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Genetic and morphological relationships of the Berlenga wall lizard (*Podarcis bocagei berlengensis*: Lacertidae)

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Abstract

Six biometric characters and 15 electrophoretic loci of three known subspecies of *Podarcis bocagei* were studied. Contrary to biometric indications, *Podarcis bocagei berlengensis* showed a closer genetic relationship with *Podarcis bocagei carbonelli* than with the nominal subspecies. The biometric results were confounded by the relative large size of *P. b. berlengensis*. Post-glacial colonization from two different areas might explain the greater genetic differentiation between the *P. b. bocagei* and *P. b. carbonelli* populations (Nei's $D = 0.158 =$ near the species threshold). It might also explain the known colonization of these two lizards through the islands off the west coast of the Iberian peninsula and their present distributions to the north of (Galicia; Northern Portugal) and to the south of the valley of river Douro (central Portugal, western and Sistema Central), respectively.

Key words: Wall lizard – speciation – biometric data – allozymes

Introduction

The Bocage wall lizard *Podarcis bocagei* Seoane 1884 occurs where Atlantic-type conditions prevail. It is an Iberian–Atlantic endemic species that probably originated in the north-western corner of Iberia (Galán 1986, 1997; Pérez-Mellado 1997). It is a ground-dwelling, insectivorous lizard, whose males show dark green, reticulated dorsal patterns, whereas the females are brown with a pair of green stripes (Pérez-Mellado 1981a, 1986). The systematics of the lizard have been revised taking only its morphology into account (e.g. Pérez-Mellado 1981b; Galán 1985, 1986; Pérez-Mellado and Galindo 1986). Until now three subspecies have been recognized (see Table 1). First, the nominal subspecies *Podarcis bocagei bocagei* is distributed in north-western Iberia, e.g. western Asturias, Cantabria, Galicia and the north of Portugal, where it occurs in sympatry with the Iberian wall lizard *Podarcis hispanicus* (Galán 1986; Sá-Sousa 1998). Second, there is the subspecies *Podarcis bocagei carbonelli* Pérez-Mellado 1981 which was initially thought to be restricted to the mountain range of western 'Sistema Central' (Pérez-Mellado 1981b, 1983, 1984, 1986). However, *P. b. carbonelli*, has also been found in other mountains and along the Atlantic coastal lowlands, particularly in Portugal (Magraner 1986; Sá-Sousa 1995, 1999). Third, there is the insular subspecies, *Podarcis bocagei berlengensis* Vicente 1985, which is described as a relative large form that is related to the populations of *P. bocagei* of NW Spain (Galicia), and is restricted to the Berlengas islands off the western coast of Portugal (Vicente 1985). This subspecies has been also found near the coast in the adjacent (pen)insula of Peniche (Sá-Sousa 1992), a fact that is consistent with the low genetic differentiation found between lizards from Berlenga and from Peniche (Almeida et al. (1992).

Electrophoretic investigations of various Lacertidae, have frequently suggested a reduction in the number of subspecies (Cirer and Guillaume 1986; Ramón et al. 1991), and clarified the relationships within some complex of species (Fu et al. 1995; MacCulloch et al. 1995; Bobyn et al. 1996). This technique can also be used to calculate estimates for evolutionary divergence (Gorman et al. 1975; Guillaume and Lanza 1982; Mayer and Tiedemann 1982; Busack 1986) or to demonstrate that some populations are less related to each other than previously sup-

posed (Guillaume et al. 1976; Busack 1987; Capula 1994; Mayer and Arribas 1996).

Guillaume and Cirer (1985) suggested that Nei's genetic distances (identities) of a magnitude of $D \leq 0.2$ ($I \geq 0.8$) are compatible with an intraspecific differentiation, whereas $0.3 \leq D \leq 0.5$ ($0.75 \leq I \leq 0.60$) indicates congeneric species, and $D \geq 0.9$ ($I \leq 0.4$) implies that species belong to different genera. Guillaume (1989) generated a chronological calibration of D for lacertids estimating that Nei's $D = 1.0$ is equivalent to 10–13 My ago, taking into account Busack's (1986) data and the age of the opening of the Strait of Gibraltar at 5.5–7.0 My ago.

We studied the differentiation of *P. b. berlengensis* in relation with the other two subspecies of *P. bocagei*, both from a morphological point of view using biometric data and from a genetic point of view using allozyme electrophoresis.

