SPECIES DISTINCTION AND RELATIONSHIPS OF THE WESTERN IBERIAN 
PODARCIS LIZARDS (REPTILIA, LACERTIDAE) BASED ON MORPHOLOGY 
AND MITOCHONDRIAL DNA SEQUENCES

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Wall lizards (Podarcis) are the dominant reptile group across most of southern Europe. Their taxonomy is complex because most species exhibit substantial intraspecific morphological polymorphisms. We have estimated the phylogeny of the particularly diverse western Iberian forms using partial cytochrome oxidase and cytochrome b mitochondrial DNA sequence data and have compared this against morphological variation. Of the two currently recognized species in the area—Podarcis hispanica and P. bocagei—neither is monophyletic, and extremely high genetic diversity between newly identified forms (up to 15% cytochrome b divergences) indicates that both are species complexes. Podarcis b. bocagei is genetically distinct from P. (b.) carbonelli which appears to be a separate species using both mtDNA and protein electrophoretic data. The insular form previously assigned to P. b. berlengensis, and sometimes argued to deserve species status is not genetically distinct from P. (b.) carbonelli using the mtDNA sequences. P. hispanica can be separated into at least four highly divergent groups, two in western Iberia, one in eastern Iberia and one in North Africa.

Key words: phylogeny, cytochrome b, cytochrome oxidase, morphology, Iberian lizards

INTRODUCTION

On the Iberian Peninsula three species of insectivorous wall lizards have been recognized—the Iberian wall lizard, Podarcis hispanica Steindachner 1870; Bocage’s wall lizard, Podarcis bocagei Seoane 1884; and Podarcis muraL Laurenti 1768. Podarcis bocagei and P. hispanica live in sympathy in large areas of NW Iberia (Galán, 1986; Pérez-Mellado & Galindo, 1986; Sá-Sousa, 1995a). Both exhibit pronounced sexual dimorphism, adult males being larger than females and having more intense colour patterns (Galán, 1986; Pérez-Mellado & Galindo, 1986).

Podarcis hispanica is a medium sized (SVL 65-70 mm), morphologically variable rock-dwelling lizard, which is found in SW France (Langedoc-Rousillon and Cévennes), the Iberian Peninsula (except the northernmost corner) and NW Africa (Galán, 1986; Guillaume, 1987, 1997). Despite taxonomic controversy about P. hispanica subspecies, two forms have been recognized in Portugal (Sá-Sousa 1995a, 2000a). First, there is a NW Iberian form (P. hispanica type 1) that resembles the “lusitanica” form of Guillaume (1987). P. hispanica type 1 is found in Galicia, in the ‘Submeseta Norte’ plateau, in the northern half of Portugal and on the ‘Sistema Central’ mountain range (Sá-Sousa, 2000a). In Portugal, P. hispanica type 1 seems to inhabit mainly highlands (>400 m) where either Atlantic or continental climatic conditions may prevail. It has been found in the northern part of Portugal, north of the Tagus river (Sá-Sousa, 2000a). P. hispanica type 1 has the following characteristics: flattened head and body; either reticulated or striped dark dorsal patterns; and whitish-pearly coloured belly (for details see Pérez-Mellado, 1981a,b; Galán, 1986; Pérez-Mellado and Galindo, 1986; Guillaume, 1987; Sá-Sousa, 1995a).

P. hispanica type 2 (SW Iberian form) has been found in Andalusia, in Extremadura, in the Madrid region, and in the western and southern parts of Portugal (Sá-Sousa, 2000a). In Portugal, P. hispanica type 2 seems to prefer lowlands (<400 m) with a Mediterranean climate (Sá-Sousa, 2000a). This form has the following characteristics: head and body moderately robust; green and/or light brown patterns, and yellow-orange belly (see Klemmer, 1957; Salvador, 1986; Guillaume, 1987; González de la Vega, 1989; Sá-Sousa, 1995a). Given several records of P. hispanica vaucheri in SW Iberia (Boulenger, 1905; Klemmer, 1957; Salvador, 1974, 1986; Busack, 1986; Guillaume, 1987) one might hypothesize that morphologically P. hispanica type 2 and P. hispanica vaucheri are the same. However, we retain the P. hispanica type 2 denomination until further studies confirm whether similarity in phenotype corresponds to a common genotype across the entire range. So far all allopatric greenish morphotypes of wall lizard found in the southern part of Iberia (e.g. southern Portugal, Andalusia and Levant) have been considered as P. h. hispanica (Pérez-Mellado, 1998). Also P. hispanica type 2 is often mistaken for P. b. bocagei, because of their green or light-brown chromatic patterns, although the species show several differences and have allopatric distributions (Sá-Sousa, 1995a, 1998, 2000a).

