2004 *Asiatic Herpetological Research* Vol. 10, pp. 208-214

Genetic Variation Among Agamid Lizards of the *Trapelus agilis* Complex in the Caspian-Aral Basin

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Abstract. - Allozyme variation is examined in eight populations of *Trapelus* from the Caspian-Aral Basin of the former USSR. Thirty one loci (15 variable) exhibit remarkably low levels of genetic variation with only a Nei's genetic distance of 0.117 across 2500 km. An isolated population on the European side of the Caspian Sea is found to phenetically cluster inside the Asian populations examined, suggesting that it should not be considered taxonomically distinct.

Key words. - Reptilia, Squamata, Agamidae, *Trapelus*, Central Asia, biogeography, allozyme electrophoresis.

Introduction

The *Trapelus agilis* complex is distributed on the Iranian Plateau and adjacent regions of southwestern Asia, as well as in the Caspian-Aral Basin to the north in the interior of Asia. Two separate populations of the *Trapelus agilis* complex are separated by the Caspian Sea in the Caspian-Aral Basin (including regions draining to Lake Balkhash) of the former USSR. One population on the eastern side of the Caspian Sea ranges from western China, Kazakhstan, and Kirgizistan in the north, to Turkmenistan (Fig. 1), Uzbekistan, and Tadjikstan in the south. This Central Asian population is continuous with Iranian, Afghan, and other southwest Asian populations referred to *Trapelus agilis*. On the western side of the Caspian Sea in Europe a small population occurs in Chechenia and Dagestan, Russia. The two populations occurring in the Caspian-Aral Basin are placed either in a separate species, *T. sanguinolentus,* or subspecies, *T. agilis sanguinolentus*. Taxonomic controversy also exists as to the status of the isolated European population of *Trapelus*. Some authors consider the European population to be a distinct subspecies of *T. sanguinolentus* (*T. s. sanguinolentus*) with the Central Asian populations being referred to *T. s. aralensis* (Ananjeva and Tsaruk, 1987). Others consider the European and Asian populations in the Caspian-Aral Basin to be a single taxon, either *T. sanguinolentus* (Bannikov et al., 1977) or *T. agilis sanguinolentus* (Wermuth, 1967). The focus of this study is on the relative position of the European and Asian populations in the Caspian-Aral Basin. The Caspian-Aral Basin populations are always grouped together either as a species or as one or two distinct subspecies relative to the southwest Asian populations referred to as *T. agilis*.

Trapelus is an old genus of Agamid lizards with an Afro-Arabian origin (Macey et al., 2000b). Sequence divergence between *Trapelus* species in Africa (*Trapelus savignii*), Arabia (*T. persicus*), the Iranian Plateau (*T. agilis*), and the Caspian-Aral Basin (*Trapelus sanguinolentus* population 6 of this study), which form a clade, is 10.7-13.9% for the mitochondrial DNA segment spanning from *nad1* to *cox1* (Macey et al., 2000b). Applying the rate of 1.3% change per million years for pairwise comparisons as calculated for this segment of mitochondrial DNA in agamid lizards of the genus *Laudakia* (Macey et al., 1998), divergence times among these species of *Trapelus* are estimated to be 8.3 to 10.7 million years before present (MYBP). These data suggest that the genus has been in Asia since the Miocene.

Allozyme data are used to distinguish hypotheses of early divergence of the European and Asian trans-Caspian populations into discrete entities, verses colonization of the European side of the Caspian Sea by western Asian populations. High mountains in the Caucasus and Elburz ranges prevent colonization of the European population from the south, where a continuous land connection does exist to *Trapelus* populations in Iran. The Caspian-Aral Basin corresponds to much of the Paratethys Sea, which during the Miocene almost completely dried up 5-6 MYBP and then returned briefly in the Pliocene, 3.0-3.5 MYBP (Steininger and Rogl, 1984). Divergence following the early period (5-6 MYBP) when much of the Caspian-Aral Basin was