Materials and methods

We collected *P. bocagei* samples from four Portuguese sites within the distribution range of the three known subspecies of this lizard (Fig. 1). *P. b. bocagei* was from Gerês (G), a mountain region in the North of Portugal; *P. b. berlengensis* came from the island of Berlenga Grande (B) – hereafter referred as Berlenga – and from the adjacent coastal area of Peniche (P) and finally, *P. b. carbonelli* came from Cabo Raso (C), a coastal region south of the Berlengas islands. Six biometric characters were measured: SVL snout-vent length, HL head (pileous) length, HW head width, HH head height, FLL forelimb length (from the axillar ring to the end of the fourth finger) and HLL hindlimb length (from the groin ring to the end of the fourth toe).

Keeping the sexes apart, two different statistical analyses allowed the evaluation of biometric data from the samples. Principal component analyses (PCA) were extracted from a standardized product-moment correlated matrix. Squared Mahalanobis distance (MahD2) was also used to obtain biometric matrices, since MahD2 takes the correlations among variables into account and is independent of their scales (Legendre and Legendre 1998). The UPGMA method was used for cluster analysis. Descriptive statistics of samples are shown in Appendix 1.

By allozyme electrophoresis, we examined a total of 15 presumptive loci: 13 loci, encoding 11 enzymes were investigated using 12% starch gel electrophoresis (standard horizontal method), and two other loci (albumin and haemoglobin) were scored through cellulose acetate gel electrophoresis. Both techniques used are described by Pasteur et al. (1987) and Rosa and Oliveira (1994). All protein systems were analysed

Table 1. External characters that distinguish lizards from the three subspecies of *Podarcis bocagei*

Characters	<i>Podarcis bocagei bocagei</i>		<i>Podarcis bocagei carbonelli</i>		<i>Podarcis bocagei berlengensis</i>	
	m	f	m	f	m	f
Body size	great	moderate	small to moderate	small	great	great
Head depth	robust	moderate robust	robust	moderate	robust	moderate robust
Dorsal pattern	striped discontinuous; thick reticulated	striped continuous	thin reticulated; striped discontinuous	striped dis/continuous	striped discontinuous; reticulated	striped dis/continuous
Dark, ventral pigmentation	intense	moderate	low	low	intense	intense
Dorsal colour*	4–6 rows intense green	2–4 rows brown; ocreous green	0–2 rows green; brownish green	0–2 rows brown; ocreous green; yellowish	4–6 rows intense green	4–6 rows brown; ocreous green
Light stripes	green	green	green	green; white; pale yellow	green	green
Flank color	hazel-brown	hazel-brown	intense green	white; ocreous	intense green	brown; ocreous white
Belly color	yellow; orange	yellow; ocreous	white	white; pale yellow	white; nacreous	white
Blue spots*	no	no	yes many	yes moderate	yes many	yes moderate

* during the mating period.

