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Molecular Phylogenetics of Iberian Wall Lizards (*Podarcis*): Is *Podarcis hispanica* a Species Complex?

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Phylogenetic relationships between species and morphotypes of *Podarcis* wall lizards from the Iberian Peninsula and north Africa were estimated using partial 12S rRNA and cytochrome *b* mitochondrial DNA sequences. All species except *Podarcis hispanica* form monophyletic units. *P. hispanica* is paraphyletic, although all identified morphotypes are monophyletic. These morphotypes represent highly divergent lineages showing 10–15% pairwise sequence divergence with the cytochrome *b* gene. The data suggest that *P. hispanica* is a species complex. We recommend using *P. hispanica until additional sampling delimits the number and ranges of species currently referred to *P. hispanica*. *P. carbonelli*, which has recently been raised to species status, is confirmed as a genetically distinct form. *P. atrata* is genetically distinct, but much more closely related to some populations of *P. hispanica* than previously thought.**

Key Words: **12S rRNA; cytochrome *b*; *Podarcis hispanica*; phylogeny.**

INTRODUCTION

Wall lizards, *Podarcis*, are the predominant reptile group in southern Europe. Their taxonomy is complex and unstable, primarily because species are morphologically similar but exhibit substantial levels of intraspecific variation (Arnold and Burton, 1978). In the Iberian Peninsula three species have been widely recognized—*Podarcis muralis* which is also found widely in central Europe and northwest Asia Minor, *Podarcis bocagei* which is a northwest Iberian endemic, and *P. hispanica*. *P. atrata* from the Columbretes islands around 50 miles from the west coast of Spain was previously described as a subspecies of *P. hispanica*, but on the basis of high genetic divergence between the populations from the islands and a population from Spain, the island form was given a specific rank (Castilla *et al.*, 1998a). *P. carbonelli*, previously described as a subspecies of *P. bocagei*, was raised to specific rank on the basis of enzymatic (Sá-Sousa *et al.*, 2000) and morphological and mitochondrial DNA (mtDNA) sequence divergences (Harris and Sá-Sousa, 2001). Phylogenies derived from mtDNA also suggested that *P. bocagei* and *P. carbonelli* were not sister taxa, but were each more closely related to different populations of *P. hispanica* (Harris and Sá-Sousa, 2001). Within *P. hispanica* subspecific taxonomy has been controversial, with most authors accepting one, admittedly highly variable form in the Iberian Peninsula and a separate subspecies, *P. h. vaucheri* in northwest Africa (Barbadillo *et al.*, 1999). Other authors recommend the recognition of multiple subspecific groups (e.g., Guillaume, 1987). On the basis of high genetic distances of the 12S rRNA gene between one individual from Morocco and one individual from Spain, Oliverio *et al.* (2000) raised the African populations to species rank as *P. vaucheri*. Using electrophoretic data Capula (1997) also suggested that the Moroccan populations might merit specific rank, but contrary to this Busack (1986) found very low genetic distances between populations from southern Spain and Morocco.

To try to resolve these discrepancies and to further examine genetic distances and distribution of these newly described species, we have sequenced *Podarcis* from populations across Spain, Portugal, and north Africa for partial 12S rRNA and cytochrome *b* gene regions and combined this with previously published sequences. The sampling covers all the major clades previously identified within the "Spanish–Maghreb" group of *Podarcis* (Harris and Arnold, 1999; Harris and Sá-Sousa, 2001; Oliverio *et al.*, 2000).

MATERIALS AND METHODS

The number and geographic locations of the specimens used in this study are given in Table 1 and Fig. 1. Within *P. hispanica*, samples were designated type 1 or 2 in western Iberia following morphological criteria

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TABLE 1

Specimen Type and Locality Data for Samples Included in This Study

Specimen type	Code	Locality	Map code
P. atrata		Columbretes Island**	1
P. bocagei	Pbb5	Vairão, Portugal*	2
	Pbb6	Vairão, Portugal*	2
	Gpb6	Malpica, Galicia, Spain	3
	MP3	Madalena Praia, Portugal	4
P. carbonelli	Pcc1	Estrela Mts, Portugal*	5
	Pcc2	Aveiro, Portugal*	6
	Pcc3	Mt Clerigo, Portugal*	7
P. hispanica 1	Ph1	Vila Real, Portugal*	8
	Mon3	Montesinho, Portugal	9
	Mon8	Montesinho, Portugal	9
	Rua1	Vila da Rua, Portugal	10
	Trj1	Trujillo, Spain	11
	Oro1	Oropesa, Spain	12
P. hispanica 2	Phv1	Leiria, Portugal	13
	Phv3	Portalegra, Portugal*	14
	Phv4	Beja, Portugal	15
	Phv6	Agueda, Portugal*	16
	Mad2	Madrid, Spain	17
P. hispanica 3	Bar1	Barcelona, Spain	18
	Bar2	Barcelona, Spain	18
	Med1	Medinaceli, Spain	19
	Pht1	Tarragona, Spain	20
	Gir1	Girona, Spain	21
	Val1	Pyrenees, Spain	22
P. hispanica Morocco		High Atlas Mts, Morocco**	23
	PhM1	Mid Atlas Mts, Morocco*	23
	PhM2	Mid Atlas Mts, Morocco*	23
	PhM3	Mid Atlas Mts, Morocco*	23
P. h. hispanica	Cue1	Cuenca, Spain	24
	Pod12	Granada, Spain	25
P. muralis		Huesca, Spain**	26
		Cannes, France**	27

