Genetic evidence for species status of some Caucasian rock lizards in the *Darevskia saxicola* group

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Abstract. Diversity at 35 allozyme loci was examined in nine populations of the taxa currently included in *Darevskia saxicola*. The data support the recognition of the subspecies *Darevskia s. saxicola* and *D. s. lindholmi* as species. The remaining subspecies *D. s. brauneri*, *D. s. darevskii* and *D. s. szczerbaki* are conspecific although they can no longer be referred to *Darevskia saxicola*, but must be referred to *Darevskia brauneri*, whose name has priority. Considerable population substructuring was observed among the subspecies of *Darevskia brauneri*.

Introduction

The Caucasian rock lizards of the genus *Darevskia* Arribas, 1997 have been the subject of extensive study and, consequently, have undergone considerable taxonomic revision. *Darevskia saxicola* (Eversmann, 1834) was the first species to be named from the region. All subsequently described taxa were considered to be subspecies of *D. saxicola* until Méhely (1909) recognised some as being distinct species. More subspecies were elevated to species rank following further detailed morphological and molecular study (Darevsky, 1958, 1967; Fu et al., 1995). A summary of these taxonomic revisions can be found in Murphy et al. (1996a). Recently Arribas (1997) removed the Caucasian rock lizards from the genus *Lacerta* and placed them in *Darevskia*.

Darevskia saxicola presently contains five subspecies. One of these, *D. s. lindholmi*, occurs in the southern Crimea, Ukraine. Three other subspecies, *D. s. szczerbaki*, *D. s. darevskii* and *D. s. brauneri*, are found in the Black Sea watershed of southern Russia, distributed from north-west to south-east, respectively. The fifth subspecies, *D. s. saxicola*, occurs inland, east of the Black Sea. Zones of contact occur among the latter four sub-

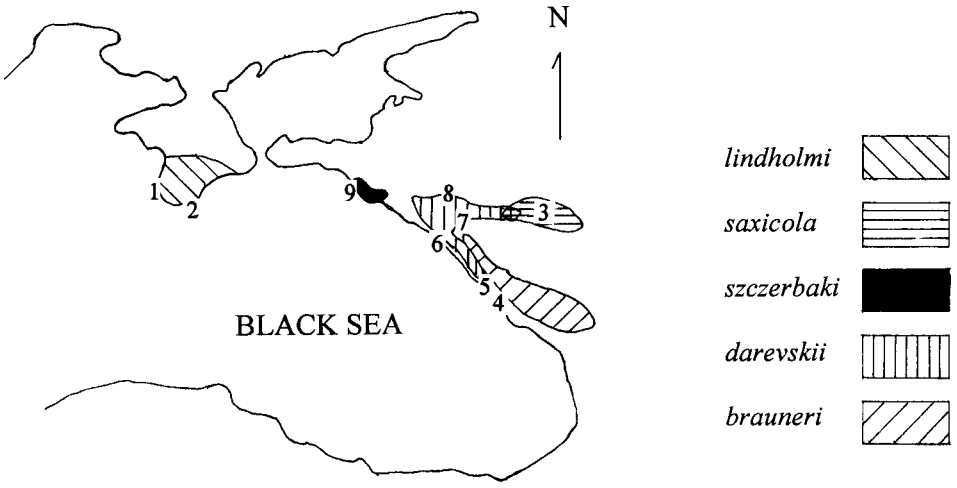

Figure 1. The distribution of *Darevskia saxicola* (s.l.). Sampling sites: 1 — Sevastopol; 2 — Yalta; 3 — Kislovodsk; 4 — Sochi; 5 — Tuapse; 6 — Dzhubga; 7 — Guzeripe; 8 — Dagomys; 9 — Anapa.

species, and between these and other species of *Darevskia*. The distributions are mapped in fig. 1.

Herein we examine the allozyme diversity among populations of *Darevskia saxicola*, and evaluate the taxonomic status of the subspecies. Recognition of taxa as unique species requires that they be distinguishable by fixed allelic differences at a minimum of two allozyme loci (Baverstock and Moritz, 1996). Our null hypothesis is that *D. saxicola*, in its present status, consists of a single species.