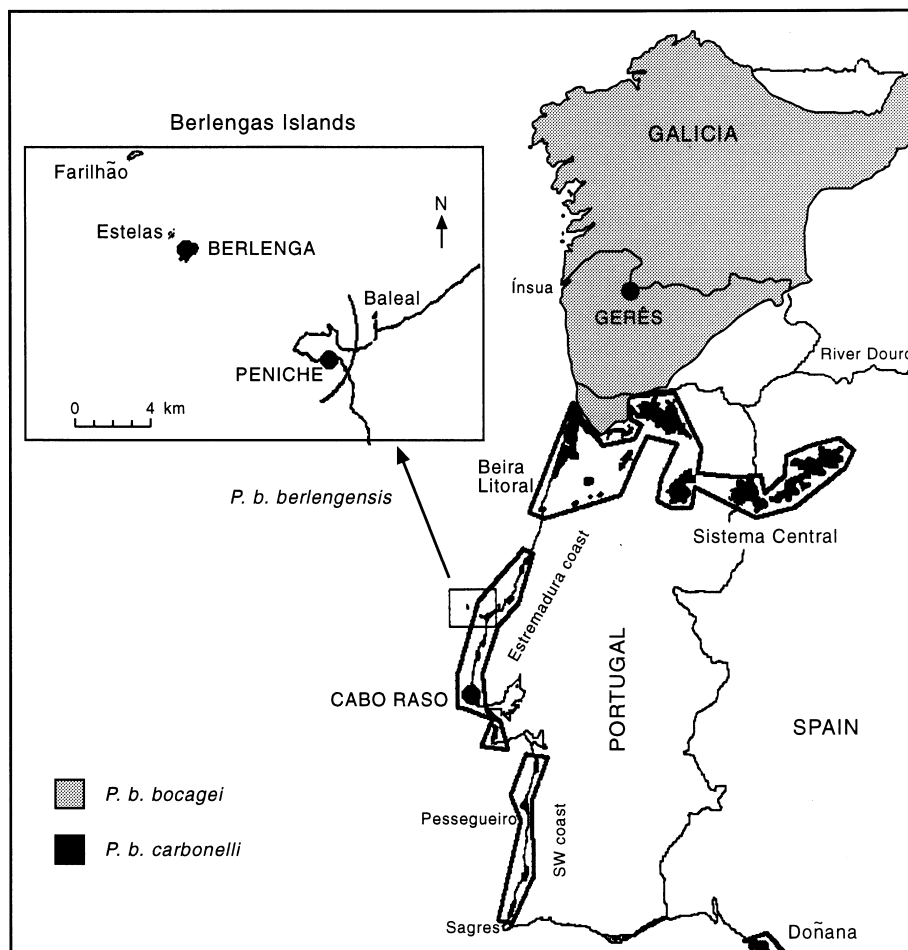


Fig. 1. Known Iberian distribution of the three subspecies of *Podarcis bocagei*: nominal subspecies (light grey shaded), *P. b. carbonelli* (dark grey shaded and border) and *P. b. berlengensis* (amplified window)

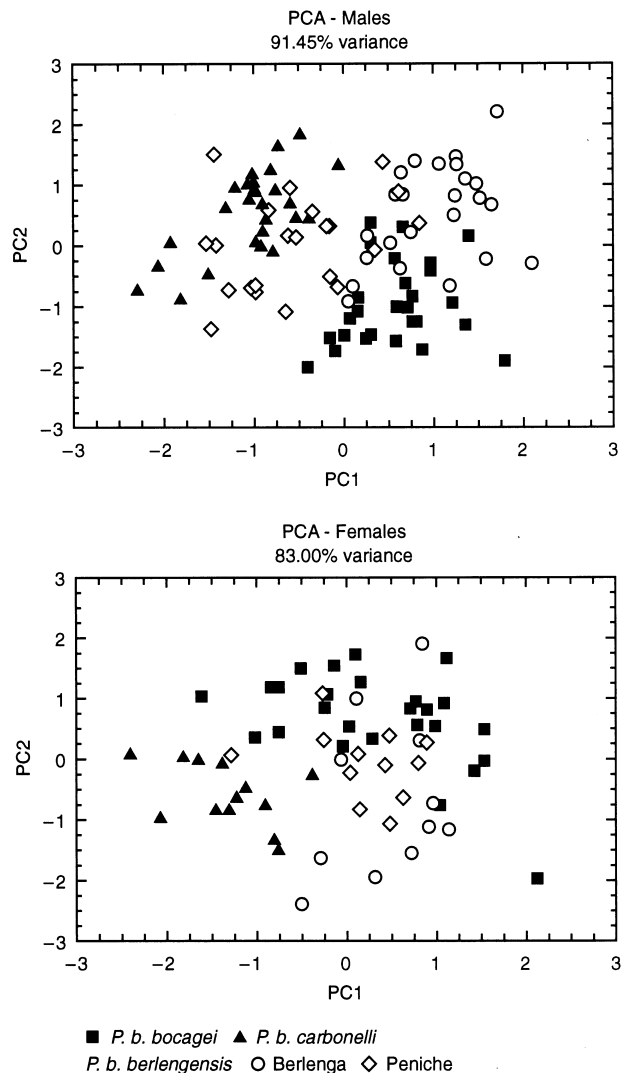


Fig. 2. Graphs of the projections of biometric data from males and females of *Podarcis bocagei* on the two first principal components (PC1 + PC2 = explained variance%): males (77.05 + 14.40 = 91.45%), females (67.17 + 17.83 = 83.00%)

using material from the liver, skeletal muscle, heart or blood, depending on the best results achieved in a pilot study. The enzymes score, the tissues used for the respective enzyme, and electrophoretic conditions applied are presented in Appendix 2. For each protein system encoded by two or more presumptive loci, the isozymes were assigned by numbers starting from the allozyme band with the greatest anodal migration. Alleles of each locus were indicated by numbers proportional to the relative migration of their allozymes, using '100' for the most frequent allele. Genetic variability was measured through mean heterozygosity (unbiased estimate, Nei 1978) and percentage of polymorphic loci, whereas the genetic differentiation was quantified through Nei's (1978) unbiased index D, from which UPGMA phenograms were constructed.

