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Toward the phylogeny of caucasian rock lizards: implications from mitochondrial DNA gene sequences (Reptilia: Lacertidae)

JINZHONG FU AND ROBERT W. MURPHY

Centre for Biodiversity and Conservation Biology, Royal Ontario Museum, 100 Queen's Park, Toronto, Ontario, Canada M5S 2C6

ILYA S. DAREVSKY

Zoological Institute, Russian Academy of Sciences, 119034 St. Petersburg, Russia

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A maximum parsimony phylogeny of 14 Caucasian species of rock lizards, genus *Lacerta*, subgenus *Archaeolacerta*, was constructed from mitochondrial cytochrome *b* and ATPase 6 partial gene sequences. Congruence analyses were carried out between the two genes. A synthesis of the data sets reveals three well supported monophyletic groups: (1) the *caucasica* group including (*Lacerta derjugini* ((*L. alpina*, *L. clarkorum*) (*L. caucasica*, *L. daghestanica*))); (2) the *rudis* group including (*L. parvula* (*L. portschinskii* (*L. valentini*, *L. rudis*))); and (3) the *saxicola* group including (*L. mixta* (*L. nairensis* (*L. saxicola*, *L. raddei*))). Despite the diagnosis of three groups, the placement of *L. praticola* as a basal taxon is uncertain, as are the relationships among the three groups. The mitochondrial DNA sequence data suggested prior hybridization between *L. mixta* and *L. alpina* and possibly between *L. saxicola* and *L. nairensis*. *Lacerta raddei* was resolved as a paraphyletic species on the mtDNA tree; this may result from either hybridization or random gene sorting.

ADDITIONAL KEY WORDS:—*Lacerta* – *Archaeolacerta* – phylogeny – Caucasus Mountains – cytochrome *b*–ATPase 6–hybridization.

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Correspondence to: J. Fu

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INTRODUCTION

The Caucasian rock lizards (genus *Lacerta*, subgenus *Archaeolacerta*), have been extensively studied, including morphological variability, ecology, allozyme variability and hybrid origins of unisexual species. However, the phylogenetic relationships among the species has been addressed only recently (Moritz *et al.*, 1992; Murphy *et al.*, 1996). Moritz *et al.* (1992) used restriction fragment mitochondrial DNA (mtDNA) data to construct a phylogenetic tree of five unisexual species (*L. dahli*, *L. armeniaca*, *L. unisexualis*, *L. uzzelli* and *L. rostombekovi*) and their potential maternal parents (*L. mixta*, *L. raddei*, *L. valentini* and *L. nairensis*). Murphy *et al.* (1996) pursued a more comprehensive study of the bisexual species using allozyme data. Thirteen ingroup species and three outgroup species were included in their analysis. A preferred tree derived from 35 loci was reported and three well-supported monophyletic groups were resolved: the *L. caucasica* group, including *L. alpina*, *L. caucasica* and *L. daghestanica*; the *L. saxicola* group, including *L. mixta*, *L. saxicola*, *L. raddei* and *L. nairensis*; and the *L. rudis* group, including *L. parvula*, *L. portschinskii*, *L. valentini* and *L. rudis*. The phyletic position of two 'ground lizards', *L. derjugini* and *L. praticola*, were considered tentative, as were the relationships among the three primary clades.

Apart from the phylogeny of the bisexual species, the origin and evolution of the unisexual species in this group have been one of the most interesting topics in herpetology. The manageable size of the Caucasian lacertas makes it possible to study the phylogenetic constraints on the evolution of unisexuality. However, a solid conclusion can only derived from a well supported and resolved phylogeny of the sexual species. Subsequent to the allozyme work, we conducted further research using mtDNA sequence data to gather more evidence for reconstructing the phylogeny. Herein, mitochondrial cytochrome *b* and ATPase 6 gene sequence data are used to further investigate the phylogeny of Caucasian *Archaeolacerta*.

MATERIAL AND METHODS

Murphy *et al.* (1996) presented a detailed taxonomic history review. All scientific names used herein follow this work.

Specimens examined

Fourteen Caucasian bisexual species of *Archaeolacerta* were used as our ingroup, and two bisexual species of Caucasian *Lacerta*, *L. media* and *L. strigata*, were used as

TABLE 1. Species of *Lacerta* examined in this study, sample size (*n*) and sample locality