Materials and Methods

A total of 99 specimens of *D. saxicola* were collected from nine locations across the species' range (see Appendix). Specimens were euthanized by an overdose of sodium pentobarbitol. Tissues (heart, liver, skeletal muscle) were removed from all specimens immediately following euthanasia and frozen in liquid nitrogen. Voucher specimens are in the collection of the Royal Ontario Museum (ROM).

Enzymes were separated by horizontal starch gel electrophoresis on 11% (w/v) gels. Homogenates of a combination of heart, liver and muscle were used. The analysis utilised 28 enzyme systems encoded by 35 loci. Electrophoresis procedures, staining protocols and enzyme and locus nomenclature follow Murphy et al. (1996b). Buffer combinations for resolving locus products are given in Fu et al. (1995), MacCulloch et al. (1995) and Bobyn et al. (1996).

Allozyme data were examined for the presence of fixed allelic differences among the subspecies. The criterion of fixed differences between taxa at two or more loci was

considered grounds for recognition of taxa as distinct species (Baverstock and Moritz, 1996). Allozyme data were further analysed using BIOSYS-1 release 1.7 (Swofford and Selander, 1989). All loci were evaluated for genetic polymorphism (mean heterozygosity, mean number of alleles per locus, percentage of loci exhibiting polymorphism) and conformity to Hardy-Weinberg expectations using a chi-square test with both correction for small sample sizes (Levene, 1949) and genotype pooling. Genetic substructuring among populations was evaluated using Wright's (1978) F-statistics. Genetic divergence among populations was examined using genetic distance coefficients (Rogers, 1972; Nei, 1978).

Results

All taxa and populations were monomorphic for 20 of the 35 loci: mAat-A, mAcoh-A, Ada-A, Cbp-1, Ck-A, Est-D, βGa-1, Gcdh-A, Gda-A, βGlur-A, βGlus-A, Gpi-B, G6pdh-A, mIdh-A, mMdh-A, sMdh-A, mMdhp-A, sMdhp-A, Pk-A and mSod-A. Genotype frequencies for the 15 polymorphic loci are shown in table 1. Alleles are listed in alphabetical order according to relative mobility, i.e., "a" represents the fastest allele. The identification of alleles as "a", "b" etc. is unique to this study; these alleles are not necessarily equivalent to those alleles similarly identified in other studies.

No fixed differences were detected among the three subspecies *D. s. brauneri*, *D. s. darevskii* and *D. s. szczerbaki*, indicating that these three taxa are conspecific. However, fixed differences occur between these three and the other two subspecies. Fixed differences at three loci (sAat-A, sAcoh-A, sSod-A) distinguish *D. s. lindholmi* from the above three taxa. *Darevskia s. saxicola* also differs from the first three taxa by fixed differences at three loci (sAcoh-A, Acp-B, sSod-A). Furthermore, fixed differences exist between, *D. s. lindholmi* and *D. s. saxicola* at five loci (sAat-A, sAcoh-A, Acp-B, Mpi-A, sSod-A). However, these latter two taxa also share the unique Pep-B(a) allele.

The fixed differences listed above provide evidence that *D. s. lindholmi* and *D. s. saxicola* merit full species status (Baverstock and Moritz, 1996). The absence of fixed differences among the remaining three taxa precludes recognition of them as separate species. Parameters of genetic variability are summarised at the bottom of table 1. Allozyme data conformed to Hardy-Weinberg predictions except for a heterozygote deficiency at Ldh-A in *D. s. lindholmi* from Sevastopol ($\chi^2 = 23.0$, $P < 0.05$) and in *D. s. szczerbaki* ($\chi^2 = 17.1$, $P < 0.05$). When these deviations were re-evaluated using genotype pooling, however, they were not significant.

Wright's index of interpopulation substructuring (F_{ST}) among all populations of all taxa is 0.686. This high value confirms that the populations are not panmictic, as shown by the fixed differences observed among some taxa. F_{ST} was therefore recalculated for each of the three taxa from which more than one population was sampled. In each of the three taxa F_{ST} was much lower than that calculated for all taxa combined (*D. s. brauneri* = 0.179, *D. s. darevskii* = 0.271, *D. s. lindholmi* = 0.020). The very low value of F_{ST} in

Table 1. Genotype frequencies for polymorphic loci in populations of *Darevskia saxicola* (s.l.). MHD = mean heterozygosity by direct count, MNA = mean number of alleles per locus, PLP = percentage of loci polymorphic (0.95 criterion).

