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Accepted: 24 September 1998
Associate Editor: Stephen Tilley

Herpetologica, 55(3), 1999, 406–417

MORPHOLOGICAL VARIATION IN BROWN FROGS FROM THE CAUCASUS AND THE TAXONOMY OF THE *RANA MACROCNEMIS* GROUP

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ABSTRACT: Multivariate analysis of body proportions, skin texture, and coloration characteristics of brown frogs (*Rana macrocnemis* and *R. camerani*) from 14 Georgian populations confirms the existence of two, geographically distinct groups of populations. Frogs in the first group (*R. macrocnemis*) are characterized by a smooth skin, a pale spotted pattern, absence of a mid-dorsal stripe, and a small inner metatarsal tubercle. Frogs in the second group (known as *R. camerani*) possess a rugose skin, a conspicuous pattern of dorsal spots, a mid-dorsal stripe with contrasting borders, and a large metatarsal tubercle. The first group occurs in the uplands of the Great Caucasus and other forested areas while the second group occurs in the treeless uplands of the Near East. A stepped cline exists between them, with parallel variation in eight morphological characters. Other characters analyzed vary independently of the cline. Fully diagnostic (fixed) morphological characters separating the groups were not observed. Some characters previously used for taxonomic purposes are shown to be associated with local ecological conditions. We conclude that *R. macrocnemis* is a single, though polytypic, species composed of two interbreeding evolutionary lineages.

Key words: Brown frogs; Caucasus; Clinal variation; Coloration; Multivariate statistics; Near East; *Rana macrocnemis*; Taxonomy; Transition zone

THE brown frogs inhabiting the Near East form a group of related taxa (Baran and Atatür, 1986; Green and Borkin, 1993; Mensi et al., 1992) that is geographically separated from other Palearctic representatives of the subgenus *Rana* (sensu Dubois, 1993) and that we refer to as the *R. macrocnemis* species group. Its distribution (Fig. 1) is restricted to the mountainous regions of Anatolia, the Caucasus, northern Iran and perhaps northern Iraq (Baran and Atatür, 1986; Tarkhnishvili, 1996; Tarkhnishvili and Thiesmeier, 1994;

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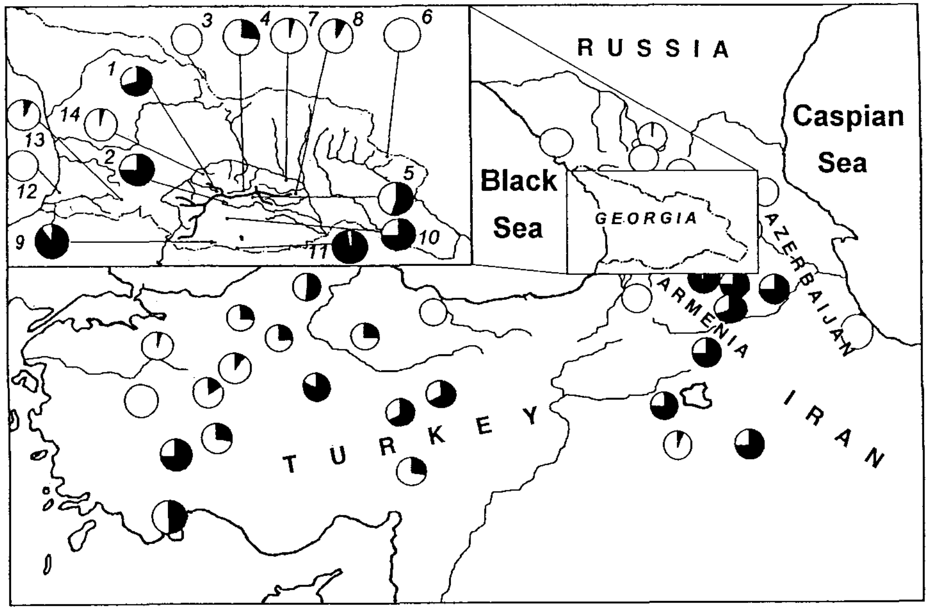

FIG. 1.—The approximate distribution of brown frogs of the *Rana macrocnemis* group in the Near East, following Baran and Atatür (1986), Ishchenko (1978, 1987), Tarkhnishvili (1996), and the present paper. Pie diagrams indicate the proportion of frogs in the population with mid-dorsal stripe present (black) and absent (white). The insert shows the study populations in Georgia, numbered 1–14 (for locality names see Table 1). The bold line indicates the watershed of the Javakheti Plateau.

Tertishnikov et al., 1979). This group is separated from *R. arvalis* Nilsson, 1842 in the north by the southern Russian steppes and *R. temporaria* Linnaeus, 1758 in the west by the Bosporus. The only other species of brown frog in the region is *R. dalmatina* Bonaparte, 1840 which occurs in western Anatolia (Sparreboom and Arntzen, 1987), and possibly all along the southern coast of the Black Sea (Yilmaz, 1989).