Figure 1. A *Trapelus* from Repetek Desert Reserve Station, Repetek (38° 34' N, 63° 11' E), Chardjou Region, Turkmenistan. The photo was taken in May, 1989. This is a representative of population 5 of this study.

available for colonization, should halt gene flow in the late Miocene or Pliocene and two discrete populations are expected to be detected, one in Europe and one in Asia. Alternatively, a more recent colonization from a founder event, when the Caspian Sea level fluctuated in the Pleistocene, should have a much later restriction in gene flow and therefore the European population may be expected to be nested within the Asian population.

Material and Methods

Laboratory Protocols. - Tissues were taken in the field, immediately frozen in liquid nitrogen, and later transferred to an ultracold freezer and maintained at -80° C. For analysis of allozymic variation, liver and muscle tissues were homogenized separately. Horizontal starch-gel electrophoresis was employed to differentiate variation in 31 presumptive loci. The 31 loci and eight buffer conditions utilized to resolve them are displayed in table 1. Allozymes were stained using standard methods (Harris and Hopkinson, 1976; Murphy et al., 1990; Richardson et al., 1986; Selander et al., 1971). Carboxylic ester hydrolase (Dimeric Esterase) was resolved using 4-methylumbelliferyl acetate as the substrate, Alcohol dehydrogenase (ADH) was resolved using *Trans*-2-Hexen-1-ol as the substrate, an unidentified peptidase (PEP-1) was resolved using L-leucyl-L-alanine as the substrate, and Peptidase D (PEP-D) and an unidentified peptidase (PEP-2) with the use of L-phenylalanyl-L-proline as the substrate. The isozymes, and loci if more than one, were labeled according to their migration from anode to cathode.

Specimen Information. - Museum numbers and localities for voucher specimens are presented below. Acronyms are CAS for California Academy of Sciences, San Francisco and MVZ for Museum of Vertebrate Zoology, University of California at Berkeley. Russia: (population 1) Tersko-Kumskaya nizmennast (the lowland between Terek and Kuma Rivers), 15 km WNW (airline) of Voskresenskaya, which is approx. 25 km NNW of Gudermes (43° 21' N 46° 06' E), Schelkovskaya District, Chechen-Ingush Autonomous Republic (CAS 182952, 183032-183038). Kazakhstan: (population 2) Almaty (43° 15' N 76° 57' E), Almaty Region (MVZ 216014-216016, CAS 183047-183051). Uzbekistan: (population 3) sand dunes on the west side of the Surkhan Darya (River), on the Kumkurgan (37° 48' N, 67° 37' E) to Denau (38° 16' N, 67° 54' E) Rd., Surkhan Darjinskaya Region (CAS 183004-183006).

Table 1. The 31 protein loci scored and the electrophoretic conditions within which they were resolved.

Enzyme or blood protein	Electrophoretic abbreviation	E. C. No.	No. of Loci	Tissue[a]	Conditions[b]
Serum albumin	AB	-	1	L	1
Aconitase hydratase	ACON	4.2.1.3	1	L	2
Adenylate kinase	AK	2.7.4.3	1	M	2
Alcohol dehydrogenase	ADH	1.1.1.1	2	L	3
Aspartate aminotransferase	AAT	2.6.1.1	1	L	4
Carboxylic ester hydrolase	EST-D[c]	3.1.1.-	2	L	1
Creatine kinase	CK	2.7.3.2	1	M	4
Fructose-bisphosphate aldolase	FBA	4.1.2.13	2	L	5
Glucose-6-phosphate isomerase	GPI	5.3.1.9	2	L	4
Glycerol-3-phosphate dehydrogenase	G3PDH	1.1.1.8	1	L	1
D-2-Hydroxy-acid dehydrogenase	HADH	1.1.99.6	1	L	5
L-Iditol dehydrogenase	IDDH	1.1.1.14	1	L	5
Isocitrate dehydrogenase	IDH	1.1.1.42	2	L	2
L-Lactate dehydrogenase	LDH	1.1.1.27	2	L	3
Malate dehydrogenase	MDH	1.1.1.37	2	L	6
Mannose-6-phosphate isomerase	MPI	5.3.1.8	1	L	6
Peptidase (unidentified 1)	PEP-1	3.4.-.-	1	L	1
Peptidase D	PEP-D	3.4.13.9	1	L	7
Peptidase (unidentified 2)	PEP-2	3.4.-.-	1	L	7
Phosphoglucomutase	PGM	5.4.2.2	1	L	8
Phosphogluconate dehydrogenase	PGDH	1.1.1.44	1	L	6
Purine-nucleoside phosphorylase	PNP	2.4.2.1	1	L	7
Pyruvate kinase	PK	2.7.1.40	1	L	8
Superoxide dismutase	SOD	1.15.1.1	1	L	4