Subspecies:	*lindholmi*	*lindholmi*	*saxicola*	*brauneri*	*brauneri*	*darevskii*	*darevskii*	*darevskii*	*szczerbaki*
Location:	Sevastopol	Yalta	Kislovodsk	Sochi	Tuapse	Dzhubga	Guzeripe	Dagomys	Anapa
sAat-A	cc(12)	cc(8)	bb(31)	ab(3)	bb(3)	bb(4)	bb(9)	bb(3)	bb(9)
EC 2.6.1.1				bb(17)					
sAcoh-A	aa(12)	aa(8)	cc(31)	bb(19)	bb(3)	bb(4)	bb(8)	bb(2)	bb(9)
EC 4.2.1.3				bd(1)					
Acp-B	bb(12)	bb(4)	cc(31)	ab(3)	bb(2)	aa(4)	aa(8)	aa(3)	aa(9)
EC 3.1.3.2				bb(15)					
Cat-A	aa(5)	aa(3)	aa(4)	ab(3)	bb(2)	bb(3)	ab(3)	bb(1)	bb(9)
EC 1.11.1.6	ab(5)	ab(2)	ab(9)	bb(11)		bc(1)	bb(5)		
	bb(1)		bb(5)	bc(2)					
Ck-C	aa(12)	aa(8)	aa(31)	ab(5)	ab(1)	ab(1)	bb(9)	ab(1)	ab(2)
EC 2.7.3.2				bb(14)	bb(2)	bb(3)		bb(2)	
Gpi-A	bb(11)	bb(8)	ab(3)	bb(20)	bb(3)	bb(4)	bb(9)	bb(3)	bb(9)
EC 5.3.1.9	bc(1)		bb(28)						
sIdh-A	ab(1)	bb(8)	ab(2)	bb(20)	aa(2)	bb(4)	ab(1)	ab(1)	ab(1)
EC 1.1.1.42	bb(11)		bb(29)		ab(1)		bb(8)	bb(2)	bb(8)
Ldh-A	ab(1)	bb(6)	bb(24)	bb(15)	bb(3)	bb(3)	ab(1)	bb(3)	ab(1)
EC 1.1.1.27	bb(10)	bc(2)	bc(5)	bc(3)		bc(1)	bb(7)		bb(7)
	cc(1)		cc(1)	cc(1)			bc(1)		cc(1)
Ldh-B	bb(11)	ab(1)	ab(1)	bb(18)	bb(3)	bb(4)	bb(9)	bb(3)	bb(8)
EC 1.1.1.27	bc(1)	bb(7)	bb(29)	bc(2)					bc(1)
Mpi-A	aa(2)	aa(1)	cc(28)	ab(2)	ab(1)	bb(3)	cc(9)	bb(3)	bb(9)
EC 5.3.1.8	ab(8)	ab(2)		bb(17)	bb(1)				
	bb(2)	bb(2)							
Pep-A	cc(12)	cc(8)	bb(24)	bb(3)	bb(1)	bc(1)	bb(3)	bb(2)	ac(3)
EC 3.4.–.–			bc(5)	bc(7)	bc(1)	cc(1)	bc(3)	cc(1)	bb(3)
			cc(2)	bd(5)	cc(1)	bd(2)	cc(3)		bc(3)
				cd(2)					
				dd(2)					

Table 1. (Continued).