Initially, all brown frogs from the Caucasus were identified as *Rana temporaria* and *R. dalmatina* (Bedriaga, 1879; Eichwald, 1841; Kessler, 1878). Boulenger (1885, 1896) described frogs from Uludag (western Turkey) and Tiflis (= Tbilisi, Georgia) as *Rana macrocnemis* Boulenger, 1885 and frogs from lake Tabatskuri (Georgia) and Tiflis as *Rana camerani* Boulenger, 1896 (according to some authors, *R. cameranoi*). For describing and distinguishing between *R. macrocnemis* and *R. camerani*, Boulenger (1896) used several morphometric characters and the presence (*Rana camerani*) and absence (*Rana macrocnemis*) of a mid-dorsal stripe with dark edges. A third representative of the group, *R. holtzi*, was described from the Taurus mountains in Turkey (Werner 1898). Because *R. macrocnemis*, *R. camerani*, and *R. holtzi* are morphologically similar, discussions on their taxonomic status continue to the present day (Borkin, 1987 1997; Ishchenko, 1987; Kuzmin, 1996 Tarkhnishvili, 1996). These taxa are generally considered separate but closely related species within the subgenus *Rana* (*Rana*) (Alekperov, 1978; Ananyeva et al. 1988; Baran, 1969; Baran and Atatür 1986; Dubois, 1993; Eiselt and Schmidtler 1971; Frost, 1985; Green and Borkin 1993; Mensi et al., 1992; Papanyan, 1961) while others perceive the differences between the forms as minor and consider *camerani* as a subspecies of *Rana macrocnemis* (Delwig, 1928; Gumilevsky, 1939

TABLE 1.—Sampling localities and characteristics of Georgian brown frog populations. S is the percentage of frogs possessing a bright mid-dorsal stripe.

Locality	Habitat	Elevation	Sample size: Males	Females	Juveniles	S
1. Gujareti	Subalpine	1850	13	6	28	75
2. Tabatskuri	Mountain steppe	2000	27	12	23	77
3. Mamisoni	Alpine	2550	21	1	0	0
4. Gostibe	Subalpine forest	1600	4	3	4	27
5. Imera	Mountain steppe	1550	2	1	7	70
6. Duruji	Subalpine	1950	21	1	0	0
7. Satovle	Beach forest	1350	33	2	0	3
8. Tskhneti	Hornbeam forest	950	15	2	1	6
9. Knachali	Mountain steppe	1950	8	4	11	91
10. Samsari	Alpine	2500	16	9	1	73
11. Kurjanchay	Mountain steppe	2200	11	18	0	97
12. Batumi	Deciduous forest	50	0	4	10	0
13. Goderdzi	Subalpine	1950	2	4	6	8
14. Nedzura	Mixed forest	1000	52	18	0	4

Ishchenko and Pyastolova, 1973; Lantz and Cyren, 1913; Logvinenko and Pryalkina, 1987; Nikolsky, 1913). According to some authors (Bannikov et al., 1977; Bodenheimer, 1944; Borkin, 1977; Ishchenko, 1978, 1987), neither the names *R. camerani* nor *R. macrocnemis camerani* represent valid taxa.

The following information underlies the present study. (1) Nominal taxa of brown frogs inhabiting the Near East are genetically closer to each other than to other species of the subgenus *Rana* (Green and Borkin, 1993; Mensi et al., 1992). (2) Two groups of populations can be distinguished that differ in the frequency of specimens with a mid-dorsal stripe (Fig. 1). The first group, with a high proportion of striped specimens (>50%), inhabits treeless uplands of the Near East, including southern Georgia, Armenia, northern Iraq and the central part of Anatolia. The second group in which striped specimens are rare or absent (<10%), inhabits western Anatolia, the Great Caucasus, and the mountains along the southern coast of the Caspian sea (Alekperov, 1978; Baran, 1969; Baran and Atatür, 1986; Borkin, 1977; Lantz and Cyren, 1913). (3) The Javakheti plateau in southern Georgia is the northernmost range for populations with a high proportion of striped specimens (Tarkhnishvili, 1996; Tarkhnishvili and Gokhelashvili, 1996). These frogs were recognized as *R. camerani* and frogs from the other parts of Georgia were recognized as *R. macrocnemis* (Boulenger, 1885, 1896; Nikolsky, 1913).

We studied frogs from all over Georgia with the aims of answering the following questions. (1) Are there pronounced and consistent morphological differences between nominal *R. camerani* and nominal *R. macrocnemis* or, alternatively, could the morphological variation between populations be explained by local ecological adaptation? (2) If there are morphological differences between the two taxa, is the transition gradual or abrupt? (3) Is the mid-dorsal stripe a taxonomically valuable character? (4) Is *R. "camerani"* a separate species?

MATERIALS AND METHODS

We obtained data on body proportions and coloration patterns for 401 frogs (225 males, 85 females, and 91 subadults and juveniles) representing 14 populations (Table 1, Fig. 1). Sample size ranged from 10–72/population. Seven populations were from the Javakheti plateau and seven populations from other parts of Georgia. Measurements were taken from freshly sacrificed adult specimens with calipers of 0.1 mm precision as follows: (1) L = snout–urostyle length, (2) Lc = head length, (3) Ltc = head width, (4) Dro = distance from eye to tip of snout, (5) Spoc = distance between the eyes, (6) Loc = diameter of the eye, (7) Ltym = diameter of the tympanum, (8) F = femur length, (9) T = tibia length, (10) Dp = length of the first toe, (11) Cint = length of the inner metatarsal tubercle (Table 2). The data were ln-transformed and the standardized residuals of the regression of each character on snout–urostyle length was calculated, thereby reducing the number of morphometric characters to 10. The transformation was done in order to reduce the effect of variation in individual size and to increase the fit to the requirements for such analysis (Sokal and Rohlf, 1995; Thorpe and Leamy, 1983). Data for adult males and females were analyzed separately to reduce possible effect of sexual dimorphism. Frogs were preserved in 5% formaldehyde and deposited as vouchers in the collection of Tbilisi State University.

