bursa) which are currently assigned to *R. macrocnemis*.

KEY WORDS

Amphibia: Anura: Ranidae; *Rana macrocnemis*, taxonomy, morphology, polyacrylamide gel disc electrophoresis; Berçelan Plateau, east Anatolia, Turkey

INTRODUCTION

Although *Rana macrocnemis* BOULENGER, 1885, *Rana holtzi* WERNER, 1898, and *Rana camerani* BOULENGER, 1886, were initially described as three separate species, different views about their systematic positions were set forth in the following years (BOULENGER 1898; WERNER 1902; LANTZ & CYRÉN 1913; BODENHEIMER 1944; MERTENS 1952; BAŞOĞLU & HELMICHH 1958, EISELT 1965; BARAN 1969; BARAN & ATATÜR, 1986; PICARIELLO et al. 1999; TARKHNI-SHVILI et al. 1999; ARIKAN et al. 2001; DÜSEN et al. 2002; BARAN et al. 2004).

Based on studies at the molecular level, VEITH et al. (2003a, 2003b) regarded the Anatolian frog species *R. m. macrocnemis*, *R. camerani* and *R. holtzi* as conspecific

and considered the taxa *tavasensis* BARAN & ATATÜR, 1986, and *pseudodalmatina* EISELT & SCHMIDTLER, 1971, as separate species, which had been classified as subspecies of *R. macrocnemis*. A recent morphological, morphometric and serological study on Anatolian mountain frogs by ÇEVİK et al. (2006) found two species (*R. camerani* and *R. holtzi*) living in Anatolia and considered *R. camerani* from Mt. Erciyes a synonym of *R. macrocnemis*.

Subsequently, the mountain frog specimens collected from new localities in Anatolia were examined in terms of their morphological characteristics and classified according to their taxonomic position, as accepted at that time (BARAN et al. 2007;

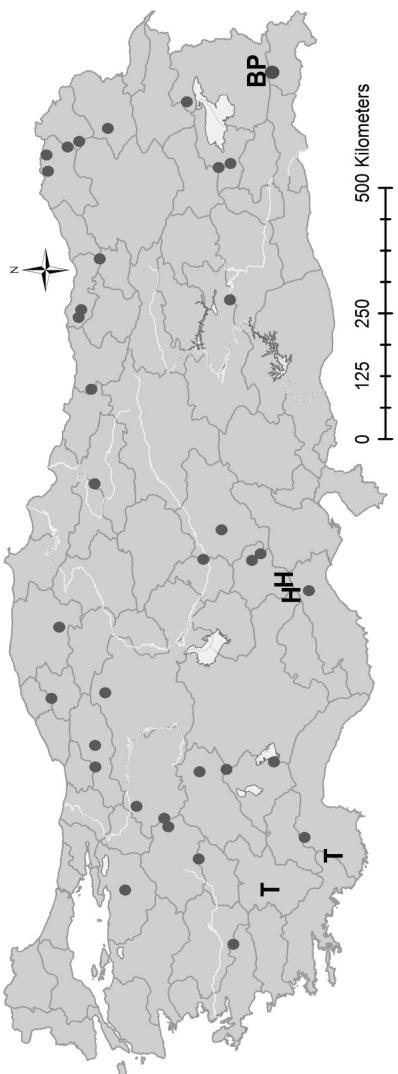


Fig. 1: Distribution of *Rana macrocnemis* BOULENGER, 1885, *Rana tavasensis* BARAN & ATATÜR, 1986, and *Rana holtzi* WERNER, 1898, in Turkey. Data according to BARAN & ATATÜR (1988), VEITH et al. (2003), BARAN et al. (2007) and BAŞKALE et al. (2012). Green dots - *R. macrocnemis*, T - *R. tavasensis*, H - *R. holtzi* (Bolkar Mountains) and red dot marked with BP - the localities of the Berçelan Plateau (Hakkari) from which the specimens of the present study were collected.

Abb. 1: Verbreitung von *Rana macrocnemis* BOULENGER, 1885, *Rana tavasensis* BARAN & ATATÜR, 1986 und *Rana holtzi* WERNER, 1898 in der Türkei nach Angaben in BARAN & ATATÜR (1988), VEITH et al. (2003), BARAN et al. (2007) und BAŞKALE et al. (2012). Grüne Punkte - *R. macrocnemis*, T - *R. tavasensis*, H - *R. holtzi* (Bolkar Gebirge), mit BP gekennzeichneter roter Punkt – die Fundorte im Berçelan Plateau (Hakkari), woher die in der vorliegenden Arbeit untersuchten Frösche stammen.

BAŞKALE et al. 2012; AFSAR et al. 2013). In summary, *R. holtzi* was detected at two localities in the Bolkar Mountains and near Eğrigöl (Çamlıayla, Mersin) (BARAN et al. 2007) and Karagöl (Çamlıayla, Mersin) (BAŞKALE et al. 2012) in the central Taurus Mountains, where its range appeared to be wider than expected. New localities of *R. macrocnemis* were determined from Konya (Ereğli, Seviçova) and Elazığ (Maden, Örtülü Village) by BARAN et al. (2007), and the southernmost record of the species was detected at the Village of Örtülü (Maden/Elazığ). However, the taxonomic position of the mountain frog populations in the Hakkari section of the east Anatolian region remained unclear due to insufficient material (see BARAN & ATATÜR 1986). In this early paper, the populations of Muş, Bitlis and Erciş were included in *R. macrocnemis*, whereas a specimen from Mt. Nemrut (Bitlis) was assigned to *R. camerani*.

In the present study, numerous mountain frog specimens collected from the Berçelan Plateau (Karadağ, Hakkari) were examined with regard to their morphology (morphometric and color-pattern characteristics) and serology, with the aim to clarify the taxonomic position of the mountain frogs in the east Anatolian region.