Results

The ordination of centroids of the male and female locality samples of *P. bocagei* in the space of the first two principal components of variation (PC1, PC2), deduced from the six biometric characters, is shown in Fig. 2. The associated factor loadings and cumulative percentages of variance they explain are shown in Table 2. PC1 (77–83%) reflects general 'size' and it is positively (and significantly > 0.70) associated with all

other characters. PC2 (14–18%) reflects intersample variation within *P. bocagei*: a clear separation occurs only for females of *P. b. carbonelli* from the other subspecies, with a large overlap of individual biometric projections, particularly within males (Fig. 2). Squared Mahalanobis distances are shown in Table 3 and their UPGMA phenograms are depicted in Fig. 3. Biometric data showed that males of *P. b. bocagei* and of *P. b. berlangensis* from Berlenga were similar and bigger than those from Peniche/Cabo Raso. For females, there were no significant differences within *P. b. berlangensis* (Berlenga and Peniche), but there was a biometric similarity between the samples of *P. b. bocagei* and *P. b. berlangensis*.

Of the 15 loci analysed, seven (47%) were monomorphic in all samples: AAT-2, AK, CK, LDH-2, MDH-1, PEP, SOD. For the remaining eight loci, at least two different alleles were detected per locus. The respective frequencies are presented in Table 4. No locus was discriminative among the four samples. EST-2 was strongly polymorphic, whereas two loci (MPI, PGM-2) were almost dimorphic in all samples. For two of the polymorphic loci, AAT-1 and PGD, there were two predominant alleles (AAT-110, PGD100) shared by *P. b. berlangensis* (Berlenga and Peniche) and *P. b. carbonelli* (Cabo Raso), whereas the other two alleles (AAT-150, PGD130) were the most frequent in *P. b. bocagei* (Gerês). For the loci LDH-1, ALB and HB, the alleles predominating in *P. b. berlangensis* (LDH-1100, ALB100, HB100) were different from those that occurred most frequent in the mainland subspecies, *P. b. bocagei* (Gerês) and *P. b. carbonelli* (Cabo Raso).

Unique rare alleles occurred at Cabo Raso (AAT-1133, EST-253), Gerês (PGD160) and Peniche (PGM-2139). Conformity to Hardy–Weinberg equilibrium expectations was found for all loci, except for the locus MPI (Cabo Raso and Peniche), for which a reduced number of heterozygotes (chi-square, $p > 0.05$) was obtained. Genetic variability measures are shown in Table 5. Mean heterozygosity was greatest for mainland subspecies *P. b. bocagei* (Gerês) and *P. b. carbonelli* (Cabo Raso) whereas a lower heterozygosity was found for the *P. b. berlangensis* (Berlenga and Peniche). Polymorphism was 1.5 to 2 times greater in mainland samples (Gerês; Cabo Raso) than in the insular ones, using both 99 and 95% criteria. Genetic distances are shown in Table 6 and the UPGMA phenograms deduced are shown in Figure 3. Within *P. b. berlangensis* (Berlenga and Peniche) there was low differentiation (Nei's $D = 0.008–0.011$). Following Guillaume and Cirer (1985) criteria, *P. b. carbonelli* (Cabo Raso) is linked either with *P. b. berlangensis* on a subspecific genetic level (Nei's $D = 0.111$) or with *P. b. bocagei* from Gerês (Nei's $D = 0.158$). Finally, *P. b. berlangensis* reached the species threshold in relations with *P. b. bocagei* (Nei's $D = 0.245–0.250$).

