

Contribution to the study of the genetic variability and taxonomic relationships among five lizard species of the family Lacertidae from Greece

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ABSTRACT. The present study examines the genetic variability and the taxonomic relationships among five lacertid species, i.e. *Podarcis taurica*, *P. milensis*, *P. peloponnesiaca*, *Lacerta graeca* and *Algyroides moreoticus*, representing the three main genera of this family in Europe. The last four of the above species are endemic to Greece and three of them live sympatrically in Peloponnisos. These relationships were studied by allozyme analysis. Of the loci analyzed, the *Mpi-1* locus was found to be a convenient molecular marker for discrimination of the genera *Podarcis* (allele a), *Lacerta* (allele b) and *Algyroides* (allele c). The values of Nei's genetic distances between the examined species ranged from 0.025 to 0.484. According to the UPGMA-dendrogram plotted using the Nei's genetic distances, two species groups are formed indicating that the genera *Lacerta* and *Algyroides* show a stronger relationship to one another than either does to *Podarcis*. These results are in agreement with DNA sequence data but are not in accordance with previous electrophoretic and immunological studies, which suggest that *Lacerta* is more closely related to *Podarcis* than to *Algyroides*. The studied *Podarcis* taxa were found to be close relatives (Nei's diastances <0.18), especially *P. taurica* and *P. milensis*. These values are lower than those usually given in the litterature for the distinction of lacertid species.

INTRODUCTION

Although many studies have been carried out on the systematics of the family Lacertidae dating from the last quarter of the 19th century, well-supported estimations of the overall phylogeny of the family on the basis of morphological and molecular evidence have only appeared quite recently (ARNOLD, 1989; HARRIS et al., 1998; HARRIS & ARNOLD, 1999). According to ARNOLD (1989) Lacertidae falls into two parts: (a) a paraphyletic Palaearctic and Oriental group of primitive forms, also including nearly all Mediterranean species, and (b) a holophyletic assemblage of Ethiopian and advanced Saharo-Eurasian taxa derived from the former part.

Among the members of the Palaearctic-Oriental group, the collective genus *Lacerta* is one of the most diverse lacertid genera, with more than 50 species. The phylogeny and

taxonomy of this widespread genus has been the subject of by far the most attempts (for a review see ARNOLD, 1989, 1993). Cladistically, it is evident that the traditional genus *Lacerta* is a paraphyletic group consisting of a number of smaller clades and species groups; so its systematics is still unclear and many conflicts occur. On the other hand *Podarcis* and *Algyroides* are much more homogeneous entities: The well-defined Mediterranean group of *Algyroides* is clearly holophyletic, and there is little or no doubt of the holophyletic character of *Podarcis* (ARNOLD, 1989; MAYER & LUTZ, 1990; HARRIS & ARNOLD, 1999). Taxonomically, *Algyroides* was long ago thought to be a clearly delineated genus while *Podarcis*, although formerly considered as a subgenus of *Lacerta* (more recently this opinion is also expressed by MAYER & TIEDEMANN, 1982 and Guillaume and Lanza, 1982), now is widely accepted as a definite genus composed of closely related species.

Within the clearly paraphyletic taxon *Lacerta* s. lat. as defined by ARNOLD (1989), *Archaeolacerta* is considered

as a rather informal and not well-defined assemblage of primitive lizards; according to this author it seems better, for the present, to treat it as a subgenus of *Lacerta* s. lat., although HARRIS et al. (1998) do not give enough evidence for the monophyly of archaeolacertas as a whole.

For the clarification of the phylogenetic relationships of the European lacertids, apart from the morphological studies, biochemical and immunological techniques have been used extensively (for a review see ARNOLD, 1989 and HARRIS et al., 1998). Recently, mt-DNA and r-RNA sequence data have also been used (GONZALEZ et al., 1996; DOURIS et al., 1997; FU et al., 1997; HARRIS et al., 1998; HARRIS & ARNOLD, 1999). In spite of all this accumulated information many ambiguities and controversies concerning the affinities of certain taxa still remain.

