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Mitochondrial DNA, allozymes, morphology and historical biogeography in the *Podarcis vaucheri* (Lacertidae) species complex

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Abstract. Mitochondrial DNA analysis indicates that *Podarcis vaucheri* is a species complex with one clade inhabiting both north and south shores of the Strait of Gibraltar and one clade restricted to North Africa. While each clade exhibits differing morphology, allozyme analysis suggests varying degrees of reproductive contact among populations within clades. Ancestral *P. vaucheri* appears to have been restricted to insular areas of the Betic-Rif Massif and mainland Africa during Miocene, with major lineage differentiation occurring during Pliocene.

Our analysis further confirms that *Podarcis atrata* warrants species status and suggests that it may be more widely distributed than previously reported.

Introduction

While herpetologists have traditionally relied upon morphological data for making phylogenetic decisions, contemporary techniques facilitate our discovery of cladistic events. Once genetically similar populations are identified, and their geographic distributions determined, analyses focused on morphological differentiation can be initiated. Iberian and North African populations of *Podarcis* provide an excellent example of a morphologically similar (some might say confusing) array of species for which a multivariate approach to understanding phylogeography and taxonomy is required.

As recently as the 1960s, lizards currently considered members of the genus *Podarcis* were considered representative of the genus *Lacerta* (Mertens and Wermuth, 1960). The primarily Iberian species *P. hispanica* was partitioned into four subspecies. Today *Podarcis hispanica* is recognized as but one representative of a species complex containing as many as seven species (Harris et al., 2002). Recent use of electrophoresis, immunology and DNA technology has clarified our understanding of relationships within the species complex and these data have also provided a basis for understanding the historical biogeography of the group.

Analyses of cytochrome *b* and 12S rRNA from *Podarcis hispanica* indicate that this taxon actually represents a species complex (Harris and Sá-Sousa, 2001, 2002). At least two former subspecies (*P. h. atrata* and *P. h. vaucheri*) have been considered candidates for independent species status (Castilla et al., 1998; Oliverio et al., 2000; Harris et al., 2002), but population structure in *P. vaucheri* remains poorly known.

Genetic differentiation in populations of *Podarcis* inhabiting both northern and southern shores of the Strait of Gibraltar, as determined by allozyme analysis, has been reported to be both substantial (Capula, 1997; Pinho et al., 2003) and minor (Busack, 1986), and Harris and Sá-Sousa (2002) suggested further sampling. We undertook morphological and mtDNA analysis of specimens collected from Spain and Morocco and supplemented these data with those from an unpublished allozyme

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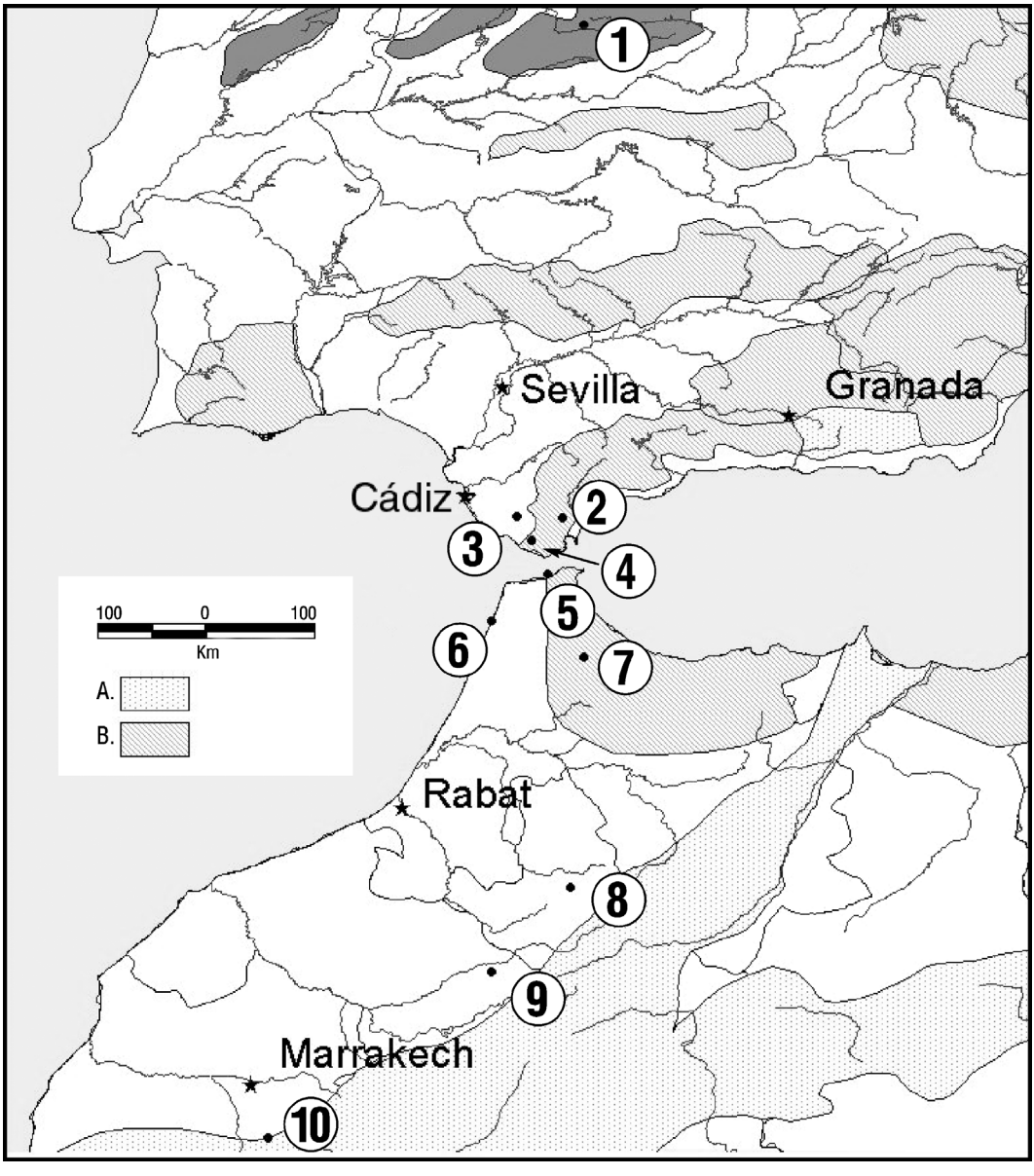

Figure 1. Study areas in Spain and Morocco. Shading indicates general topography; A. areas of moderate to high elevation, B. areas of low to moderate elevation. Specimens examined from the vicinity of San Martín del Pimpollar and Arenas de San Pedro (1), the Río Hozgarganta (2), Benalup de Sidonia (3), Facinas (4), Ksar-es-Seghir (5), Asilah (6), Chechaouèn (7), Âïn Leuh (8), El Ksiba (9), and Oukaïmedèn (10).

study of *Podarcis* from this region (fig. 1). In many cases, the same specimens provided data for each of the three data sets. James Harris (CIBIO/UP, Vila do Conde, Portugal) graciously supplied cytochrome *b* sequences so that we might associate our samples from the *P. hispanica* species complex with his, thereby insuring concordance between our taxonomy and that suggested by recent workers. We present this synthesis of morphological, allozyme, and mtDNA data as a supplement to our understanding of population structure and biogeographic history of *P. vaucheri.*

Material and methods

DNA Analysis

Genomic DNA was isolated from liver tissue from 1 *Podarcis muralis*, 1 *P. atrata* (the northeastern Spanish form of Harris and Sá-Sousa [2002:77]; because of its close genetic relationship to the Columbretes population, our sample is referred to as *P. atrata* throughout this discussion), 2 *P. hispanica* Type I (see Harris and Sá-Sousa, 2002:77), and 2 *P. vaucheri* collected in Spain and 5 *P. vaucheri* collected in Morocco (see Specimens Examined for precise locality data). Tissue was digested for 3-4 h at 65°C with constant motion in 2 ml of lysis buffer (Tris HCl 100 mM at pH 8.0, EDTA 50 mM at pH 8.0, NaCl 10 mM, SDS 0.5%) containing 60 μg of proteinase K per ml. Extraction twice with phenol/$CHCl_3$ at pH 7.3, then once with $CHCl_3$, followed digestion. DNA was precipitated from the aqueous layer with 2.5 volumes of pure ethanol and the precipitated DNA was then washed in 80% ethanol, dried and redissolved in TE buffer (Tris 10 mM, EDTA 1 mM, pH 8.0).

Template DNA for the polymerase chain reaction (PCR) was prepared by diluting stock DNA with TE buffer to give spectrophotometric absorption readings between 0.2 A and 0.7 A at A260. Mitochondrial DNA was amplified from template DNA in 100 μl reactions using a hot start method in a thermal cycler with a 7-min denaturing step at 94°C followed by 40 cycles of denaturing for 40 sec at 94°C, primer annealing for 30 sec at 46°C and elongation for one min at 72°C with a final 7-min elongation step at 72°C. PCR products were purified using Promega Wizard® PCR Preps DNA Purification System (Promega, Madison, WI, USA) according to manufacturer's instructions.