We made qualitative descriptions of coloration pattern and skin structure for both adult and juvenile specimens. The following characters were scored as present or absent: (1) S = presence versus absence of mid-dorsal stripe with contrasting dark edges, (2) DC = dark brown or almost black dorsal coloration versus olive to mid-brown colored specimens, (3) BS = dark and sharp dorsal spots versus no spots or spots with vague borders, (4) R = rugose skin versus smooth skin, (5) DT = spotted or dark-gray throat versus unspotted white throat, (6) DB = spotted belly versus unspotted belly, (7) P = presence versus absence of dark speckles on the dorsum (independently of character BS), (8) V = presence versus absence of V-shaped spot on the back of the head, (9) DS = presence versus absence of dorsolateral dark stripes, (10) NS = the number of dark dorsal spots higher versus lower than 9, (11) OS = oblong versus round dorsal spots, (12) SS = dark dorsal spots placed symmetrically versus asymmetrically, and (13) LS = presence versus absence of light dorsolateral stripes (Table 3). None of these characters displayed significant sexual or ontogenetic differences and the data were pooled for sex and size classes.

In order to estimate morphological differentiation between populations, we analyzed the continuous and qualitative characters by principal component analysis (PCA) using population means for each character. For coloration characters, the PCA was done with the mid-dorsal stripe excluded, to avoid circularity in the interpretation of the results (i.e., the stripe was used to assign populations to one of the two nominal taxa). The analysis was done with the SPSS software package, version 6.1 (1994).

In the analysis of geographic variation, populations were arranged according to their distance from the mountain range bordering the Javakheti plateau. Individual PCA scores for animals collected at different distances from the watershed were compared using one-way ANOVA. Interpopulation differences in the arcsine-transformed frequencies (Sokal and Rohlf, 1995) of qualitative characters were tested by Fisher's angular test (Fisher, 1954; Zaitsev, 1984). Prior to the analysis, proportions were arcsine-transformed, in order to reduce the dependence of dispersion on the mean (Sokal and Rohlf, 1995).

The association between two data sets arranged in a matrix (e.g., geographic versus morphological distances between populations) can be tested by a matrix correspondence or Mantel test (Manly, 1986; Smouse and Long, 1992). In this study, a morphological difference among geographic populations was simultaneously tested for an association with "nominal taxonomic group", local ecological conditions, and geographic proximity using a partial regression Mantel test (Thorpe et al., 1996). This method aims to partial out the interrelation that might exist among patterns generated by the alternative hypotheses. The dependent variables (morphological Euclidean distances between populations, estimated for individual morphological characters and for multivariate generalizations) were compared with three independent variables: (1) the difference between populations in the frequency of striped specimens (i.e., the reference character identifying nominal taxa), (2) ecological dissimilarity, expressed as the difference in elevation between populations (altitude in the study area varies from sea-level to 2700 m and largely determines local ecological conditions), and (3) geo-

TABLE 2.—Morphometric characteristics of adult brown frogs from Georgia. Indicated are the mean ($\bar{x}$), standard deviation (SD), and range. Measurements are in millimetres. For population names and sample size see Table 1. L = snout–urostyle length; Lc = head length; Ltc = head width; Dro = distance from eye to tip of snout; Spoc = distance between the eyes; Loc = diameter of the eye; Ltym = diameter of the tympanum; F = femur length; T = tibia length; Dp = length of the first toe; Cint = length of the inner metatarsal tubercle; m = males; f = females.