MATERIALS AND METHODS

Study area and individuals studied.- The mountain frog specimens examined were obtained from the Berçelan Plateau, located at an altitude of 2,700 m on Mt. Karadağ in the north of Hakkari (Fig. 1). The frogs were collected on the moist

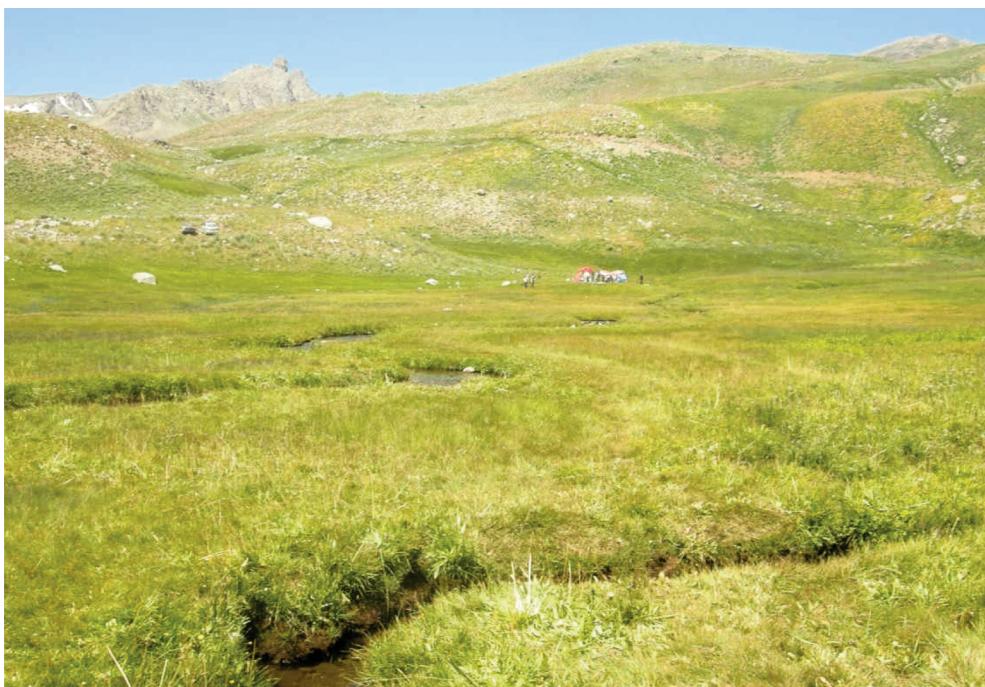


Fig. 2: Habitat of *Rana macrocnemis* BOULENGER, 1885, in the Berçelan Plateau (Hakkari).

Abb. 2: Lebensraum von *Rana macrocnemis* BOULENGER, 1885 im Berçelan Plateau (Hakkari).

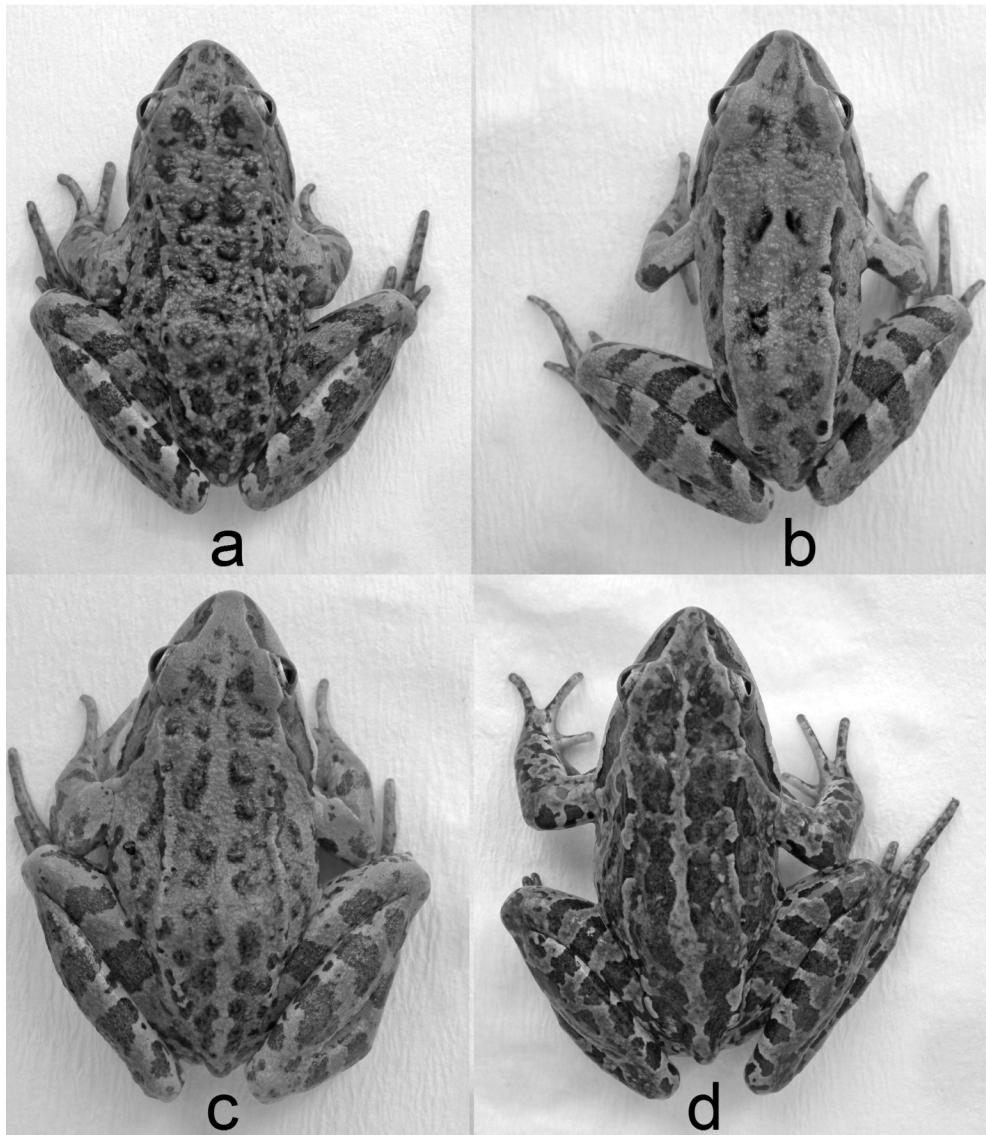
soil of the meadowland along the banks of small streams generated by the melting snow in open fields, and by spring waters (Fig. 2).

The color-pattern characteristics of those specimens were noted, which were brought alive to the laboratory, and color photographs taken. The specimens were preserved at the Department of Zoology at Celal Bayar University (CBU, Manisa). m – males, f – females.