* Indicates cytochrome *b* gene region previously published.
** Indicates both gene regions previously published.

(Sá-Sousa, 2000; Harris and Sá-Sousa, 2001). For most samples voucher specimens were taken and kept at the University of Évora. Total genomic DNA was extracted from small pieces of tail using standard methods (Sambrook *et al.*, 1989). Polymerase chain reaction primers used in both amplification and sequencing were cytochrome *b*1 and 2 and 12Sa and 12Sb from Kocher *et al.* (1989). Amplification conditions were the same as described by Harris *et al.* (1998). Amplified fragments were sequenced on a 310 Applied Biosystem DNA Sequencing Apparatus. Two individuals of *P. muralis* (Harris *et al.*, 1998; Fu, 2000) were included in the analysis and designated as an outgroup. Two sequences from *P. hispanica* and *P. atrata* for both genes (Harris and Arnold, 1999) were also included. For 11 individuals the cytochrome *b* gene region has already been published (Harris and Sá-Sousa, 2001), and for these the 12S rRNA from the same individual was sequenced for this study. In all cases sequences from the cytochrome *b* and 12S rRNA belonging to the same individual were merged in the subsequent analysis and aligned using Clustal W (Thompson *et al.*, 1994). Cytochrome *b* and 12SrRNA sequences were, respectively, 306 and 414 bp long. The cytochrome *b* sequences contained no indels. Alignment of the 12S rRNA required insertions in six places. Assessment of saturation in each gene by plotting numbers of transitions and transversions against uncorrected distances indicated that they were not saturated (Fig. 2). Therefore all positions were included in the analysis. The data were imported into PAUP* 4.0b5 (Swofford, 2001) for phylogenetic analysis. When estimating phylogenetic relationships among sequences, one assumes a model of evolution. We used the approach outlined by Huelsenbeck and Crandall (1997) to test 56 alternative models of evolution, employing PAUP* 4.0b5 and Modeltest (Posada and Crandall, 1998) described in detail in Harris and Crandall (2000). Once a model of evolution was chosen, it was used to estimate a tree using maximum-likelihood (ML) (Felsenstein, 1981) with random sequence addition. Support for nodes was estimated using the bootstrap (Felsenstein, 1985) technique, with 1000 replicates. A maximum-parsimony (MP) analysis was also carried out (100 replicate heuristic search) with random sequence addition, and support for nodes was estimated using decay analysis

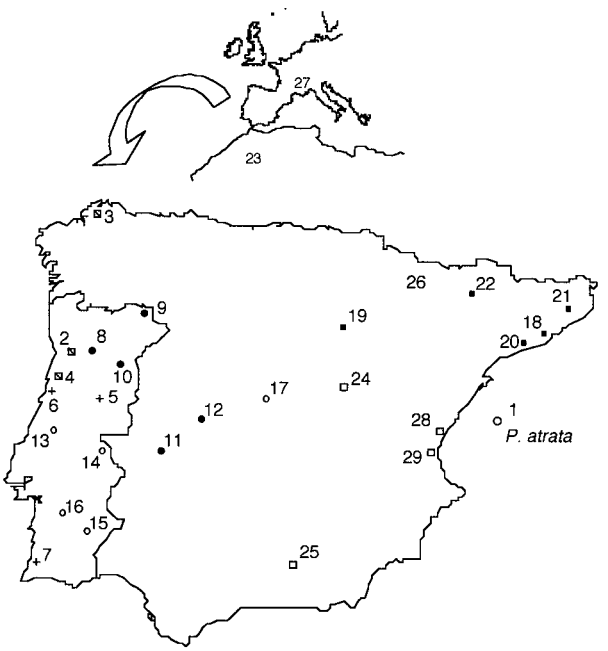

FIG. 1. Map showing the localities of specimens included in this study. Different species and morphotypes are represented as *P. atrata* (circle, endemic to Columbretes islands), *P. bocagei* (square with stripe), *P. carbonelli* (crosses), *P. hispanica* 1 (black circle), *P. hispanica* 2 (open circle), *P. hispanica* 3 (black square), and *P. h. hispanica* (open square). Numbers refer to Table 1.