Species	*n*	Localities
L. alpina	1	Russia, Krasnodar, Aisho Mountains, 45°02′N, 039°00′E
L. caucasica	1	Russia, Daghestan, Khvarshi, 42°21′N, 046°06′E
L. clarkorum	1	Turkey, Mahden, 41°12′N, 041°42′E
L. daghestanica	1	Russia, Daghestan, Kuli, 42°01′18″N, 047°14′42″E
L. derjugini	1	Georgia, Achaldaba, 41°54′24″N, 043°30′05″ E
L. media	1	Armenia, Abovyan, Arailer Mt., 40°24′N, 044°29′E
L. mixta	2	Georgia, Achaldaba, 41°54′24″N, 043°30′05″E
L. nairensis	1	Armenia, Aragatz Mt, Bjurakan, 40°21′54″N, 044°15′12″E
L. parvula	1	Georgia, Achaldaba, 41° 54′24″N, 043°30′05″E
L. portschinskii	1	Armenia, Stepenavan, 41°01′15″N, 044°22′54″E
L. praticola	1	Russia, vicinity of Sochi, 43°35′N, 039°46′E
L. raddei	2	Armenia, Gosh, 40°44′51″N, 045°01′26″E
	2	Armenia, Geghart, 40°08′15″N, 044°49′06″E
L. rudis	1	Georgia, Achaldaba, 41°54′24″N, 043°30′05″E
L. saxicola	1	Russia, Dombay, 43°15′N, 041°45′E
L. valentini	1	Armenia, Sevan, 40°30′58″N, 044°56′26″ E

the outgroup (Table 1). Fieldwork was conducted during 1992–5. Live specimens were euthanized by an overdose of sodium pentobarbital before processing, and following accepted animal welfare protocols. Blood, heart, liver and tail muscle tissues were collected and frozen in liquid nitrogen for use in multiple molecular investigations. All tissue samples were kept at −75°C in the laboratory. Tail muscle was used for DNA extraction. All voucher specimens were deposited in the herpetological collections of the Royal Ontario Museum (specific data available on request). Both Moritz *et al.* (1992) and Bobyn *et al.* (1996) questioned the monophyly of *L. raddei* and suggested that the population from Gosh, Armenia may represent a separate lineage. Consequently, we used two populations of *L. raddei* (Gosh and Geghart, Armenia).

Amplification and sequencing protocol

The phenol extraction method was used for obtaining the whole DNA from the muscle tissues following Murphy *et al.* (1995). The extraction solutions were directly used for polymerase chain reaction (PCR) without ethanol precipitation. A cytochrome *b* gene fragment of 658 bp was amplified and sequenced using primers B1 (L16346 5′cca tcc aac atc tca gca tga tga aa3′; Kocher *et al.* 1989), B2 (H16654 5′gcc cct cag aat gat att tgt cct ca3′; Kocher *et al.*, 1989), and B4 (H17005 5′ttg ctg ggg tga agt ttt ctg ggt c3′; Birt *et al.*, 1992). An ATPase 6 gene fragment of 402 bp was amplified and sequenced using primer A1 (L10056 5′atg aac cta agc ttc gac caa tt3′) and A2 (H10459 5′ata aaa agg cta att gtt tcg at3′), both were designed by Haddrath (Upton & Murphy, in press). The numbers correspond to the 3′ end with homologous positions relative to *Xenopus laevis* (Roe *et al.*, 1985). PCR products were isolated by electrophoresis on 1.5% agarose gels followed by purification using GeneClean (Bio101). Double stranded DNA was sequenced directly largely following the protocol recommended by the manufacturer (US Biochemical, 1994). This

method was modified in having 10 min of denaturation at 100°C and annealing on ice. The first part of cytochrome *b* gene (from B1 to B2) and ATPase 6 gene were sequenced in both directions with about 90% overlap to minimize errors. The second part of cytochrome *b* was sequenced only in one direction. The reaction products were separated by 5% Long Ranger gel (JT Baker) and the gel was subsequently exposed to autoradiographic film to visualize the bands. We re-sequenced the cytochrome *b* fragment from B1 to B4 in an ALF Automated DNA Gene Sequencer using AutoLoad Solid Phase Sequencing (Pharmacia). Consequently, the entire cytochrome *b* fragment was sequenced in both directions.

Sequences were aligned using ESEE (version 3.0; Cabot & Beckenbach, 1989) and analysed using MacClade (version 3.04; Maddison & Maddison, 1992), PAUP (version 3.1; Swofford, 1993), and RandomCladistics (version 3.0; Siddall, 1995) which interfaces with Hennig86 (version 1.5; Farris, 1988).

Phylogenetic analysis

Cytochrome *b* and ATPase 6 genes were analyzed both separately and combined. The corroboration from independent data sets provides strong evidence for the reliability of phylogenetic trees (Hillis, 1987; Cracraft & Helm-Bychowski, 1991). A congruence analysis was carried out for the mtDNA data, and the allozyme data of Murphy *et al.* (1996).

To test the presence of character covariance in the data we examined skewness (g_1 statistics) using PAUP based on 1000 randomly chosen trees (Huelsenbeck, 1991; Hillis & Huelsenbeck, 1992), and permutation tail probability (PTP) (Faith & Cranston, 1991; Siddall, 1995).

For each gene, an initial analysis was conducted treating each base position as an unordered character and equally weighting all characters. The maximum parsimony criterion was used for selecting trees. Following the initial analysis, we used five techniques to evaluate both resolved nodes and ambiguities.