[a]Tissue abbreviations are: L = liver; M = skeletal muscle.
[b]Electrophoretic conditions: (1) Lithium-borate/Tris-citrate pH 8.2, 250 v for 6 h (Selander et al., 1971); (2) Amine-citrate (Morpholine) pH 6.0, 250 v for 6 h (Clayton and Tretiak, 1972); (3) Tris-citrate/borate pH 8.7, 250 v for 5 h (Selander et al., 1971); (4) Histidine-citrate pH 7.8, 150 V for 8 hours (Harris and Hopkinson, 1976); (5) Phosphate-citrate pH 7.0, 120 v for 7 h (Selander et al., 1971); (6) Tris-citrate II pH 8.0, 130 v for 8 h (Selander et al., 1971); (7) Tris-HCL pH 8.5, 250 v for 4 1/2 h (Selander et al., 1971); (8) Tris-maleate-EDTA pH 7.4, 100 v for 10 h (Selander et al., 1971).
[c]EST-D = Dimeric Esterase

Turkmenistan: (population 4) SW bank of the Amur Darya (River), approx. 2 km NE of Nephtezavodsk which is 30 km WNW of Deynau (39° 15' N, 63° 11' E), Chardjou Region (CAS 179552-179559); (population 5) 1 km north of Repetek Desert Reserve Station, Repetek (38° 34' N, 63° 11' E), Chardjou Region (CAS 179199-179203, 179416-179420), and Repetek Desert Reserve Station, Repetek (38° 34' N, 63° 11' E), Chardjou Region (CAS 179331); (population 6) 55 km north of Ashgabat (37° 57' N, 58° 23' E) on the Ashgabat - Bakhardok (38° 46' N, 58° 30' E) Rd. then 21 km WNW on dirt Rd., Ashgabat Region (CAS 179758-179767); (population 7) Ashgabat (37° 57' N, 58° 23' E), Ashgabat Region (MVZ 216087-216092); (population 8) near Iolotan' [Yolotan] (37° 18' N, 62° 21' E), Mary Region (MVZ 216013).

Data Analysis. - Nei's (1978) unbiased genetic distance and Rogers (1972) genetic similarity were calculated using BIOSYS-1 (Swofford and Selander, 1981). Phenetic clustering was constructed using the neighbor-joining algorithm (Saitou and Nei, 1987), which does not require rate uniformity, using PAUP* 4.0 (Swofford, 1999) and Nei's (1978) unbiased genetic distance.

Results

Variable Loci. - Fifteen of the 31 loci screened show variation among the sampled populations (Table 2). Up to five different allelic states are recognized per loci among populations with no more than four allelic states being present within a population.