The present article constitutes a contribution intended to reveal or confirm the relationships among *Lacerta* (*Archaeolacerta*), *Podarcis* and *Algyroides*. Such a direct, simultaneous comparison of these three genera by means of allozyme electrophoresis has been lacking from the literature. It is well known theoretically that a large number of species (if not the total) of the three genera compared should be studied in order to acquire definitive conclusions on their relationships. However, in practice, acceptable results have been obtained by many authors who used smaller numbers of species. In the present work five species (*L. graeca*, *P. peloponnesiaca*, *P. taurica*, *P. milensis* and *A. moreoticus*) were considered as representatives of the above three genera because they are interesting members of the Balkan lacertids. Apart from *P. taurica* all others are endemic to confined continental and/or insular parts of Greece (CHONDROPOULOS, 1986; VALAKOS & MYLONAS, 1992). These particular species were studied since it is generally known that the relationships of the Mediterranean lacertids have been greatly influenced by the major palaeogeographic events in the area (BOHME & CORTI, 1993); so endemism and other biogeographical consequences of those events constitute very useful material to illuminate phylogenetic affinities and test the taxonomic hypotheses proposed. On the other hand the faunistic importance of the endemic Mediterranean lizards is obvious as they largely contribute to the local and the whole European biodiversity.

MATERIAL AND METHODS

A total of 53 lizards of the five studied species were used. The sampling areas and the respective sample size of each species are shown in Table 1. The collection of this material has been carried out on the basis of rules and limitations of the Greek legislation (Presidential Decree 67/1981). For the electrophoretic procedure liver, kidneys and muscles were taken from the properly anesthetized animals. The electrophoretic analysis was carried out on already prepared cellulose acetate plates using the set of devices, both provided by Helena Laboratories house. The preparation of the tissue samples, the electrophoretic con-

TABLE 1
The sampling areas and the sample size of the species examined

Taxa	Sample size	sampling area
<i>Podarcis peloponnesiaca</i> (Bibron & Bory, 1833)	15	Stymfalia, Korinthia Pref.
<i>Podarcis milensis</i> (Bedriaga, 1882)	10	Milos archipelago, Kyklades Pref.
<i>Podarcis taurica</i> (Pallas, 1814)	7	Stymfalia, Korinthia Pref.
<i>Lacerta graeca</i> (Bedriaga, 1886)	6	Stymfalia, Korinthia Pref.
	4	Kardamyli, Messinia Pref.
<i>Algyroides moreoticus</i> (Bibron & Bory, 1833)	3	Zarouchla, Korinthia Pref.
	8	Vouraikos Gorge, Achaia Pref.

ditions and the protein-specific staining were performed according to SEARLE (1983) modified by Fragedakis-Tsolis. In the present study 17 putative genetic loci also used in previous studies on lacertid lizards, were examined (Table 2). For technical reasons in *P. milensis* only 8 loci were examined. The electrophoretic results were treated with the biostatistical package BIOSYS-1 (SWOFFORD & SELANDER, 1981).

TABLE 2
The loci examined, the respective enzymes and the tissues from which they were extracted

Locus	Enzyme	Tissue
Aco-1,2	Aconitase	Liver
Ak-1	Adenylate kinase	Liver
Ck-1	Creatine kinase	Liver
Got-1,2	Glutamate oxaloacetate transaminase	Liver
Gpi-1s	Glucose phosphate isomerase	Muscle
Idh-1,2	Isocitrate dehydrogenase	Kidneys
Ldh-1,2	Lactate dehydrogenase	Muscle
Mod-1,2	Malic enzyme	Kidneys
Mor-1	Malate dehydrogenase	Kidneys
Mpi-1	Mannose phosphate isomerase	Liver
Np-1	Nucleoside phosphorylase	Liver
Pgm-1	Phosphoglycomutase	Muscle

RESULTS

As a consequence of factors mentioned above for the material of *P. milensis*, there are two sets of results stemming from the statistical treatment of the electrophoretic raw data: the first set includes the results obtained from the comparison of 4 species (*P. milensis* is excluded), on the basis of all the 17 loci, while the second one includes

the results derived from the comparison of all the 5 species, taking into account 8 loci.

In the case of the first data set 13 of the 17 loci examined were polymorphic (Table 3). The polymorphic locus Mpi-1 was fixed for three different alleles, one for each of the three lacertid genera examined: namely allele a in *Podarcis*, allele b in *Lacerta (Archaeolacerta)* and allele c in *Algyroides* (Table 3). The mean value of the fixation index $F_{(ST)}=0.618$ implies that 61.8% of the total genetic variability observed was due to differences between the species studied, and the remaining 38.2% was due to intraspecific differences. The values of the Nei's genetic distances between the species studied ranged from 0.123 to 1.426, while those of Roger's genetic identity ranged

TABLE 3

Allele frequencies of the 17 genetic loci examined in 5 of the lacertid species studied (dashes indicate the loci not examined in *P. milensis* – see Materials and Methods).