Hillis, Huelsenbeck & Cunningham (1994) suggested that weighting characters may result in a more accurate phylogeny. Thus, we initially inversely weighted transversions (TV) over transitions (TS) according to the ratio of changes (Williams & Fitch, 1991); all changes, including autapomorphic changes, were used for calculating the ratio.

Second, the functional ingroup/outgroup method (FIG/FOG; Watrous & Wheeler, 1981; Fu & Murphy, 1997) was used for examining ambiguous nodes. Considering the predictably high levels of homoplasy in DNA sequence data, the divergence between the outgroup and ingroup is critical. A highly diverged outgroup may result in long branch attraction on the tree (Felsenstein, 1978; Swofford *et al.*, 1996).

Third, taxon pruning was used to evaluate nodal stability. Because of their ambiguous placement on the tree, Murphy *et al.* (1996) deleted the 'ground lizards', *L. derjugini* and *L. praticola*, both individually and then together, as a way of evaluating nodal stability.

Fourth, decay analysis (Bremer, 1988) was used for evaluating the nodes. Because sequence data sets are usually large and highly homoplastic, the difference between the optimal trees and the suboptimal trees with one or few steps more are fairly small. Thus, it is advisable to examine the suboptimal, alternative trees (Smith,

1989; Swofford, 1991; Cracraft & Helm-Bychowski, 1991). We examined all suboptimal trees that were less than or equal to 1% longer than the most parsimonious unweighted solution.

Fifth, two pseudostatistical methods, bootstrap (BS; Felsenstein, 1985; Hillis & Bull, 1993; Trueman, 1993) and permutation tail probability (PTP; Faith & Cranston, 1991) were used for inferring confidence limits of the recovered nodes. Despite the wide criticisms of BS (e.g. Jones *et al.*, 1993; Trueman, 1993), we considered the method as a one way estimation; a high BS value indicates strong support, but the low value does not mean poor support. Our PTP analysis followed the suggestions of Fu & Murphy (unpublished). Nodes having significant covaried data were regarded as well supported.

A congruence analysis was carried out using both character congruence (total evidence or combined data; Kluge, 1989; Barrett, Donoghue & Sober, 1991; Kluge & Wolf, 1993) and taxonomic congruence (or consensus approach; Mickevich, 1978; Swofford, 1991; de Queiroz, 1993; Lanyon, 1993). For the second approach, the commonalties between the well supported elements were first compared (Lanyon, 1993). Suboptimal trees were considered when searching for congruence (Swofford, 1991; Omland, 1994).

The congruence between the cytochrome *b* and ATPase 6 trees was evaluated by searching for common nodes. A globally parsimonious tree from the combined data was obtained for comparison with the commonalties from the separate analyses.

Our gene trees were compared with the allozyme analysis of Murphy *et al.* (1996) using taxonomic congruence for two reasons. First, because of documented hybridizations among species (Darevsky 1967, 1993), it is important to potentially identify maternal (mtDNA) contributions in hybridizations. Second, we do not believe that combining two different type of data sets would give an efficient estimation of congruence, without considering both the quality and quantity of the data. Although the allozyme data are far less homoplastic, this smaller data set may be easily overwhelmed by the larger data set (DNA sequence data; Miyamoto, 1985). Alternatively, the allozyme data represent 35 genes whereas the mtDNA sequence data represent only 2. If genes were used as the characters, then the data sets would not contribute equally to the phylogenetic estimation.

RESULTS

Cytochrome b

A total of 625 base pair sequences were resolved and aligned for all 16 species. No gaps, insertions or deletions were found, although some ambiguities remained. The largest pairwise difference between outgroup and ingroup was 19.8% (*L. strigata* vs. *L. portschinskii*), and the smallest was 15.84% (*L. media* vs. *L. caucasica*). The largest pairwise difference between ingroup members was 15.84% (*L. saxicola* vs. *L. portschinskii*), the smallest was 1.44% (*L. mixta* vs. *L. alpina*).

A g_1 value of -0.862 resulted from 1000 random tree replicates, which is lower than the critical value of 0.15 suggested by Hillis & Huelsenbeck (1992). PTP analysis yielded a value of 0.001 from 999 replicates. Both analyses indicated that there was significant character covariance in the data set.

The initial analysis of 189 potentially phylogenetically informative characters resulted in four most parsimonious trees (MPTs) with CI = 0.453, RI = 0.539 and 525 steps. Four monophyletic groups were resolved on all trees including (1) ((*L. caucasica*, *L. daghestanica*) (((*L. alpina*, *L. mixta*) *L. clarkorum*) *L. derjugini*)), (2) ((*L. rudis*, L. *valentini*), *L. portschinskii*), (3) ((*L. saxicola*, *L. nairensis*) *L. raddei*-Geghart), and (4) (*L. raddei*-Gosh, *L. praticola*). *Lacerta parvula* was resolved as the sister group of all other ingroup members. Three regions of the tree were responsible for the occurrence of multiple trees. First, the *L. raddei*-Gosh–*L. praticola* clade was grouped with either the *rudis* or *saxicola* clade. Second, the *saxicola* clade was associated with either the *caucasica* or *rudis* clade. Third, *L. derjugini* was ambiguously resolved as either the sister group of (*L. caucasica*, *L. daghestanica*), ((*L. mixta*, *L. alpina*) *L. clarkorum*), or basal within the *caucasica* clade.