Site	Sex		L	Lc	Ltc	Dro	Spoc	Loc	Ltym	F	T	Dp	Cint
1	m	$\bar{x}$	70.42	20.33	23.01	9.26	9.05	6.25	4.41	37.55	35.62	8.20	4.39
		SD	50.70	3.06	5.09	0.74	0.76	0.69	0.32	18.89	10.92	1.70	0.46
		min	56.1	16.1	18.7	7.8	7.1	4.9	3.4	28.8	28.0	5.3	3.1
		max	82.9	23.9	26.6	11.0	10.4	7.3	5.1	44.9	40.0	10.2	5.3
	f	$\bar{x}$	72.42	20.65	23.47	9.60	8.93	5.87	4.02	36.15	35.97	7.33	4.35
		SD	24.35	1.88	4.38	0.55	0.93	0.09	0.24	22.34	2.73	0.15	0.06
		min	64.7	18.9	20.6	8.7	7.7	5.4	3.5	29.4	34.0	6.6	4.0
		max	77.8	22.3	26.7	10.5	10.2	6.3	4.9	42.1	38.4	7.6	4.7
2	m	$\bar{x}$	62.65	19.04	20.85	8.97	8.09	5.53	3.84	29.06	31.61	7.03	3.81
		SD	20.25	1.13	2.55	0.37	0.35	0.17	0.21	7.06	4.32	0.43	0.26
		min	54.0	16.6	17.4	8.0	6.9	4.4	2.6	22.9	27.6	5.5	2.8
		max	73.8	21.1	24.4	10.7	9.2	6.2	4.7	35.4	35.1	8.3	4.8
	f	$\bar{x}$	62.33	18.23	20.39	8.78	7.61	5.10	3.61	28.72	30.50	6.85	3.53
		SD	17.29	0.91	2.66	0.35	0.31	0.18	0.19	3.97	6.93	0.46	0.27
		min	55.5	16.5	17.1	7.8	6.5	4.3	3.1	24.9	23.8	5.6	2.5
		max	68.1	20.0	23.1	9.8	8.3	5.8	4.3	33.1	33.5	7.8	4.3
3	m	$\bar{x}$	71.88	20.12	23.64	9.61	9.21	6.23	4.11	35.43	35.50	7.66	4.10
		SD	4.38	0.48	0.63	0.43	0.45	0.17	0.29	2.68	1.79	0.96	0.17
		min	67.0	18.9	22.3	8.5	7.0	5.7	3.4	32.7	32.9	5.9	3.0
		max	75.4	21.5	25.5	10.8	10.2	7.0	5.5	39.2	37.7	10.6	4.8
	f		72.4	20.4	24.3	10.1	8.3	6.3	4.1	34.5	33.0	7.6	3.7
4	m	$\bar{x}$	59.83	18.65	21.00	8.70	8.05	6.25	3.93	32.38	34.65	7.40	3.60
		SD	81.82	4.33	2.15	0.69	1.52	0.74	0.29	19.44	10.92	1.67	0.41
		min	50.7	16.6	19.1	7.8	7.4	5.5	3.3	28.3	31.4	6.3	3.0
		max	69.5	21.4	22.3	9.7	9.9	7.4	4.6	36.9	38.4	9.0	4.2
	f	$\bar{x}$	71.70	20.85	24.95	9.75	9.30	6.25	4.25	34.85	36.60	8.00	3.90
		SD	0.18	2.20	0.40	0.41	0.02	0.41	0.00	1.81	0.32	0.98	0.00
		min	71.4	19.8	24.5	9.3	9.2	5.8	4.2	33.9	36.2	7.3	3.9
		max	72.0	21.9	25.4	10.2	9.4	6.7	4.3	35.8	37.0	8.7	3.9
5	m	$\bar{x}$	64.80	18.45	22.40	9.20	8.50	6.05	3.85	33.25	35.50	8.05	3.95
		SD	33.62	0.00	12.50	0.18	0.18	0.84	0.13	9.24	28.88	1.44	0.85
		min	60.7	18.4	19.9	8.9	8.2	5.4	3.6	31.1	31.7	7.2	3.3
		max	68.9	18.5	24.9	9.5	8.8	6.7	4.1	35.4	39.3	8.9	4.6
	f		63.7	19.7	22.6	9.5	8.9	6.3	4.5	33.7	35.2	7.9	3.2
6	m	$\bar{x}$	62.41	17.90	18.31	8.73	7.93	5.97	3.94	32.55	33.06	7.05	2.82
		SD	14.44	1.14	1.61	0.34	0.12	0.20	0.12	4.53	4.08	0.73	0.16
		min	58.0	16.1	16.5	7.6	7.4	5.3	3.5	29.1	30.0	5.6	2.0
		max	72.9	20.5	22.0	9.5	8.5	6.9	4.7	36.9	38.2	8.5	3.6
	f		66.3	17.8	19.0	9.1	8.1	6.0	4.2	31.5	32.2	8.2	3.1
7	m	$\bar{x}$	66.19	19.61	21.19	9.35	8.63	6.58	4.02	34.12	36.81	7.58	3.29
		SD	18.11	2.34	2.61	0.58	0.74	0.38	0.19	6.94	6.02	0.60	0.24
		min	57.9	16.4	17.5	7.4	7.0	5.4	3.1	29.3	30.8	5.9	2.4
		max	76.1	24.8	23.5	11.2	10.4	7.5	5.1	40.3	40.7	9.2	4.1
	f	$\bar{x}$	76.40	22.60	24.15	10.00	9.05	6.50	3.70	35.45	39.70	8.25	3.45
		SD	14.58	13.52	1.12	0.50	0.12	0.50	0.50	0.84	2.42	0.61	0.13
		min	73.7	20.0	23.4	9.5	8.80	6.0	3.2	34.8	38.6	7.7	3.2
		max	79.1	25.2	24.9	10.5	9.30	7.0	4.2	36.1	40.8	8.8	3.7
8	m	$\bar{x}$	73.47	20.77	22.81	9.29	8.73	6.85	4.54	38.50	43.05	9.53	3.96
		SD	15.82	1.23	1.79	0.48	0.75	0.12	0.21	8.83	7.13	0.59	0.37
		min	67.3	19.0	20.0	7.9	7.3	6.3	3.8	35.0	38.8	8.5	2.7
		max	82.8	23.1	24.7	10.1	10.0	7.4	5.3	45.2	48.5	10.9	4.9

TABLE 2.—Continued.