Cycle sequencing was performed on PCR products using the Big Dye® (Perkin-Elmer, Norwalk, CT) reaction premix for 50 cycles of 96°C for 10 sec, 45°C for 5 sec, and 60°C for four min. The nucleotide sequence was determined by using an ABI model 3100 Genetic Analyzer (Applied Biosystems, Norwalk, CT, USA). Oligonucleotide primers for amplification and sequencing were taken from the literature or designed for this project and are listed in table 1.

The entire cytochrome *b* gene (1143 bps) was amplified using primers L14910 and H16064. The sequence of single stranded DNA was obtained by using primers L14761, H14892 and H15149 for cycle sequencing. If ambiguous sites were found both strands were sequenced using primer L14919 in addition to those mentioned above.

Subunit 2, NADH dehydrogenase (ND2; 1038 bps) was amplified using primers L4437b and H5877. DNA sequence was obtained by using primers L4411 and H5877.

Table 1. Primers used for *Podarcis* DNA amplification and sequencing. Primers from the current study are named to indicate position of the 3′ nucleotide in the mitochondrial genome of *Eumeces egregious* (Kumazawa and Nishida, 1999).

Primer	Primer Sequence	Use	Location	Reference
L14910	5′-GAC CTG TGA TMT GAA AAA CCA YCG TTG T-3′	Amp.	tRNA-glu	de Queiroz et al., 2002
L14919	5′-AAC CAC CGT TGT TAT TCA ACT-3′	Amp./Seq.	tRNA-glu	Burbrink et al., 2000
L14761	5′-MTC HAA CAA CCC AAY MGG-3′	Seq.	Cyt *b*	This study
H14892	5′-TGC NGG KGT RAA KTT TTC-3′	Seq.	Cyt *b*	This study
H16064	5′-CTT TGG TTT ACA AGA ACA ATG CTT TA-3′	Amp./Seq.	tRNA-thr	Burbrink et al., 2000
H15149	5′-CCC TCA GAA TGA TAT TTG TCC TCA-3′	Seq.	Cyt *b*	Kocher et al., 1989
L4437b	5′-CAG CTA AAA AAG CTA TCG GGC CCA TAC C-3′	Amp./Seq.	tRNA-met	Kumazawa et al., 1996
L4411	5′-AAC CAA ACM CAM CTA CG-3′	Seq.	ND2	This study
H5877	5′-AAA CTA GKA GCC TTG AAA GCC-3′	Amp./Seq.	tRNA-trp	de Queiroz et al., 2002
ND4	5′-CAC CTA TGA CTA CCA AAA GCT CAT GTA GAA GC-3′	Amp./Seq.	ND4	Arévalo et al., 1994
Leu	5′-CAT TAC TTT TAC TTG GAT TTG CAC CA-3′	Amp./Seq.	tRNA-leu	Arévalo et al., 1994

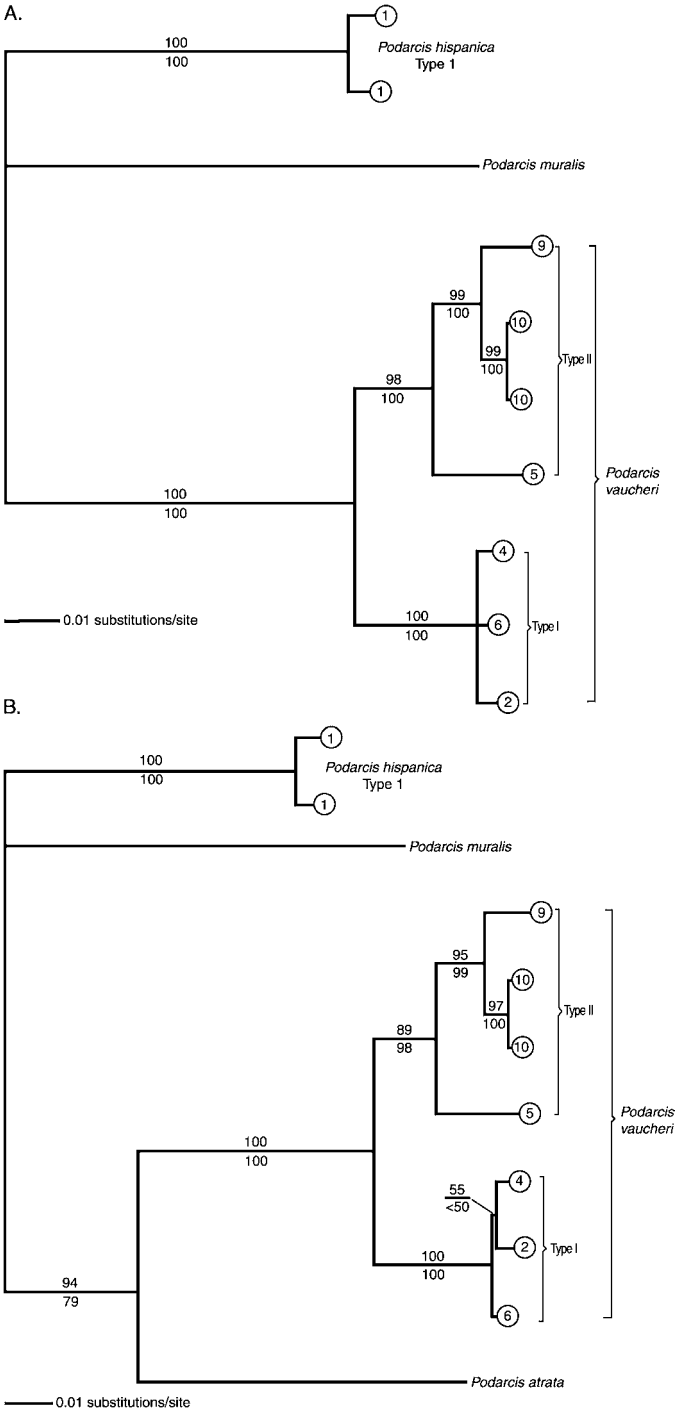

Figure 2. Maximum-likelihood (ML) trees derived from combined cytochrome *b*, ND2, and ND4 gene sequences ("A" does not include *Podarcis atrata*; "B" includes only data from cytochrome *b* and ND4 [see text]). Trees were rooted using *P. muralis*. Numbers above nodes indicate bootstrap support (>50%, 1000 replicates) from the ML analysis; numbers below nodes indicate bootstrap support from the maximum-parsimony analysis. Numbers in circles are keyed to localities in fig. 1.

Subunit 4, NADH dehydrogenase (ND4; 709 bps) was amplified using primer ND4 and leu; sequences of both strands of DNA were obtained by cycle sequencing with primers ND4 and leu.

Nucleotide sequences were aligned using the program Sequencher™ version 4.0 (Gene Codes Corp., Ann Arbor, MI, USA). All sequences are deposited in Genbank (AY234144-AY234174).

The optimality criterion maximum parsimony, executed in PAUP* version 4.0b4a (Swofford, 2000), was used to recover phylogenetic data for *Podarcis hispanica*, *P. muralis* and *P. vaucheri* from combined cytochrome *b*, ND2 and ND4 (2890 bps) sequence data (see de Queiroz et al., 2002, regarding the efficacy of this procedure). Because we could not sequence ND2 from *P. atrata*, only data from cytochrome *b* and ND4 (1852 bps) were used to recover phylogenetic information involving this taxon. Maximum parsimony analyses were conducted using the heuristic search mode with 100 random stepwise addition replicates, tree-bisection-reconnection (TBR) branch swapping, all characters treated as unordered, and all character state changes weighted equally. Support for clades in 1,000 pseudoreplicates of the maximum parsimony analysis was evaluated by nonparametic bootstrapping (Felsenstein, 1985) using the heuristic search mode with simple stepwise addition and TBR branch swapping. Phylogenetic estimates suggested by these procedures (fig. 2) provided the basis for assignment of specimens to Type I or Type II populations.

Allozyme analysis

Thirteen individuals (5 *Podarcis hispanica* Type I and 8 *P. vaucheri*) collected in Spain and 23 specimens (all *P. vaucheri*) collected in Morocco (fig. 1; see Specimens Examined for precise locality data) were sacrificed in the field, and samples of heart and liver were removed, frozen, and stored in liquid nitrogen (−196°C). In the laboratory, tissues were transferred to a freezer (−76°C) until used in allozyme analysis (heart and liver) two to 12 months later. Tissue samples were pooled for each animal. Proteins were separated electrophoretically in horizontal starch gels (11.5% hydrolyzed starch, Sigma Chemical Co.) and localized by standard histochemical staining procedures (Ayala et al., 1972; Harris and Hopkinson, 1976; Selander et al., 1971; table 2).

Allozymic data for each protein system were obtained through "side by side" comparisons of Spanish and Moroccan material, and genetic interpretations of these data were based on criteria developed by Selander et al. (1971). Multiple loci within a protein system were numbered with "1" designating the most anodally migrating set of allelic products. Alleles of a locus were lettered, with "a" representing the most anodally migrating product (table 3). The unbiased minimum genetic distance between populations ($\hat{D}$), recommended by Nei (1978) for comparisons utilizing small sample sizes was computed from allele frequencies using algorithms provided by Nei (1978).