Genetic Distances. - Allozymic variation among sampled populations of *Trapelus* is surprisingly low (Table 3). The two geographically most distant samples, the European side of the Caspian-Aral Basin (West Caspian) and Kazakhstan (Almaty), have a Nei's (1978) unbiased genetic distance of only 0.117 across 2500 km. The highest Nei's (1978) unbiased genetic distances recov-

Table 2. Electromorph frequencies for the 15 polymorphic loci from eight populations of *Trapelus* sampled. Localities are West Caspian (WCA), Almaty (ALM), Uzbekistan (UZB), Nephtezavodsk (NEP), Repetek (REP), 70 km NW Ashgabat (NWA), Ashgabat (ASH), Iolotan' (IOL). See text for complete localities of all populations used.

Locus	Electromorph	1-WCA	2-ALM	3-UZB	4-NEP	5-REP	6-NWA	7-ASH	8-IOL
AK	a	0.125							
	b	0.875	0.063	0.167	0.938	0.909	1.000	0.929	1.000
	c		0.938	0.667	0.063	0.091		0.071	
	d			0.167					
EST-D-2	a	1.000	0.313	1.000	1.000	1.000	0.950	1.000	1.000
	b		0.688				0.050		
CK	a	1.000	1.000	1.000	1.000	0.909	1.000	1.000	1.000
	b					0.091			
FBA-1	a								0.500
	b				0.063				
	c		0.125		0.188				
	d	1.000	0.875	1.000	0.750	1.000	1.000	0.929	0.500
	e							0.071	
FBA-2	a	1.000							
	b			1.000	0.875	0.818	0.600	0.929	1.000
	c		1.000		0.125	0.182	0.400	0.071	
GPI-2	a	0.313				0.045	0.150		
	b	0.688	1.000	1.000	1.000	0.864	0.850	1.000	1.000
	c					0.091			
HADH	a	1.000	1.000	0.667	1.000	1.000	1.000	0.929	1.000
	b							0.071	
	c			0.333					
IDDH	a					0.182	0.050	0.071	
	b	1.000	1.000	1.000	1.000	0.818	0.950	0.929	1.000
IDH-1	a	1.000	1.000	0.833	1.000	1.000	1.000	1.000	1.000
	b			0.167					
LDH-2	a				0.375	0.091			
	b	1.000	1.000	1.000	0.625	0.909	1.000	1.000	1.000
MPI	a	0.063						0.071	
	b	0.938	1.000	1.000	1.000	0.955	1.000	0.929	1.000
	c					0.045			
PEP-1	a					0.045	0.100		0.500
	b	1.000	1.000	0.500	1.000	0.727	0.800	0.857	0.500
	c			0.500		0.227	0.100	0.143	
PGM	a				0.063	0.091			
	b	0.125			0.125		0.150		
	c	0.875	1.000	1.000	0.813	0.909	0.850	1.000	1.000
PGDH	a					0.091			
	b		1.000		0.063	0.091			
	c			0.333	0.125	0.045			
	d	1.000		0.667	0.813	0.773	1.000	1.000	1.000
PNP	a	1.000	1.000	1.000	1.000	1.000	0.950	1.000	0.500
	b						0.050		0.500

Table 3. Matrix of genetic distance and identity coefficients from the eight populations of *Trapelus* sampled. Nei's unbiased genetic distance (Nei, 1978) is above the diagonal, Rogers genetic similarity (Rogers, 1972) is below the diagonal and sample sizes are on the diagonal. See text for specimen deposition and complete localities of all populations used.

	1-WCA	2-ALM	3-UZB	4-NEP	5-REP	6-NWA	7-ASH	8-IOL
1. West Caspian (WCA)	**8**	0.117	0.066	0.039	0.034	0.026	0.035	0.064
2. Almaty (ALM)	0.865	**8**	0.088	0.102	0.094	0.094	0.108	0.145
3. Uzbekistan (UZB)	0.886	0.873	**3**	0.031	0.019	0.032	0.021	0.048
4. Nephtezavodsk (NEP)	0.927	0.872	0.910	**8**	0.006	0.009	0.006	0.029
5. Repetek (REP)	0.928	0.865	0.920	0.952	**11**	0.002	0.001	0.026
6. 70 km NW Ashgabat (NWA)	0.949	0.875	0.909	0.947	0.958	**10**	0.003	0.026
7. Ashgabat (ASH)	0.939	0.873	0.931	0.957	0.963	0.964	**7**	0.022
8. Iolotan' (IOL)	0.899	0.836	0.900	0.925	0.920	0.932	0.943	**1**