Materials.- CBU 9/2011, 1-12 m, f, Berçelan, Hakkari, 18.08.2011, leg. M. AFSAR & B. AFSAR, 37°39.407'N, 43°46.560'E. CBU 9/2011, 1-42 m, f, Berçelan, Hakkari, 17.07.2012, leg. M. AFSAR & B. AFSAR, 37°39.555'N, 43°46.341'E. CBU 1/2013, 1-8 m, f, Uludağ, Bursa, 10.07.2013, leg. M. AFSAR & B. AFSAR, 40°07.905'N, 29°06.733'E (The 8 Uludağ specimens were used for blood plasma electrophoresis only). The specimens willulti-

mately be incorporated into the collection of the Zoology Museum of Ege University (ZDEU). Comparative data were obtained from specimens of the type localities of *R. macrocnemis* (ZDEU 162/2002-1-12, f, m, Uludağ, AFSAR & AFSAR leg. 17.07.2002) and *Rana holtzi* (ZDEU 141/1970-1-18 f, m, Karagöl, leg. BARAN 08.06.1970).

Morphometric characters and ratios.- The following morphometric measurements were taken: Snout-vent length (SVL), head length (HL), head width (HW), internarial distance (ID), interorbital distance (IO), femur length (FL), tibia length (TL), hind leg length (AAU) and metatarsal tubercle length (CAL) were measured using a compass sensitive to 0.01 mm, according to TERENTJEV & CHERNOW (1965) and BARAN (1969). The ratios HL/SVL, HW/SVL, ID/SVL, IO/SVL, FL/SVL, TL/SVL, AAU/SVL and CAL/SVL were calculated from the morphological data.



Figs. 3a-3d: Four types of dorsal pattern found in *Rana macrocnemis* BOULENGER, 1885, from the Berçelan Plateau (Hakkari).

Abb. 3a-3d: Die vier Typen der Rückenfleckung wie sie bei *Rana macrocnemis* BOULENGER, 1885 vom Berçelan Plateau (Hakkari) gefunden wurden.

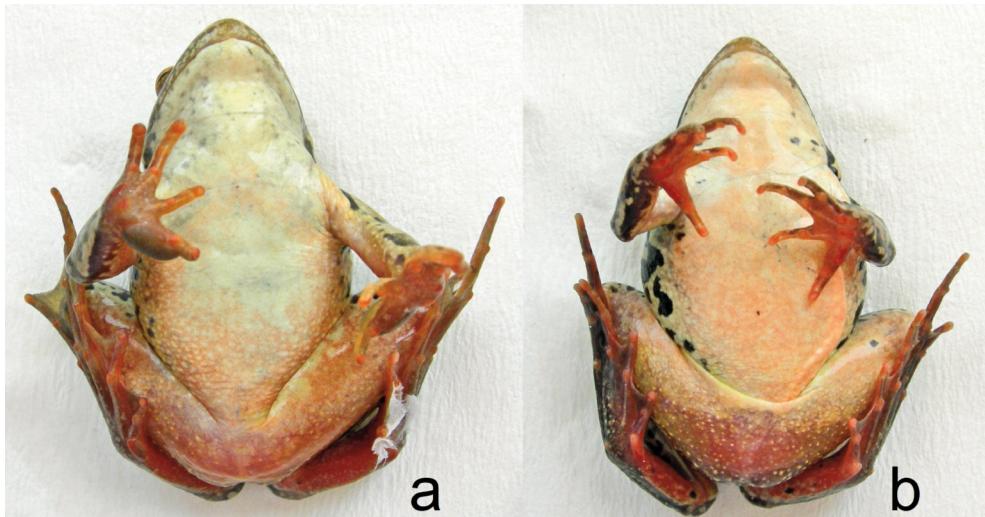
Statistical analysis.- The data under study was normally distributed according to the Kolmogorov-Smirnov test ($p > 0.05$). Adult specimens were sexed with

the help of the presence/absence of a callus on the thumb as an indicator. The morphometric data of males and females was compared by using the independent samples t-

Table 1: Descriptive statistics of the morphometric data [mm] and ratios of the examined mountain frog populations from the Berçelan Plateau (Hakkari), Uludağ (Bursa) and Karagöl, Bolkar Mountains (Nigde). N - number of specimens, M - mean; SD - standard deviation; SE - standard errors of the mean. See Materials and Methods for abbreviations in column one.

Tab 1: Deskriptive Statistiken der morphometrischen Daten (in mm) und Quotienten der untersuchten Braunfrosch-Populationen vom Berçelan Plateau (Hakkari), Uludağ (Bursa) und von Karagöl, Bolkar Gebirge (Nigde). N – Anzahl Exemplare, M – Mittelwert, SD – Standardabweichung, SE – Standardfehler des Mittelwertes. Die Abkürzungen in Spalte eins sind im Abschnitt Materials and Methods erklärt.

Characters	Berçelan (Hakkari)						Uludağ (Bursa)						Karagöl, Bolkar Mountains (Nigde)					
	N = 54			N = 12			N = 18			N = 12			N = 18			N = 18		
	Min	M	Max	SD	Min	M	Max	SD	Min	M	Max	SD	Min	M	Max	SD	Min	M
SVL	48.69	57.50	70.68	4.43	51.46	56.73	61.35	2.84	43.03	47.29	52.50	2.56						
HL	15.34	17.53	20.19	1.24	14.41	17.26	19.58	1.30	14.17	14.93	16.39	0.69						
HW	16.94	19.89	23.67	1.41	17.36	19.86	22.09	1.52	15.66	17.70	19.29	0.97						
ID	3.40	4.21	5.10	0.42	3.84	4.31	5.04	0.38	2.86	3.53	4.10	0.33						
IO	3.04	3.88	4.72	0.38	3.21	3.71	4.19	0.33	3.03	3.46	3.76	0.23						
FL	24.01	28.88	36.20	2.52	24.26	28.45	31.26	2.14	20.68	22.32	24.77	1.26						
TL	27.87	31.34	39.49	2.57	29.01	31.77	34.46	1.74	20.54	24.01	26.57	1.83						
AAU	27.14	32.93	41.34	2.76	29.12	31.47	35.42	1.98	22.19	25.69	29.87	2.02						
CAL	2.42	3.01	4.05	0.39	2.12	2.86	3.39	0.35	1.68	2.07	2.49	0.23						
HL/SVL	0.28	0.31	0.33	0.01	0.28	0.30	0.32	0.01	0.30	0.32	0.34	0.01						
HW/SVL	0.32	0.35	0.38	0.01	0.33	0.35	0.38	0.01	0.35	0.37	0.40	0.013						
ID/SVL	0.06	0.07	0.09	0.008	0.07	0.08	0.08	0.004	0.06	0.07	0.08	0.006						
IO/SVL	0.05	0.07	0.08	0.005	0.06	0.07	0.07	0.004	0.06	0.07	0.08	0.006						
FL/SVL	0.44	0.50	0.55	0.02	0.46	0.50	0.53	0.02	0.45	0.47	0.49	0.01						
TL/SVL	0.49	0.55	0.61	0.02	0.52	0.56	0.59	0.01	0.46	0.51	0.55	0.02						
AAU/SVL	0.52	0.57	0.65	0.02	0.50	0.56	0.60	0.03	0.49	0.54	0.57	0.02						
CAL/SVL	0.04	0.05	0.08	0.007	0.04	0.05	0.06	0.005	0.03	0.04	0.05	0.005						