Site	Sex		L	Lc	Ltc	Dro	Spoc	Loc	Ltym	F	T	Dp	Cint
	f	$\bar{x}$	62.60	17.85	20.25	8.10	8.00	5.95	2.80	31.40	33.15	7.85	3.25
		SD	0.18	1.13	1.13	0.00	0.02	0.13	0.02	0.32	0.00	0.25	0.41
		min	62.3	17.1	19.5	8.1	7.9	5.7	2.7	31.0	33.1	7.5	2.8
		max	62.9	18.6	21.0	8.1	8.1	6.2	2.9	31.8	33.2	8.2	3.7
9	m	$\bar{x}$	59.16	18.73	20.09	8.53	8.25	5.90	4.03	29.71	31.61	6.61	3.63
		SD	16.51	2.60	1.88	0.34	0.37	0.22	0.02	4.40	3.38	0.43	0.37
		min	52.0	16.2	18.0	7.5	7.7	5.0	3.9	26.9	27.9	6.0	3.0
		max	65.6	21.3	21.8	9.4	9.1	6.5	4.3	32.5	33.6	7.7	5.0
	f	$\bar{x}$	60.53	18.35	20.48	8.55	8.20	5.78	3.80	29.60	30.78	6.95	3.45
		SD	7.01	0.68	2.21	0.22	1.13	0.11	0.05	6.27	3.68	0.22	0.16
		min	57.6	17.3	19.4	8.0	7.4	5.4	3.60	27.2	29.1	6.5	3.1
		max	63.4	19.1	22.6	9.1	9.7	6.2	4.0	33.0	33.5	7.6	4.0
10	m	$\bar{x}$	58.43	18.26	19.93	8.86	7.72	6.07	4.08	29.78	32.72	6.98	3.63
		SD	22.42	2.29	2.59	0.38	0.45	0.20	0.13	12.96	10.79	0.77	0.30
		min	50.6	15.7	17.0	7.7	7.0	5.2	3.5	21.7	26.0	5.7	2.6
		max	65.1	20.6	22.5	9.8	9.2	7.0	5.0	34.9	38.5	8.4	4.6
	f	$\bar{x}$	64.18	19.48	21.52	9.12	8.22	6.18	4.14	31.57	32.72	7.14	3.60
		SD	25.88	1.59	6.24	0.65	0.88	0.10	0.30	11.00	6.17	0.75	0.18
		min	55.6	17.5	18.3	8.1	7.1	5.7	3.4	25.6	27.8	6.0	3.1
		max	70.5	21.3	25.1	10.1	10.3	6.6	5.1	35.0	35.5	8.5	4.1
11	m	$\bar{x}$	54.89	17.77	20.08	8.71	7.59	5.93	3.60	27.31	28.83	6.35	3.48
		SD	5.38	0.98	0.89	0.41	0.55	0.05	0.18	3.04	2.84	0.27	0.27
		min	51.0	15.7	18.1	7.1	6.0	5.6	2.9	25.5	26.5	5.5	2.9
		max	58.0	18.9	21.5	9.5	8.6	6.3	4.4	29.7	31.7	7.0	4.5
	f	$\bar{x}$	56.53	18.07	20.49	8.72	7.64	5.70	3.69	27.28	28.40	6.41	3.46
		SD	28.85	1.61	4.98	0.30	0.80	0.35	0.12	9.25	4.46	0.46	0.12
		min	49.1	16.0	17.0	7.6	6.1	4.9	3.1	21.7	24.7	5.3	3.0
		max	65.9	20.1	24.5	10.1	9.1	6.8	4.2	32.6	32.2	7.4	4.0
12	f	$\bar{x}$	59.80	18.83	20.13	9.10	8.93	6.55	3.85	32.73	34.88	7.30	3.23
		SD	1.19	0.96	0.74	0.01	0.36	0.23	0.25	2.55	1.62	0.03	0.06
		min	59.1	17.6	19.0	9.0	8.1	6.1	3.3	31.7	33.1	7.1	3.1
		max	61.4	20.0	21.1	9.2	9.5	7.1	4.5	35.1	36.1	7.5	3.6
13	m	$\bar{x}$	54.45	17.10	18.25	8.20	7.70	5.30	4.00	25.50	29.10	6.40	3.05
		SD	49.00	2.42	3.13	0.98	2.00	0.18	0.50	28.88	32.00	0.50	0.41
		min	49.5	16.0	17.0	7.5	6.7	5.0	3.5	21.7	25.1	5.9	2.6
		max	59.4	18.2	19.5	8.9	8.7	5.6	4.5	29.3	33.1	6.9	3.5
	f	$\bar{x}$	57.80	17.23	19.13	8.33	7.95	5.48	4.03	27.18	28.18	6.50	3.08
		SD	22.80	0.67	2.68	0.97	0.42	0.20	0.08	5.42	8.09	0.21	0.24
		min	51.2	16.2	17.1	7.2	7.4	5.1	3.6	23.7	25.4	5.9	2.6
		max	61.6	18.1	20.6	9.2	8.8	6.1	4.2	28.5	31.1	6.9	3.7
14	m	$\bar{x}$	69.26	19.61	21.11	8.92	8.99	6.40	4.08	35.73	40.45	8.34	3.83
		SD	13.81	1.19	2.25	0.28	0.44	0.27	0.17	6.09	9.68	0.76	0.28
		min	60.5	16.7	18.1	7.7	7.2	5.1	3.0	30.5	33.9	6.0	2.9
		max	79.2	22.9	25.0	10.2	10.3	7.5	4.8	41.1	45.9	10.0	4.9
	f	$\bar{x}$	69.81	19.77	21.37	8.77	8.79	6.52	3.87	33.64	37.26	7.72	3.85
		SD	36.00	1.46	3.87	0.54	0.63	0.81	0.24	6.52	11.54	1.19	0.44
		min	55.2	17.9	18.1	7.3	7.2	5.5	3.2	28.0	28.1	5.0	2.5
		max	77.6	22.1	25.2	10.1	10.0	9.6	5.0	37.3	41.1	9.2	4.9

graphical distance. The software used was R. S. Thorpe's version of the Mantel-test, originally written by B. Manly (1986). The sequential Bonferroni correction procedure (Rice, 1989) was applied in correlation tables across rows to adjust for the testing of multiple hypotheses. The strength of association between frequencies of qualitative variables was expressed with Spearman's nonparametric correlation coefficient (r_s). This was done separately for localities close to the Javakheti orogenic border (<25 km) and for populations further away (>25 km). Sets of r_s

TABLE 3.—Percent frequencies of qualitative characters observed in Georgian brown frogs. For population names and sample size see Table 1. S = light mid-dorsal stripe; DC = dark dorsal coloration; BS = sharp-edged dorsal spots; R = rugose skin; DT = spotted throat; DB = spotted belly; P = dark speckles on dorsum; V = V-shaped spot on the back of the head; DS = dorso-lateral dark stripes; NS = dorsal dark spots count; OS = dorsal spot shape; SS = dorsal spots orientation, and LS = dorso-lateral light stripes.