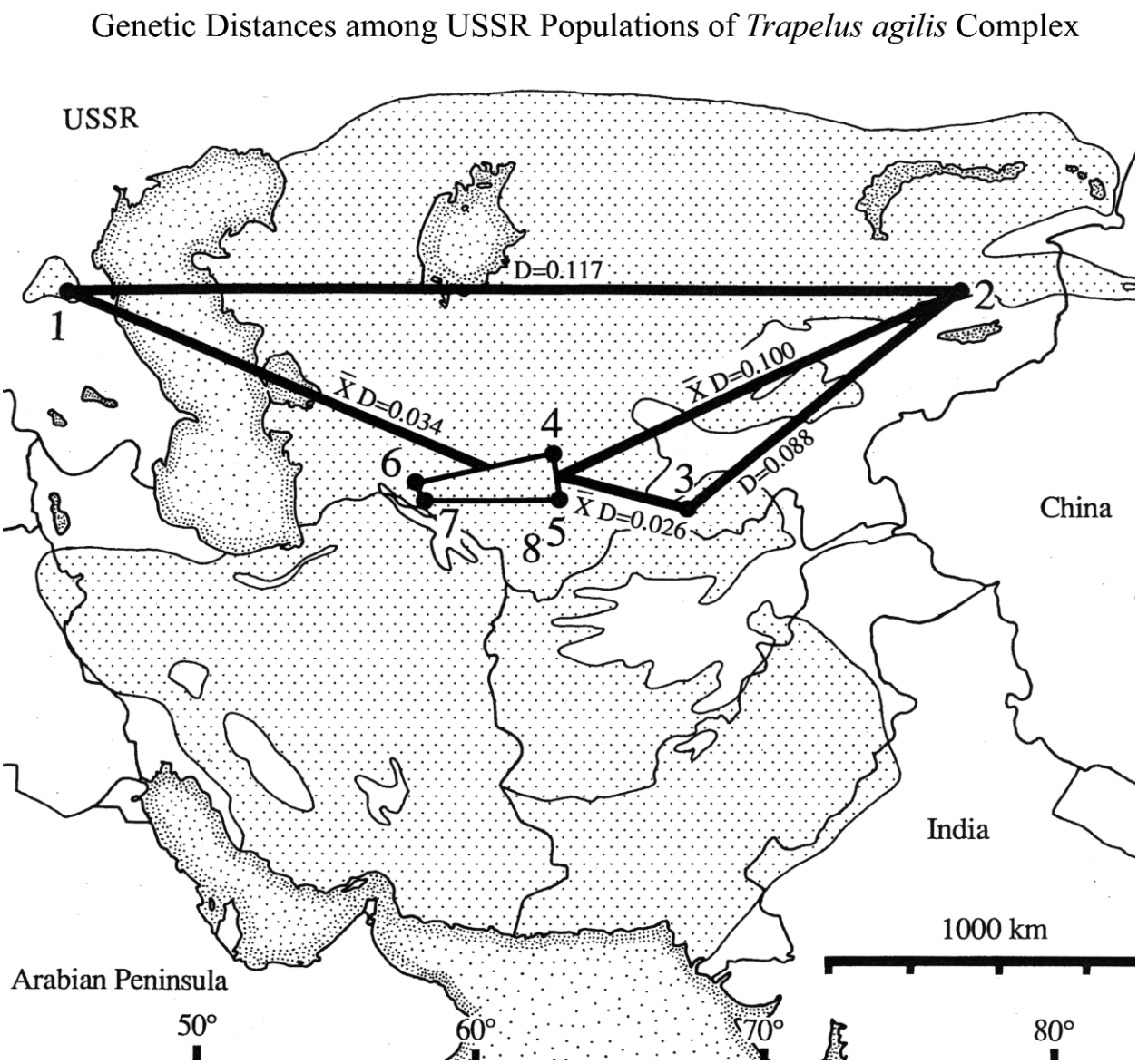

Figure 2. Map of the Caspian-Aral Basin and southwest Asia showing the distribution of the *Trapelus agilis* complex. Dots depict populations sampled. Lines connect populations in major areas, West Caspian, Kazakhstan, Uzbekistan and Turkmenistan. Nei's unbiased genetic distance (Nei 1978) is plotted between areas. The Turkmen populations are averaged. The western most sample on the west side of the Caspian Sea is population 1 (table 3). The eastern most sample is Almaty in Kazakhstan (population 2). To the southwest of this sample is the Uzbekistan population (population 3). Four of the Turkmen samples are connected by lines. The most northeastern is population 4 from Nephtezavodsk and the most southeastern is population 5 from Repetek. The most northwestern is population 6 from 70 km NW Ashgabat and the most southwestern is population 7 from Ashgabat. Population 8 from Iolotan' is not included in the average of Turkmen populations because of the low sample size of one and it is distributed between populations 5 and 7.

ered are 0.088-0.145 (note that the highest value is with a sample size of one) between the northwestern population in Kazakhstan (Almaty) and all other populations sampled in the Caspian-Aral Basin. The European population