Figs. 4a-4b: Ventral aspects of a male (a) and female (b) of *Rana macrocnemis* BOULENGER, 1885, from the Berçelan Plateau (Hakkari).

Abb. 4a-4b: Ventralansichten einer männlichen (a) und weiblichen (b) *Rana macrocnemis* BOULENGER, 1885 vom Berçelan Plateau (Hakkari).

test. Results with $p < 0.05$ point to a statistically significant difference in the compared characteristics. The morphometric measurements obtained from the specimens of the Berçelan Plateau (Hakkari) as well as from the comparative specimens of Uludağ (*R. macrocnemis*) and Mt. Bolkar, Karagöl (*Rana holtzi*) were subject to independent samples *t*-test and Discriminant Function Analysis (DFA) using \log_{10} transformed values.

Electrophoretic analysis.— By the help of a hematocrit capillary tube, blood samples were taken from the cardiac ventricle of adult animals that had been anesthetized with ether. The blood was centrifuged at 600 g for 5 min, separated from the cellular section, and serum preserved at -20 °C. Separation was carried out at a laboratory temperature of 20 to 25 °C by using 5 µl of serum per sample. A Canalco® model 1200 was used as the electrophoresis bath, and a Gelman® Deluxe model served as power supply. Glass gel columns (4.7 mm x 75 mm) were utilized. Amperage per column was adjusted to 0.85 mA initially and gradually increased to 1.75 mA (36 mA,

370 volts for 12 gel columns) until the tracking dye (bromphenol blue) moved about 53 mm from the start.

The blood-serum proteins were separated according to the methods given by DAVIS (1964) and slightly modified by ÖZETİ & ATATÜR (1979). Accordingly, a buffer system containing the stacking gel (2.5 %, pH 6.7) at the top and a mixture of the separation gel (7.5 %, pH 9.0) and tris-glycine (pH 8.3) at the bottom was used. The electrophoretic separations were carried out at room temperature (approx. 20-25 °C). The separation gels were stained with 0.5 % Amido Black (Naphthol Blue Black 10-B) and then destained passively with repeated 7 % acetic acid baths. Later on, the stained gels were photographed. Qualitative evaluation of the gels was made directly from the electropherograms, and the densitometric curves of the separations were obtained by means of a Gelman® ACD-15 Model 39430 densitometer at a wavelength of 500 nm. The images of the stained gels were taken with a Nikon® Coolpix P1 digital camera.

RESULTS

Color-pattern.- The dorsal skin is granulated in all Hakkari specimens, and the head, the back and the dorsal side of the legs are mostly of light brownish green, greyish green or yellowish green color. A light brick-red is seen in some specimens with a light brownish ground color. The dorsolateral folds are well developed. There is a swelling (mating callus) on the first finger in all male specimens. The vertebral band is clearly present in 27 specimens (50 %) and hardly visible in two specimens (3.70 %). The population of the Berçelan Plateau (Hakkari) was divided into four groups according to the presence and arrangement of dorsal spots. A large number of individual spots of various shapes are irregularly scattered in 17 of 25 specimens lacking a dorsal band, while few spots are irregularly scattered in 8 of them (Figs. 3a, 3b). In 17 of 29 specimens showing a vertebral band, individual spots are scattered in an almost symmetrical order to the sides of the vertebral band. In 12 specimens, however, the spots join to form aggregates on the sides of the vertebral band and extend along the paravertebral area (Figs. 3c, 3d). The spots in the upper part of the hind legs are arranged in the form of transverse bands. The throat region bears sparsely or clearly visible dark spots in 30 specimens but is spotless in 24 specimens. The ventral side is spotless, off-white or slightly pinkish gray in males and light pink in females (Fig. 4). The underside of the femur is pinkish or pinkish-gray in males and females.

Morphometric measurements and ratios.- The descriptive statistics of the body measurements and ratios of the populations are provided in Table 1. Sexual dimorphism was expressed by the femur length (FL) in the studied population of the Berçelan Plateau (Hakkari) ($t = 2.026, df = 53, p = 0.048$) (Table 2). Independent-samples t-tests did not find differences between the Hakkari and Uludağ [i.e., *R. macrocnemis*] populations, but did so between the populations of Hakkari and the Bolkar Mountains [i.e., *R. holtzi*] in all the characteristics measured (Table 3). The specimens in the Hakkari population are larger than those in the Bolkar population ($t = 9.95, df$

= 70, $p < 0.05$). No differences were found in the measurement ratios between the populations of Hakkari and Uludağ [i.e., *R. macrocnemis*], whereas there were differences in all ratios obtained except for ID/SVL between the populations of Hakkari and the Bolkar Mountains [i.e., *R. holtzi*] (Table 3). In the Hakkari population, the values for CAL, TL and AAU are bigger than in the Bolkar population ($df = 70, p < 0.05, t = 4.88, 5.71$ and 3.81, respectively), whereas those for HW and IO are smaller ($df = 70, p < 0.05, t = 8.33$ and 3.79, respectively).