As in Moritz *et al.* (1992) and Bobyn *et al.* (1996), the Gosh population of *L. raddei* was not resolved as the sister group of other populations of *L. raddei*. A taxon pruning trial excluding *L. raddei*-Gosh resulted in one MPT. *Lacerta praticola* was resolved as the sister species of *L. parvula* and was located at the base of the tree. *Lacerta derjugini* formed the sister group of (*L. mixta*, *L. alpina*, *L. clarkorum*). Finally, the *caucasica* clade was resolved as the sister group of the *rudis* clade.

Although *L. derjugini* was always resolved as a member of the *caucasica* clade, its relationships within the clade were ambiguous. When *L. saxicola* and *L. nairensis* were used as the FOG, we resolved *L. derjugini* as the sister group of all other members in the *caucasica* clade.

Weighting transversions four times greater than transitions resulted in one MPT. *Lacerta praticola* appeared as the basal taxon and *L. parvula* branched next. The *saxicola* clade grouped with the *caucasica* clade. *Lacerta derjugini* was resolved as the sister group of *L. caucasica*. *Lacerta raddei*-Gosh joined the *rudis* clade at the base of the tree.

We accepted the weighted cladogram with two exceptions. First, *L. raddei*-Gosh could not be placed on the tree with any certainty. Second, the sister group relationship of *L. derjugini* and *L. caucasica* was never resolved on the unweighted tree, and is not supported by any other evaluation, including allozyme data (Murphy *et al.* 1996). A functional outgroup evaluation supported a sister relationship of *L. caucasica* and *L. daghestanica*, and this alternative relationship is assumed to be true (Fig. 1).

Decay analyses strongly supported the monophyly of the *rudis*, *saxicola*, and (*L. alpina*, *L. mixta*, *L. clarkorum*) clades. These clades were maintained in all 360 trees whose steps were equal to or less than 530 steps. The *caucasica* clade collapsed on the strict consensus tree when all 90 trees of 528 or fewer steps were kept. The association of *L. praticola* and *L. raddei* from Gosh was retained on the strict consensus of 22 trees with 526 or fewer steps.

BS trials found nine nodes with values greater than 0.5. Our PTP analyses supported four clades, the three major clades, and the (*L. mixta*, *L. alpina*) clade (Fig. 1).

ATPase 6

A 338 base pair sequence was resolved and aligned for 12 species. No deletions, insertions or gaps were found, although some ambiguities remained. We failed to sequence five species (*L. parvula*, *L. raddei*-Geghart, *L. nairensis*, *L. rudis*, *L. valentini*).

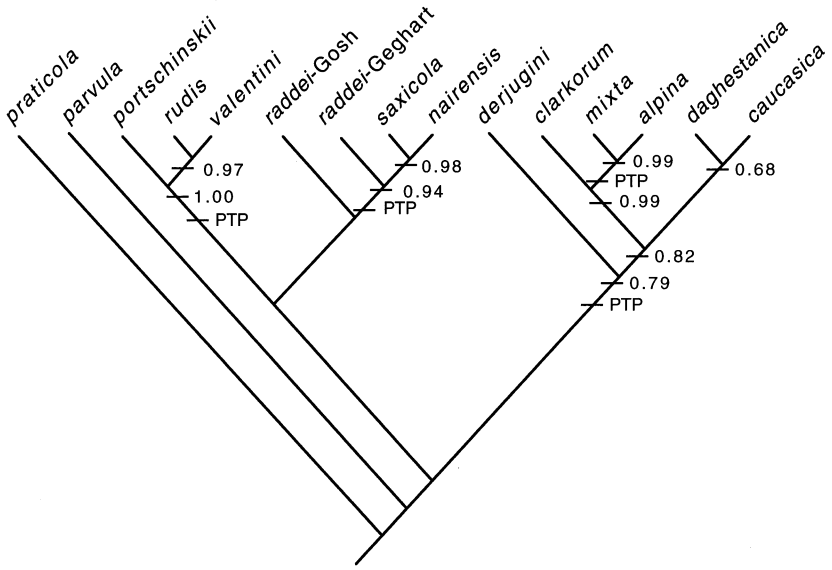

Figure 1. Maximum parsimony solution for bisexual Caucasian rock lizards (*Lacerta*) derived from cytochrome *b* partial gene sequences. Numbers on the cladogram indicate bootstrap values greater than 0.50. 'PTP' marks nodes well-supported by permutation tail probabilities.