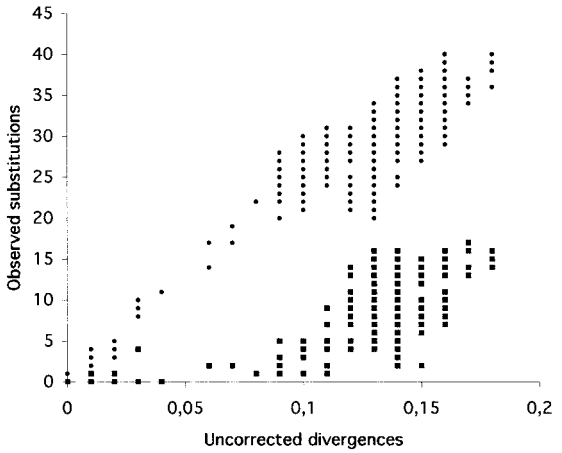

FIG. 2. Patterns of nucleotide substitution between cytochrome *b* sequences. Transitions (dots) and transversions (squares) are plotted against uncorrected distances. The pattern of substitution was similar for 12S rRNA sequences, but with a much lower level of sequence divergence (see Table 2).

(Bremer, 1988) and bootstrapping with 1000 replicates.

RESULTS

Including the two outgroups, 33 taxa were analyzed from 28 populations. Aligned sequences of the combined gene regions were 720 bp long. New sequences were deposited in GenBank, Accession Nos. AF469415–AF469461. The data appear to be mitochondrial DNA sequences and not nuclear integrated copies (see Nielson and Arctander, 2001) for several reasons. The protein-coding gene contains no introns or stop codons, and the free energy of the secondary structure of the 12S rRNA is similar to that of other lacertid species (data not shown; see Harris, 2001). Also, the strong strand bias in the third position of the cytochrome *b* gene is typical in reptiles (A 29% C 38% G 02% T 31%, compared to average in lacertids of A 31% C 39% G 03% T 27%; Harris, 2002). Using Modeltest (Posada and Crandall, 1998) we concluded that the TRN model (base frequencies A 0.31, C 0.26, G 0.15, T 0.28, equal transversion ratios A/G 5.7, C/T 14.8), with a gamma-distributed rate heterogeneity model (four rate categories, G = 0.1460; Yang, 1994), was the most appropriate model of evolution for this data. A 10-replicate heuristic search with this model produced three equally likely trees with likelihoods of −ln 2794.5 (Fig. 3). Maximum-parsimony analysis found three trees of 374 steps that were similar to the ML trees. All branches with bootstrap support >50% were congruent with the ML tree (Fig. 3); 153 characters were parsimony informative.

DISCUSSION

Identification of Major Lineages within Iberian Podarcis

Our analyses support the previous hypothesis that *P. hispanica* is paraphyletic (Harris and Sá-Sousa, 2001). Constraining *P. hispanica* to be monophyletic, performing a heuristic search to find the shortest tree with this constraint, and comparing this to the optimal tree using the Shimodaira and Hasegawa (SH) (1999) test shows that the difference is significant (SH test using 1000 RELL bootstraps, diff –ln L = 42.5, $P > 0.001$). Apart from the outgroup, six major clades can be identified. Samples of *P. bocagei* strongly form a monophyletic group (100% bootstrap support in the ML analysis). Similarly, samples from *P. carbonelli* are a monophyletic group (100% support). In western and central Iberia two allopatric morphotypes have been identified in *P. hispanica* (Sá-Sousa, 2000). In the northwest of Iberia exists morphotype 1 (corresponding to *P. h. "lusitanica"* described by Guillaume, 1987), which has a very depressed head and body and is mainly dark dorsally with a whitish belly. In southwestern Iberia there is morphotype 2, first described as *Lacerta muralis* (= *P. hispanica*) *vaucheri* by Boulenger (1905). This form is more robust and often has a pale greenish dorsal pattern. In our analysis, sequences from these morphotypes form monophyletic units, although with lower levels of bootstrap support (56 and 72%, respectively). However, the specimens from Morocco that are typically also assigned to *P. h. vaucheri* and that have been argued to deserve specific rank as *P. vaucheri* (Oliverio *et al.*, 2000) are a separate clade (100% support), which is the sister taxa (95% support) to sequences from the nominal type *P. hispanica hispanica* from southeastern Spain. Finally, an additional clade can be identified which includes specimens from northeastern Spain (morphotype 3, which may correspond to *P. h. "liolepis;"* Guillaume, 1987) and that is most closely related to the single individual of *P. atrata* from the Columbretes islands (98% support).