Alleles of each variable locus were also numbered (with "1" representing the most anodally migrating product; see table 3) and examined by Discriminant Function Analysis (DFA). Results from DFA are presented graphically (fig. 3).

Table 2. Protein systems examined by electrophoresis; enzymes arranged by Enzyme Commission number.

Protein (abbreviation)	Enzyme Commission Number	Electrophoretic conditions
General Proteins (Gp) (Oxidoreductases)	–	B
Alcohol dehydrogenase (Adh)	1.1.1.1	A
Glycerol-3-phosphate dehydrogenase (G3pdh)	1.1.1.8	D
L-Lactate dehydrogenase (Ldh)	1.1.1.27	F
Malate dehydrogenase (Mdh)	1.1.1.37	F
Malate dehydrogenase (Nadp+) (Mdhp)	1.1.1.40	F
Isocitrate dehydrogenase (Idh)	1.1.1.42	E
Phosphogluconate dehydrogenase (Pgdh)	1.1.1.44	E
Glucose-6-phosphate dehydrogenase (G6pdh)	1.1.1.49	D
Aldehyde dehydrogenase (Aldh)	1.2.1.3	F
Glutamate dehydrogenase (Gtdh)	1.4.1.3	D
Superoxide dismutase (Sod) (Transferases)	1.15.1.1	D
Aspartate aminotransferase (Aat)	2.6.1.1	D
Hexokinase (Hk)	2.7.1.1	G
Creatine kinase (Ck)	2.7.3.2	G
Adenylate kinase (Ak) (Hydrolases)	2.7.4.3	G
Esterase (nonspecific) (Est)	3.1.-.-	B
Acid phosphatase (Acp)	3.1.3.2	G
Fructose-bisphosphatase (Fbp)	3.1.3.11	D
N-Acetyl-β-glucosaminidase (βga)	3.2.1.30	G
Peptidase (PEP), L-Leucylglycylglycine (Pep-B)	3.4.-.-	C
Proline dipeptidase (Pep-D)	3.4.13.9	B
Dipeptidase I, L-Leucyl-L-Alanine (La)	3.4.13.11	B
Adenosine deaminase (Ada) (Lyases)	3.5.4.4	A
Aconitase hydratase (Acoh) (Isomerases)	4.2.1.3	E
Mannose-6-phosphate isomerase (Mpi)	5.3.1.8	E
Glucose-6-phosphate isomerase (Gpi)	5.3.1.9	F
Phosphoglucomutase (Pgm)	5.4.2.2	E

A: Histidine, pH 7.8 gel & electrode buffer (Harris and Hopkinson, 1976), 150v/3h. B: LiOH A + B, pH 8.2 gel & LiOH A, pH 8.1 electrode buffer (Selander et al., 1971), 300v/3h. C: Poulik, pH 8.7 gel & borate, pH 8.2 electrode buffer (Selander et al., 1971), 250v/3h. D: Tris citrate II, pH 8.0 gel & electrode buffer (Selander et al., 1971), 130v/4h. E: Tris citrate II, pH 8.0 + NADP gel & tris citrate II, pH 8.0 electrode buffer (Selander et al., 1971), 130v/4h. F: Tris citrate III, pH 7.0 gel & electrode buffer (Ayala et al., 1972), 180v/3h. G: Tris citrate III, pH 7.0 + 15% glycerine gel & tris citrate III, pH 7.0 electrode buffer (Ayala et al., 1972), 180v/3h.

Table 3. Allele frequencies of *Podarcis hispanica* Type I and *Podarcis vaucheri*. *Podarcis vaucheri* arranged by mtDNA type; population numbers (in parenthesis) coincide with designations in figure 1.

	Podarcis hispanica Type I	*Podarcis vaucheri*						
		Type unknown		**Type I**		**Type II**		
		Benalup de Sidonia	Checha-ouèn	Facinas	Asilah	Ksar-es-Seghir	Âïn Leuh	Oukaï-medèn
Population:	(1)	(3)	(7)	(4)	(6)	(5)	(8)	(10)
N*	5	3	4	5	5	5	5	5
H**	0.16	0.09	0.15	0.18	0.17	0.15	0.13	0.16
% Polymorphic	30.3	18.2	36.4	36.4	36.4	30.3	30.3	30.3
Loci:								
Aat1	a (0.80) b (0.20)	c	c	a (0.40) c (0.60)	c	c	c	c
Aat2	b	b	b	b	b	b	a (0.20) b (0.80)	b
Acoh1	a (0.20) b (0.80)	a	a (0.75) c (0.25)	a	a	a	a	a (0.60) c (0.40)
Acp1	b	a (0.333) b (0.667)	b	a (0.40) b (0.60)	b	b	b	b
Ada	a	b	a	b	b	b	b (0.80) c (0.20)	*** b
Adh	a	a (0.833) b (0.167)	a (0.75) b (0.25)	a	a	a	a	a
Aldh	b	b	b	b	a (0.20) b (0.80)	a (0.20) b (0.80)	b (0.80) c (0.20)	b
βga	c (0.60) e (0.40)	c	c (0.25) d (0.75)	*** b (0.75) c (0.25)	c (0.60) d (0.40)	c (0.60) d (0.40)	*** b (0.50) c (0.25) d (0.25)	a (0.20) b (0.80)
Ck	b	b (0.667) a (0.333)	b	c	b	b (0.80) c (0.20)	b	b
Est	a	a	a (0.25) b (0.75)	a	a	a	a	a
Fbp	c	b (0.333) c (0.667)	b (0.375) c (0.500) e (0.125)	a (0.10) b (0.70) c (0.20)	b (0.10) c (0.60) d (0.30)	c	b (0.20) c (0.80)	b (0.70) c (0.10) e (0.20)
Gp	a	a	a	a	a	a	a (0.80) b (0.20)	a (0.60) b (0.40)
G6pdh	a	b	a (0.75) b (0.25)	a (0.80) b (0.20)	a (0.60) b (0.40)	a (0.50) b (0.50)	a (0.60) b (0.40)	a (0.60) b (0.40)
Hk	a	a	a	a	a	b	a	a
Idh1	b (0.20) c (0.80)	b	b	a (0.20) b (0.50) c (0.30)	a (0.20) b (0.80)	b	b	a (0.20) b (0.80)
La	a (0.60) c (0.20) d (0.20)	c	c	b (0.20) c (0.80)	b (0.40) c (0.60)	c	c	c
Ldh1	b	b	a (0.25) b (0.75)	a (0.20) b (0.80)	b	b	b	b
Ldh2	b (0.40) c (0.60)	c	b (0.25) c (0.75)	a (0.20) b (0.20) c (0.60)	a (0.40) b (0.60)	a (0.60) b (0.20) c (0.20)	c	a (0.20) b (0.20) c (0.60)
Mdh	a (0.20) c (0.80)	c	b (0.25) c (0.75)	b (0.40) c (0.60)	b (0.60) c (0.40)	a (0.20) b (0.60) c (0.20)	b (0.20) c (0.80)	a (0.20) b (0.20) c (0.60)

Table 3. (Continued).

	Podarcis hispanica Type I	*Podarcis vaucheri*						
		Type unknown		**Type I**		**Type II**		
		Benalup de Sidonia	Checha-ouèn	Facinas	Asilah	Ksar-es-Seghir	Âïn Leuh	Oukaï-medèn
Population:	(1)	(3)	(7)	(4)	(6)	(5)	(8)	(10)
Mdhp	a (0.10) b (0.50) c (0.20) d (0.20)	c (0.667) d (0.333)	a (0.125) b (0.875)	d (0.70) e (0.30)	b (0.30) c (0.70)	b (0.10) c (0.10) d (0.20) e (0.60)	b	b
Mpi	**** c	b	b (0.875) d (0.125)	b	b (0.90) d (0.10)	a (0.10) b (0.70) d (0.20)	b	b
Pep-B	b (0.40) c (0.60)	c	b	c	b (0.50) c (0.50)	b (0.40) c (0.60)	a (0.20) b (0.80)	b
Pep-D	a (0.20) b (0.20) c (0.40) d (0.20)	c (0.333) d (0.667)	b (0.75) d (0.25)	b (0.60) c (0.20) d (0.20)	a (0.40) b (0.60)	c (0.80) d (0.20)	a (0.40) b (0.60)	a (0.60) c (0.20) d (0.20)
Pgdh	b	b	b	b	b	b	b	a (0.40) b (0.60)

*Number of animals sampled per protein. **Average heterozygosity for all proteins (Nei, 1978). ***Based on 4 specimens. ****Based on 3 specimens.