Site	S	DC	BS	R	DT	DB	P	V	DS	NS	OS	SS	LS
1	75	28	68	10	6	0	0	2	0	36	26	19	21
2	76	15	89	37	21	35	0	0	0	19	50	0	35
3	0	0	19	19	62	24	0	19	0	19	24	0	0
4	27	10	70	60	10	0	0	20	0	40	30	10	10
5	70	0	50	50	0	20	0	10	0	40	30	10	90
6	0	5	40	0	9	14	0	23	0	14	9	0	5
7	3	9	59	29	37	0	0	0	0	3	11	0	17
8	6	11	78	11	28	17	0	0	0	11	17	72	11
9	91	0	74	91	17	0	13	0	0	22	43	35	61
10	70	27	77	85	42	0	4	8	8	42	62	54	54
11	97	14	76	79	24	0	10	0	0	10	83	90	76
12	0	7	13	60	60	0	0	40	13	0	13	13	0
13	8	8	17	8	83	8	0	17	0	8	25	25	0
14	4	37	14	10	29	0	3	4	9	6	13	7	23

values were compared by the t-test for paired samples.

RESULTS

The raw morphometric and qualitative measurements are summarized in Tables 2 and 3.

Principal Component Analysis

The second and third principal axes for body proportions, together with the first principal component axis for coloration pattern, separated the populations from the Javakheti plateau and populations from the rest of Georgia (Fig. 2). Especially clear is the separation between males from these two areas. The second axis for body proportions was dominated by the length of the first toe and the length of the inner metatarsal tubercle, and the third axis by head length and head width. High loadings on the first axis were observed for the diameter of the eye and tympanum. This axis, however, did not help to distinguish between populations from different geographic areas. The highest loading on the first principal axis for coloration pattern was observed for the character "oblong spot shape" (Table 4).

Analysis of Clinal Variation

Marked differences were observed in the percentage of striped frogs around the geographic borders of the Javakheti plateau. The frequency of seven other characters also varied across this region. The pattern of spatial variation became clear when the populations were pooled into four groups, according to their geographic distance from the plateau (Fig. 3). Comparisons between groups of populations at either side of the watershed (i.e., groups B and C as defined in the legend of Fig. 3) demonstrated a significant difference for most characters, (metatarsal tubercle and frequency of striped specimens, $P < 0.001$; spot orientation and skin type, $P < 0.01$; presence/absence of speckles, sharp-edged spots and dorsolateral stripes, $P < 0.05$). The mean values of individual scores along the first principal axis significantly decreased with the distance from the Javakheti plateau both for body proportions and coloration characters (one-way ANOVA; $P < 0.001$ for both sets of data), although individual scores showed substantial overlap for different geographic areas (Fig. 4A,B).

Mantel Tests

The statistical probabilities of association between morphological distance and "taxonomy", geographic distance and "ecology" are presented in Table 5, for each of the morphological characters. Six characters were significantly associated

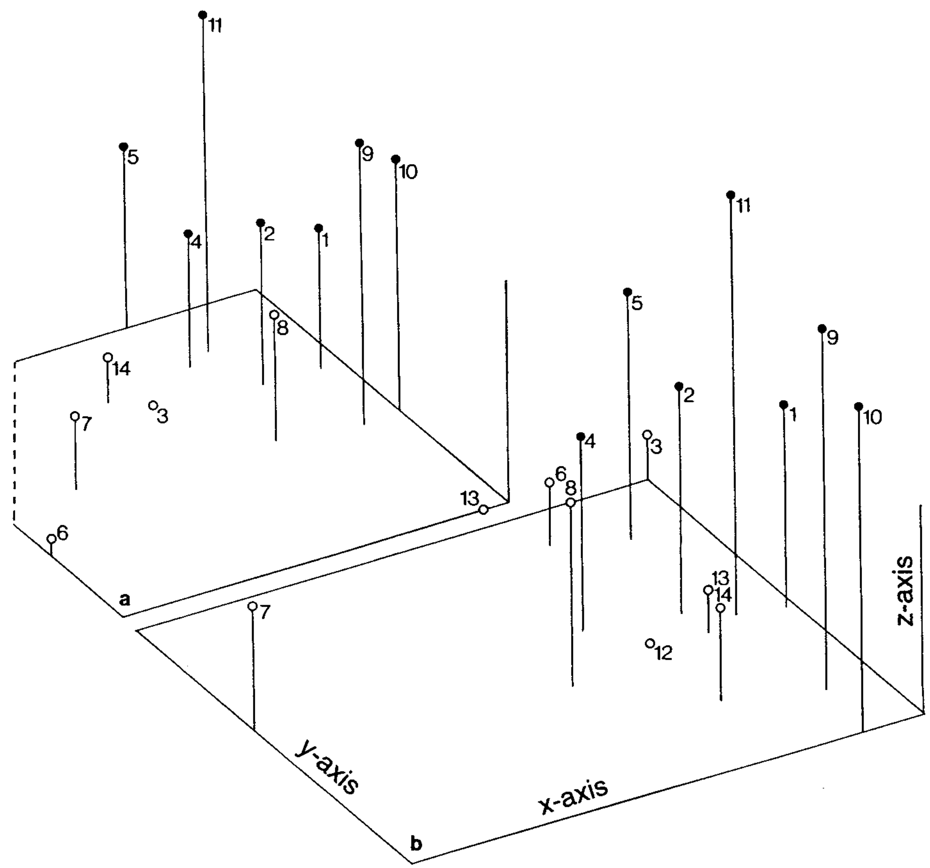

FIG. 2.—Multivariate analysis of body proportions by principal component analysis in brown frogs from Georgia: (a) males, (b) females. The x- and y-axis represent the second and third principal component axis for the body proportion characters, while the z-axis represents the first principal axis for the coloration pattern characters. The axes are in arbitrary units. Populations from the Javakheti plateau (solid dots) are positioned in the deep and upper part of the figures, away from populations from the rest of Georgia (open dots). Note that the first axis for body proportions (not shown) reflects variability in the size of the eye and tympanum, which characters do not help to explain the observed variation in a taxonomically relevant manner.

with taxonomy. Nominal *R. camerani*, apart from a dorsal stripe, possessed a significantly larger inner metatarsal tubercle, a longer first toe, and more frequently had a rugose skin, oblong dorsal spots, light lateral stripes and a speckled dorsum, than nominal *R. macrocnemis*. Characters significantly associated with ecology were the diameter of the eye and the tympanum. Frogs living at high altitudes had a smaller tympanum and smaller eyes than their low altitude counterparts. No characters were significantly associated with geographical distance between populations (Table 5).