Discriminant function analysis distinguished two distinct groups when the data of the mountain frogs from Hakkari ($n = 54$), Uludağ ($n = 12$) and the Bolkar Mountains ($n = 18$) was processed. One cluster was formed by the populations of Hakkari and Uludağ and the other group comprised the individuals of the Bolkar Mountains. The first canonical variate explained 92.5 % of the total variance and separated the population of the Bolkar Mountains from the other two populations (Hakkari and Uludağ). The first two discriminant functions explained 100 % of the total variance (Table 4). In this analysis, 100 % of the Bolkar Mountains specimens were classified correctly and formed a cluster, 11 specimens (20.80 %) of the Hakkari population were classified incorrectly and lumped together with the Uludağ specimens, whereas two specimens from the Uludağ population (16.70 %) were classified incorrectly and placed with most Hakkari (Berçelan Plateau) specimens (Table 5 and Fig. 5). Thereupon, with the aim to further increase the discriminating power and improve correct classification, another analysis was made to discriminate the populations of the Bolkar Mountains from the combined populations "Hakkari plus Uludağ". In this analysis, 100 % of both the Bolkar Mountains individuals and the combined "Hakkari plus Uludağ" individuals were classified correctly (Tables 6 and 7).

Electrophoretic analysis.- Sex-dependent variation was not observed in the electropherograms obtained from the blood

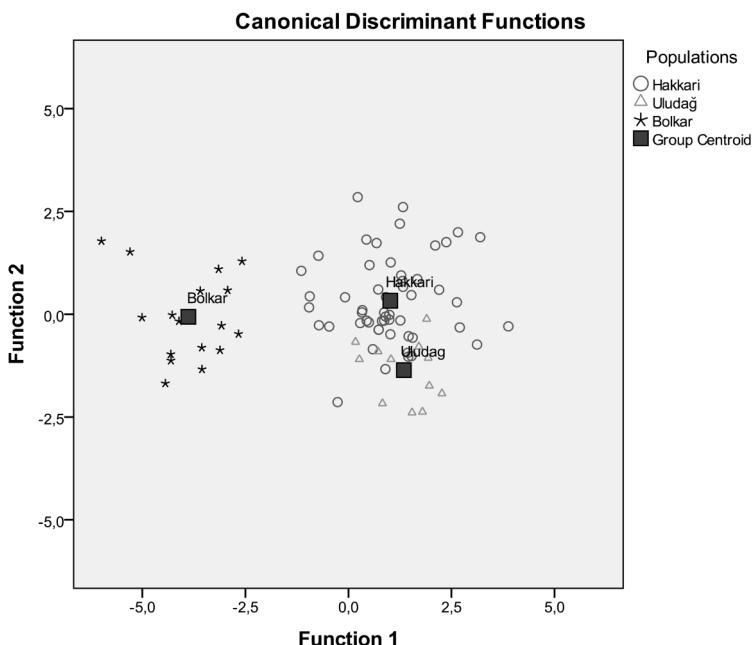


Fig. 5: Graph visualizing the result of a Discriminant Function Analysis based on eight morphological characteristics of the mountain frog populations of the Berçelan Plateau (Hakkari) [circles], Uludağ (Bursa) [triangles], and Karagöl, Bolkar Mountains (Niğde) [asterisks].

Abb. 5: Graphische Darstellung des Ergebnisses der Diskriminanzanalyse von acht morphologischen Merkmalen der Braunfrösche des Berçelan Plateaus (Hakkari) [Kreise], des Uludağ (Bursa) [Dreiecke] und von Karagöl, Bolkar Gebirge (Niğde) [Sternchen].

plasma samples of the Berçelan Plateau (Hakkari) and Sarialan population (Uludağ). Based on the gel photograph and densitometric curve of the electrophoretic separation of the blood-plasma proteins in an adult

male of the Hakkari population (Fig. 6) and the Uludağ population (Fig. 7), the proteins can be divided into 13 fractions or fraction groups (one in the albumin region and 12 in the region of globulins).

DISCUSSION

Previous studies on the morphology of various Anatolian mountain frog populations (BARAN 1969; BARAN & ATATÜR 1986; ÇEVİK et al. 2006; BARAN et al. 2007; BAŞKALE et al. 2012) agree on the observations that in *R. holtzi* (i) the dorsal skin is smooth and soft, (ii) no vertebral band is present and (iii) a ring lighter than the ground color surrounds each dorsal spot, unlike *R. macrocnemis*.

In the present study, the frogs of the Berçelan Plateau (Hakkari) were distinguished easily from *R. holtzi* by their (i) granulated skin, (ii) the high rate (53.70 %) of a vertebral band present and (iii) the absence of a ring, lighter than the ground color, encircling the spots on the dorsum.

Rana macrocnemis pseudodalmatina EISELT & SCHMIDTLER, 1971 was described

Table 2: Independent samples t-test comparisons of male and female mountain frogs of the Berçelan Plateau (Hakkari), based on measurements of nine morphometric variables. The statistically significant ($p < 0.05$) difference in femur length (FL) was indicated with an asterisk. See Material and Methods for abbreviations.

Tab. 2: T-Test-Vergleich männlicher und weiblicher Braunfrösche des Berçelan Plateaus (Hakkari) hinsichtlich neun morphometrischer Variablen. Der signifikante ($p < 0,05$) Unterschied in der Femurlänge (FL) ist mittels Asterisk gekennzeichnet. Die Abkürzungen in Spalte eins sind im Abschnitt Materials and Methods erklärt.

Characters Merkmale	Males vs. Females		
	Männchen	gegenüber	Weibchen
	t	df	p
SVL	0.29	53	0.766
HL	-0.15	53	0.885
HW	-0.43	53	0.667
IO	0.91	53	0.366
FL	2.03	53	0.048*
TL	1.04	53	0.304
AAU	1.76	52	0.085
CAL	-0.36	53	0.719
ID	0.24	53	0.815

from the Mazandaran province (north Iran), a region relatively close to the present study area. In the above study, *pseudodalmatina* was distinguished from the nominate subspecies by many morphological characteristics, including the thin and petite body structure, smoother skin, more barely perceptible dorsolateral folds, barely perceptible dorsal pattern, and the absence of a light vertebral band in the middle of the dorsum. Furthermore, different from *R. macrocnemis* or *R. camerani*, the white or whitish upper labial stripe was thinner and more petite and extended in cranial direction only as far as under the eye.

The mountain frog specimens of the Berçelan Plateau can be distinguished easily from *pseudodalmatina* by morphological traits such as the granulated skin, the extension of the white or whitish upper labial stripe up to the tip of the chin, the evident dorsolateral folds, the distinct dorsal pattern, and the high rate of a vertebral band present. Thus, the population of the Berçelan Plateau substantially resembles the characteristics given for *R. macrocnemis* in the literature.