The largest pairwise difference between the outgroup and ingroup was 22.49% (*L. strigata* vs. *L. daghestanica*), and the smallest 14.20% (*L. media* vs. *L. clarkorum*). The largest pairwise difference between ingroup members was 16.57% (*L. praticola* vs. *L. caucasica* and *L. daghestanica*) and the smallest 0.59% *(L. alpina* vs. *L. mixta*).

A g_1 value of -0.822, which is lower than the critical value of 0.30 suggested by Hillis & Huelsenbeck (1992), resulted from 1000 replicates. PTP analyses resolved a value of $P=0.001$ from 999 replicates. Thus, both analyses indicated that the ATPase 6 sequence data contain significant structure.

Our initial analysis included 86 potentially phylogenetically informative characters and resulted in four MPTs (CI = 0.562, RI = 0.509, and 197 steps). The *caucasica* clade appeared on all four trees, as well as the *saxicola* clade, including *L. saxicola* and *L. raddei*-Gosh. *Lacerta praticola* resolved at the base of our trees as the sister group of all other members. The relationships among the *caucasica* and *saxicola* clades and *L. portschinskii* were ambiguous; the *saxicola* clade grouped with both the *rudis* and the *caucasica* clades. In the *caucasica* clade, *L. alpina* and *L. mixta* formed a sister group relationship, as did *L. caucasica* and *L. daghestanica*. The positions of *L. derjugini* and *L. clarkorum* were unresolved.

Weighting transversions 2 times more than transitions resulted in a single solution, which was one of the 4 MPTs (Fig. 2). *Lacerta clarkorum* and *L. derjugini* formed the sister group of (*alpina*, *mixta*). The *saxicola* clade formed the sister group of the *caucasica* clade. No further resolution was achieved using the *saxicola* clade as FOG to the *caucasica* clade.

Our decay analyses revealed only two well-supported nodes. Upon retaining all trees of less than or equal to both 198 and 199 steps, the strict consensus tree resolved two nodes: *L. mixta* with *L. alpina*, and *L. saxicola* with *L. raddei*-Gosh.

BS evaluations indicated six nodes with a value over 0.5. Four nodes received

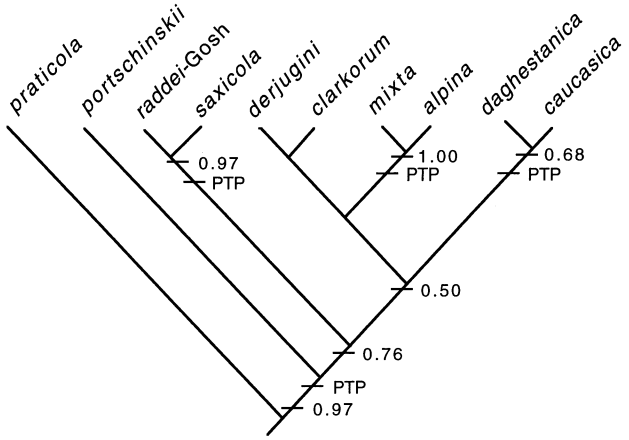

Figure 2. Maximum parsimony solution for bisexual Caucasian rock lizards (*Lacerta*) derived from ATPase 6 partial gene sequences. Numbers on the cladogram indicate bootstrap values greater than 0.50. 'PTP' marks nodes well-supported by permutation tail probabilities.

PTP support, including (*L. saxicola*, *L. raddei*-Gosh), (*L. alpina*, *L. mixta*), (*L. caucasica*, *L. daghestanica*), and all taxa excluding basal *L. praticola* (Fig. 2).

Combined cytochrome b *and ATPase 6 sequences*

Trees derived from the two genes had no fundamental differences. Differences occurred in the placement of *L. derjugini* in the *caucasica* clade and *L. raddei*-Gosh. Neither gene confidently resolved the relationships of *L. derjugini*. The cytochrome *b* data strongly supported the clade (*L. clarkorum*, *L. alpina*, *L. mixta*), and consequently *L. derjugini* is not likely to be the sister group of *L. clarkorum*. The Gosh population of *L. raddei* was consistently grouped with *L. saxicola* by ATPase 6, but was unresolved on the cytochrome *b* tree.

The combined data sets resulted in two MPTs (CI = 0.479, RI = 0.526, 727 steps). The instability was due to *L. praticola*, which was resolved as either the sister group of *L. parvula* or the sister group of all other ingroup members except *L. parvula*. This analysis grouped the *saxicola* and *rudis* clades, and *L. derjugini* with ((*L. alpina*, *L. mixta*) *L. clarkorum*). Placement of the *caucasica* clade with either the *saxicola* clade, or the *rudis* clade, required only 4 additional steps out of 727 steps, indicating little support for the relationships among the three major clades. This is also true for the placement of *L. derjugini*.