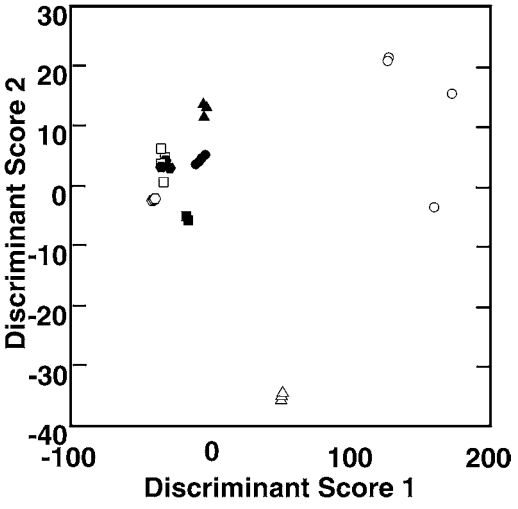

Figure 3. Discriminant function analysis of allele frequency data. *Podarcis vaucheri* Type I represented by closed circles (population 4, fig. 1) and closed hexagons (population 6, fig. 1); *P. vaucheri* Type II by open squares (population 5, fig. 1) and closed triangles (population 10, fig. 1); *P. hispanica* from population 1 (fig. 1) are represented by open circles. Phylogenetic affinity of populations from Benalup de Sidonia, Spain (open hexagons; population 3, fig. 1), Âïn Leuh (closed squares; population 8, fig. 1) and Chechaouèn (open triangles; population 7, fig. 1), Morocco, were not assessed by mtDNA analysis.

Morphology

One hundred nine specimens (60 males and 49 females) provided data for morphological analysis (see Appendix). To assess morphology, we examined 17 characters generally utilized for systematic assignment within the family Lacertidae. We purposely omitted coloration because color and pattern are of limited use within this species complex (Arnold, 1973: 345-346) and coloration in *Podarcis bocagei*, a closely-related species, may vary seasonally and be related to the reproductive cycle (Galán, 2000).

Continuously distributed characters (body length [posterior margin of front limb to anterior margin of rear limb]; forearm length; length of fourth toe on front foot; head length [posterior margin of occipital to anterior edge of rostral]; hind leg length; snout length [anterior margin of eye to anterior edge of rostral]; snout-vent length; and head width [anterior margin of tympanum]) were measured to 0.1 mm with dial calipers. Numbers of scales comprising the collar; chin shields (in contact behind the mental); dorsal scales (mid-body); enlarged (= broader than long) forearm scales (axilla to wrist); femoral pores; infralabials; sub-digital lamellae (fourth digit, front foot); supralabials and ventral scales (midline, collar to vent) were also recorded.

Specimens were assigned to Type I or Type II following results from the mtDNA analysis (see above) and data representing *Podarcis vaucheri* Type I from localities 4 (fig. 1; 26 males, 23 females) and 6 (fig. 1; 12 males, 9 females), and *P. vaucheri* Type II from localities 5 (fig. 1; 2 males, 5 females) and 10 (fig. 1; 20 males, 12 females)

Table 4. Summary of character values, males. (Type I, $n = 38$; Type II, $n = 22$).

	Collar scales		Infralabials		Supralabials		Enlarged forearm scales		Dorsal scales		Ventral scales		Femoral pores		Sub-digital lamellae	
Type	**I**	**II**	**I**	**II**	**I**	**II**	**I**	**II**	**I**	**II**	**I**	**II**	**I**	**II**	**I**	**II**
Mean	11.16	10.41	6.08	6.05	8.11	8.00	10.58	10.55	61.84	56.05	26.74	27.14	18.61	17.64	17.13	16.14
95% Confidence limits	10.78-11.54	10.08-10.73	5.94-6.22	5.95-6.14	8.00-8.21	7.81-8.19	10.37-10.79	10.25-10.84	60.23-63.46	54.91-57.18	26.24-27.24	26.49-27.78	18.04-19.17	17.03-18.24	16.73-17.54	15.72-16.55
Range	9-16	9-12	5-8	6-7	8-9	7-9	9-12	10-12	52-71	51-62	23-30	25-31	15-22	14-20	14-20	14-18
C.V.	0.10	0.07	0.07	0.04	0.04	0.05	0.06	0.06	0.08	0.05	0.06	0.05	0.09	0.08	0.07	0.06
	Snout-Vent Length		Body Length		Forearm Length		Hind Leg Length		Fourth Toe Length		Head Width		Head Length		Snout Length	
Mean	48.51	45.52	21.10	21.49	6.34	5.78	7.17	6.50	5.95	4.91	6.86	6.44	12.38	11.42	5.11	4.80
95% Confidence limits	46.81-50.22	42.78-48.25	20.40-21.80	20.19-22.78	6.06-6.62	5.41-6.14	6.88-7.47	6.13-6.87	5.69-6.21	4.64-5.19	6.65-7.07	6.03-6.85	11.97-12.79	10.76-12.08	4.92-5.29	4.48-5.12
Range	37.8-60.0	33.5-54.5	14.7-25.0	16.1-25.6	4.4-8.1	4.2-7.0	5.6-9.2	4.9-8.3	4.4-7.6	3.7-6.0	5.6-8.0	5.0-7.9	10.3-15.1	9.0-13.5	4.0-6.6	3.6-5.9
C.V.	0.11	0.14	0.10	0.14	0.13	0.14	0.12	0.13	0.13	0.13	0.09	0.14	0.10	0.13	0.11	0.15

Table 5. Summary of character values, females. (Type I, $n = 32$; Type II, $n = 17$).

	Collar scales		Infralabials		Supralabials		Enlarged forearm scales		Dorsal scales		Ventral scales		Femoral pores		Sub-digital lamellae	
Type	**I**	**II**	**I**	**II**	**I**	**II**	**I**	**II**	**I**	**II**	**I**	**II**	**I**	**II**	**I**	**II**
Mean	11.13	10.41	6.09	6.24	7.94	7.76	10.59	10.59	60.44	56.35	30.53	30.29	17.22	17.00	16.91	16.29
95% Confidence limits	10.80-11.45	9.86-10.96	6.00-6.20	6.01-6.46	7.85-8.03	7.54-7.99	10.31-10.88	10.27-10.91	58.82-62.05	54.28-58.43	30.04-31.02	29.51-31.08	16.57-17.86	16.27-17.73	16.47-17.34	15.70-16.89
Range	10-13	8-12	6-7	6-7	7-8	7-8	9-12	9-11	53-70	50-65	28-33	28-33	12-21	14-19	15-20	14-19
C.V.	0.08	0.10	0.05	0.07	0.03	0.06	0.08	0.06	0.07	0.07	0.04	0.05	0.10	0.08	0.07	0.07
	Snout-Vent Length		Body Length		Forearm Length		Hind Leg Length		Fourth Toe Length		Head Width		Head Length		Snout Length	
Mean	46.06	45.36	22.75	23.76	5.33	5.25	6.15	5.88	5.09	4.47	5.87	5.69	10.48	10.26	4.35	4.19
95% Confidence limits	43.74-48.37	43.19-47.54	21.26-24.25	22.69-24.83	5.02-5.65	4.96-5.53	5.91-6.38	5.67-6.08	4.84-5.34	4.29-4.66	5.63-6.11	5.41-5.97	10.09-10.87	9.74-10.78	4.13-4.56	3.91-4.46
Range	31.5-57.3	38.5-52.7	12.7-30.6	18.8-26.4	3.6-7.2	4.3-6.5	4.2-7.5	5.3-6.7	3.2-6.3	4.0-5.1	4.3-7.2	4.7-7.0	8.2-12.0	8.6-12.4	3.1-5.7	3.3-5.1
C.V.	0.14	0.09	0.18	0.09	0.16	0.11	0.11	0.07	0.14	0.08	0.11	0.10	0.10	0.10	0.14	0.13

were subjected to Discriminant Function Analysis (tables 4 and 5). Males and females were assessed both separately, and together; untransformed data from all 17 variables were utilized in the stepwise analysis.

Miscellaneous

Airline distances between localities from which samples were collected were computed from 1:1,000,000 scale road maps published by Michelin for Morocco (1977, Number 169) and by Firestone Hispania for Spain (1969, España y Portugal). We assumed a non-parametric distribution of airline distances, Nei genetic distances, and *p*-distances and assessed correlation with Spearman's rank correlation procedure.

We used the computer-based statistical package SYSTAT 7.0.1 for Windows (SPSS, 1997) for all data analysis. Statistical significance was assumed when $\alpha = 0.05$, and all reported probabilities are those of committing a Type I error in a two-tailed test.

Results

mtDNA

In total, 2,890 aligned nucleotide positions from the cytochrome *b*, ND2 and ND4 genes were resolved for *Podarcis hispanica* Type I, *P. muralis* and *P. vaucheri*; the ND2 gene was not resolved for our sample of *P. atrata* and comparisons involving this taxon include only 1852 aligned nucleotide positions. Cytochrome *b* sequences contained 1,143 total characters (300 variable, 197 parsimony-informative), ND2 sequences contained 1038 total characters (284 variable, 156 parsimony-informative) and ND4 sequences contained 709 total characters (174 variable, 117 parsimony-informative).