Table 3: Independent samples t-test comparisons of mountain frogs of the Berçelan Plateau (Hakkari) versus specimens of Mt. Uludağ (Bursa) and Karagöl, Bolkar Mountains (Niğde) regarding eight morphometric variables and seven derived ratios. Significant p-values are indicated by an asterisk. See Materials and Methods for abbreviations in column one.

Tab. 3: T-Test-Vergleich der Braunfrösche des Berçelan Plateaus (Hakkari) gegenüber Fröschen des Mt. Uludağ (Bursa) sowie von Karagöl, Bolkar Gebirge (Niğde) in acht morphometrischen Variablen und sieben abgeleiteten Quotienten. Signifikante p-Werte sind mittels Asterisk gekennzeichnet. Die Abkürzungen in Spalte eins sind im Abschnitt Materials and Methods erklärt.

Character Merkmäl	Berçelan vs. Uludağ			Berçelan vs. Karagöl		
	t	df	p	t	df	p
SVL	0.51	64	0.613	9.95	70	0.000*
HL	0.70	64	0.488	9.01	70	0.000*
HW	0.09	64	0.930	6.38	70	0.000*
IO	1.36	64	0.180	4.403	70	0.000*
TL	-0.63	64	0.529	12.33	70	0.000*
AAU	1.73	63	0.089	11.15	69	0.000*
CAL	1.19	64	0.240	10.97	70	0.000*
ID	-0.84	64	0.406	6.56	70	0.000*
HW/SVL	-0.87	64	0.387	-8.33	70	0.000*
HL/SVL	0.31	64	0.76	-3.19	70	0.002*
ID/SVL	-1.19	64	0.23	-0.72	70	0.472
IO/SVL	1.25	64	0.21	-3.79	70	0.000*
TL/SVL	-1.91	64	0.06	5.71	70	0.000*
AAU/SVL	1.83	63	0.07	3.81	69	0.000*
CAL/SVL	0.96	64	0.34	4.88	70	0.000*

Total length (SVL) (BARAN et al. 2007) and the relative size of the inner metatarsal tubercle (CAL) (derived from data in ÇEVİK et al. 2006) were found to be smaller in *R. holtzi* than in other mountain frog species in Anatolia. BAŞKALE et al. (2012) detected statistically significant differences in all 12 metric characteristics measured when he compared *R. holtzi* from Karagöl, Çamlıayla (Mersin) and *R. macrocnemis* from Seviçova (Ereğli) and Uludağ (Bursa). In the present study, the independent samples t-test detected differences in eight metric characteristics between the populations of the Berçelan Plateau (Hakkari) and Mt. Bolkar (Karagöl, Niğde) (Table 3). The discriminant analysis, distinguished clearly the populations of the Berçelan Plateau (Hakkari) from the population of *R. holtzi* in the Bolkar Mountains (Karagöl). The plot shows a

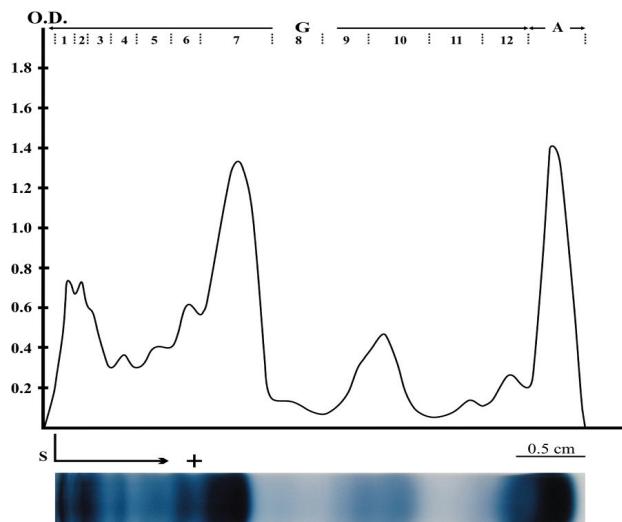


Fig. 6 : Gel photograph (below) and densitometric tracing curve (above) showing the electrophoretic separation of the blood plasma proteins obtained from an adult male specimen representing the population of the Berçelan Plateau (Hakkari). O.D. - Optical density, S - start, A - albumin, G₍₁₋₁₂₎ - Globulins.

Abb. 6: Gel (unten) und densitometrische Verteilungskurve (oben) der elektrophoretischen Auf trennung der Blutplasmaproteine eines adulten männlichen Braunfrosches vom Berçelan Plateau (Hakkari). O.D. – optische Dichte, S – Startpunkt, A Albumin, G₍₁₋₁₂₎ – Globuline.

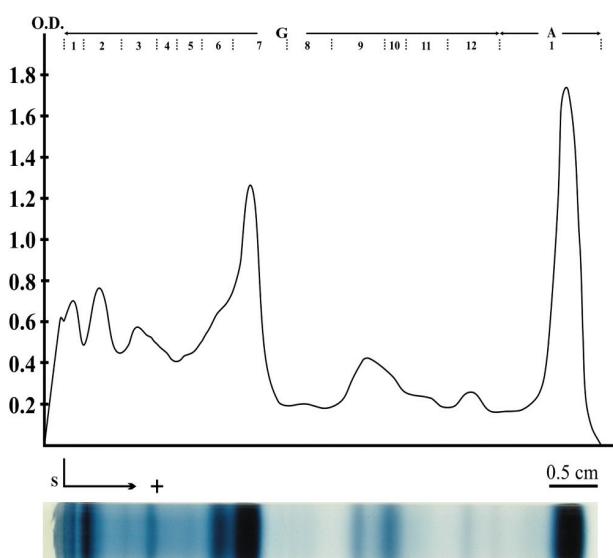


Fig. 7: Gel photograph (below) and densitometric tracing curve (above) showing the electrophoretic separation of the blood-plasma proteins obtained from an adult male specimen representing the Sarialan / Uludağ (Bursa) population. O.D. - Optical density, S - start, A - albumin, G₍₁₋₁₂₎ - globulins.

Abb. 7: Gel (unten) und densitometrische Verteilungskurve (oben) der elektrophoretischen Auf trennung der Blutplasmaproteine eines adulten männlichen Braunfrosches der Sarialan / Uludağ (Bursa) population. O.D. – optische Dichte, S – Startpunkt, A Albumin, G₍₁₋₁₂₎ – Globuline.