Discussion

Biometric data (PCA, MahD2 clusters) mask the inferred closer genetic relationship between *P. b. berlangensis* and *P. b. carbonelli*, although the morphological resemblance based on external characters (e.g. chromatic patterns, see Table 1) agrees with the genetic data. In contrast to *P. b. bocagei*, both *P. b. berlangensis* and *P. b. carbonelli* show individuals with blue spots on the outer ventral scales and a white belly; the males always have green flanks independently of the greenish to brownish coloration of the mid-back and they share similar thin reticulated patterns; with the exception of the trait 'size', females of both forms also resemble each other in their chromatic patterns (Table 1). Thus, the biometric clusters found

Table 2. Factor loadings and cumulative percents of explained variance for the three first principal components of six biometric characters measured among four samples of *Podarcis bocagei*. Biometric characters are described in the text

Variable	PC1	Males n = 104			Females n = 63		
		PC2	PC3	PC1	PC2	PC3	
SVL	0.9650	-0.0758	-0.0385	0.8692	-0.0174	0.4483	
HL	0.9595	0.0811	-0.0683	0.9048	-0.1951	0.2535	
HW	0.8765	0.2846	-0.3418	0.8133	-0.3866	-0.1818	
HD	0.6891	0.6617	0.2886	0.7444	-0.4632	-0.3515	
FLL	0.8823	-0.3736	0.1635	0.6933	0.6374	-0.2158	
HLL	0.8658	-0.4395	0.0682	0.7998	0.5114	-0.0749	
cum. % expl. variance	77.05	91.45	95.41	65.17	83.00	90.90	

PCA loadings extracted from product-moment correlation matrices.

Table 3. Squared Mahalanobis distance matrix for four samples of *Podarcis bocagei* (males above diagonal, females below diagonal) * not significantly different at $p < 0.05$

Subspecies	Sample	G	B	P	C
<i>P. b. bocagei</i>	Gerês	–	5.084	11.657	23.998
<i>P. b. berlengensis</i>	Berlenga	4.822	–	8.360	16.579
<i>P. b. berlengensis</i>	Peniche	2.786*	1.686*	–	2.901
<i>P. b. carbonelli</i>	Cabo Raso	14.189	11.038	10.527	–

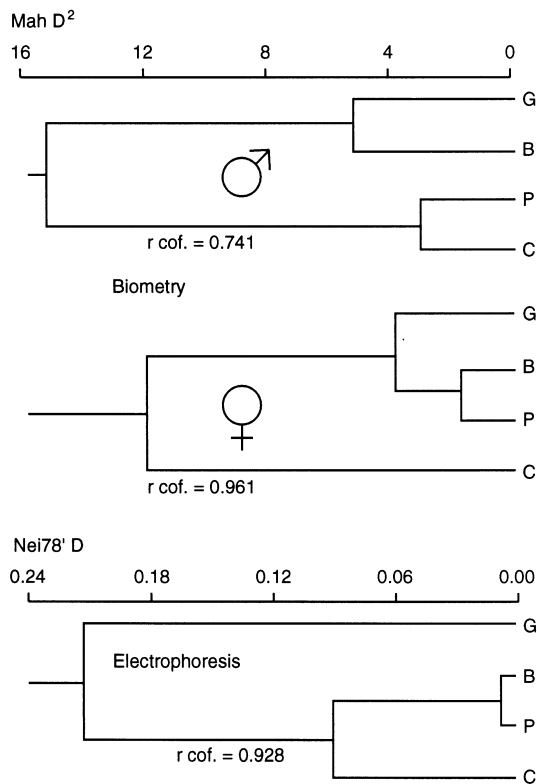


Fig. 3. Distance phenograms resulting from UPGMA clustering based on squared Mahalanobis distance (morphology) and Nei's (1978) unbiased distance (electrophoresis). Letters at the end of branches correspond to abbreviations of sampling sites (see legend in Tables 3,4,5,6). r cof. is the cophenetic correlation

illustrate the relative large size of *P. b. berlengensis*, not in comparison with *P. b. bocagei*, but with *P. b. carbonelli* (see genetic data below). Lizard gigantism is a phenomenon that

is widespread among insular populations of *Podarcis*, e.g. in *Podarcis lilfordi*, *Podarcis muralis*, *Podarcis pityusensis*, *Podarcis tiliguetus* (Cirer 1981, 1997; Salvador 1986; Cheylan 1988; Pérez-Mellado and Salvador 1988; Delauguerre and Cheylan 1992). Lizards tend to show a size increase and a greater morphological homogeneity (by genetic drift) on smaller and older islands (Cheylan 1988; Cirer and Martínez-Rica 1990). The reduction of predator pressure and the simultaneous occurrence of high population density of insular lizards leads to a shortage of resources and the increase of intraspecific competition and sexual dimorphism linked with agonistic behaviour of males, thus creating a trend towards gigantism (Thorpe 1985; Cheylan 1988; Vicente 1989; Cirer and Martínez-Rica 1990; Case and Schwaner 1993). Conversely, we found less overlap of biometric characters in all females, probably reflecting indirectly the selective pattern that prevails in the males of *P. b. berlengensis* (Fig. 2).

