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Allozyme differentiation and relationship among the Iberian-Pyrenean Mountain Lizards
(Squamata: Sauria: Lacertidae)

Allozym-Differenzierung und Verwandtschaftsbeziehungen zwischen den Iberisch-Pyrenäischen Gebirgseidechsen
(Squamata: Sauria: Lacertidae)

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KURZFASSUNG

Sechs nominelle Taxa (*"Lacerta" monticola cantabrica, "L." monticola monticola, "L." aranica, "L." aurelioi, "L." bonnali, "L." cyreni*) der Iberisch-Pyrenäischen Gebirgseidechsen wurden mittels Allozym-Elektrophorese hinsichtlich 15 enzymatischer Loci untersucht; *"L." horvathi* wurde als Outgroup für die phylogenetische Analyse verwendet.

Nach unseren Ergebnissen, die völlig mit karyologischen Befunden übereinstimmen (ODIERNA & al. im Druck a, b), können fünf Taxa, die wir als Arten auffassen (*"L." monticola, "L." aranica, "L." aurelioi, "L." bonnali, "L." cyreni*), unterschieden werden.

ABSTRACT

Six nominal taxa (*'Lacerta' monticola cantabrica, 'L.' monticola monticola, 'L.' aranica, 'L.' aurelioi, 'L.' bonnali, 'L.' cyreni*) of the Iberian-Pyrenean Mountain Lizards were investigated by allozyme electrophoresis with regard to 15 enzymatic loci. *'L.' horvathi* was used as an outgroup for phylogenetic analysis.

On the basis of our results - which are in perfect agreement with karyological data (ODIERNA & al. in press a, b) - five taxa can be distinguished (*'L'. monticola, 'L'. aranica, 'L'. aurelioi, 'L'. bonnali, 'L'. cyreni*) which we consider to be species.

KEY WORDS

Archaeolacerta, 'Lacerta' horvathi, 'L.' monticola cantabrica, 'L.' monticola monticola, 'L.' aranica, 'L.' aurelioi, 'L.' bonnali, 'L.' cyreni; Iberian-Pyrenean Mountain Lizards, systematics, taxonomy; Spain, Portugal

INTRODUCTION

Ingroup and outgroup relationships of the Mountain Lizards ('Archaeolacertae') - lacertid lizard species which inhabit the mountains of southern Europe and western Asia - are far from being well understood. There are doubts concerning their monophyly (MAYER & LUTZ, 1989; MAYER & BENYR, 1994); additionally, we do not know the extent of the group. Therefore, at the moment, we should not treat this group as a genus (*Archaeolacerta*) but as an assemblage within the collective genus *Lacerta*. It seems to be clear that *Lacerta* is a paraphyletic group with most of its members not closely related to the Green Lizards of the subgenus *Lacerta* s. str. That is why we use '*Lacerta*' for the species dealed with in this paper.

A few years ago, all Iberian-Pyrenean taxa among the 'Archaeolacertae' were considered to be members of only one species, '*Lacerta*' *monticola*. Only in 1993, the Pyrenean '*L.*' *m. bonnali* was recognized as a species different from '*L.*' *monticola* (ARRIBAS 1993a; PEREZ-MELLADO & al. 1993). Moreover, the Pyrenean 'Archaeolacertae' proved to be a heterogenous assemblage of three taxa, *bonnali*, *aranica*, and *aurelioi* (ARRIBAS 1993b, 1994). From a karyological point of view, all Iberian-Pyrenean Mountain Lizards (formerly '*L.*' *monticola*) are characterized by the lack of microchromosomes (ODIERNA & al. in press a, b). They share this feature with another 'Archaeolacerta', the Dinaric-Alpine '*L.*' *horvathi* (DE LUCA

Table 1: Lizard specimens examined.
Tab. 1: Untersuchtes Eidechsenmaterial.