Correlation between the Coloration Characters

The values of Spearman's correlation coefficient (r_s) between the frequencies of seven qualitative characters displaying a stepped clinal variation ranged from 0.26–0.88 ($\bar{x}$ = 0.54). For populations at large

TABLE 4.—Percent variance explained and scores of the first three axes of a principal component analysis for (a) body proportions and (b) coloration pattern in brown frogs from Georgia. Character codes as in Tables 2 and 3. Boldface type indicates characters demonstrating highest loadings on individual axes.

a

Axis	Males First	Males Second	Males Third	Females First	Females Second	Females Third
% variance	46	22	18	37	19	16
Lc	0.39	0.53	**−0.72**	−0.41	0.45	0.35
Ltc	0.39	0.52	**−0.72**	−0.01	0.13	**0.93**
Dro	0.71	−0.25	0.29	0.71	0.22	−0.45
Spoc	0.71	−0.25	0.29	0.83	0.04	−0.04
Lo	**0.92**	−0.11	0.01	0.13	0.58	0.22
Ltym	**0.92**	−0.11	0.01	**0.85**	0.21	0.23
F	0.88	0.18	0.14	0.83	−0.35	−0.05
T	0.88	0.18	0.14	0.80	−0.24	0.36
Dp	−0.11	**0.85**	0.50	−0.19	0.66	−0.36
Cint	−0.11	**0.85**	0.50	0.44	**0.80**	−0.07

b

Axis	First	Second	Third
% variance	37	19	12
DC	0.11	0.27	**−0.91**
BS	0.79	−0.36	−0.12
R	0.70	0.44	0.36
DT	−0.50	0.50	0.29
DB	−0.16	−0.69	0.32
P	0.73	−0.42	0.20
V	−0.65	0.32	0.39
DS	−0.28	**0.77**	−0.20
NS	0.47	−0.36	−0.11
OS	**0.84**	0.18	0.17
SS	0.64	0.36	−0.00
LS	0.84	−0.02	0.12

distances from the watershed (3, 6, 9, 10, 11, 12, and 13), values of r_s ranged from 0.57–0.94 ($\bar{x}$ = 0.78); for populations close to the watershed (1, 2, 4, 5, 7, 8, and 14), r_s ranged from −0.61–0.88 ($\bar{x}$ = 0.04). The difference between these averages was significant (*t*-test, $P < 0.001$).

DISCUSSION

Brown frogs from the Javakheti plateau in Georgia (nominal *R. camerani*) differ from frogs inhabiting other parts of the country (nominal *R. macrocnemis*). Frogs of the first group have a mid-dorsal stripe, large metatarsal tubercles, long first toes, a rugose skin, often with sharp-edged, elongated and symmetrically placed dark dorsal spots, dark speckles on the dorsum and light lateral stripes. These characters decrease in frequency from the Javakheti plateau towards the Great Caucasus mountains and the Black Sea, producing congruent stepped clines between the forms.

In his original descriptions, Boulenger (1885, 1896) used relative leg length and the shape of the snout for describing and distinguishing between *R. macrocnemis* and *R. camerani*. We have shown that these characters do not display a clear geographic pattern. Ishchenko (1978, 1987) studied five populations of nominal *R. macrocnemis* from the Great Caucasus and six populations of nominal *R. camerani* from the Minor Caucasus and showed that the differences in body proportions between populations within "taxa" were comparable, or exceeded, those between the "taxa". He concluded that *R. camerani* does not represent a valid taxon at any level and considered *R. macrocnemis* as a single, monotypic, though variable, species. Insofar as the results of the present study show geographic categories, they do not

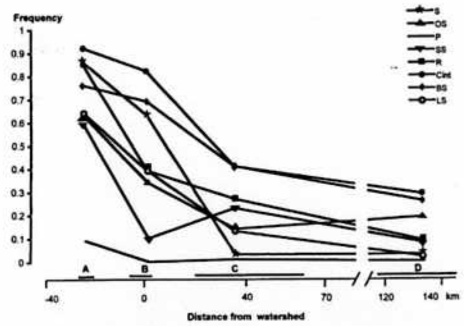

FIG. 3.—Spatial variation in brown frogs from Georgia for eight characters that display clinal variation. The vertical axis expresses the observed frequency of character states. The horizontal axis indicates the distance from the Jakavethi watershed (with negative values refering to southern locations; see Fig. 1). Populations at similar distances from the watershed are combined as follows: (A) −32 to −26 km (populations 9, 10, 11), (B) −8 to +5 km, (populations 1, 2, 4, 5), (C) +20 to +65 km (populations 7, 8, 13, 14), and (D) +110 to +150 km (populations 3, 6, 12). Character codes are: S = mid-dorsal stripe; OS = spot shape; P = dorsal speckles; SS = spot location; R = skin type; Cint = metatarsal tubercle; BS = spot border; LS = dorsolateral stripes.