Both, the results of Almeida et al. (1992) and of this study show that genetic differentiation among lizards from the Berlengas islands (e.g. Berlenga, Estelas, Farilhão Grande) and from Peniche are low (Nei's $D = 0.008-0.046$). This is in accordance with their taxonomic status pertaining to *P. b. berlengensis*. However, we also found an accidental introduction of *P. b. berlengensis* from Berlenga into Peniche harbour and its surroundings. Thus, a process of intergradation may occur there between the introduced lizards and those from the aboriginal population of Peniche.

The greater value of genetic distance (Nei's $D = 0.158$) between *P. b. carbonelli* and *P. b. bocagei* probably means that these two lizards subspecies evolved in two different refugia during glaciations. We and other authors presume that the great morphological homogeneity reported within mainland populations of *P. b. bocagei* may reflect their recent postglacial expansion from a north-west Galician refugium (Galán 1986; Pérez-Mellado and Galindo 1986; Pérez-Mellado 1997). Thus, former populations of *P. bocagei* might have occupied western Iberia more or less extensively until the climatic changes of

Table 4. Allelic frequencies calculated for all 15 presumptive *loci* in each sample

<i>Locus</i>	Allele	n	<i>P. b. b</i>	<i>P. b. be</i>	<i>P. b. c</i>
			Gerês G	Berlenga B	Peniche P
AAT-1	133		–	–	0.045
	100		0.100	1.000	0.955
	50		0.900	–	–
AAT-2	100	n	20	20	19
	100		1.000	1.000	1.000
AK	100	n	19	20	19
	100		1.000	1.000	1.000
CK	100	n	20	17	15
	100		1.000	1.000	1.000
EST-2	173	n	20	20	19
	147		0.421	–	–
	100		0.579	0.575	0.237
	100		–	0.425	0.763
	53		–	–	–
LDH-1	150	n	20	20	20
	100		0.725	0.350	0.425
LDH-2	100	n	20	20	20
	100		0.275	0.650	0.575
MDH-1	100	n	20	20	20
	100		1.000	1.000	1.000
MPI	111	n	20	19	19
	100		–	–	–
	88		0.975	1.000	0.947
PEP	100	n	20	19	19
	100		0.025	–	0.053
PGD	160	n	20	20	20
	130		1.000	1.000	1.000
	100		–	–	–
PGM-2	139	n	20	20	20
	100		0.056	–	–
SOD	100	n	20	20	20
	100		0.944	–	–
ALB	102	n	20	20	20
	100		–	–	0.025
HB	100	n	20	20	20
	82		1.000	1.000	0.975

n = number of individuals scored successfully for each *locus*.

Table 5. Genetic variability measures at 15 presumptive *loci* in all samples

Subspecies	Sample		H	P 99%	P 95%	A
<i>P. b. bocagei</i>	Gerês	G	0.126	40.0	40.0	1.4
<i>P. b. berlengensis</i>	Berlenga	B	0.080	20.0	20.0	1.2
<i>P. b. berlengensis</i>	Peniche	P	0.095	33.3	26.7	1.3
<i>P. b. carbonelli</i>	Cabo Raso	C	0.127	46.7	40.0	1.6

H = mean heterozygosity, unbiased estimate; A = mean number of alleles per *locus*.
P 99%, P 95% = percentage of polymorphic *loci*, 99% and 95% criteria.