Taxon		n	Locality/Fundort
'Lacerta' horvathi MÉHELY, 1904	hor	3	Austria, Carinthia
'Lacerta' m. monticola BOULENGER, 1905	mmc	2	Portugal, S[a] de Estrela
'Lacerta' m. cantabrica MERTENS, 1929	mmc	3	Spain, Pto. Vegarada
'Lacerta' cyreni MÜLLER & HELLMICH, 1937	mcy	3	Spain, S[a] Guadarrama
'Lacerta' bonnali LANTZ, 1927	bon	5	Spain, Monte Perdido
'Lacerta' bonnali LANTZ, 1927	bon	3	Spain, Posets Massif
'Lacerta' aranica ARRIBAS, 1993	ara	3	Spain, North Aran Mts.
'Lacerta' aurelioi ARRIBAS, 1994	aur	4	Spain, Pica d'Estats

& DULIC 1988; CAPULA & al. 1989). In contrast to the more or less karyological uniformity of the major part of lacertid lizards, the Pyrenean ones are characterized by drastic reduction of chromosome number (by Robertsonian fusion) and - in part - by partic-ular sex chromosome differentiation (ODIERNA & al. in press a, b).

In this paper, allozyme features are reported as a contribution to the understanding of the relationship within the Iberian-Pyrenean Mountain Lizards.

MATERIAL AND METHODS

Fifteen presumptive genetic loci have been investigated. Skeleton muscle, heart, and liver were frozen (-80°C) until usage. The samples were homogenized with little water and put on the gel by the aid of small filter paper inserts. Electrophoresis was carried out on 12% starch gel and Tris-citrate buffer (pH 7). Enzyme specific staining was done according to standard methods. For the lizards and enzymes examined see tables 1 and 2.

RESULTS

LDH proved to be monomorphic at both loci in all specimens studied. Concerning four loci, the results have been very unsatisfactory (SDH, IcDH-2, MPI, and ADA) and have not been included in the analysis. Hence, the analysis is limited to 11 proteins, 9 of them turned out to be polymorphic.

No differences were found between *'Lacerta' m. monticola* and *'L.' m. cantabrica* on one hand and between the two populations of *'L.' bonnali* on the other hand. This is why they are referred as 'mmc' and 'bon', respectively: all specimens proved to be monomorphic in all loci. The results are given in table 3. The pairwise differences (table 4) formed the basis for the construction of all 28 possible dendrograms according to the Fitch-Margoliash-algorithm (program FITCH in FEL-

Table 2: Enzymes examined.
Tab. 2: Untersuchte Enzyme.

Enzyme/Enzym		# Loci	EC numbers/Nummern
Sorbitol dehydrogenase	SDH	1	EC 1.1.1.14
Lactate dehydrogenase	LDH	2	EC 1.1.1.27
Malate dehydrogenase	MDH	2	EC 1.1.1.37
Isocitrate dehydrogenase	IcDH	2	EC 1.1.1.42
Glutamate-oxalacetate transaminase	GOT	2	EC 2.6.1.1
Creatine phosphokinase	CPK	1	EC 2.7.3.2
Adenylate kinase	AK	1	EC 2.7.4.3
Phosphoglucomutase	PGM	2	EC 2.7.5.1
Mannose phosphate isomerase	MPI	1	EC 5.3.1.8
Adenosine desaminase	ADA	1	EC 3.5.4.4

Table 3: Allozyme variants found in our sample. The abbrevations (f - fast; m - intermediate; s - slow; v - very slow) refer to the relative anodic electrophoretic mobitities of the allozymes. For abbreviations of taxa and enzymes see tables 1 and 2.

Tab. 3: Allozym-Varianten in unserer Stichprobe. Die Abkürzungen (f - schnell; m - mittelschnell; s - langsam v - sehr langsam) beziehen sich auf die relative elektrophoretische Wanderungsgeschwindigkeit der Allozyme. Abkürzungen der Taxa und Enzyme siehe Tabellen 1 und 2.