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**Podarcis b. bocagei** is a medium-sized (adult snout-vent length, SVL=65-70 mm), ground-dwelling lizard, the males of which show green dorsal patterns, while females are brown with a pair of green stripes (Galán, 2000; Pérez-Mellado, 1981a,b, 1986; Sá-Sousa, 1995a). This species is an Iberian-Atlantic endemic occurring in W Asturias, Cantabria, Galicia and north of Portugal (Galán, 1986, 1997; Sá-Sousa, 1998). On a coarse scale, the distribution of *P. b. bocagei* can be largely explained by macroenvironmental variables and type of climate (Sá-Sousa, 2000b).

It has been suggested that *P. b. carbonelli* Pérez-Mellado 1981 merits species distinction (Sá-Sousa, in prep.; Sá-Sousa et al. 2000). *P. b. carbonelli* (SVL=50-55 mm) is a small, green ground-dwelling lizard, initially thought to be restricted to the W Sistema Central range (Pérez-Mellado, 1981a,b, 1986). However, it has been found in other mountain systems as well as along the Atlantic lowlands, particularly in Portugal (Magraner, 1986; Sá-Sousa, 1995a, 1999, 2000b).

There, the type of climate – but also the balance between the number of frost days per year and the degree of aridity – appear important to explain the distribution of *P. b. carbonelli* (Sá-Sousa, in prep.).

Morphological characters and the existence of parapatric zones of contact between different types (i.e. without interbreeding) suggested that *P. bocagei* and *P. hispanica* might in fact be species complexes (Sá-Sousa, 2000a and in prep.). To resolve relationships between and within these groups, and to determine whether recognized clades based on morphological characters are genetically distinct, a phylogenetic analysis was conducted using DNA sequences derived from two mitochondrial genes, cytochrome b and cytochrome oxidase I. Populations across the range of the accepted subspecies of *P. bocagei* were sampled. For the cytochrome b data sets previously published sequences of *P. hispanica* from eastern Spain and Morocco (Castilla et al., 1998; Harris & Arnold, 1999) were included in the analyses.

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**TABLE 1.** List of lizards examined in the mtDNA phylogeny. * indicates previously published data was included in the analysis. Sequences are deposited in Genbank (accession numbers AF372051 to AF372089). Map codes are shown in Fig. 1.