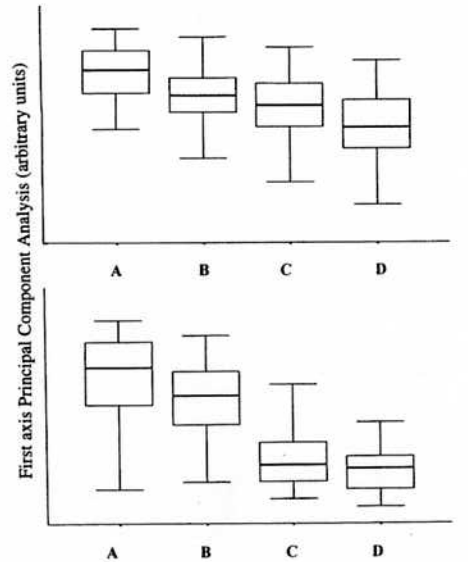

FIG. 4.—Spatial variation in brown frogs from Georgia for individual scores along the first principal axis for (top) body proportions and (bottom) coloration. The boxplots indicate the 75% and 100% range. Data for populations with similar distance to the Jakavethi watershed are pooled as in Fig. 3.

support Ishenko's (1978, 1987) conclusion. For eight characters (size of the metatarsal tubercle, presence of the mid-dorsal stripe, dark speckles and light dorsolateral stripes, rugose skin, and distribution, shape and type of dark dorsal spots), we have demonstrated the existence of congruent stepped clines between geographic forms. Consequently, we adhere to the view that brown frogs from Georgia and the Near East represent a polytypic group, characterized by bimodal state distributions in several characters. Interestingly, only two of these characters (rugose skin and the mid-dorsal stripe) were used in the original description of *R. camerani*.

The question remains whether "*camerani*" and "*macrocnemis*" should be classified as separate species. One or two questions are to be solved prior to taking the taxonomic decision. (1) Are "*macrocnemis*" and "*camerani*" reproductively isolated [different biological species sensu Mayr (1969); see Arntzen and Bauer (1996) for a recent discussion among herpetologists]? (2) Are there fixed morphological or genetic differences between "*macrocnemis*" and "*camerani*" (suggesting that they are evolutionary species sensu Frost and Hillis, 1990; Wiley, 1978)?

The existence of a transition zone indicates that "*macrocnemis*" and "*camerani*" are not reproductively isolated. Indeed, Ishchenko and Pyastolova (1973) demonstrated that "*macrocnemis*" and "*camerani*" interbreed freely under laboratory conditions. In natural populations from the transition zone, specimens possessing different combinations of clinally varying characters do not obviously differ in reproductive biology (i.e., phenology of spawning, breeding sites or mating behavior: Tarkhnishvili and Gokhelashvili, 1996; Tarkhnishvili, unpublished data). The inferred interbreeding within the transition zone indicates that "*macrocnemis*" and "*camerani*" are not biological species. Also, no single morphological character consistently separates "*camerani*" from "*macrocnemis*." This is in line with the morpho-

TABLE 5.—Mantel test for association of morphological distance between frog populations with taxonomic dissimilarity (TAXDIS), geographical distance (GEODIS), and ecological dissimilarity (ECODIS). Upper panel: *P*-values for individual morphometric characters (males only) and for pooled characters. Lower panel: *P*-values for qualitative characters, separately and combined.

Body proportions	TAXDIS	GEODIS	ECODIS
Lc	0.228	0.584	0.491
Ltc	0.226	0.580	0.501
Dro	0.191	0.896	0.318
Spoc	0.196	0.893	0.312
Lo	0.925	0.302	0.004**
Ltym	0.910	0.303	0.004**
F	0.301	0.688	0.143
T	0.684	0.299	0.144
Dp	0.009**	0.033	0.163
Cint	0.009**	0.033	0.165
All characters, males	0.206	0.842	0.497
All characters, females	0.602	0.640	0.949
Qualitative characters			
DC	0.912	0.244	0.906
BS	0.030	0.022	0.862
R	0.013*	0.831	0.835
DT	0.892	0.025	0.104
DB	0.697	0.936	0.695
P	0.006**	0.668	0.597
V	0.688	0.146	0.131
DS	0.755	0.234	0.046
NS	0.138	0.940	0.630
OS	0.002**	0.684	0.924
SS	0.079	0.715	0.937
LS	0.004**	0.984	0.545
All characters	0.040	0.107	0.737

* $P < 0.05$, ** $P < 0.01$ (Bonferroni correction applied).

logical results obtained by Baran (1969), Baran and Atatür (1986), and Mensi et al. (1992). Biochemical genetic studies do not contradict these interpretations. Across 34 allozyme loci surveyed (Green and Borkin, 1993; Mensi et al., 1992), only at one locus, *Icd* (coding for the enzyme isocitrate dehydrogenase), a marked genetic difference was observed between "*macrocnemis*" and "*camerani*" (Green and Borkin, 1993). For *Icd*, three alleles were described, one of which was shared between the taxa, at a frequency of 88% in "*macrocnemis*" and 5% in "*camerani*." However, the material available for study was limited to four specimens of "*macrocnemis*" and 10 of "*camerani*," from a single population each. In conclusion, no convincing evidence is available for classifying "*macrocnemis*" and "*camerani*" as separate species, according to either the biological or evolutionary species concepts.

Acknowledgments.—We thank R. Gokhelashvili for assistance in the field and J. Wiens for constructive criticisms on the manuscript. The work was completed whilst D. N. Tarkhnishvili was in receipt of a NATO/Royal Society Post-Doctoral Fellowship.

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Accepted: 12 August 1998
Associate Editor: John Wiens