Subspecies: Location:	*lindholmi* Sevastopol	*lindholmi* Yalta	*saxicola* Kislovodsk	*brauneri* Sochi	*brauneri* Tuapse	*darevskii* Dzhubga	*darevskii* Guzeripe	*darevskii* Dagomys	*szczerbaki* Anapa
Pep-B	ab(2)	aa(1)	aa(31)	bb(15)	bb(2)	bb(2)	bb(9)	bb(1)	bc(1)
EC 3.4.–.–	bb(10)	ab(2)		bc(4)	bc(1)	bc(2)		bc(2)	cc(8)
		bb(5)							
Pnp-A	ab(1)	bb(6)	ab(1)	ab(1)	ab(2)	ab(1)	ab(1)	bb(3)	ab(6)
EC 2.4.2.1	bb(9)		bb(28)	bb(19)	bb(1)	bb(3)	bb(8)		bb(3)
sSod-A	cc(12)	cc(8)	aa(31)	bb(20)	bb(2)	bb(4)	bb(9)	bb(3)	bb(8)
EC 1.15.1.1									
Tpi-A	ab(1)	bb(8)	bb(31)	aa(3)	bb(3)	bb(4)	bb(9)	bb(3)	bb(9)
EC 5.3.1.1	bb(10)			ab(3)					
	bc(1)			bb(14)					
MHD	0.054	0.041	0.031	0.070	0.071	0.064	0.033	0.038	0.079
MNA	1.31	1.14	1.23	1.40	1.17	1.20	1.17	1.11	1.26
PLP	17	14	9	29	17	17	14	11	20

Table 2. Matrix of genetic distance coefficients among populations of *Darevskia saxicola* (s.l.). Above diagonal: Rogers (1972) distance. Below diagonal: Nei's (1978) distance.

Subspecies: Location:	*lindholmi* Sevastopol	*lindholmi* Yalta	*saxicola* Kislovodsk	*brauneri* Sochi	*brauneri* Tuapse	*darevskii* Dzhubga	*darevskii* Guzeripe	*darevskii* Dagomys	*szczerbaki* Anapa
D. s. lindholmi Sevastopol		0.020	0.202	0.200	0.221	0.231	0.233	0.241	0.247
D. s. lindholmi Yalta	0.000		0.199	0.204	0.225	0.229	0.242	0.239	0.247
D. s. saxicola Kislovodsk	0.209	0.200		0.225	0.251	0.224	0.195	0.221	0.221
D. s. brauneri Sochi	0.187	0.190	0.228		0.070	0.052	0.086	0.066	0.094
D. s. brauneri Tuapse	0.200	0.208	0.246	0.023		0.082	0.102	0.074	0.100
D. s. darevskii Dzhubga	0.223	0.225	0.228	0.024	0.047		0.056	0.027	0.051
D. s. darevskii Guzeripe	0.242	0.248	0.202	0.059	0.073	0.030		0.060	0.091
D. s. darevskii Dagomys	0.229	0.231	0.220	0.028	0.040	0.000	0.032		0.050
D. s. szczerbaki Anapa	0.242	0.241	0.217	0.055	0.066	0.015	0.066	0.012	

D. s. lindholmi indicates near-panmixis, while the higher values in *D. s. brauneri* and *D. s. darevskii* are indicative of substructuring among populations.

The lowest values of genetic distance were found among populations of the same taxa (table 2). In general, distances roughly correspond to the species differences determined by fixation of alternate alleles.

Discussion

The fixed differences in the allozyme data among some of the taxa examined refute our null hypothesis that all taxa are conspecific. These results constitute grounds for the elevation of *D. s. saxicola* and *D. s. lindholmi* to species rank. The former should be referred to as *Darevskia saxicola* (Eversmann, 1834). The latter should hereafter be referred to as *Darevskia lindholmi* (Lantz and Cyrén, 1936). This name has an unusual history. Lantz and Cyrén (1936, p. 164) examined specimens from Crimea but were unable to obtain specimens from Kislovodsk, the type locality of *D. saxicola*, for comparison with the Crimean specimens. Nonetheless, they gave the name *lindholmi* to the Crimean specimens "in case, when specimens from Kislovodsk are examined, they are found to be different from the Crimean form."

The restriction of the name *D. saxicola* to the former subspecies *D. s. saxicola* has repercussions for the remaining three taxa. Differences among these three do not support their elevation to full species. However, they can no longer be referred to *D. saxicola* and must therefore be referred to another name. The name *D. s. brauneri* (Méhely, 1909) has priority and the three subspecies must hereafter be referred to as *Darevskia brauneri brauneri* (Méhely, 1909), *Darevskia brauneri darevskii* (Szczerbak, 1962) and *Darevskia brauneri szczerbaki* (Lukina, 1963).