Table 4: Descriptive statistics of the first and second canonical discriminant functions of an analysis in which mountain frog populations of the Berçelan Plateau (Hakkari), Mt. Uludağ (Bursa) and Karagöl, Bolkar Mountains (Niğde) were analyzed with regard to eight morphometric variables.

Tab. 4: Deskriptive Statistiken der ersten und zweiten kanonischen Diskriminanzfunktion aus einer Analyse, in welcher Braunfrösche des Berçelan Plateaus (Hakkari), des Uludağ (Bursa) und von Karagöl, Bolkar Gebirge (Niğde) hinsichtlich acht morphometrischer Variablen untersucht wurden.

Canonical Function	Eigenvalue	Canonical Correlation	Test of Function(s)	df	Significance
			Wilks' Lambda	Chi-square	
1	4.349	0.902	0.138	154.255	10
2	0.351	0.510	0.740	23.453	4

Table 5: Classification results (predicted group memberships) of the discriminant function analysis of eight morphometric variables from mountain frogs of the Berçelan Plateau (Hakkari), Mt. Uludağ (Bursa) and Karagöl, Bolkar Mountains (Niğde).

Tab. 5: Die Zuordnungsergebnisse (vorhergesagte Klassenzugehörigkeit) aus der Diskriminanzanalyse acht morphologischer Variablen von Braunfröschen des Berçelan Plateaus (Hakkari), des Uludağ (Bursa) und von Karagöl, Bolkar Gebirge (Niğde).

	Groups / Klassen	Predicted Group Membership / vorhergesagte Klassenzugehörigkeit			Σ
		Berçelan	Uludağ	Karagöl	
N	Berçelan (Hakkari)	42	11	0	53
	Uludağ (Bursa)	2	10	0	12
	Karagöl (Bolkar Mt.)	0	0	18	18
%	Berçelan (Hakkari)	79.2	20.8	0.0	100.0
	Uludağ (Bursa)	16.7	83.3	0.0	100.0
	Karagöl (Bolkar Mt.)	0.0	0.0	100.0	100.0

Table 6: Descriptive statistics of the first canonical discriminant function of an analysis, in which the combined mountain frog populations “Berçelan Plateau (Hakkari) plus Mt. Uludağ (Bursa)” and the individuals from Karagöl, Bolkar Mountains (Niğde) were analyzed with regard to eight morphometric variables.

Tab. 6: Deskriptive Statistiken der ersten kanonischen Diskriminanzfunktion aus einer Analyse, in welcher die vereinigten Braunfrosch-Stichproben “Berçelan Plateau (Hakkari) plus Uludağ (Bursa)” und die Individuen von Karagöl, Bolkar Gebirge (Niğde) hinsichtlich acht morphometrischer Variablen untersucht wurden.

Canonical Function	Eigenvalue	Canonical Correlation	Test of Function(s)	df	Significance
			Wilks' Lambda	Chi-square	
1	4.301	0.901	0.189	130.936	5

Table 7: Classification results (predicted group memberships) of the discriminant function analysis of eight morphometric variables from the combined mountain frog populations “Berçelan Plateau (Hakkari) plus Mt. Uludağ (Bursa)” and the individuals from Karagöl, Bolkar Mountains (Niğde). One hundred percent of the cases were classified correctly.

Tab. 7: Die Zuordnungsergebnisse (vorhergesagte Klassenzugehörigkeit) aus der Diskriminanzanalyse acht morphologischer Variablen von Braunfröschen der vereinigten Stichproben “Berçelan Plateau (Hakkari) plus Uludağ (Bursa)” und der Individuen von Karagöl, Bolkar Gebirge (Niğde).

	Groups / Klassen	Predicted Group Membership / vorhergesagte Klassenzugehörigkeit		Σ
		Berçelan + Uludağ	Karagöl	
N	Berçelan + Uludağ	65	0	65
	Karagöl (Bolkar Mt.)	0	18	18
%	Berçelan + Uludağ	100.0	0.0	100.0
	Karagöl	0.0	100.0	100.0

distinct separation between the populations of the Berçelan Plateau and the Bolkar Mountains (Karagöl); however, the population of the Berçelan Plateau cannot be distinguished from the population of *R. macrocnemis* from Sarialan (Uludağ) (Figure 5 and Table 5).

As regards the blood-plasma proteins, significant quantitative differences are present within the regions of globulins of the population belonging to the Berçelan Plateau (Hakkari) and the Sarialan population (Uludağ). According to DESSAUER (1956), CHEN (1967), FERGUSON (1980) and ARIKAN et al. (1998, 1999), who worked on the blood-plasma proteins of amphibians, factors such as age, as well as physiological and environmental conditions, cause quantitative differences in the blood-plasma pro-

teins, while only genetic variations cause qualitative differences. Interpopulational quantitative differences were in fact evident, especially among the globulins, but are not of taxonomical significance. This result is in agreement with the result provided for the Uludağ mountain frogs (*R. macrocnemis*) by ÇEVİK et al. (2006).

In conclusion, the population of the Berçelan Plateau (Hakkari) substantially resembles the Sarialan population (Uludağ) when both morphological characteristics and the results of the electrophoretic analysis of the blood-plasma proteins are taken into consideration. Hence, the authors are convinced that it is appropriate to assign this east Anatolian mountain frog population to the taxon *R. macrocnemis*.