P. atrata was raised to specific rank on the basis of high genetic divergences between them and *P. hispanica hispanica*. In our analysis some *P. hispanica* from the mainland are more closely related to *P. atrata* than the populations of *P. hispanica hispanica* that were compared to them previously (Castilla *et al.*, 1998a,b). Overall uncorrected pairwise genetic divergences for representative taxa from each main clade are given in Table 2. 12S rRNA sequence divergence between *P. atrata* and *P. hispanica hispanica* is 6%, compared to 1.2% between *P. atrata* and *P. hispanica* type 3. Therefore although the genetic distance between *P. atrata* and mainland populations is still high, this study shows the importance of wide sampling of populations when comparing island taxa to mainland

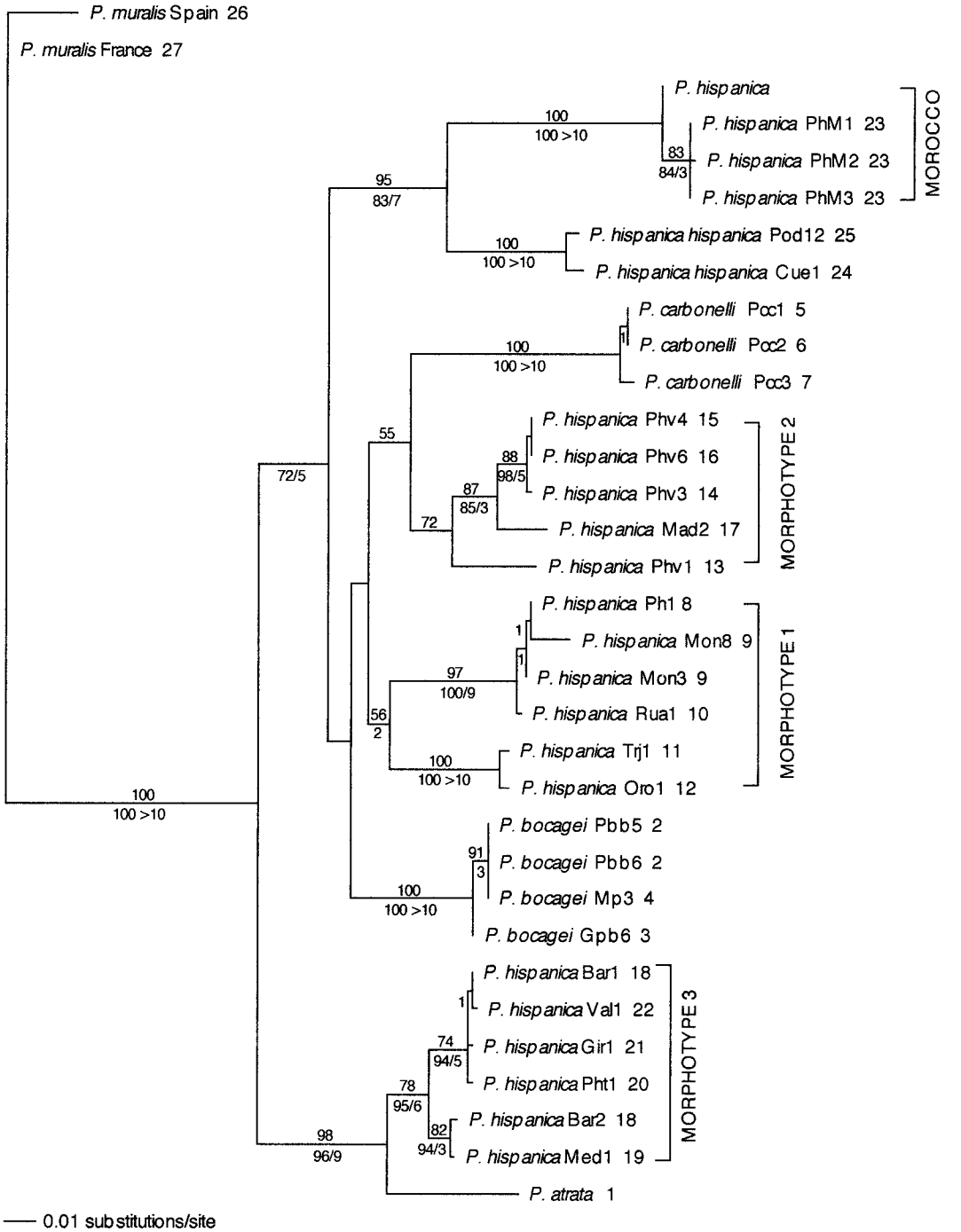

FIG. 3. One of three maximum-likelihood (ML) trees derived from the combined 12S rRNA and cytochrome *b* sequences. The model used was the Tamura–Nei model (different transition rates), including a discrete approximation of the gamma distribution (0.146). The tree was rooted using *Podarcis muralis*. The other ML trees differed in the positions of the short branches within *P. carbonelli* and *P. hispanica* (Moroccan). Numbers above nodes indicate bootstrap support (>50%, 1000 replicates) from the ML analysis; numbers below nodes indicate bootstrap support from the maximum-parsimony (MP) analysis followed by decay values. The MP strict consensus differed only in being less resolved. After the species names are specimen numbers (Table 1) followed by map codes (Fig. 1).