<table>
<thead>
<tr>
<th>Species</th>
<th>mtDNA Code</th>
<th>Locality</th>
<th>COI/ Cyt b</th>
<th>Map Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gallotia galloti</td>
<td></td>
<td>Gran Canaria</td>
<td>ipe</td>
<td>B</td>
</tr>
<tr>
<td>Lacerta dugesii dugesii</td>
<td></td>
<td>Madeira</td>
<td>1/*</td>
<td></td>
</tr>
<tr>
<td>Lacerta perspicillata</td>
<td></td>
<td>Mallorca</td>
<td>1/*</td>
<td></td>
</tr>
<tr>
<td>Podarcis hispanica ‘litoilepis’</td>
<td></td>
<td>Castellón, Spain</td>
<td>1/*</td>
<td></td>
</tr>
<tr>
<td>Podarcis hispanica “type 1”</td>
<td>P. h.1</td>
<td>Vila Real, Pt.</td>
<td>1/1</td>
<td>B</td>
</tr>
<tr>
<td></td>
<td>P. h.2</td>
<td>Montesinho, Pt.</td>
<td>1/*</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>P. h.3</td>
<td>Montesinho, Pt.</td>
<td>1/*</td>
<td>A</td>
</tr>
<tr>
<td>Podarcis hispanica “Moroccan”</td>
<td>P. h.m1</td>
<td>High Atlas, Morocco</td>
<td>1/*</td>
<td>T</td>
</tr>
<tr>
<td></td>
<td>P. h.m2</td>
<td>High Atlas, Morocco</td>
<td>1/*</td>
<td>T</td>
</tr>
<tr>
<td></td>
<td>P. h.m3</td>
<td>High Atlas, Morocco</td>
<td>1/*</td>
<td>T</td>
</tr>
<tr>
<td>Podarcis hispanica “type 2”</td>
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<td>Leiria, Pt.</td>
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<td>C</td>
</tr>
<tr>
<td></td>
<td>P. h.v2</td>
<td>Portalegre, Pt.</td>
<td>1/*</td>
<td>D</td>
</tr>
<tr>
<td></td>
<td>P. h.v3</td>
<td>Beja, Pt.</td>
<td>1/*</td>
<td>E</td>
</tr>
<tr>
<td></td>
<td>P. h.v4</td>
<td>Marvao, Pt.</td>
<td>1/*</td>
<td>F</td>
</tr>
<tr>
<td></td>
<td>P. h.v5</td>
<td>Águeda, Pt.</td>
<td>1/*</td>
<td>G</td>
</tr>
<tr>
<td>Podarcis bocagei bocagei</td>
<td>P. b.1</td>
<td>Montesinho, Pt.</td>
<td>1/*</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>P. b.2</td>
<td>Montesinho, Pt.</td>
<td>1/*</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>P. b.3</td>
<td>Vila Pouca d Aguiar, Pt.</td>
<td>1/*</td>
<td>K</td>
</tr>
<tr>
<td></td>
<td>P. b.4</td>
<td>Serra do Gerês, Pt.</td>
<td>1/*</td>
<td>H</td>
</tr>
<tr>
<td></td>
<td>P. b.5</td>
<td>Vairão, Pt.</td>
<td>1/*</td>
<td>J</td>
</tr>
<tr>
<td></td>
<td>P. b.6</td>
<td>Vairão, Pt.</td>
<td>1/*</td>
<td>K</td>
</tr>
<tr>
<td></td>
<td>P. b.7</td>
<td>Vairão, Pt.</td>
<td>1/*</td>
<td>J</td>
</tr>
<tr>
<td></td>
<td>P. b.8</td>
<td>Braga, Pt.</td>
<td>1/*</td>
<td>I</td>
</tr>
<tr>
<td></td>
<td>P. b.9</td>
<td>Viana do Castelo, Pt.</td>
<td>1/*</td>
<td>L</td>
</tr>
<tr>
<td></td>
<td>P. b.10</td>
<td>Viana do Castelo, Pt.</td>
<td>1/*</td>
<td>L</td>
</tr>
<tr>
<td></td>
<td>P. b.11</td>
<td>Viana do Castelo, Pt.</td>
<td>1/*</td>
<td>L</td>
</tr>
<tr>
<td>Podarcis carbonelli carbonelli</td>
<td>P. c.1</td>
<td>Serra da Estrela, Pt.</td>
<td>1/*</td>
<td>M</td>
</tr>
<tr>
<td></td>
<td>P. c.2</td>
<td>Torreira, Aveiro, Pt.</td>
<td>1/*</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td>P. c.3</td>
<td>Monte Clérigo, Pt.</td>
<td>1/*</td>
<td>Q</td>
</tr>
<tr>
<td></td>
<td>P. c.4</td>
<td>Peniche, Pt.</td>
<td>1/*</td>
<td>P</td>
</tr>
<tr>
<td>Podarcis carbonelli beriengensis</td>
<td>P. c.b</td>
<td>Berlenga isle, off Peniche</td>
<td>1/*</td>
<td>O</td>
</tr>
</tbody>
</table>
amplified regions of approximately 350 bp and 550 bp respectively. Thermocycling consisted of 30 cycles of 93°C for 30 secs, 55°C for 1 min and 72°C for 1 min, followed by a single cycle at 72°C for 5 min. Successful PCR bands were purified using a QIAEX II kit (Quiagen) and sequenced on an Applied Biosystems Model 373A DNA Sequencing System, using a PRISM Ready Reaction DyeDeoxy Terminator Cycle Sequencing kit. Centrisep spin columns (Princeton Separations Inc.) were used for excess dye extraction.