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REFERENCES

- AFSAR, M. & AFSAR, B. & ARIKAN, H. (2013): *Rana macrocnemis* BOULENGER, 1885, from the Emir Mountains in western inner Anatolia (Turkey).- Herpetozoa, Wien; 26 (3/4): 115-123.
- ARIKAN, H. & ATATÜR, M. K. ÇEVİK, İ. E. & KUMLUŞAŞ, Y. (1999): A serological investigation of *Lacerta viridis* (LAURENTI, 1768) (Sauria: Lacertidae) populations in Turkey.- Turkish Journal of Zoology, Ankara; 23: 227-230.
- ARIKAN, H. & ATATÜR, M. K. & MERMER, A. (1998): An investigation on the blood-serum proteins of *Chalcides ocellatus* (Sauria: Scincidae) populations from Southern Anatolia.- Turkish Journal of Zoology, Ankara; 22: 175-177.
- ARIKAN, H. & OLGUN, K. & TOK, C. V. & ÇEVİK İ. E. (2001): Morphological and serological investigation on the Mountain Frogs of the Mid-Taurus Range between East longitudes 33° and 36°.- Turkish Journal of Zoology, Ankara; 25: 141-145.
- BARAN, İ. (1969): Anadolu dağ kurbağaları üzerinde sistematik araştırma.- Ege Üniversitesi Fen Fakültesi İlimi Raporlar Serisi, İzmir; 80: 1-178.
- BARAN, İ. & ATATÜR, M. K. (1986): A taxonomical survey of the mountain frogs of Anatolia.- Amphibia-Reptilia, Leiden; 7: 115-133.
- BARAN, İ. & ILGAZ, Ç. & KUMLUŞAŞ, Y. & OLGUN, K. & AVCI, A. & İRET, F. (2007): On new populations of *Rana holtzi* and *Rana macrocnemis* (Ranidae: Anura).- Turkish Journal of Zoology, Ankara; 31: 1-7.
- BARAN, İ. & KUMLUŞAŞ, Y. & TOK, C. V. & ILGAZ, Ç. & KASKA, Y. & OLGUN, K. & TURKOZAN, O. & İRET, F. (2004): On two herpetological collections made in East Anatolia (Turkey).- Herpetozoa, Wien; 16 (3/4): 99-114.
- BAŞKALE, E. & ÇEVİK, İ. E. & KAYA, U. & KUMLUŞAŞ, Y. & OLGUN, K. (2012): New data on the distribution of the Taurus Frog, *Rana holtzi* WERNER, 1898, in the Bolkar Mountains of Turkey.- Herpetozoa, Wien; 24 (3/4): 113-120.
- BAŞOĞLU, M. & HELLMICH, W. (1958): Auf herpetologischer Forschungsfahrt in Ost-Anatolien.- Die Aquarien-und Terrarien- Zeitschrift (Datz), Stuttgart; 12: 118-121.
- BODENHEIMER, F. S. (1944): Introduction into the knowledge of the Amphibia and Reptilia of Turkey.- İstanbul Üniversitesi Fen Fakültesi Mecmuası, İstanbul; (B) 9: 1-78.
- BOULENGER, G. A. (1898): The tailles batrachians of Europe. Part II, London (The Ray Society), pp. 211-376.
- ÇEVİK, İ. E. & ARIKAN, H. & KAYA, U. & ATATÜR, M. K. (2006): Comparative morphological and serological studies of three Anatolian Mountain frogs, *Rana macrocnemis*, *R. camerani* and *R. holtzi* (Anura, Ranidae).- Amphibia-Reptilia, Leiden; 27: 63-71.
- CHEN, P. S. (1967): Separation of serum proteins in different amphibian species by polyacrylamide gel electrophoresis.- Experientia, Basel; 23: 483-485.

- DAVIS, B. J. (1964): Disc electrophoresis - II. Method and application to human serum proteins.- Annals of the New York Academy of Sciences, Hoboken; 121: 404-427.
- DESSAUER, H. C. (1956): Characteristic electrophoretic patterns of orders of Amphibia and Reptilia.- Science, Washington; 124: 225-226.
- DÜŞEN, S. & ÖZ, M. & TUNÇ, M. R. (2002): A new locality for *Rana camerani* BOULENGER, 1886 (Anura: Ranidae) in Turkey.- Russian Journal of Herpetology, Moskva; 9: 135-136.
- EISELT, J. (1965): Einige Amphibien und Reptilien aus der nordöstlichen Türkei, gesammelt von Herrn H. Steiner.- Annalen des Naturhistorischen Museums, Wien; 68: 387-399.
- EISELT, J. & SCHMIDTLER, J. F. (1971): Vorläufige Mitteilung über zwei neue Subspezies von Amphibia Salientia aus dem Iran.- Annalen des Naturhistorischen Museums, Wien; 75: 383-385.
- FERGUSON, A. (1980): Biochemical systematics and evolution. New York (Halstead Press, John Wiley and Sons), pp. ix, 194.
- LANTZ, L. A. & CYRÉN, O. (1913): Über die Identität von *Rana macrocnemis* und *Rana camerani*.- Zoologischer Anzeiger, Jena, Leipzig; 43: 214-220.
- MERTENS, R. (1952): Amphibien und Reptilien aus der Türkei.- İstanbul Üniversitesi Fen Fakültesi Mecmuası, İstanbul; (B) 17: 41-75.
- ÖZETİ, N. & ATATÜR, M. K. (1979): A preliminary survey of the serum proteins of a population of *Mertensiella luschani finikensis* BAŞOGLU and ATATÜR from Finike in southwestern Anatolia.- İstanbul Üniversitesi Fen Fakültesi Mecmuası, İstanbul; 44: 23-79.
- PICARIELLO, O. & FELICIETTO, I. & SCILLITANI, G. & CATAUDO, A. & MARESCA, I. B. & CHINALLI, G. (1999): Morphological and molecular evidence supporting the taxonomic identity of *R. macrocnemis*, *R. camerani* and *R. holtzi* (Anura: Ranidae).- Hydrobiologia, Dordrecht; 38: 167-182.
- TARKHNISHVILI, D. & ARNTZEN, W. & THORPE, R. (1999): Morphological variation in Brown Frogs of the Caucasus and the taxonomy of the *Rana macrocnemis* group.- Herpetologica, Lawrence; 55 (3): 406-417.
- TERENT'EV, P. V. & CHERNOV, S. A. (1965): Key to amphibians and reptiles. 3rd edition. Jarusalem (Israel program for Scientific Translations Ltd., translated from Russian), pp. 315.
- VEITH, M. & KOSUCH, J. & VENCES, M. (2003b): Climatic oscillations triggered post-Messinian speciation of Western Palearctic brown frogs (Amphibia, Ranidae).- Molecular Phylogenetics and Evolution, San Diego, etc.; 26: 310-327.
- VEITH, M. & SCHMIDTLER, J. & KOSUCH, J. & BARAN, İ. & SEITZ A. (2003a): Palaeoclimatic changes explain Anatolian mountain frog evolution. A test for alternating vicariance and dispersal events.- Molecular Ecology, Oxford; 12: 185-199.
- WERNER, F. (1902): Die Reptilien- und Amphibienfauna von Kleinasiens.- Sitzungsberichte der Akademie der Wissenschaften, mathematisch-naturwissenschaftliche Klasse, Wien; (Abtheilung I) 111: 1057-1121.

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