Locus	<i>P. pel/siaca</i>	<i>P. taurica</i>	<i>P. milensis</i>	<i>L. graeca</i>	<i>A. moreoticus</i>
Aco-1	b	.214a .786b	b	b	.091a .909b
Aco-2	b	.071a .857b .071c	.150a .600b .250c	.050a .200b .750c	.545b .455c
Ak-1	a	a	a		.818a .182a
Ck-1	a	.429a .571b	.800a .200b	b	.364b .636c
Got-1	.643a .357b	c	c	a	b
Got-2	.214a .786b	b	b	b	b
Gpi-1s	a	.786a .214b	-	a	.875a .125b
Idh-1	.233a .767b	.857a .143b	-	a	a
Idh-2	a	a	-	.950a .050b	a
Ldh-1	a	a	-	a	a
Ldh-2	a	a	-	a	a
Mod-1	.466a .267b .267c	.357a	-	.400a	.125a
Mod-2	b	.643c .286a .714b	-	a	a
Mor-1	a	a	-	a	a
Mpi-1	a	a	a	b	c
Np-1	a	a	a	a	a
Pgm-1	b	b	-	a	.938a .063c

from 0.597 to 0.796 (Table 4). According to the UPGMA-dendrogram representing the taxonomic relationships between the four taxa considered in this case (Fig.1), the lizards *P. peloponnesiaca* and *P. taurica* are the most closely related, while *L. (A.) graeca* and *A. moreoticus* form another group of more remote relatives.

TABLE 4

Values of Nei's genetic distances (above diagonal) and values of Roger's genetic identity (below diagonal) between the 5 lacertid species studied. In parentheses are indicated the respective Nei's and Roger's values between 4 lacertid species studied (except *P. milensis*).

Species	<i>P. pel/siaca</i>	<i>P. taurica</i>	<i>P. milensis</i>	<i>L. graeca</i>	<i>A. mor/ticus</i>
<i>P. pel/siaca</i>	***	0.179 (0.123)	0.142 (-)	0.466 (0.426)	0.437 (0.426)
<i>P. taurica</i>	0.750 (0.796)	***	0.025 (-)	0.451 (0.318)	0.449 (0.308)
<i>P. milensis</i>	0.795 (-)	0.898 (-)	***	0.484 (-)	0.471 (-)
<i>L. graeca</i>	0.582 (0.619)	0.586 (0.678)	0.593 (-)	***	0.437 (0.181)
<i>A. mor/ticus</i>	0.567 (0.597)	0.597 (0.684)	0.601 (-)	0.596 (0.780)	***

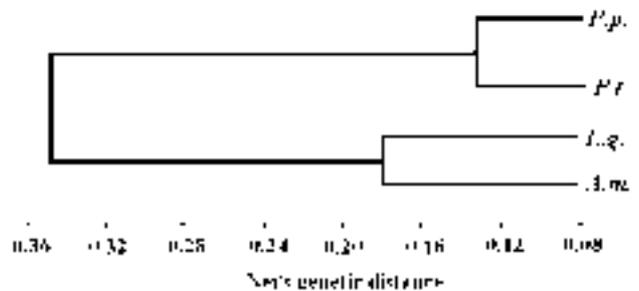


Fig. 1. – The UPGMA-dendrogram representing the relationships of 4 of the species studied, based on the Nei's genetic distances (*P.p.*: *Podarcis peloponnesiaca*, *P.t.*: *Podarcis taurica*, *L.g.*: *Lacerta graeca*, *A.m.*: *Algyroides moreoticus*).

In the second case seven of the eight presumptive loci analyzed were polymorphic (Table 3). The Mpi-1 locus in *P. milensis* was found monomorphic for the same 'a' allele as in the other *Podarcis* taxa examined. The mean value of the fixation index $F_{(ST)}=0.646$ implies that 64.6% of the total genetic variability observed was due to interspecific differences, while the remaining 35.4% was due to intraspecific ones. The values of the Nei's genetic distances between the taxa examined ranged from 0.025 to 0.484, while those of Roger's genetic identity ranged from 0.582 to 0.898 (Table 4). The UPGMA-dendrogram expressing the relationships between the 5 species studied is shown in Fig.2. This dendrogram clearly indicates that *P. milensis* and *P. taurica* are the most closely related species and *P. peloponnesiaca* is the next most related one