Weighting transversions 3 times more than transitions resulted in a single MPT, which was one of the unweighted MPTs (Fig. 3). A clade consisting of *L. praticola* and *L. parvula* was resolved as the sister group of all other Caucasian *Archaeolacerta*.

Decay analysis strongly supported the monophyly of the *rudis* group, the *caucasica* group, and the *saxicola* group, excluding *L. raddei* from Gosh. These clades were maintained in all 122 trees equal to or less than 734 steps. The association of *L. derjugini* with (*L. caucasica*, *L. daghestanica*), collapsed on the strict consensus tree when all 6 trees of less than or equal to 728 steps were kept. *Lacerta raddei*-Gosh was

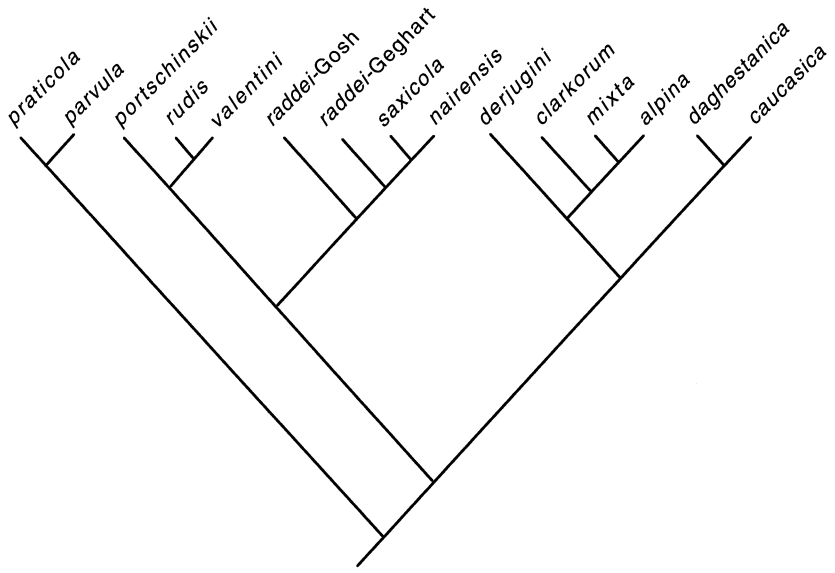

Figure 3. Maximum parsimony solution for bisexual Caucasian rock lizards (*Lacerta*) derived from the combined cytochrome *b* and ATPase 6 partial gene sequences. Transversions were weighted three times as heavily as transitions.

separated from the *saxicola* clade, and the association of the *saxicola* and *rudis* clades collapsed, when 39 trees equal to or less than 731 steps were kept.

DISCUSSION

Congruence of DNA and allozyme data

We recently found a typographical error in the data file used by Murphy *et al.* (1996) in their phylogenetic analysis of Caucasian lacertas using allozymes. Our re-analysis of these allozyme data resulted in 6 MPTs, with 83 steps. Instabilities in tree shape were restricted to the relationships of the three major speciose clades, and among the three members of the *saxicola* clade. The strict consensus tree is shown in Figure 4. The associations of ((*caucasica, daghestanica*) *alpina*) and (*valentini*, *rudis*) held on the strict consensus tree of 107 trees with 84 or fewer steps. The results are generally congruent with the mtDNA sequence trees (Figs 1–3). Both evaluations resolved the three major speciose clades, although differences were observed in membership within and among the three clades. The lack of congruence with DNA data is attributable, in part, to insufficient allozyme data.

The allozyme and sequence analyses differed most with respect to the *caucasica* clade. As previously noted, the sequence data unambiguously placed *L. derjugini* in the *caucasica* clade whereas the allozyme data placed it basally to all other Caucasian *Archaeolacerta.* However, moving *L. derjugini* to the base of the *caucasica* clade on the allozyme tree required only one additional step and we prefer this arrangement (Fig. 4). Although the DNA sequence data suggest the placement of *L. derjugini* in