forms. In this case the populations on the mainland closest to the islands are not the most closely related group genetically. The Columbetes could have been colonized by populations from more northern coastal Spain, following the southerly sea currents (National Geographic Atlas, 7th Edition). This is similar to the situation of *Tarentola* geckos from the Cape Verde islands, which are more closely related to the distant

TABLE 2

Pairwise Comparison of Cytochrome *b* (above Diagonal) and 12S rRNA (below) Sequences among the Major Clades Included in This Study

Name	1	2	3	4	5	6	7	8	9	10	11
1. *P. muralis*	—	0.167	0.173	0.144	0.173	0.154	0.163	0.147	0.152	0.147	0.160
2. PhM1	0.059	—	0.163	0.137	0.124	0.157	0.176	0.127	0.129	0.127	0.141
3. *P. carbonelli*	0.052	0.039	—	0.136	0.142	0.152	0.148	0.135	0.137	0.115	0.104
4. *P. bocagei*	0.054	0.051	0.027	—	0.128	0.131	0.118	0.095	0.102	0.088	0.098
5. Phh Cue1	0.050	0.042	0.037	0.042	—	0.147	0.137	0.144	0.142	0.114	0.127
6. Ph Gir1	0.042	0.056	0.034	0.039	0.055	—	0.098	0.137	0.132	0.137	0.154
7. *P. atrata*	0.049	0.054	0.041	0.043	0.060	0.012	—	0.137	0.145	0.127	0.131
8. Ph1 Mon3	0.042	0.056	0.042	0.039	0.050	0.042	0.044	—	0.092	0.101	0.108
9. Ph2 Oro1	0.037	0.054	0.032	0.039	0.050	0.037	0.043	0.024	—	0.090	0.116
10. Ph2 Phv1	0.054	0.056	0.029	0.042	0.055	0.049	0.053	0.046	0.032	—	0.062
11. Ph2 Phv6	0.034	0.049	0.029	0.034	0.039	0.039	0.046	0.029	0.024	0.027	—

Canary Islands rather than to the closer mainland African forms (Carranza *et al.*, 2000). In both examples patterns of colonization seem to be related to prevailing sea currents rather than to simple distance.

For the cytochrome *b*, these can be compared to the average genetic distance between congeneric species for reptiles, 13.6% (Harris, 2001b). In a comparison for cytochrome *b*, no sequences from the same species of reptile, for which data was on GenBank, had uncorrected cytochrome *b* divergence of greater than 8.5% (Hendry *et al.*, 2000). In Iberian *Podarcis* all currently recognized species have pairwise genetic divergences higher than this. Pairwise comparisons between the three morphotypes included in this study and the samples from Morocco are also all higher than 9%. Even within morphotype 1, the two samples from Spain show genetic divergence of 9% from the samples from Portugal. This implies that there are additional isolated genetic units even within the presently distinguished forms. Contrary to this, genetic divergences within *P. bocagei* and *P. carbonelli* are much lower (Harris and Sá-Sousa, 2001).

Geographic Distribution of the Major Lineages

Distributions of *P. bocagei* (Sá-Sousa, 1998) and *P. carbonelli* (Sá-Sousa, 1999) are relatively well known, although new records do occur (Malkmus and Schwarzer, 2000) and confusion in the past, especially between *P. carbonelli* and *P. hispanica vaucheri*, means that some older records are uncertain. In particular *P. carbonelli* in the south of Spain may well have been overlooked.

The two forms of *P. hispanica* in Portugal have allopatric ranges. Type 1 is found in Galicia, northern Portugal, and the "Sistema Central" range, which corresponds with *P. h. lusitanica* described by Guillaume (1987). Distribution models suggest that *P. hispanica* type 1 predominates in highlands and where Atlantic environmental conditions prevail, while *P. hispanica* type 2 occurs where Mediterranean conditions are typical, at least in Portugal (Sá-Sousa, 2000). The sample of *P. hispanica* type 1 from Spain (Fig. 1, position 11) is from one of the most southerly populations where this form has been reported. *P. hispanica* type 2 occurs in central and southern Portugal and Spain. Populations reported from the province of Cadiz and Gibraltar appear to be this morphotype (Sá-Sousa, 2000), although this has not been confirmed by molecular sampling to date. Both morphotypes have been reported from the region of Madrid in central Spain (García-Paris *et al.*, 1989). The sample included in this study from Madrid corresponds to morphotype 2, but further sampling would be needed to determine whether there is a contact zone in this region.