Mean transition:transversion ratios defined among all taxa ranged from 2.83-7.74 in cytochrome *b*, 2.63-9.79 in ND2, and 2.66-5.90 in ND4. Within *Podarcis hispanica* and *P. vaucheri*, mean transition:transversion ratios for cytochrome *b* ranged between 11:0 ($n = 1$) and 2:1 to 39:1 ($n = 8$), respectively. Transition:transversion ratios varied between 5:3 ($n = 1$) and 7:0 to 33:1 ($n = 8$), respectively, for ND2, and between 5:1 ($n = 1$) and 4:0 to 11:2 ($n = 8$), respectively, for ND4. Because transitions outnumber transversions in every comparison, there is no evidence for saturation within or between these taxa.

Our data compliment those of Castilla et al. (1998) with regard to recognition for the specific status of *Podarcis atrata*, those of Harris and Sá-Sousa (2001, 2002) with regard to the species-complex status of *P. hispanica*, and support the recommendation for species status of *P. vaucheri* (Oliverio et al., 2000); pairwise sequence polymorphism (*p*-distance) among these taxa ranged from 12.7% to 13.7%. The lowest *p*-distance we found was in the comparison between *P. atrata* and *P. vaucheri* and the highest *p*-distance was found between *P. hispanica* and *P. vaucheri* (table 6).

Two clades were identified within *Podarcis vaucheri*, each containing populations in Morocco. Intrapopulation *p*-distance within the clade inhabiting southern Spain and northwestern coastal Morocco (*P. vaucheri* Type I; fig. 1: localities 2, 4, 6) varied between 0.3% and 0.5% among populations separated by airline distances varying between 32 km and 113 km. Within the clade inhabiting the area from north coastal Morocco to the Atlas Mountains (*P. vaucheri* Type II; fig. 1: localities 5, 9 and 10), intrapopulation *p*-distance varied between 1.4% and 3.5% among populations separated by airline distances varying between 230 km and 555 km. In contrast, the population of *P. vaucheri* Type I at Asilah, Morocco (fig. 1: 6), separated from the population of *P. vaucheri* Type II at Ksar-es-Seghir, Morocco (fig. 1: 5), by approximately 61 km demonstrated 5.2% pairwise sequence polymorphism (table 6). There is no statistically detectable relationship between *p*-distance and geographic distance, however ($n = 15$, $r_s = 0.09$, $P \gg 0.05$).

Configurations of parsimonious trees generated from cytochrome *b*, ND2, and ND4 gene data for *Podarcis vaucheri* are generally concordant (fig. 2A) and bootstrap analyses utilizing combined gene data indicate strong support for identified clades (fig. 2B).

Table 6. Pairwise percent sequence differences between samples when 2884* base pairs representing complete gene sequences for cytochrome *b*, ND2, and ND4 are compared. Localities numbered (in parenthesis) to coincide with designations in figure 1 (*Podarcis atrata* and *P. muralis* not mapped). * 1852 base pairs for comparisons involving *P. atrata*; ND2 sequence was not available for this taxon.

	Podarcis atrata	*Podarcis hispanica*		*Podarcis vaucheri*						
	Type I	Type I	Type I	**Type I**			**Type II**			
				Río Hozgar-ganta	Facinas	Asilah	Ksar-es-Seghir	El Ksiba	Oukaï-medèn	Oukaï-medèn
Locality	–	(1)	(1)	(2)	(4)	(6)	(5)	(9)	(10)	(10)
P. muralis	13.51	13.35	13.32	14.56	14.71	14.60	14.46	14.46	14.01	13.91
P. atrata	–	12.70	12.60	12.80	12.65	12.70	12.16	12.64	12.32	12.32
P. hispanica	–	–	0.87	13.20	13.13	13.13	12.92	13.37	12.99	12.89
P. hispanica	–	–	–	12.99	12.86	12.86	12.86	13.27	12.92	12.82
P. vaucheri										
Río Hozgar-ganta	–	–	–	–	0.62	0.55	5.16	5.20	4.85	4.88
Facinas	–	–	–	–	–	0.48	5.09	5.16	4.92	4.89
Asilah	–	–	–	–	–	–	5.02	5.09	4.85	4.82
Ksar-es-Seghir	–	–	–	–	–	–	–	3.12	2.94	2.91
El Ksiba	–	–	–	–	–	–	–	–	1.42	1.39
Oukaïmedèn	–	–	–	–	–	–	–	–	–	0.10

Table 7. Nei (1978) unbiased genetic distances (above diagonal) and airline distances (km; below diagonal) between sampled populations. Numbers in parentheses coordinated with locality designations in figure 1.

				Podarcis vaucheri				
				Type I		**Type II**		
	Podarcis hispanica Type I	Benalup de Sidonia	Checha ouèn	Facinas	Asilah	Ksar-es-Seghir	Âïn Leuh	Oukaï-medèn
Locality	(1)	(3)	(7)	(4)	(6)	(5)	(8)	(10)
(1)		0.250	0.197	0.199	0.202	0.262	0.215	0.258
(3)	450		0.178	0.083	0.070	0.111	0.106	0.149
(7)	575	140		0.161	0.101	0.185	0.064	0.109
(4)	470	22	117		0.067	0.126	0.095	0.104
(6)	552	101	78	84		0.077	0.041	0.085
(5)	506	62	79	40	61		0.125	0.159
(8)	990	340	206	317	247	282		0.036
(10)	1045	600	500	583	500	555	332	

Allozymes

The products (81 alleles) of 33 presumptive gene loci were resolved; Acoh2, Acp2, Ak, Gtdh, Gpi, G3pdh, Idh2, Pgm, and Sod were monomorphic. Allele frequency differences, coupled with the distribution of unique alleles, contribute to genetic distances ($\hat{D}$) between 0.20 and 0.26 between the *Podarcis vaucheri* species complex and *P. hispanica* Type I (table 7).

Because *Podarcis vaucheri* from Benalup de Sidonia, Spain, and Chechaouén, Morocco (populations 3 and 7, respectively; fig. 1), were not examined in the mtDNA analysis, we did not assign either to Type I or II. A generally low genetic distance ($\hat{D} = 0.07$) was calculated between *P. vaucheri* Type I populations (table 7)

but the distribution of 21 alleles may separate Spanish from Moroccan populations (table 3). The broad range in genetic distances ($\hat{D}$ between 0.04 and 0.16) among three populations of Type II *P. vaucheri* is reflected in the distribution of thirty (57%) alleles not shared by all populations (table 3).

Discriminant function analysis of the allozyme data also demonstrates that *Podarcis hispanica* and *P. vaucheri* are representative of different species complexes (fig. 3). Further, this analysis suggests that *Podarcis* inhabiting the area around Chechaouén, Morocco (population 7, fig. 1), may represent an undescribed species. The affinity of the population of *P. vaucheri* inhabiting the area around Benalup de Sidonia, Spain (population 3, fig. 1), remains unclear.

Morphology

Sexes combined. Specimens representing mtDNA Type I (populations 4 and 6; figs. 1 and 2) were correctly assigned to Type I populations by Discriminant Function Analysis (DFA) with 93% accuracy. Five of 70 Type I individuals were classified as more closely resembling individuals drawn from a Type II population (2 individuals from population 4 assigned to population 5; 1 individual from population 6 assigned to population 10). Within Type I, two individuals from population 4 were classified as more closely resembling individuals from population 6 (figs. 1 and 4a).

Representatives of mtDNA Type II (populations 5 and 10; figs. 1 and 2) were correctly assigned by DFA to Type II populations with 97% accuracy. Only one of 39 individuals was classified as more closely resembling an individual drawn from a Type I population; this individual (from population 10) was classified as more closely resembling an individual from population 4. Within Type II, one individual from population 5 was classified as more closely resembling an individual from population 10 (figs. 1 and 4a).

Males. Specimens representing mtDNA Type I (populations 4 and 6; figs. 1 and 2) were correctly assigned to Type I populations with 97% accuracy by DFA. Only one of 38 individuals was classified as more closely resembling an individual drawn from a Type II population. Within Type I, no individuals were classified as more closely resembling individuals from another Type I population (fig. 4b).

Representatives from Type II populations (5 and 10; figs. 1 and 2) were correctly classified by DFA with 100% accuracy (fig. 4b).

Females. Specimens representing mtDNA Type I (populations 4 and 6; figs. 1 and 2) were correctly assigned to Type I populations with 100% accuracy by DFA. Within Type I, no individuals were classified as more closely resembling individuals from another Type I population (fig. 4c).

Representatives from Type II populations (5 and 10; figs. 1 and 2) were correctly classified by DFA with 94% accuracy (fig. 4c). One individual from population 10 was classified as more closely resembling an individual from Type I population 4 (figs. 1 and 4c).