Pleistocene, an event that provoked the fragmentation and sub-speciation of their populations. During the last glacial period of Pleistocene (18–20000 years ago) a large refugium of deciduous oak forests was present in western Iberia, as a consequence

of the warm Gulf Stream (Zagwijn 1992). After the last glacial period (Würm) both *P. b. bocagei* and *P. b. carbonelli* could expand from their refugia. In accordance with the genetic distance found, the hypothesis of different glacial refugia for each

Table 6. Nei's (1978) genetic matrices for four samples of *Podarcis bocagei*: genetic distance above diagonal and genetic identity below diagonal

Subspecies	Sample	G	B	P	C
<i>P. b. bocagei</i>	Gerês	–	0.245	0.250	0.158
<i>P. b. berlengensis</i>	Berlenga	0.783	–	0.011	0.111
<i>P. b. berlengensis</i>	Peniche	0.779	0.989	–	0.080
<i>P. b. carbonelli</i>	Cabo Raso	0.854	0.895	0.923	–

lizard form might explain why so many islands off north-west Galicia were exclusively colonized by *P. b. bocagei* instead, for instance, by *P. hispanicus* which is also found in the adjacent mainland nowadays. Conversely, south-west Galician islands were only colonized by *P. hispanicus* (north-west Iberian form), to the detriment of *P. b. bocagei* or *P. b. carbonelli* (see data from Galán 1985, 1986, 1987; Mateo 1990, 1997; Pérez-Mellado 1997; Sá-Sousa 1995, 1998, 2000). The chance of one island being colonized by nonflying vertebrates might have been dependent on the abundance of the species that occurred in the adjacent mainland synchronically and with the formation of land-bridges (Cox and Moore 1993). Thus, *P. b. bocagei* may occur in the north-west Galician islands now for the simple reason that it colonized them first from its nearby refugium, at the same time as *P. hispanicus* started to colonize south-west Galicia (see Bas 1984; Galán 1986).

Probably, *P. b. carbonelli* had a Würmian refugium somewhere further south, towards the river Douro (see Fig. 1), a hypothesis that might explain its genetic differentiation from *P. b. bocagei*, and also their parapatric distributions at present. The genetic and morphological resemblance found between and within *P. b. berlengensis/P. b. carbonelli* populations seems compatible with the assumption of a recent postglacial separation, whereas other insular *Podarcis* populations were separated by eustatic rising of sea level (see Guillaume and Lanza 1982; Cirer and Guillaume 1986; Ramón et al. 1991; Capula 1994). There are a few islands off the west coast of Portugal on which only *P. b. berlengensis* (e.g. Berlengas islands) or *P. b. carbonelli* (e.g. Baleal, near Peniche; and Pessegueiro off the south-west coast) are present, although nowadays *P. hispanicus* (south-west Iberian form) is the wall lizard species that is more abundant on the adjacent mainland (Sá-Sousa 1992, 1995, 2000; cf. Fig. 1). Both postglacial warming and the progressive aridity in central and southern Iberia confined the areas influenced by the Atlantic during the Quaternary (Zagwijn 1992). This phenomenon should have led to a contraction of the range of *P. b. carbonelli* towards areas where Atlantic conditions still predominated. However, the known distribution of *P. b. carbonelli* shown in Fig. 1 is still patchy, e.g. in Beira Litoral, Sistema Central range and other mountain areas (Sá-Sousa 1995, 1999), as a consequence of the discontinuity of environmental conditions required by this lizard (e.g. Estremadura and south-western coastlands) and the real difficulties encountered when trying to find them in the field.

In general, we found some parallelism between *P. bocagei* and Schreiber's green lizard (*Lacerta schreiberi*) with respect to distribution and climatic requirements. This latter species is also an Iberian-Atlantic endemism whose area has contracted but which still occurs in some isolated locations in south-west Iberia (De la Riva 1987; Brito et al. 1996; Brito et al. 1998).

Finally, taking into account the genetic differentiation between *P. b. bocagei* and *P. b. berlengensis/P. b. carbonelli*, the morphological resemblance of the two latter semi-species or the allopatric distribution (and presumed glacial refugia), we may hypothesize a species rank for *P. carbonelli*, with the Berlenga wall lizard as its insular subspecies, *P. c. berlengensis*. The comparatively lower genetic polymorphism (1.5 to 2 times) that we found for *P. b. berlengensis* could be the result of the founder effect.

The hypothesis proposed above seems a little speculative using only our present data, but it is clearly supported by evidence from mitochondrial DNA analyses on these lizards conducted by James D. Harris (personal communication).

Acknowledgements

We thank all colleagues and technicians who helped us in various ways, either in the field or in the laboratory. Lizards were captured and handled under permit from the Instituto de Conservação da Natureza (ICN). P. Sá-Sousa was supported by a grant of Fundação para Ciência e Tecnologia (FCT) Praxis XXI-BD/5268/95, while L. Vicente and E. G. Crespo were supported by (FCT)PBIC/C/BIA/2095/95.