**PHYLOGENETIC ANALYSES**

Sequences were aligned using Clustal W (Thompson et al., 1994). There were no insertions or deletions. They were then imported into PAUP* (Swofford, 2001) for phylogenetic analyses. When estimating phylogenetic relationships among sequences, one assumes a model of evolution regardless of the optimality criteria employed. Determining which model to use given one’s data is a statistical problem (Goldman, 1993). We used the approach outlined by Huelsenbeck and Crandall (1997) to test alternative models of evolution, employing PAUP* and Modeltest (Posada & Crandall, 1998). A starting tree was obtained using neighbour-joining. With this tree, likelihood scores were calculated for various models of evolution and compared using a chi-square test, with degrees of freedom equal to the difference in free parameters between the models being tested. The null hypotheses tested in this way included: (1) nucleotide frequencies are equal; (2) transition rates are equal to transversion rates; (3) transition rates are equal,; (4) transversion rates are equal; (5) rate homogeneity within the data set; and (5) no significant proportion of invariable sites (Table 2). Once a model of evolution was chosen, it was used to estimate a tree using maximum likelihood. Ten replicate heuristic searches were made with random sequence addition. Confidence in resulting nodes was assessed using the bootstrap technique (Felsenstein, 1985) with 1000 replicates. Genes were analysed separately and in combination.

**BIOMETRICS**

Eleven biometric variables were obtained from 12 females and 24 males from each of 20 populations (exact localities available on request), using 0.05 mm callipers (see procedure in Pérez-Mellado & Gosá, 1988): (1) snout-vent length; (2) head length; (3) head width; (4) inter-orbital width; (5) frontal width; (6) inter-nasal width; (7) head depth; (8) orbital depth; (9) frontoparietal depth; (10) nasal depth; and (11) hind limb length (Fig. 2). Sexes were analysed separately. Squared Mahalanobis distance between centroids was used since it takes into account the correlations among biometric variables and is independent of the relative scales of the various variables (Legendre & Legendre 1998). UPGMA clustering was applied to the distance matrix to assess the lizard phenetic relationships (Rohlf, 1993; Sokal & Rohlf, 1995).
TABLE 2. Tests of hypotheses relating to the model of evolution appropriate for phylogeny reconstruction (Huelsenbeck & Crandall, 1997). P-values were obtained with ModelTest (Posada & Crandall, 1998). For each hypothesis the data set with cytochrome oxidase (top), cytochrome b (middle) and then with the combined regions (below) is tested. Due to the performance of multiple tests, the significance level of rejection of the null hypothesis was adjusted via the Bonferroni correction to α=0.01.

<table>
<thead>
<tr>
<th>Null hypothesis</th>
<th>Models compared</th>
<th>-lnL0</th>
<th>-lnL1</th>
<th>df</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Equal nucleotide frequencies</td>
<td>H0: JC69, H1: F81</td>
<td>2035</td>
<td>2009</td>
<td>3</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1558</td>
<td>1509</td>
<td></td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2843</td>
<td>2822</td>
<td></td>
<td>0.000</td>
</tr>
<tr>
<td>Equal ti and tv rates</td>
<td>H0: F81, H1: HKY85</td>
<td>2009</td>
<td>1907</td>
<td>1</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1509</td>
<td>1439</td>
<td></td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2822</td>
<td>2684</td>
<td></td>
<td>0.000</td>
</tr>
<tr>
<td>Equal ti rates</td>
<td>H0: HKY85, H1: TrN</td>
<td>1907</td>
<td>189</td>
<td>1</td>
<td>0.000</td>
</tr>
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<td></td>
<td></td>
<td>1439</td>
<td>1425</td>
<td></td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2684</td>
<td>2684</td>
<td></td>
<td>0.581</td>
</tr>
<tr>
<td>Equal tv rates</td>
<td>H0: TrN, H1: TIM</td>
<td>1896</td>
<td>1896</td>
<td>1</td>
<td>0.275</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1425</td>
<td>1425</td>
<td></td>
<td>0.322</td>
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<td></td>
<td>H0: HKY85, H1: K81uf</td>
<td>2684</td>
<td>2684</td>
<td>1</td>
<td>0.380</td>
</tr>
<tr>
<td>Equal rates among sites</td>
<td>H0: TrN, H1: TrN+G</td>
<td>1896</td>
<td>1817</td>
<td>1</td>
<td>0.000</td>
</tr>
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<td></td>
<td></td>
<td>1425</td>
<td>1357</td>
<td></td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>H0: HKY85, H1: HKY85+G</td>
<td>2684</td>
<td>2591</td>
<td>1</td>
<td>0.000</td>
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<tr>
<td>Proportion of invariable sites</td>
<td>H0: TrN+G, H1: TrN+G+i</td>
<td>1817</td>
<td>1817</td>
<td>1</td>
<td>0.999</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1357</td>
<td>1355</td>
<td></td>
<td>0.057</td>
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<tr>
<td></td>
<td>H0: HKY85+G, H1: HKY85+G+i</td>
<td>2591</td>
<td>2589</td>
<td>1</td>
<td>0.032</td>
</tr>
</tbody>
</table>