Discussion

In a survey of cytochrome *b* *p*-distances within reptiles, Harris (2002: fig. 1) reported a range between 5.6 and 19.7 among 13 species within the genus *Podarcis*. Taxa we examined that are currently considered different species were very well differentiated, and all comparisons were above Harris' illustrated midpoint of 12.7 *p*-distance units. The *p*-distance between *P. muralis* and *P. atrata* we derived solely from cytochrome *b* was 14.1 ($n = 1$), while *p*-distances between *P. muralis* and *P. hispanica* Type I ranged from 13.0 to 13.1 ($n = 2$, $\bar{x} = 13.0$). *Podarcis hispanica* Type I and *P. atrata* *p*-distances ranged from 13.0 to 13.3 ($n = 2$, $\bar{x} = 13.1$) and two populations of *P. hispanica* Type I were separated by a *p*-distance of 1.0. Clearly *P. atrata*, *P. muralis* and *P. hispanica*

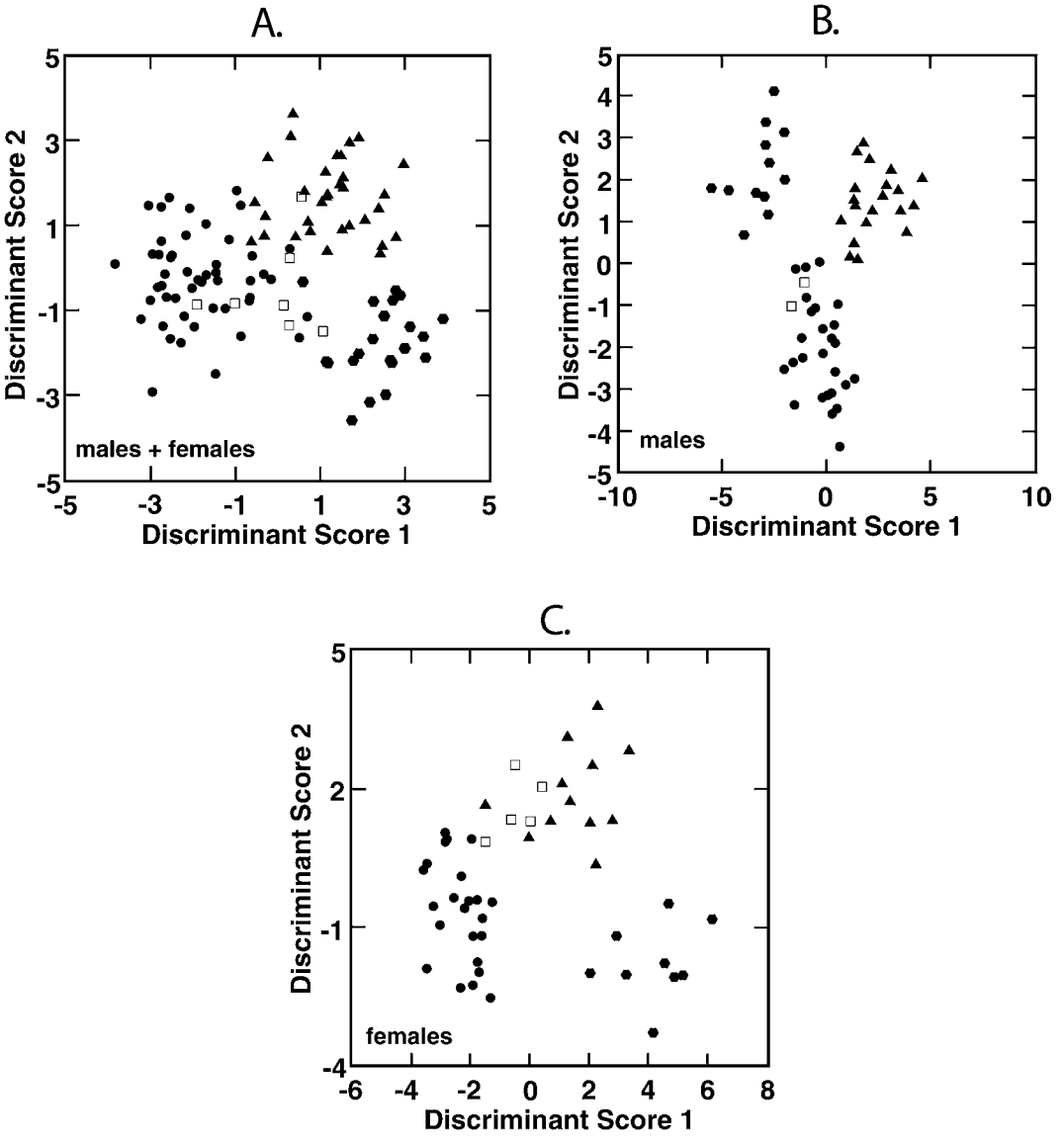

Figure 4. Discriminant function analysis results; canonical discriminant scores from 17 morphological characters (see Material and methods). The percentage of *Podarcis vaucheri* correctly associated with clade of origin was 92% in A, 98% in B, and 98% in C (see Results). *P. vaucheri* Type I: closed circles population 4, closed hexagons population 6; *P. vaucheri* Type II: open squares population 5, closed triangles population 10 (fig. 1).

Type I are independent phylogenetic units, all of which are deserving of species status.

Podarcis vaucheri Type I appears well differentiated from *P. atrata* (p-distances between 12.7 and 12.8; $n = 3$, $\bar{x} = 12.8$), *P. hispanica* Type I (13.0-13.7; $n = 6$, $\bar{x} = 13.4$) and *P. muralis* (15.0-15.2; $n = 3$, $\bar{x} = 15.2$). *Podarcis vaucheri* Type II is also well differentiated from *P. atrata* (p-distances between 12.8 and 13.5; $n = 4$, $\bar{x} = 13.0$), *P. hispanica* Type I (12.9-13.6; $n = 8$, $\bar{x} = 13.1$) and *P. muralis* (13.7-14.3; $n = 4$, $\bar{x} = 14.0$).

Interspecific allozymic differentiation has also been surveyed within the genus *Podarcis*, and estimates of Nei genetic distances between species range from 0.19 (between

P. taurica and *P. erhardii*; Mayer and Tiedemann, 1980) to 0.47 (between *P. peloponnesiaca* and *P. melisellensis* and between *P. muralis* and *P. peloponnesiaca*; Mayer and Tiedemann, 1982). While genetic distances computed from different, and differing numbers, of allozymic loci among representatives of different taxa may not be directly comparable (Busack, 1986), these data provide an indication of the range in values expected between species within the genus *Podarcis*. Nei genetic distances ($\hat{D}$ = 0.20-0.26, $n = 7$, $\bar{x} = 0.23 \pm 0.01$[SE]) computed in a comparison between *P. hispanica* Type I and *P. vaucheri* (table 7) are similar in magnitude to interspecific *p*-distances identified through cytochrome *b* analysis. *Podarcis vaucheri*, as with *P. atrata*, *P. muralis* and *P. hispanica* Type I, is an independent phylogenetic unit deserving of species status.

While it is clear that *Podarcis atrata*, *P. hispanica* Type I, *P. muralis* and *P. vaucheri* are differentiated at the species level, the status of both *P. vaucheri* Type I and *P. vaucheri* Type II remains less obvious. *Podarcis vaucheri* Types I and II are clearly differentiated from each other (*p*-distances between 5.2 and 5.9, $n = 12$, $\bar{x} = 5.4$; $\hat{D}$ values between 0.04 and 0.18, $n = 12$, $\bar{x} = 0.11$), but with mean cytochrome *b* *p*-distances and mean allozyme $\hat{D}$ values either below or barely above minima discovered for species pairs within *Podarcis* by Harris' survey (2002: fig. 1) or established by Mayer and Tiedemann (1980, 1982), respectively.

Podarcis vaucheri Types I and II also differ morphologically. While there is considerable overlap in actual values, Type I males, on average, tend to have more collar scales ($\bar{x} = 11.2$ vs 10.4), more dorsal scales ($\bar{x} = 61.8$ vs 56.1), more sub-digital lamellae ($\bar{x} = 17.1$ vs 16.1), longer hind legs ($\bar{x} = 7.2$ mm vs 6.5 mm) and longer fourth toes ($\bar{x} = 6.0$ mm vs 4.9 mm) than Type II males (table 4). Females also show considerable overlap in actual values, but Type I specimens, on average, tend to have more dorsal scales ($\bar{x} = 60.4$ vs 56.4) than Type II females (table 5). When subjected to Discriminant Function Analysis, 98% of males and 98% of females were correctly assigned to populations representing Type I or Type II *P. vaucheri* (fig. 4).

While recognizing genetic and morphological differentiation within and among *Podarcis vaucheri* Types I and II, we remain conservative with regard to taxonomy. As our analysis addresses only a sampling within this species group, we do not find taxonomic changes advisable at this time. Genetic differentiation among populations we sampled is less substantial than that currently reported among species in the genus *Podarcis*, and allozymic and morphological differentiation within *P. vaucheri* may be related to microclimate (Busack and Arjo, unpublished).

Podarcis vaucheri in the vicinity of the Strait of Gibraltar experienced geographic change similar to that experienced by *P. erhardi* in the area of the Aegean Sea. Correlating cytochrome *b* sequence differences in *P. erhardi* to Late Cenozoic geographic change in the Aegean area, Poulakakis et al. (2003) calculated sequence change at 1.45% to 1.59% per million years. If we apply these estimates to cytochrome *b* *p*-distances between *P. vaucheri* Type I and Type II populations (5.2-5.9; this study), Type I and Type II populations would last have been in reproductive contact between 3.27 and 4.07 million years ago.