Zusammenfassung

Genetische und morphologische Verwandtschaftsbeziehungen bei der Berlenga Mauer-Eidechse (Podarcis bocagei berlengensis: Lacertidae)

Sechs biometrische Merkmale und 15 Allozym-Loci wurden bei den drei bekannten Unterarten von *Podarcis bocagei* untersucht. Im Gegensatz zu den biometrischen Ergebnissen, erweist sich *P. b. berlengensis* genetisch näher verwandt mit *P. b. carbonelli* (Nei's D = 0.074–0.106) als mit der nominellen Unterart *P. b. bocagei*. Die biometrischen Ergebnisse sind wahrscheinlich durch den relativen Großwuchs von *P. b. berlengensis* verzerrt. Die postglaziale Besiedlung von zwei verschiedenen Refugien könnte vielleicht die genetische Differenzierung zwischen *P. b. bocagei* und *P. b. carbonelli* (Nei's D = 0.158) erklären. Sie könnte auch die bekannte Kolonisation dieser Formen über küstennahe Inseln im Westen der Iberischen Halbinsel und deren gegenwärtige Verbreitung im Norden (Galizien, N Portugal) bzw. im Süden (Zentral-Portugal, W Sistema Central) des Flußtales des Douro verständlich machen.

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Appendix 1

Descriptive statistics for six biometric characters measured in four samples of *Podarcis bocagei* (m, males; f, females). Biometric characters are described in the text

		<i>Podarcis b. bocagei</i>		<i>Podarcis b. berlengensis</i>		<i>Podarcis b. carbonelli</i>			
		Gerês G		Berlenga B		Peniche P			
n	f	26		12		12		13	
	m	29		24		24		27	
Variables	x	SD	x	SD	x	SD	x	SD	
SVL	f	55.12	3.44	56.25	4.39	56.17	2.44	50.38	2.02
	m	60.07	1.98	61.06	2.7	54.02	2.99	51.81	2.4
HL	f	11.22	0.54	11.48	0.44	11.2	0.41	10.68	0.34
	m	13.98	0.63	14.46	0.64	12.96	0.61	12.55	0.55
HW	f	5.53	0.41	5.64	0.28	5.45	0.24	5.16	0.23
	m	6.71	0.33	7.2	0.45	6.26	0.43	6.19	0.35
HD	f	5.08	0.29	5.37	0.3	5.19	0.21	4.8	0.22
	m	6.12	0.37	6.78	0.46	6.14	0.45	6.05	0.4
FLL	f	16.28	0.83	14.93	1.59	15.24	0.6	13.67	0.58
	m	19.37	0.92	19.14	0.84	17.45	1.32	16.14	0.68
HLL	f	24.43	1.19	23.58	1.71	23.65	1.26	20.2	0.82
	m	30.94	1.59	30.05	1.62	27.11	1.66	24.91	1.12

Appendix 2

Protein systems studied in the electrophoresis and respective *loci* and buffer systems

Protein system	E.C. number	<i>Loci</i> scored	Organic tissues	Buffer system
Adenylate kinase	2.7.4.3	AK	L	I
Aspartate aminotransferase	2.6.1.1	AAT-1,2	H, M	I
Creatine kinase	2.7.3.2	CK	L	I
Esterases	3.1.1.1/2	EST-2	L	II
Lactate dehydrogenase	1.1.1.27	LDH-1,2	H, M	I
Leucyl-tyrosine peptidase	3.4.1.1	PEP	L	III
Malate dehydrogenase	1.1.1.37	MDH-1	L	II
Mannose phosphate isomerase	5.3.1.8	MPI	L	III
Phosphogluco-mutase	2.7.5.1	PGM-2	L	I
Phosphogluconate dehydrogenase	1.1.1.43	PGD	L	I
Superoxide dismutase	1.15.1.1	SOD	L	I
Albumin	–	ALB	B	IV
Hemoglobin	–	HB	B	IV

B = blood; H = heart; L = liver; M = muscle.

I = tris-citrate pH 8.0; II = tris-citrate pH 6.7; III = tris-borate-LiOH pH 8.3; IV = tris-barbital pH 8.8.