Genetic variability parameters (MHD, MNA, PLP) found in this study are comparable with those found in other Caucasian *Darevskia* (Fu et al., 1995; MacCulloch et al., 1995, 1997a, b; Bobyn et al., 1996). Genetic distance values (table 2) among the taxa and populations are similar to those found in groups of closely related species (the *D. rudis* group, MacCulloch et al., 1995, and the *D. caucasica* group, Fu et al., 1995). While the genetic distances in table 2 roughly correspond to the species differences determined by fixation of alternate alleles, these do not represent the phylogenetic relationships among the taxa. A phylogenetic analysis of these relationships is outside the scope of the present study. A more comprehensive analysis, including all other Caucasian rock lizard species, must be conducted to determine the relationships among these lizards.

A number of individuals heterozygous at Ck-C were found in our study (table 1). All of these individuals belonged to *D. b. brauneri*, *D. b. darevskii* and *D. b. szczerbaki*. Heterozygosity at Ck-C is rare in squamate reptiles (Buth et al., 1985). Occurrence of Ck-C heterozygotes in *Darevskia* has been attributed to hybridisation with sympatric taxa expressing alternative alleles (Murphy et al., 1996a). Because the three taxa containing

heterozygotic individuals are sympatric (or nearly so) with species bearing the Ck-C(a) allele (*D. caucasica*, *D. rudis*), hybridisation is a likely source of heterozygosity in Ck-C. Hybridisation may also be the source for other alleles as well.

Although no fixed allelic differences were found among the three subspecies of *D. brauneri*, some results (shown in table 1) merit further mention. The Mpi-A(c) allele appears to be fixed in the specimens of *D. b. darevskii* from Guzeripe and is absent from all other specimens of *D. b. darevskii*. In *D. s. szczerbaki* the Pep-B(c) allele is unusually common and it is the only taxon to possess the Pep-A(a) allele. In *D. b. brauneri* from Tuapse, the sIdh-A(a) allele is atypically common. Although these phenomena may be artefacts of sampling, they may also reflect unique mutations in isolated populations.

The amount of genetic substructuring (F_{ST}) in the two subspecies *D. b. brauneri* and *D. b. darevskii* was similar to that in some other Caucasian *Darevskia* (*D. daghestanica*, Fu et al., 1995; *D. raddei*, Bobyn et al., 1996; *D. derjugini*, MacCulloch et al., 1997a; *D. portschinskii*, MacCulloch et al., 1997b; *D. valentini*, MacCulloch et al., 1995). In all taxa examined the substructuring appears to be the result of loss of alleles among populations, rather than the presence of unique mutations. However, sampling error cannot be completely ruled out as a contributor to the high amount of substructuring. In contrast, the F_{ST} in *D. lindholmi* indicates ongoing gene flow between the two sampled populations, which are some 50 km apart. A similar low F_{ST} was measured in *D. praticola* (MacCulloch et al., 1997a). There is no obvious reason why some taxa exhibit greater or lesser gene flow than others. Clearly, the question of gene flow and intraspecific substructuring requires more study.

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Appendix. Origins of specimens used in this study.

Darevskia b. brauneri:	Russia, Krasnodar District, vicinity of Sochi, 43°35′N, 039°46′E (n = 20)
	Russia, Krasnodar District, Tuapse, 44°06′N, 039°05′E (n = 3)
Darevskia b. darevskii:	Russia, Krasnodar District, Dzhubga, 44°20′N, 038°44′E (n = 4)
	Russia, Krasnodar District, Dagomys, 44°40′N, 039°50′E (n = 3)
	Russia, Krasnodar District, Guzenpe, 43°59′N, 040°09′E (n = 9)
Darevskia b. szczerbaki:	Russia, Anapa, 44°54′N, 037°20′E (n = 9)
Darevskia lindholmi:	Ukraine, Sevastopol, 44°36′N, 033°31′E (n = 12)
	Ukraine, Yalta, 44°30′N, 034°09′E (n = 8)
Darevskia saxicola:	Russia, Kislovodsk, 43°56′N, 042°44′E (n = 31)

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