Fu (2000:212), in his analysis of the family Lacertidae, found that 4708 base pairs of mtDNA failed to clarify phylogeny among Eurasian lacertinae, including those in the genus *Podarcis*. While the mtDNA sequence data collected in his study recovered the deep divergence of lacertids with confidence, it failed to decipher more recent divergence and Fu attributed the lack of structure in his data to recent explosive speciation events. The complex mid- and late-Miocene geologic and geographic history of northern Morocco (Gomez et al., 2000; de Jong, 1998), coupled with mid-Pliocene climate change (Street and Gasse, 1981), may have

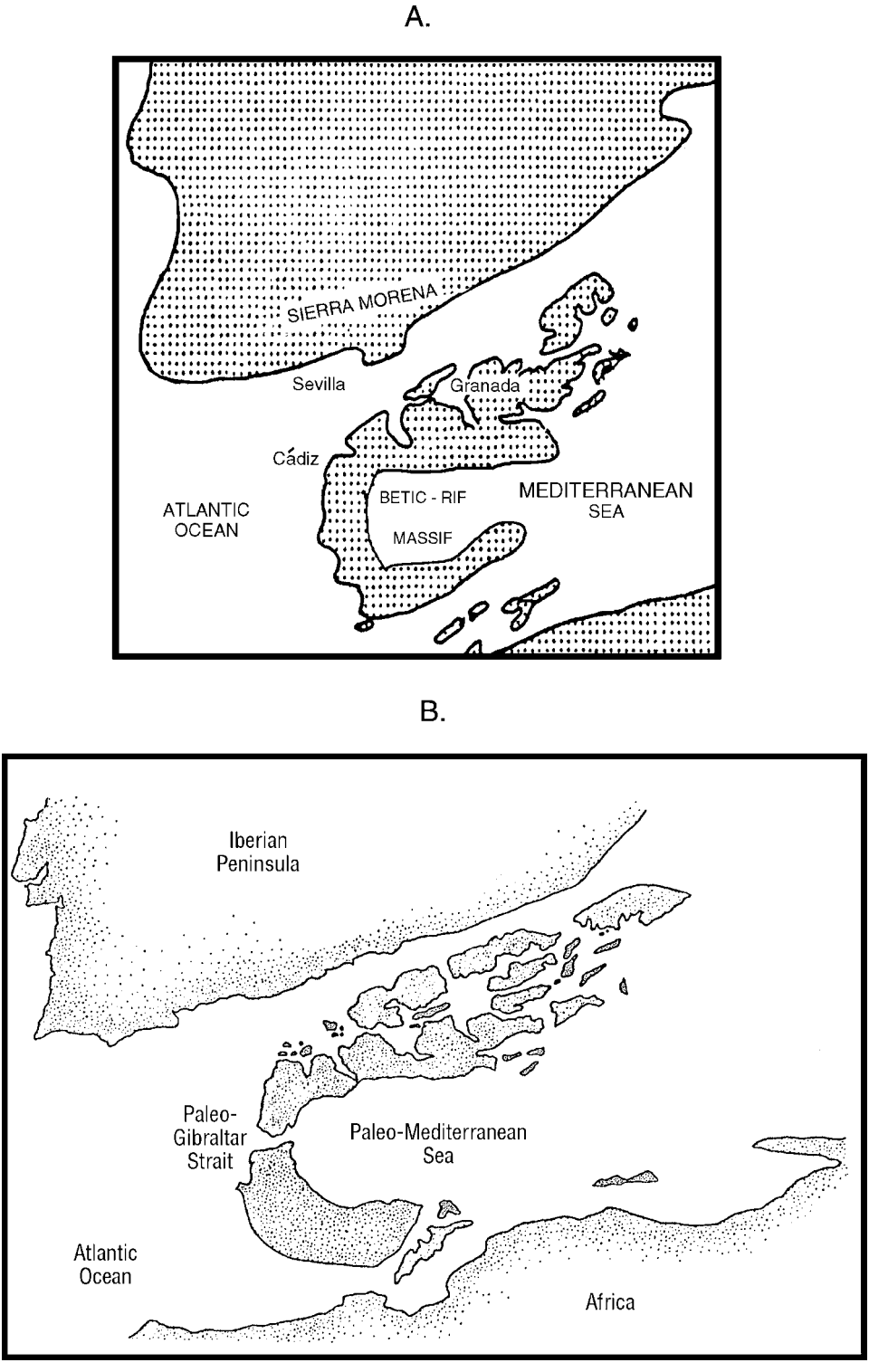

Figure 5. Paleogeographic reconstructions of the Strait of Gibraltar. A. approximately 14 mybp (after Fernex et al., 1967), B. approximately 7 mybp (after Benson et al., 1991 and 1992).

precipitated the differentiation we report within *P. vaucheri*.

In a study of Iberian herpetofaunal diversity, Busack and Jaksić (1982: table 1, reported as *Podarcis hispanica*) and Busack (unpublished field data) found that Spanish *P. vaucheri* inhabiting the province of Cádiz selected microclimates where humidity was low, maximum temperatures averaged 23.4°C, and minimum temperatures averaged 10°C. Street and Gasse (1981) and Biberson (1970) provide considerable evidence to show that most of the region under consideration was much wetter and more densely vegetated 2.3 to 0.9 million years before the present (mid-Pliocene to Pleistocene). If sex-limited mitochondrial markers reflect deep phylogenetic history and bi-parentally inherited allozymic markers accurately reflect more recent movement and assembly, differentiation within *P. vaucheri* is likely related to post-Pliocene or Pleistocene habitat modification in northern Africa (fig. 2, table 3).

Historical geographical change involving the area surrounding the Strait of Gibraltar is now fairly well understood (fig. 5). If ancestral *Podarcis hispanica* and *P. vaucheri* inhabited Iberia and North Africa while the Strait of Gibraltar was being formed and stabilized (Miocene-Pliocene), associated physiogeographic changes probably influenced the evolution of the two species. *Podarcis hispanica* may have become isolated on the Iberian mainland while ancestral *P. vaucheri* was restricted to insular areas of the Betic-Rif Massif and mainland Africa (fig. 5A). Trans-Gibraltar *p*-distances based on cytochrome *b*, ND2 and ND4 (table 6) among comparisons of *Podarcis vaucheri* Types I and II range between 4.86 and 5.20 (corresponding to *p* distances of 5.2-5.9 obtained from cytochrome *b* alone, see above) suggesting initiation of lineage differentiation during Pliocene. As the Paleo-Gibraltar Strait formed, *P. vaucheri* Type I populations likely remained on insular areas currently representing both north (to the Río Guadalquivir) and south (to the Rif) shores of the paleo-Strait. *Podarcis vaucheri* Type II populations likely inhabited only areas to the south of the paleo-Strait (fig. 5A). Low level differentiation (fig. 2) between representatives of *P. vaucheri* Type II from Ksar-es-Seghir (fig. 1: 5) and those from more southern populations (fig. 1: 9 and 10) suggests that the Ksar-es-Seghir population may have migrated northward as landforms continued to evolve (fig. 6B; Gomez et al., 2000: fig. 2C).

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References

Arnold, E.N. (1973): Relationships of the Palaearctic lizards assigned to the genera *Lacerta*, *Algyroides* and *Psammodromus* (Reptilia:Lacertidae). Bulletin of the British Museum (Natural History), Zoology **25**: 291-366.

Arévalo, E., Davis, S.K., Sites, J.W., Jr. (1994): Mitochondrial DNA sequence divergence and phylogenetic relationships among eight races of the *Sceloporus grammicus* complex (Phrynosomatidae) in Central Mexico. Systematic Biology **43**: 387-418.

Ayala, F.J., Powell, J.R., Tracey, M.L., Mourão, C.A., Perez-Salas, S. (1972): Enzyme variability in the *Drosophila willistoni* group. IV. Genic variation in natural populations of *Drosophila willistoni*. Genetics **70**: 113-139.

Benson, R.H., Rakic-El Beid, K., Bonaduce, G. (1991): An important current reversal (influx) in the Rifian Corridor (Morocco) at the Tortonian-Messinian boundary: the end of the Tethys Ocean. Paleoceanography **6**: 164-192.

Benson, R.H., Rakic-El Beid, K., Bonaduce, G. (1992): An important current reversal (influx) in the Rifian Corridor (Morocco) at the Tortonian-Messinian boundary: the end of the Tethys Ocean. Notes et Mémoires du Service Géologique du Maroc **366**: 115-146.

Biberson, P. (1970): The problem of correlations between south Europe and North Africa during the Pleistocene. Palaeogeography, Palaeoclimatology, Palaeoecology **8**: 113-127.

Burbrink, F.T., Lawson, R., Slowinski, J.B. (2000): MtDNA phylogeography of the North American rat snake (*Elaphe obsoleta*): a critique of the subspecies concept. Evolution **54**: 2107-2118.

Busack, S.D. (1986): Biogeographic analysis of the herpetofauna separated by the formation of the Strait of Gibraltar. National Geographic Research **2**: 17-36.

Busack, S.D., Jaksić, F.M. (1982): Ecological and historical correlates of Iberian herpetofaunal diversity: an analysis at regional and local levels. Journal of Biogeography **9**: 289-302.