RESULTS

Twenty-six individuals from 15 populations of *P. hispanica* or *P. bocagei* were sequenced for the cytochrome oxidase gene. The closely related *L. dugesii* (Harris et al., 1998) was sequenced as an outgroup, and the sequence from the more distantly related *Gallotia galloti* was also included in the analyses. The most appropriate model of evolution for this data set was the Tamura-Nei model (TrN) model with a discrete approximation of a gamma distribution of variable sites (base frequencies A: 0.31, C: 0.16, G: 0.24, T: 0.29, equal transversion ratios and A/G 13.8, C/T 8.3, gamma shape parameter 0.18 - Table 2). Using this model, a ten replicate heuristic search found a single most likely tree, with a log likelihood of -1811 (Fig. 3A). Twelve individuals from nine populations were also sequenced for the cytochrome b gene. This was combined with four previously published sequences for *P. hispanica* (Harris & Arnold, 1999), and two additional outgroups, *Lacerta dugesii* and *L. perspicillata*. For this data set the most appropriate model of evolution was again the TrN model with a discrete approximation of a gamma distribution of variable sites (base frequencies A: 0.26, C: 0.29, G: 0.13, T: 0.32, equal transversion ratios and A/G 3.5, C/T 9.7, gamma shape parameter 0.26 - Table 2). Using this model, a ten replicate heuristic search found a single most likely tree, with a log likelihood of -1348 (Fig. 3B). Since both genes are mitochondrial and therefore inherited as a single locus, a combined analysis was also carried out. *L. dugesii* was used as the outgroup, and 12 individuals of *P. hispanica* or *P. bocagei* were included. For this data set the most appropriate model of evolution was the HKY model (transition/transversion ratio of 4.99) with a discrete approximation of a gamma distribution of variable sites (Table 2). Using this model, a ten replicate heuristic search found a single most likely tree, with a likelihood of -2586 (Fig. 3C). This ML-based hypothesis of relationships was compared against two alternatives, with *P. bocagei* monophyletic, and *P. hispanica* monophyletic respectively, and other relationships unrestrained. Using the same model of evolution, ten replicate heuristic searches found short-
FIG. 3. A, maximum likelihood tree derived from cytochrome oxidase sequences. The tree was rooted using Gallotia galloti and Lacerta dugesii as outgroups. Numbers above nodes indicate bootstrap support (1000 replicates). B, maximum likelihood tree derived from cytochrome b sequences. The tree was rooted using previously published sequences of L. perspicillata and L. dugesii sequences as outgroups. Numbers above nodes indicate bootstrap support (1000 replicates). C, maximum likelihood tree derived from combined cytochrome b and cytochrome oxidase sequences. The tree was rooted using L. dugesii as an outgroup. Numbers above nodes indicate bootstrap support.
TABLE 3. Maximum-Likelihood Tests (Shimodaira & Hasegawa, 1999) of alternative tree topologies for Podarcis lizards. Trees compared were the maximum likelihood tree based on the combined DNA sequence data (Fig. 2C), and those based on alternative hypotheses where either Podarcis bocagei or Podarcis hispanica are monophyletic. *P is the probability of obtaining a more extreme t-value under the null hypothesis of no difference between trees. Both these hypotheses show significantly decreased fit relative to the maximum likelihood tree.

<table>
<thead>
<tr>
<th>Tree</th>
<th>Log likelihood</th>
<th>Δ Log likelihood</th>
<th>*P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Max. likelihood tree</td>
<td>-2586</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monophyletic P. bocagei</td>
<td>-2598</td>
<td>12</td>
<td>0.029</td>
</tr>
<tr>
<td>Monophyletic P. hispanica</td>
<td>-2599</td>
<td>13</td>
<td>0.021</td>
</tr>
</tbody>
</table>

FIG. 4. UPGMA cluster analysis of the biometric variables. Included are P. hispanica type 1 (P.h. 1) and type 2 (P.h. 2), P. bocagei (P.b.) and P. (b.) carbonelli (P.c.). Separation of the four forms is evident in the females, but less so in the males. The trees of -ln 2599 and -ln 2598 respectively. These were compared against the ML tree with the likelihood variance test of Shimodaira and Hasegawa (1999) using 1000 RELL bootstraps. Both were significantly less likely (Table 3).