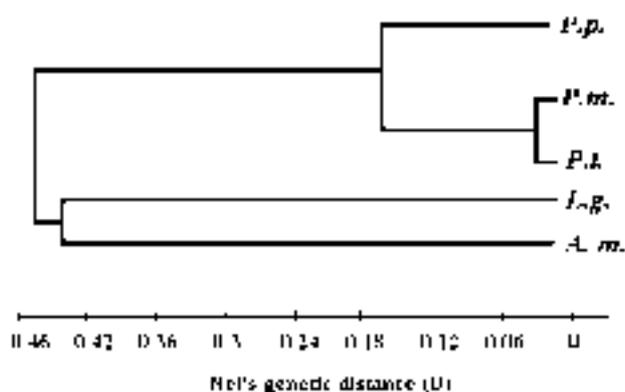


Fig. 2. – The UPGMA-dendrogram representing the relationships of all 5 species studied, based on the Nei's genetic distances (*P.p.*: *Podarcis peloponnesiaca*, *P.m.*: *Podarcis milensis*, *P.t.*: *Podarcis taurica*, *L.g.*: *Lacerta graeca*, *A.m.*: *Algyroides moreoticus*).

to them. The two remaining taxa, *L. (A.)graeca* and *A. moreoticus*, show much more remote affinities to the previous species, being more closely related to each other than to any of the *Podarcis* species.

DISCUSSION

Morphological analysis indicates that *Lacerta* (*Archaeolacerta*), *Podarcis* and *Algyroides* are closely interrelated taxa as compared to other, more distant members of the primitive Palaearctic and Oriental lacertids (ARNOLD, 1989). Such a close phylogenetic affinity of the above three taxa is also corroborated by karyological and DNA sequence data (ODIERNA et al., 1993; OLMO et al., 1993; HARRIS et al., 1998). BOHME & CORTI (1993) suggest that *Archaeolacerta*, *Podarcis* and *Algyroides* belong to the radiation that also produced the taxa *Teira* and *Timon*, about 18-20 million years ago, but these authors also stress that the interrelations of the above taxa are unresolved.

According to the dendrogram of Fig.1 our data seems to properly separate the three genera examined. This separation is emphasized by the existence of a genus-specific molecular marker, i.e. the protein coded by the *Mpi-1* locus where a different allele (a) was found to be fixed in each genus. On the basis of at least the representative species studied it was found that the relationships among *Lacerta* (*Archaeolacerta*) and *Algyroides* appear to be stronger than the relationships of each of them to *Podarcis*. Morphology does not give evidence for an unequivocal pattern of relations among most of the Palaearctic lizard taxa on either generic or specific level (ARNOLD, 1989). Molecular data (electrophoretic, immunological and DNA sequencing) offer additional information but they are often in conflict each other and frequently also with the morphological data.

Our results are in accordance with DNA sequence data of HARRIS et al. (1998) who found that archaeolacertas (*L.*

(A.) graeca included) are closer to *Algyroides* (represented in their material by *A. marchi* only) than to *Podarcis* (represented by *P. muralis* and *P. taurica*). On the contrary the data of the present study are not supported by chromosomal and immunological data, which suggest that archaeolacertas are closer to *Podarcis* than to *Algyroides* (MAYER & LUTZ, 1990; ODIERNA et al., 1993; OLMO et al., 1993).

The addition of another species, *Podarcis milensis*, to interspecific comparison procedure of our electrophoretic data let us confirm and strengthen our results of the first data set concerning the interrelationships among archaeolacertas, *Algyroides* and *Podarcis* (Fig.1). Furthermore, it makes clear that within *Podarcis* there are stronger relationships of *P. milensis* to *P. taurica* than to *P. peloponnesiaca*; this finding corroborates previous data for the close relations of the former two species and agrees with the opinion that the third one is taxonomically closer to *P. erhardii* than to *P. taurica* (ARNOLD, 1973; MAYER & TIEDEMANN, 1982; LUTZ & MAYER, 1985; MAYER, 1986).

Concluding this paper we have to underline the frequently conflicting results of studies on the lacertid interrelationships, even within relatively well-studied lineages of this family. This fact is indicative of the complicated taxonomic relations of Lacertidae and makes necessary more detailed and exhaustive studies in order to elucidate the pattern of the lacertid affinities, especially those of the Mediterranean basin.

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