Castilla, A.M., Fernandez-Pedrosa, V., Harris, D.J., Gonzalez, A., Latorre, A., Moya, A. (1998): Mitochondrial DNA divergence suggests that *Podarcis hispanica atrata* (Squamata:Lacertidae) from the Columbretes Islands merits specific distinction. Copeia **1998**: 1037-1040.

Capula, M. (1997): Genetic divergence and phylogenetic inferences in the genus *Podarcis*. Abstracts of the Third Herpetological Congress of Herpetology, Prague (not seen, cited from Harris and Sá-Sousa, 2002).

de Jong, H. (1998): In search of historical biogeographic patterns in the western Mediterranean terrestrial fauna. Biological Journal of the Linnean Society **65**: 99-164.

de Queiroz, A., Lawson, R., Lemos-Espinal, J.A. (2002): Phylogenetic relationships of North American garter snakes (*Thamnophis*) based on four mitochondrial genes: how much DNA sequence is enough? Molecular Phylogenetics and Evolution **22**: 315-329.

Felsenstein, J. (1985): Confidence limits on phylogenies: an approach using the bootstrap. Evolution **39**: 783-791.

Fernex, F., Magné, J., Mongin, D. (1967): The paleogeography of the eastern Betic Range of southern Spain during the Caenozoic. In: Aspects of Tethyan biogeography, p. 239-246. Adams, C.G., Ager, D.V., Eds, London, Systematics Association.

Fu, J. (2000): Toward the phylogeny of the family Lacertidae — Why 4708 base pairs of mtDNA sequences cannot draw the picture. Biological Journal of the Linnean Society **71**: 203-217.

Galán, P. (2000): Females that imitate males: dorsal coloration varies with reproductive stage in female *Podarcis bocagei* (Lacertidae). Copeia **2000**: 819-825.

Gomez, F., Beauchamp, W., Barazangi, M. (2000): Role of the Atlas Mountains (northwest Africa) within the African-Eurasian plate-boundary zone. Geology **28:** 775-778.

Harris, D.J. (2002): Reassessment of comparative genetic distance in reptiles from the mitochondrial cytochrome *b* gene. Herpetological Journal **12**: 85-86.

Harris, D.J., Carranza, S., Arnold, E.N., Pinho, C., Ferrand, N. (2002): Complex biogeographical distribution of genetic variation within *Podarcis* wall lizards across the Strait of Gibraltar. Journal of Biogeography **29**: 1257-1262.

Harris, D.J., Sá-Sousa, P. (2001): Species distinction and relationships of the western Iberian *Podarcis* lizards (Reptilia, Lacertidae) based on morphology and mitochondrial DNA sequences. Herpetological Journal **11**: 129-136.

Harris, D.J., Sá-Sousa, P. (2002): Molecular phylogenetics of Iberian wall lizards (*Podarcis*): Is *Podarcis hispanica* a species complex? Molecular Phylogenetics and Evolution **23**: 75-81.

Harris, H., Hopkinson, D.A. (1976): Handbook of enzyme electrophoresis in human genetics. Amsterdam, North Holland Publishing Company.

Kocher, T.D., Thomas, W.K., Meyer, A., Edwards, S.V., Paabo, S., Villablanca, F.X., Wilson, A.C. (1989): Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. Proceedings of the National Academy of Sciences **86**: 6196-6200.

Kumazawa, Y., Ota, H., Nishida, M., Ozawa, T. (1996): Gene arrangements in snake mitochondrial genomes: highly concerted evolution of control-region-like sequences duplicated and inserted into a tRNA gene cluster. Molecular Biology and Evolution **13**: 1242-1254.

Kumazawa, Y., Nishida, M. (1999): Complete mitochondrial sequences of the green turtle and blue-tailed mole skink: Statistical evidence for archosaurian affinity of turtles. Molecular Biology and Evolution **16**: 284-292.

Mayer, W., Tiedemann, F. (1980): Elektrophoretische Untersuchungen an europäischen Arten der Gattungen *Lacerta* und *Podarcis* I. Die *Podarcis*-Formen der griechischen Inseln Milos und Skiros. Zeitschrift für zoologische Systematik und Evolutionsforschung **18**: 147-152.

Mayer, W., Tiedemann, F. (1982): Chemotaxonomical investigations in the collective genus *Lacerta* (Lacertidae;Sauria) by means of protein electrophoresis. Amphibia-Reptilia **2**: 349-355.

Mertens, R., Wermuth, H. (1960): Die Amphibien und Reptilien Europas. Frankfurt am Main, Waldemar Kramer.

Nei, M. (1978): Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics **89**: 583-590.

Oliverio, M., Bologna, M.A., Mariottini, P. (2000): Molecular biogeography of the Mediterranean lizards *Podarcis* Wagler, 1830 and *Teira* Gray, 1838 (Reptilia, Lacertidae). Journal of Biogeography **27**: 1403-1420.

Pinho, C., Harris, D.J., Ferrand, N. (2003): Genetic polymorphism of 11 allozyme loci in populations of wall lizards (*Podarcis* sp.) from the Iberian Peninsula and North Africa. Biochemical Genetics **41**:343-359.

Poulakakis, N., Lymberakis, P., Antoniou, A., Chalkia, D., Zouros, E., Mylonas, M., Valakos, E. (2003): Molecular phylogeny and biogeography of the wall-lizard *Podarcis erhardii* (Squamata: Lacertidae). Molecular Phylogenetics and Evolution **28**: 38-46.

Selander, R.K., Smith, M.H., Yang, S.Y., Johnson, W.E., Gentry, J.B. (1971): Biochemical polymorphism and systematics in the genus *Peromyscus*. I. Variation in the old-field mouse (*Peromyscus polionotus*). Studies in Genetics VI, University of Texas Publications **7103**: 49-90.

SPSS, Inc. (1997): SYSTAT 7.0.1 for Windows. Chicago, Illinois 60606-6307, U.S.A. (233 South Wacker Drive, 11th Floor; *http://www.spss.com/software/science*).

Street, A., Gasse, F. (1981): Recent developments in research into the Quarternary climatic history of the Sahara. In: The Sahara. Ecological change and early economic history, p. 7-28. Allan, J.A., Ed., Outwell, near Wisbech, Cambridgeshire, United Kingdom. Middle East & North African Studies Press Ltd.

Swofford, D.L. (2000): PAUP*: phylogenetic analysis using parsimony. Version 4.0b4a. Sunderland, Massachusetts, U.S.A., Sinauer Associates, Inc.

Appendix: Specimens examined

DNA Analysis. *Podarcis atrata* — SPAIN (Valladolid): Mayorga (42°10′N, 5°16′W), MNCN 11094. *P. hispanica* — SPAIN (Ávila): San Martín del Pimpollar (40°22′N, 5°02′W), MVZ 232040; Arenas de San Pedro (40°12′N, 5°05′W), MNCN 11095. *P. muralis* — SPAIN (Huesca): Benasque (42°36′N, 0°32′W), Baños de Benasque, MNCN 23636. *P. vaucheri* — MOROCCO (Beni Mellal): El Ksiba (32°35′N, 6°02′W), MVZ 178291; (Marrakech): Oukaïmedèn (31°13′N, 7°52′W), MVZ 178295 and 178297; (Tétouan): Asilah (35°28′N, 6°02′W), MVZ 186228; Ksar-es-Seghir (35°51′N, 5°34′W), MVZ 186233. SPAIN (Málaga): Río Hozgarganta at km 68 on roadway C-3331, MNCN 11088; (Cádiz): vicinity of Facinas (36°08′N, 5°42′W), Busack field series SDB 1531 (voucher specimen apparently lost, population represented by MNCN 11078-11082).

Allozyme Analysis. *Podarcis hispanica* — SPAIN (Ávila): San Martín del Pimpollar, MNCN 11083-11087. *P. vaucheri* — MOROCCO (Marrakech): Oukaïmedèn, MVZ 178292-178294, 178296, and 178299; (Meknès): Âïn Leuh (33°17′N, 5°23′W), MVZ 178319-178323; (Tétouan): Asilah, MVZ 178334-178338; Chechaouèn (35°10′N, 5°16′W), MVZ 178339-178342; Ksar-es-Seghir, MVZ 178346-178350. SPAIN (Cádiz): vicinity of Benalup de Sidonia (36°20′N, 5°49′W), MNCN 11107-11109; vicinity of Facinas, MNCN 11078-11082.

Morphological Analysis. *Podarcis vaucheri* — MOROCCO (Marrakech): Oukaïmedèn, Carnegie Museum of Natural History (CM) 55221-55225; MVZ 178292-178318; (Meknès): Âïn Leuh, MVZ 178319-178323; (Tétouan): Asilah, MVZ 178324-178333, 178335-178338, 186226-186232; Chechaouèn, MVZ 178339-178342; Ksar-es-Seghir, MVZ 178343-178348, 186233. SPAIN (Cádiz): vicinity of Facinas, CM 53213 (5 specimens), 53407, 53415-53417, 54203, 54232, 54563 (10), 54569 (18), 54603, 54796 (3), 55494 (5), 55665.

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