on the western side of the Caspian Sea is separated from all other populations except the Kazakhstan (Almaty) population by Nei's (1978) unbiased genetic distances of 0.026-0.066. The population in Uzbekistan is distinct from those in Turkmenistan by Nei's (1978) unbiased genetic distances of 0.019-0.048.

Mapping these genetic distances on geography reveals a pattern of isolation by distance in which all distances appear relatively additive (Fig. 2). Clustering of these data in a neighbor-joining phenogram and rooting the tree on the longest path places the Kazakhstan (Almaty) population and Uzbekistan sample in a basal

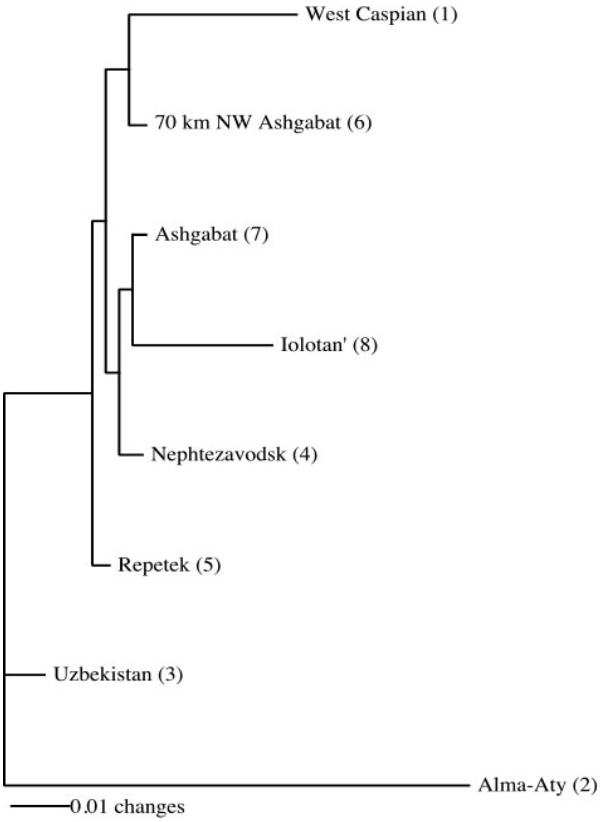

Figure 3. Neighbor-joining phenogram rooted on the longest path. Note that the European population on the western side of the Caspian Sea is nested inside the Asian populations sampled and appears as the sister population to its nearest Asian population (70 km NW Ashgabat). Population numbers corresponding to figure 1, and tables 2 and 3 are given adjacent to locality names.

position. The European (West Caspian) population appears nested within the Turkmen populations and as the sister population to its nearest Asian population (70 km NW Ashgabat), (Fig. 3). This pattern is consistent with the observed genetic distances.