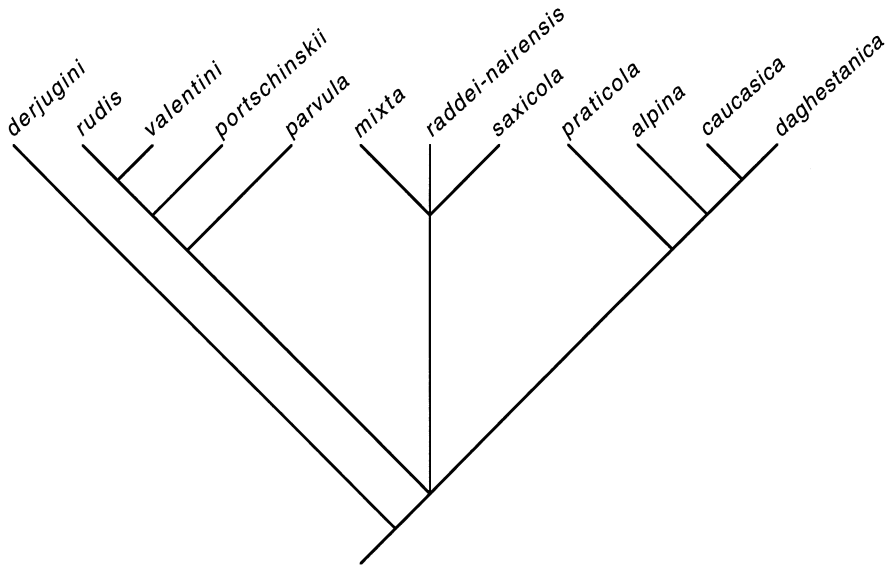

Figure 4. The revised strict consensus tree for the genealogical relationships of bisexual Caucasian rock lizards (*Lacerta*) derived from allozyme data (Murphy *et al.*, 1996).

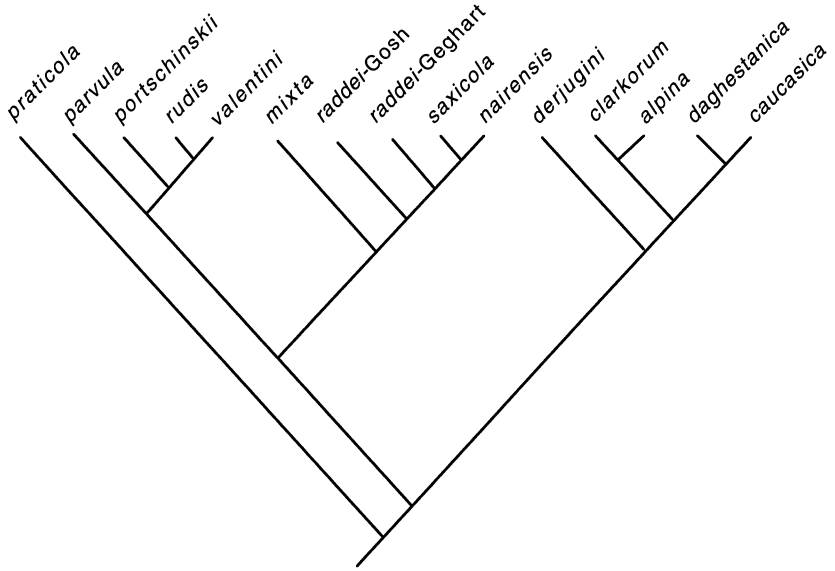

Figure 5. Our preferred phylogeny of the Caucasian rock lizards (*Lacerta*) based on congruence of allozyme and DNA sequence data.

the *caucasica* clade, this association is not strongly supported; its association with other branches requires only a few additional steps. The placement of *L. mixta* also differed between the two analyses. However, the placement of *L. mixta* in the *caucasica* clade as ((*L. alpina*, *L. mixta*) *L. clarkorum*) required four additional steps on the allozyme tree (see below).

The basal position of *L. praticola* is well supported by the ATPase 6 sequence data and by transversional weighting of cytochrome *b*. This change requires 1 additional step on the allozyme tree.

Neither the allozyme nor mtDNA data provided a robust explanation about the relationship among the three major clades. The combined mtDNA data set associated the *saxicola* and *rudis* clades. This association was also supported by our character weighting trials. However, uniting either *saxicola* or the *rudis* clades with the *caucasica* clade required only four additional steps. All three possible combinations resulted in equally parsimonious solutions on the allozyme tree. Herein, we consider the association of the *saxicola* and *rudis* clades to be tentative. The difficulty in determining the relationships among the three clades with confidence may reflect near simultaneous speciation events of the common ancestors of the clades.

The mtDNA data placed *L. parvula* basally whereas the allozyme evaluation placed it in the *L. rudis* group. Any other rearrangement required at least two additional steps in the allozyme tree. We tentatively accept the allozyme results because the combined mtDNA data require an increase of six steps, which is comparatively fewer than the increase of two steps with the allozyme data.

As expected, the results of the combined mtDNA and allozyme data hardly differed from that of the mtDNA data alone. The single MPT showed *L. derjugini* as the sister group of all other *caucasica* group members, and *L. praticola* as the sister group of *caucasica* group. All other relationships were the same as derived from the mtDNA data alone.

The position of L. mixta

The most striking conflict between the mtDNA and allozyme analyses was the position of *L. mixta*. The allozyme data unambiguously grouped it in the *saxicola* clade whereas the mtDNA data resolved it as the sister species of *L. alpina* in the *caucasica* clade. For the allozyme data, any other solution required at least three extra steps and the monophyly of ((*caucasica*, *daghestanica*) *alpina*) is well-supported by decay analysis. The mtDNA phylogeny was well supported by PTP, decay analysis, and all other trials. Accepting the mtDNA solution would increase the allozyme tree by six steps and the converse increases the mtDNA tree by 64 steps. Directly comparing the sequences, there is a 1.44% divergence of cytochrome *b* and only 0.59% at ATPase 6 between the two species. These divergences are lower than the intraspecific variation in *L. raddei* and *L. nairensis* (Fu *et al.*, unpublished data).