In this study two samples of *P. h. hispanica* were included, from Granada and Cuenca (Fig. 1, localities 24 and 25). Populations have also been sampled from Castelló de la Plana and Valencia (localities 28 and 29) on the east coast of Spain (Castilla *et al.*, 1998a,b) for cytochrome *b*. Using only this partial gene region, these populations are all a closely related form (analysis not shown). Therefore at present only *P. h. hispanica* has been reported from southeastern Iberia.

Samples from the five populations included in this study from northeastern Spain form a monophyletic unit. These might correspond to *P. h. "liolepis"* (Guillaume, 1987), who suggests that there is a contact zone between this form and *P. h. hispanica* in the region of Valencia. The sample from Medinaceli from this study is on the westerly limit for this morphotype, but additional sampling would be needed to confirm this.

Relationships between the Major Lineages

Although all of the species and morphotypes included in this study form monophyletic units based on mtDNA sequences, only some relationships between them are well supported. The samples of morphotype 3 from northeastern Spain are strongly supported as the sister taxa to *P. atrata* in both MP and ML analyses (98 and 96% support). Similarly, the samples from Morocco

are sister taxa to *P. hispanica hispanica* (95 and 83% support). Less strongly supported is a relationship between *P. hispanica* morphotype 2 and *P. carbonelli* (55% support in the ML analysis). In both the ML and the MP consensus trees the northeastern group (including *P. atrata*) comes out as basal to the other taxa, although bootstrap support is low (48 and 72% support). No other relationships between groups appear to be well supported.

Taxonomic Recommendations

A combination of high genetic divergences between clades and its apparent paraphyly suggests that *P. hispanica* is a species complex. Where the morphology of *P. hispanica* has been studied in detail, morphotypes correspond to the same phylogenetic clades identified from mtDNA. Preliminary analyses of allozyme variation also infer that *P. hispanica* is paraphyletic (Pinho and Ferrand, 2001). The current taxonomy is therefore inappropriate. If *P. h. lusitanica* and *P. hispanica* "morphotype 2" are raised to full species, *P. hispanica* "morphotype 3" would also need to be raised to specific rank to avoid paraphyly of the species. It is not unfeasible that other genetically distinct but morphologically cryptic forms also exist. Furthermore, genetic divergences within some of these morphotypes are still higher than those typically found within species. Therefore to avoid taxonomic confusion we suggest using phylocodes (e.g., Schulte *et al.*, 1998; review by Pennisi, 2001) and refer to *P. hispanica* as *P. hispanica** until additional sampling and nuclear markers delimit the numbers and ranges of the cryptic species.

CONCLUSIONS

*P. hispanica** is paraphyletic with respect to *P. bocagei*, *P. carbonelli*, and *P. atrata* and appears to be a species complex. Most *P. atrata* are closely related to a northeastern form of *P. hispanica**, although two of the individuals sampled by Castilla *et al.* (1998b) were more closely related to *P. hispanica* hispanica*. Genetic divergence of *P. atrata* between islands is very high (Castilla *et al.*, 1998b), and this could be the result of colonization of different islands from genetically distinct but morphologically cryptic species from the mainland. This phenomenon deserves further investigation. The existence of cryptic species in Spain also could explain the discrepancies between studies of allozyme variation across the Straits of Gibraltar in *Podarcis*. Capula (1997) found very high divergence between populations, but Busack (1986) reported very low divergence. This could be explained by differential sampling, since *P. hispanica* hispanica* is much more closely related to the Moroccan populations than *P. hispanica* morphotype 2*. Additional sampling in southern Spain will be necessary to determine the distributions of these two forms. Relatively high genetic divergences (up to 3.6%) between individuals for a different portion of the 12S rRNA gene have been reported in *P. sicula* from Italy which may also be a species complex (Oliverio *et al.*, 1998, 2000). Both the Iberian Peninsula and Italy served as refugia for many taxa during glacial periods (Hewitt, 2000), so it is not unexpected that higher genetic diversity compared to that in northern Europe is found in these areas. Weisrock *et al.* (2001) suggested that tectonic collision zones such as Anatolia are regions in which high genetic diversity among faunal elements is more likely. The southern part of the Iberian Peninsula was an archipelago of disjunct islands from the Oligocene to around 5 million years ago (Smith *et al.*, 1994). This could have led to genetic isolation and speciation in *Podarcis* and other similar fauna. It is clear that for *Podarcis* further sampling is essential to delimit these genetically distinct groups as a first step toward their recognition and conservation. Also, preliminary data from nuclear markers (Pinho and Ferrand, 2001) need to be expanded to ensure that the mitochondrial gene trees correspond with the species tree. This work is currently in progress.