Enzyme / Enzym	Taxon					
	hor	mmc	mcy	bon	ara	aur
CPK	f	f	f	s	s	s
AK	s	s	s	s	f	s
IcDH-1	s	f	f	f	f	f
MDH-1	f	f	f	f	s	f
MDH-2	s	f	f	f	f	f
GOT-1	m	s	vs	f	m	f
GOT-2	s	f	f	f	f	f
PGM-1	f	f	s	f	f	f
PGM-2	f	s	s	f	s	s

SENSTEIN's 1988 PHYLIP 3.1 package). As maximum differences within this group are shown by '*L.*' *horvathi* (the only non-Iberic species), this taxon was chosen as outgroup to root the tree with the best fit (fig. 1).

From outgroup comparison we can assume the following apomorphies with regard to the enzymatic electromorphs (fig. 1):

CPK: 's' is a synapomorphy of the Pyrenean group.

AK: 'f' is an autapomorphy of '*L.*' *aranica*.

MDH-1: 's' is an autapomorphy of '*L.*' *aranica*.

PGM-1: 's' is an autapomorphy of '*L.*' *cyreni*.

PGM-2: We take the electrophoretic similarity of the electromorphs of '*L.*' *horvathi* and '*L.*' *bonnali* for a homoplasy; therefore, 'f' must be an autapomorphy of '*L.*' *bonnali*.

GOT-1: We take the electrophor-

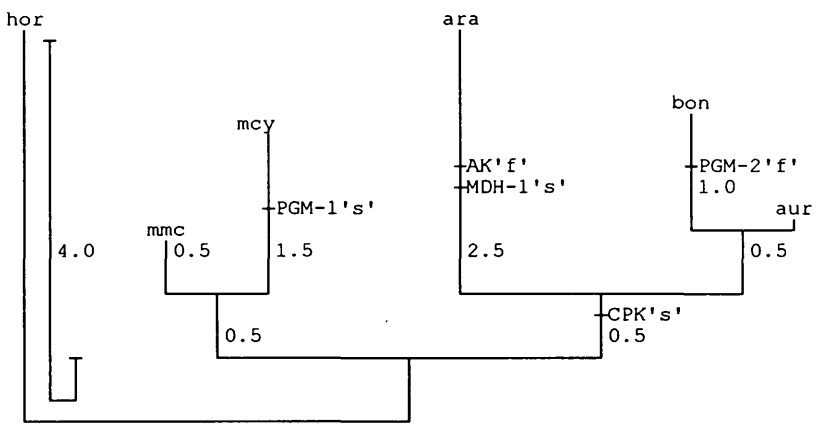

Fig. 1: Relationship among the Iberian-Pyrenean Mountain Lizards based on their allozyme features (calculated according to FITCH & MARGOLIASH 1967).
Abbrevations see 'Material and Methods'. Numbers given are branch lengths. Presumtive allozyme apomorphies are indicated on the respective branches (protein variants which cannot be assigned as apomorphies to definite branches are not indicated). For abbreviations of taxa and enzymes see tables 1 and 2.

Abb. 1: Verwandtschaftsbeziehungen der Iberisch-Pyrenäischen Gebirgseidechsen nach ihren Allozym-Merkmalen (berechnet nach FITCH & MARGOLIASH 1967).
Abkürzungen siehe 'Material and Methods'. Die angegebenen Zahlen geben die entsprechenden Astlängen wieder; Angenommene Allozym-Apomorphien sind an den entsprechenden Ästen angegeben.
Proteinvarianten, die nicht eindeutig als Apomorphien einem Ast zugewiesen werden konnten, sind nicht eingetragen. Abkürzungen der Taxa und Enzyme siehe Tabellen 1 und 2.

Table 4: Allozyme differences between the taxa investigated (referring to 11 genetic loci analyzed). For abbreviations of taxa see table 1.

Tab. 4: Allozym-Unterschiede zwischen den untersuchten Taxa (bezüglich 11 genetischer Loci, die ausgewertet werden konnten). Abkürzungen der Taxa siehe Tabelle 1.

Taxon	Taxon					
	hor	mmc	mcy	bon	ara	aur
hor	0	5	6	5	7	6
mmc	5	0	2	3	4	2
mcy	6	2	0	4	5	4
bon	5	3	4	0	4	1
ara	7	4	5	4	0	3
aur	6	2	4	1	3	0

etic similarity of the electromorphs of '*L.*' *horvathi* and '*L.*' *aranica* for a homoplasy; therefore, no decision is possible.