No acceptable suboptimal solutions can resolve the conflict, and it is highly unlikely that the conflict results from homoplastic changes. The only practicable explanation requires hybridization; either hybridization occurred between *L. mixta* and *L. alpina*, or *L. mixta* had its origin from a hybridization event. Doyle (1992) discussed how gene introgression could obscure a phylogeny. In the first scenario, *L. mixta* and *L. alpina* hybrids continued to backcross with pure *L. mixta*. However, the maternally inherited mtDNA of *L. alpina* would have become fixed in *L. mixta*. Thus, extant populations of *L. mixta* are actually the hybrid offspring of *L. mixta* and *L. alpina*. We believe that *L. mixta* shares the majority of its nuclear genome with the *saxicola* clade; its mitochondrial genome is shared with *L. alpina*. Considering the low mtDNA divergence between *L. mixta* and *L. alpina*, the hybridization likely occurred very recently.

Méhely (1909), Lantz & Cyrén (1936) and later Darevsky (1967) believed that *L. mixta* may have originated from hybridization between *L. derjugini* and *L. saxicola*, although, using allozymes, Uzzell & Darevsky (1973) and Murphy *et al.* (unpublished data) showed that *L. mixta* was not. Our mtDNA data imply that the earlier speculations of hybridization were correct. However, instead of *L. derjugini*, the mtDNA data unambiguously require that *L. alpina* was involved in hybridization with, if not in the origin of, *L. mixta*. Although *L. mixta* was associated with the *saxicola* group based on allozyme data (Murphy *et al.*, 1996), the data did not show high heterozygosity in *L. mixta* relative to other Caucasian lacertas. Consequently, we do not believe that *L. mixta* had a hybrid origin, but rather that it received its mitochondrial genome relatively recently through a hybridization event. Furthermore, the association of *L. mixta* and *L. saxicola* indicates one hazard of estimating phylogeny solely using mtDNA data. The maternal relationships resolved from the mitochondrial genome may not accurately reflect genealogical history because paternal contributions will be lost. Indeed, if hybridization is commonplace, as it is within the Caucasian *Archaeolacerta*, then the phylogeny inferred from mtDNA may be misleading.

The paraphyly of L. raddei

We resolved paraphyletic relationships among *L. raddei* and *L. nairensis*. These two taxa could not be differentiated on the basis of fixed allozyme markers (Bobyn *et al.*, 1996) and their validity as species has been questioned (Moritz *et al.*, 1992; Bobyn *et al.*, 1996). In contrast, our mtDNA data resolved *L. nairensis* as the sister species of *L. saxicola*, and the two populations of *L. raddei* were sequentially resolved as their sister taxa (Fig. 3).

Lacerta raddei formed a paraphyletic group on the cytochrome *b* tree (Fig. 1). Moritz *et al.* (1992) similarly found paraphyly in this species. However, in their case *L. raddei*-Gosh was more closely related to *L. nairensis* than to *L. raddei* populations from Yegehnadzor and Geghart. They assumed that this paraphyly reflected the random sorting of an ancestral polymorphism. Although this explanation is possible, our data suggest that hybridization is involved. On the cytochrome *b* tree, *L. raddei*-Gosh boomeranged in relationships between *L. praticola* and the *saxicola* clade. Significantly, unpublished allozyme data show frequent hybridization among the species at Gosh, Armenia (Murphy *et al.*, unpublished data). Considering commonplace hybridization among the bisexual species of Caucasian rock lizards (Murphy *et al.*, 1996), gene introgression in the Gosh species might also explain the observed paraphyly.

The preferred phylogeny

Our final preferred tree reflects both allozyme (Fig. 4) and mtDNA data (Fig. 3). The tree requires 85 steps for allozyme data, compared with 83 steps of Murphy et al. (1996), and 799 steps for the combined mtDNA data, compared to 727 steps for the MPT. The major increase of steps in the DNA data is due to the association of *L. mixta* with the *saxicola* clade. If *L. mixta* were considered the sister of *L. alpina*, then the preferred mtDNA tree would have 735 steps.

The composition of three species groups is quite unambiguous. The *caucasica* clade includes (*L. derjugini* ((*L. alpina*, *L. clarkorum*), (*L. caucasica*, *L. daghestanica*))), the *rudis* group includes (*L. parvula* (*L. portschinskii* (*L. valentini*, *L. rudis*))), and the *saxicola* group includes (*L. mixta* (*L. nairensis* (*L. saxicola*, *L. raddei*))). More data are obviously required to confidently resolve relationships among the three major clades. Although this conclusion is tentative, *L. praticola* appears to be the sister group of all other Caucasian *Archaeolacerta*.

Sequence availability

All sequence data have been deposited in GenBank (accession numbers U88589-U88617).

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