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REFERENCES

Arnold, E. N., and Burton, J. A. (1978). "A Field Guide to the Reptiles and Amphibians of Britain and Europe," Collins, London.

Barbadillo, L. J., Lacomba, J. I., Pérez-Mellado, V., Sancho, V., and López-Jurado, L. F. (1999). "Anfibios y Reptiles de la Península Ibérica, Baleares y Canarias," Editorial Planeta, Barcelona, Spain.

Boulenger, G. A. (1905). A contribution to our knowledge of the varieties of the wall lizard (*Lacerta muralis*) in Western Europe and North Africa. *Trans. Zool. Soc. Lond.* **17:** 351–436.

Bremer, K. (1988). The limits of amino acid sequence data in angiosperm phylogenetic reconstruction. *Evolution* **42:** 795–803.

Busack, S. D. (1986). Biogeographic analysis of the herpetofauna separated by the formation of the Strait of Gibraltar. *Nat. Geogr. Res.* **2:** 17–36.

Capula, M. (1997). Genetic divergence and phylogenetic inferences in the genus *Podarcis*. *In* "Abstracts of the Third Herpetological Congress of Herpetology" (Z. Rocek and S. Hart, Eds.), Third World Congress of Herpetology, Prague.

Carranza, S., Arnold, E. N., Mateo, J. A., and Lopez-Jurado, L. F. (2000). Long-distance colonization and radiation in gekkonid lizards, *Tarentola* (Reptilia: Gekkonidae), revealed by mitochondrial DNA sequences. *Proc. R. Soc. Lond. B.* **267:** 637–649.

Castilla, A. M., Fernandez-Pedrosa, V., Harris, D. J., Gonzalez, A., Latorre, A., and Moya, A. (1998a). Mitochondrial DNA divergence suggests *Podarcis hispanica atrata* from the Columbretes Islands merits specific distinction. *Copeia* **4:** 1037–1040.

Castilla, A. M., Fernandez-Pedrosa, V., Backeljau, T., Gonzalez, A., Latorre, A., and Moya, A. (1998b). Mitochondrial DNA variability and conservation of an endangered lizard from the Columbretes islands. *Mol. Ecol.* **7:** 1047–1051.

Felsenstein, J. (1981). Evolutionary trees from DNA sequences: A maximum likelihood approach. *J. Mol. Evol.* **17:** 368–376.

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

Fu, J. (2000). Toward the phylogeny of the family Lacertidae—Why 4,708 base pairs of mtDNA sequences cannot draw the picture. *Biol. J. Linn. Soc.* **71:** 203–217.

García-Paris, M., Martin, C., Dorda, J., and Esteban, M. (1989). "Los Anfibios y Reptiles de Madrid," Ministerio de Agricultura, Pesca y Alimentación de Madrid, Madrid.

Guillaume, C.-P. (1987). "Les Petits Lacertidés du Bassin Mediterranéen Occidental (Genera *Podarcis* et *Archaeolacerta* Essentiellement)," Doctoral thesis, Univ. Sciences et Techniques du Languedoc, Montpellier.

Harris, D. J. (2001). Re-evaluation of 16S ribosomal RNA variation in *Bufo* (Anura: Amphibia). *Mol. Phylogenet. Evol.* **19:** 326–329.

Harris, D. J. (2002). Reassessment of comparative genetic distance in reptiles from the mitochondrial cytochrome *b* gene. *Herpetol. J.*, in press.

Harris, D. J., Arnold, E. N., and Thomas R. H. (1998). Relationships of the lacertid lizards (Reptilia: Lacertidae) estimated from mitochondrial DNA sequences and morphology. *Proc. R. Soc. Lond. B.* **265:** 1939–1948.

Harris, D. J., and Arnold, E. N. (1999). Relationships and evolution of wall lizards, *Podarcis* (Reptilia, Lacertidae) based on partial mitochondrial DNA sequences. *Copeia* **3:** 749–754.

Harris, D. J., and Crandall, K. A. (2000). Intragenomic variation within ITS1 and ITS2 of crayfish (Decapoda, Cambaridae): Implications for phylogenetic and microsatellite studies. *Mol. Biol. Evol.* **17:** 284–291.

Harris, D. J., and Sá-Sousa, P. (2001). Species distinction and relationships of the western Iberian *Podarcis* lizards (Reptilia, Lacertidae) based on morphology and mitochondrial DNA sequences. *Herpetol. J.*, in press.