IcDH-1, MDH-2, GOT-2: '*L.*' *horvathi* is different from all Iberic-Pyrenean taxa. Therefore, no decision is possible.

DISCUSSION

The Mountain lizards that inhabit the mountains of the northern half of the Iberian Peninsula and the Pyrenees, very probably constitute a natural group, whose affinities to other European 'Archaeolacertae' are far from being well established.

'*L.*' *horvathi*, treated here as an outgroup for the phylogenetic analysis, shares the lack of microchromosomes with the Iberian-Pyrenean species group (DE LUCA & DULIC 1988; CAPULA & al. 1989; ODIERNA & al. in press a, b), a feature very rare in lacertid lizards and, therefore, judged as a probable synapomorphy. But albumin-immunological investigations indicate a closer relationship between '*L.*' *bedriagae* and '*L.*' *horvathi* in respect to '*L.*' *oxycephala* (LUTZ & MAYER 1985) and a very early split off of '*L.*' *cyreni* from the European 'Archaeolacertae' branch (represented by '*L.*' *bedriagae*) (MAYER & BENYR 1994). Additionally, all Iberian-Pyrenean species show a very similar distance to '*L.*' *bedriagae* (about 14 units) in contrast to the somewhat smaller distance of '*L.*' *horvathi* to '*L.*' *bedriagae* (about 8 units), indicating a closer relationship among the taxa of the Iberian-Pyrenean species group in respect to other European 'Archaeolacertae' and a sister group position of '*L.*' *horvathi* to '*L.*' *bedriagae* (MAYER unpublished). On the other hand, according to allozyme-electrophoretic results, '*L.*' *horvathi* shows more similarity to '*L.*' *oxycephala* than to '*L.*' *bedriagae* (MAYER & TIEDEMANN 1982). So, the relationships within the European 'Archaeolacertae' and especially the systematic position of '*L.*' *horvathi* remain still uncertain.

Among the Iberic-Pyrenean species, two groups could be separated clearly:

(1) Two species form the the Central-North Iberian group that is distributed along the mountains surrounding the Iberic North Plateau ('Meseta del Duero'): '*L.*' *monticola* (S^a de Estrella, Galicia, and Cantabrian Mts.) and '*L.*' *cyreni*, formerly regarded as a subspecies of '*L.*' *monticola*, (Spanish 'Sistema Central'). They differ from each other in the studied enzymes (two differences), in karyology (heterochromatinization of sex chromosome, localization of the NOR (ODIERNA & al. in press a) and in the pattern of adults and hatchlings (ARRIBAS 1993a; PEREZ-MELLADO & al 1993) suggesting that they are different species. The first one includes two recognized subspecies ('*L.*' *m. monticola* and '*L.*' *m. cantabrica*) that we are unable to discriminate by the studied set of enzymes as well as by karyotype (ODIERNA & al. in press a).

(2) The second group of species is the Pyrenean one with three taxa clearly distinguished by morphology (pholidosis, coloration, postorbital bones, thoracic girdle) and karyology (chromosome number,

sex chromosome system, chromosome morphology, heterochromatine distribution) (ARRIBAS 1993b, 1994; ODIERNA & al. in press a, b). According to morphological features, *'L.' bonnali* and *'L.' aranica* are the more similar taxa. Therefore they were regarded as subspecies of one species, *'L.' bonnali* (ARRIBAS 1993b). But according to karyological (ODIERNA in press a, b) and allozyme analyses, *'L.' aranica* is not only a well differenciated species but even the sister taxon of both *'L.' bonnali* and *'L.' aurelioi*. Probably the three species have been differenciated by isolation in different mountain massifs in the Pyrenees during the Pleistocene.

Investigation of additional features and of a complete set of 'Archaeolacertae' species is needed to ascertain the relationships between these taxa as well as their position within the western Palearctic lacertids.

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