The UPGMA trees derived from the morphological data show that each major cluster corresponds to one of the four forms of wall lizard (Fig. 4). Clear separation is found in females, while some populations of males belonging to one group cluster with other forms. P. bocagei bocagei clusters with P. hispanica type 2; both grouped in the next step with P. hispanica 1; and finally, P. bocagei carbonelli is the most dissimilar.

DISCUSSION

The reciprocal monophyly of the designated subspecies P. b. bocagei and P. (b.) carbonelli, is strongly supported by the mtDNA analysis. Specimens from the ranges form monophyletic groups in all analyses and bootstrap support is strong, especially in the combined analysis - 99% for P. (b.) carbonelli, 100% for P. b. bocagei. The degree of genetic differentiation between P. b. bocagei and P. (b.) carbonelli is high: 9.9-6.6% between the COI sequences and 13.5-15.5% between the cytochrome b sequences. The mean cytochrome b genetic distance for congeneric reptile species is 13.6% (Harris, in press), and lower levels of COI divergence in the iguanian lizards of the genus Tropidurus have been used to recommend species candidates (Frost et al., 1998). Further, within these two groups genetic distances are very low - 0-0.06% within P. (b.) bocagei and 0.004-0.09% within P. (b.) carbonelli. Maintenance of the present taxonomic system is further complicated by the rejection of P. bocagei monophyly using both morphological and molecular data. We recommend raising P. (b.) carbonelli to species status, following Sá Sousa et al. (2000) using protein electrophoretic data.

P. b. berlengensis shows no genetic differentiation from mainland P. carbonelli using the COI sequence data, and so should be referred to as P. c. berlengensis. This has previously been suggested based on the low genetic distance found between these groups, D=0.08 (Sá Sousa et al., 2000). P. c. berlengensis does show some distinct morphological features primarily associated with an increased mean body size (Vicente, 1985). A similar case has been shown for Gallotia simonyi simonyi and G. s. machadoi, where an extinct subspecies from a small island showed no difference in mtDNA sequences from the mainland form, despite morphological differences (Carranza et al., 1999). It is, however, markedly different from the example of Podarcis atrata from the Columbretes Islands, where inter-island cytochrome b divergence is high (Castilla et al., 1998). Much has been made of the expected decrease in genetic diversity of organisms on small islands, and the associated increased risks of extinction. Given the large numbers of insular subspecies of Podarcis lizards (nearly 300; Böhme, 1986), it is important to determine which of these phenomena is more common.

Subspecies-level taxonomy within P. hispanica has been controversial. Some authors accept one subspecies in Iberia, P. h. hispanica and one in North Africa, P. h. vaucheri (e.g. Pérez-Mellado, 1986, 1998). Others argue that P. h. vaucheri is also found in the southern Iberian Peninsula, and more separate forms within the Iberian Peninsula are to be recognized, though as yet with undetermined taxonomic status (e.g. Guillaume, 1987, 1997). Electrophoretic analyses have given conflicting results - Busack (1986) found a low genetic distance (D=0.07) between Andalusian and Moroccan populations of P. hispanica, while Capula (1997) suggests they are well differentiated (D=0.237), and could represent sibling species.

Our analyses support the conclusions of Sá-Sousa (2000a) that P. hispanica in Portugal is composed of
two genetically distinct clades. However the southern form (either *P. hispanica* type 2 or *P. hispanica vaucheri*) is also distinct from the population sampled from Morocco. Whether there are multiple cryptic African species, as has been suggested – based on immunological data (Joger & Bischoff, 1989) cannot be assessed from the present data. It is clear, however, that *P. hispanica* taxonomy needs to be reassessed – our data indicate that *P. hispanica* is made up of multiple genetically distinct clades that do not form a monophyletic group relative to *P. bocagei* and *P. carbonelli*. Additional data from nuclear loci will be needed to confirm this finding based on mtDNA, and to determine whether introgression occurs. Only extensive sampling across the remainder of the range, especially central and eastern Spain and North Africa will allow a more appropriate assessment of the status of these clades.

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