Discussion

The Age of *Trapelus* in the Caspian-Aral Basin. - The European population of *Trapelus* is found to cluster phenetically within the Asian populations with little genetic differentiation, suggesting that these taxa do not represent distinct forms. The low genetic diversity in *Trapelus* of the Caspian-Aral Basin probably indicates a recent dispersion of *Trapelus* throughout the Caspian-Aral Basin. Nei's (1978) unbiased genetic distances do not exceed 0.117 (with the exception of Kazakhstan to Iolotan' Turkmenistan with a sample size of one). A very approximate estimate of divergence time and genetic distance is 14 million years for a Nei's D of 1.0 (Maxson and Maxson, 1979). Given this rate these data suggest a divergence of *Trapelus* populations in the Caspian-Aral Basin at around 1.6 MYBP. This result suggests that *Trapelus* did not diverge in the Caspian-Aral Basin until the Pleistocene, well after the last drying of the Paratethys Sea 3.5 MYBP (Steininger and Rogl, 1984).

Comparison to Other Taxa. - One additional genus of lizard has been sampled from the Caspian-Aral Basin for allozyme variation. The northern populations of the gekkonid genus *Mediodactylus* from Almaty and the Junggar Depression of China show a minimum of two fixed differences when compared to a southern population in the Kara Kum Desert (Macey et al., 2000a). This divergence is greater than those observed among *Trapelus* where no fixed differences are detected between Almaty (population 2) and the Kara Kum Desert (populations 4-8). Because the Caspian-Aral Basin has had periods of inundation followed by drying over the last 6 million years, and the surrounding mountains of the Pamir-Tien Shan are older providing a land refuge (10 million years old; Abdrakhmatov et al., 1996), taxa in the Caspian-Aral Basin may show different levels of divergence.

Taxonomic Recommendations. - Because *Trapelus sanguinolentus* in the Caspian-Aral Basin is distinguished from *Trapelus agilis* of the Iranian Plateau by 10.9% sequence divergence for the mitochondrial DNA segment spanning from *nad1* to *cox1* (Macey et al., 2000b), we consider them separate species. No more than a single fixed difference is observed between populations of *Trapelus* in the Caspian-Aral Basin. Therefore, we interpret these populations to be a single taxon, *T. sanguinolentus*. Further work comparing populations in Southwest Asia is needed in order to determine the specific status of these populations.

Acknowledgments

This work is LBNL-54654 and was performed under the auspices of the U.S. Department of Energy, Office of Biological and Environmental Research, under contract No. DE-AC03-76SF00098 with the University of California, Lawrence Berkeley National Laboratory. This work was also supported by grants from the National Geographic Society (4110-89 and 4872-93 to Theodore J. Papenfuss and J.R.M.), Russian Foundation of Basic Research (N 02-04-48720 to N.B.A.), Scientist School (NS 1647.2003.4 to N.B.A.), the California Academy of Sciences and the Museum of Vertebrate Zoology. We thank Tatjana N. Duysebayeva for tissue specimens. Nikolai Orlov, Theodore J. Papenfuss, Sakhat M. Shammakov, and Boris S. Tuniyev aided with field work. Kraig Adler and Allan Larson provided valuable comments on an earlier draft of the manuscript. The first author thanks David B. Wake and Margaret F. Smith

for the opportunity to collect allozymic data at the Museum of Vertebrate Zoology.

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