Hendry, A. P., Vamosi, S. M., Latham, S. J., Heilbuth, J. C., and Day, T. (2000). Questioning species realities. *Cons. Genet.* **1:** 67–76.

Hewitt, G. M. (2000). The genetic legacy of the Quaternary ice ages. *Nature* **405:** 907–913.

Hulsenbeck, J. P., and Crandall, K. A. (1997). Phylogeny estimation and hypothesis testing using maximum likelihood. *Annu. Rev. Ecol. Syst.* **28:** 437–466.

Kocher, T. D., Thomas, W. K., Meyer, A., Edwards, S. V., Pääbo, S., Villablanca, F. X., and Wilson, A. C. (1989). Dynamics of mitochondrial evolution in animals: Amplification and sequencing with conserved primers. *Proc. Natl. Acad. Sci. USA* **86:** 6196–6200.

Malkmus, R., and Schwarzer, U. (2000). Die verbreitung der amphibien und retilien in Südwest-Portugal. *Zeitschrift für Feldherpetologie* **7(112)** 37–75.

Nielsen, K. K., and Arctander, P. (2001). Recombination among multiple mitochondrial pseudogenes from a passerine genus. *Mol. Phylogenet. Evol.* **18:** 362–369.

Oliverio, M., Bologna, M. A., Monciotti, A., Annesi, F., and Mariottini, P. (1998). Molecular phylogenetics of the Italian *Podarcis* lizards (Reptilia, Lacertidae). *Ital. J. Zool.* **65:** 315–324.

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

Pennisi, E. (2001). Linnaeus's last stand? *Science* **291:** 2304–2307.

Pinho, C., and Ferrand, N. (2001). Differentiation within and between forms of *Podarcis* in the Iberian Peninsula: Evidence from protein electrophoretic data. *In* "Abstracts of the Fourth Symposium on Lacertids of the Mediterranean Basin, Minorca, Govern de les Illes Balearg, Minorca."

Posada, D., and Crandall, K. A. (1998). Modeltest: Testing the model of DNA substitution. *Bioinformatics* **14:** 817–818.

Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989). "Molecular Cloning: A Laboratory Manual," Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Sá-Sousa, P. (1998). Distribución de la lagartija *Podarcis b. bocagei* en Portugal. *Bol. Asoc. Herpetol. Esp.* **9:** 2–4.

Sá-Sousa, P. (1999). New data on the distribution of *Podarcis bocagei carbonelli* Pérez-Mellado, 1981 in Portugal. *Herpetozoa* **12:** 87–90.

Sá-Sousa, P. (2000). A predictive distribution model for the Iberian wall lizard (*Podarcis hispanicus*) in Portugal. *Herpetol. J.* **10:** 1–11.

Sá-Sousa, P., Almeida, A. P., Rosa, H., Vicente, L., and Crespo, E. G. (2000). Genetic and morphological relationships of the Berlenga wall lizards (*Podarcis bocagei berlengensis*: Lacertidae). *J. Zool. Syst. Evol. Res.* **38:** 95–102.

Schulte, J. A., II, Macey, J. R., Larson, A., and Papenfuss, T. J. (1998). Molecular tests of phylogenetic taxonomies: A general procedure and example using four subfamilies of the lizard family lguanidae. *Mol. Phylogenet. Evol.* **10:** 367–376.

Shimodaira, H., and Hasegawa, M. (1999). Multiple comparisons of log-likelihoods with applications to phylogenetic inference. *Mol. Biol. Evol.* **13:** 964–969.

Smith, A. G., Smith, D. G., and Funnell, B. M. (1994). "Atlas of Mesozoic and Cenozoic Coastlines," Cambridge Univ. Press, Cambridge, UK.

Swofford, D. L. (2001). PAUP*: Phylogenetic Analysis Using Parsimony (and other methods) 4.0.b5. Sinauer, Sunderland, MA.

Thompson, J. D., Higgins, D. G., and Gibson, T. J. (1994). Clustal W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice. *Nucleic Acids Res.* **22:** 4673–4680.

Weisrock, D. W., Macey, J. R., Ugurtas, I. H., Larson, A., and Papenfuss, T. J. (2001). Molecular phylogenetics and historical biogeography among salamandrids of the "true" salamander clade: Rapid branching of numerous highly divergent lineages in *Mertensiella luschani* associated with the rise of Anatolia. *Mol. Phylogenet. Evol.* **18:** 434–448.

Yang, Z. (1994). Maximum likelihood phylogenetic estimation from DNA sequences with variable rates over sites: approximate methods. *J. Mol. Evol.* **39:** 306–314.